Food Standards Agency
food.gov.uk

Modelling framework to quantify the risk of AMR exposure via food products: example of chicken and lettuce

January 2022

Authors: Ausvet Europe (Céline Faverjon, Angus Cameron), Safoso (Marco De Nardi)

DOI: 10.46756/sci.fsa.qum110

Contents

List of figures

List of tables

1. Acknowledgements

The project consortium (AUSVET and SAFOSO) would like to thank Prof. Jaap A. Wagenaar (Utrecht University) for his contribution and the members of the advisory panel Dr. Lucie Collineau (French Agency for Food, Environmental, and Occupational Health and Safety, ANSES), Dr. Daniel Parker (veterinary advisor to the British Poultry Council) and Prof. Jim Monaghan, (director of the Fresh Produce Centre at Harper Adams University) who all provided advice on the models and reviewed the interim reports and the representatives of the UK industry who helped to define the critical steps in the risk pathways.

SAFOSO would like to thank Violeta Munoz for her contributions during the initial phase of the project.

AUSVET would like to acknowledge the technical inputs of Dr. Anne Meyer and Dr. Rohan Sadler during the development of the model.

The consortium is also grateful to the project team of the Food Standard Agency (FSA) for the profitable discussions and technical exchanges and feedback.

2. Lay Summary

Antimicrobial resistance (AMR) is a major global public health challenge. It is a complex issue driven by a variety of interconnected factors enabling microorganisms to survive antimicrobial treatments thus making such infections more difficult to treat. Unless urgent action is taken to reduce AMR globally, the number of deaths caused by AMR is predicted to increase to an estimated 10 million each year by 2050. Addressing the public health threat posed by AMR is a national strategic priority for the UK and for FSA. The food chain of various food products (i.e., chicken, vegetables) may be important transmission route of antimicrobial resistant bacteria to humans.

AMR related to bacteria present in food poses a health risk for UK consumers. The current project developed an advanced tool (for risk assessment) that will help FSA to

assess the risk for UK consumers with regards to AMR associated to bacteria in food. To test the adaptability of the tool to various pathogens and different food production chains, two combinations of microorganisms (*E. Coli* and *Campylobacter* spp.) and two very different food productions were used: the chicken and the lettuce production chains.

The results showed that the model outputs were consistent with the existing scientific literature and therefore provided reliable results. One of the major strengths of the tool is certainly represented by its adaptability and flexibility to test new microorganisms and/or to change some attributes, steps of the food value chains.

During the development of the tool, it was clear that for some of variables used in the tool there was scarce availability of data especially for a number of AMR-related parameters. However, this did not represent a major obstacle towards the development of the tool which was the main objective of the project. Still, future studies should focus on improving the amount of data available on these parameters to be able to obtain more accurate outputs from the tool particularly for antimicrobial resistant microorganisms.

3. Executive Summary

Introduction and objective

Antimicrobial resistance (AMR) is a major global public health challenge. It is a complex issue driven by a variety of interconnected factors enabling microorganisms to survive antimicrobial treatments thus making such infections more difficult to treat. Unless urgent action is taken to reduce AMR globally, the number of deaths caused by AMR is predicted to increase to an estimated 10 million each year by 2050. Addressing the public health threat posed by AMR is a national strategic priority for the UK and for FSA.

The food chain is one important transmission route of antimicrobial resistant bacteria to humans. AMR related to hazards present in food poses a health risk for UK consumers. The presence of antimicrobial resistant genes (ARGs) in food can amplify the burden of foodborne AMR in the UK population. Quantifying consumers' exposure to specific AMR bacteria and ARGs from different food sources can elucidate the relative importance of food production value chains on the AMR transmission.

To protect UK consumers, the Microbiological Risk Assessment team in FSA is required to provide microbial risk assessments with a quick turnaround. However due the complex nature of AMR, providing quantitative AMR risk assessments can be both time consuming and labour-intensive. To assist with the creation of quantitative risk assessments in the short timescales required, there is a need to develop easily adaptable 'off-the-shelf' modular farm-to-fork AMR templates for key products and production processes such as for AMR bacteria in poultry and fresh produce.

The principal objective of this project was the development of a stochastic and modular modelling framework, and its user-friendly interface, to quantify consumer's exposure to AMR bacteria and ARGs that can be adapted to different microorganisms and ARGs in different value chains. To fulfil the principal objective, the project team agreed with FSA to develop the modelling framework using as case studies two important production systems in the UK, chicken and lettuce value chains.

The modelling framework

The prevalence and concentration of antimicrobial resistant bacteria possessing AMR genes on a unit of interest (i.e., either birds, or lettuce) originating from positive and negative production units (i.e., either a poultry flock or production field) was followed from farm-to-fork. For each value chain (chicken and lettuce) the modelling framework is organized in 4 distinct modules that represent steps in the risk pathway:

- Production module: includes all the relevant on-farm practices having an influence on the probability of presence of bacteria carrying AMR genes in food.

- Processing module: includes all the food transformation processes from raw product to manufactured product including packaging and their associated probabilities of reducing or increasing bacteria load and AMR genes contamination in food.

- Post-processing module: focuses on transport and storage practices at retail having an influence on bacteria load and AMR genes contamination level

- Home preparation module: includes the key consumers behaviour (for example, washing lettuce or cooking meat) having an influence on the final AMR exposure which is a function of the prevalence and level of contamination of food units at the time of consumption.

Cross contamination between positive and negative production units was assumed to occur only in the production and processing modules. The modelling framework ends with estimates of the probability of consumption and amount consumed of antimicrobial resistant bacteria via two routes: direct ingestion of contaminated product, and ingestion by cross-contamination.

The case studies

The chicken and lettuce value chains were investigated by means of a literature review and a stakeholder elicitation workshop with the UK poultry and lettuce industry representatives which contributed to define the risk pathways of the models and to discuss the effectiveness of intervention measures influencing AMR and ARG in bacteria contamination in each specific food chain.

To test the adaptability of the modelling framework to different pathogens and value chains, two combinations of microorganisms and ARGs (defined as AMR1 and AMR2 in this project) were selected to test and validate the models over 3 case studies defined by a combination of the following elements: a type of food product, a microorganism, and a resistance gene.

- The first case study focused on the microorganism *E. coli*, the resistance gene “ampC beta-lactamase gene CMY-2”, and the food product “fresh skin off portioned chicken”.

- The second case study focused on the microorganism *Campylobacter spp*., and the food product “fresh skin off portioned chicken”. It was initially planned to investigate *Campylobacter spp*. carrying the mutated GyrA gene. However, based on the output of the initial literature review and the results of the first case study, the amount of data currently available on *Campylobacter spp*. carrying GyrA gene was considered not sufficient to properly validate the results of the model with published evidence.

- The third case study looked at the risk of consumer exposure to *E. coli* and the food product “pre-washed outdoor grown bagged lettuce”. For the same reasons as for the second case study, it was decided together with the FSA to only investigate the risk of bacteria exposure and not the risk of AMR gene exposure.

Because of the lack of relevant data on genotypic antimicrobial resistance, two out of three of the cases studies were only based on phenotypic data. However, it should be noted that the model was constructed with sufficient details such that new information obtained from whole genome sequencing could be integrated in future iterations as they become available.

The variables in the risk assessment framework

Each module was built with four different types of variables: selected variables, estimated variables, calculated variables, and output variables. The relationship between the different set of variables and the exact list of variables used in the model depends on the food production chain investigated. The numeric value of some of the variables used in the model depends on each specific case study investigated.

The selected and estimated variables represent the model input variables: the **selected variables** are variables defined by the model user before running the analysis. They are used to define a particular model scenario, including the value chain and the hazard risk pathway considered in the risk analysis (for example, food product, farm typology, microorganism and resistance gene investigated). The **estimated variables** are estimated based on the literature. They are often expressed as probability distributions.

The **calculated variables** are defined as variables calculated based on the value of the selected and estimated variables previously defined. The **output variables** are a special kind of calculated variables used to estimate the risk of AMR bacteria/gene exposure at the end of each module. They are the key variables used as results of the risk analysis. Their value is presented in terms of probability distribution, median and 95% prediction intervals. As key variables of interest, the output variables are also the target for the correlation analysis.

Computational aspects and correlation analysis

The stochastic model was built in R (R Development Core Team 2019) and uses several R packages to simulate probability distribution, compute and visualize the results. The framework allows the model user to perform a number of Monte Carlo simulations of their choice. Briefly, Monte Carlo simulation randomly samples values from each estimated variable distribution and provides outputs as distributions for each parameter.

The modelling framework supports a global sensitivity analysis, or correlation analysis, to evaluate the impact of variability and uncertainty in the estimated variables on the uncertainty in the output variables. The correlation between the values of the estimated variables and the outcome variables was calculated using Spearman's rank correlation coefficients. Because this is a stochastic model, all the outcome variables are probability distributions.

Results and critical aspects

The specific results for each case study are described in Annex 4, 5 and 6 and not summarized here.

The validation process for both models, through comparison with available evidence and the internal and external peer reviewing process confirmed the robustness and quality of the overall modelling framework. The results of the internal model validations showed that the model outputs were consistent with the existing scientific literature. In addition, the results of the external quality check done in parallel by external reviewers and FSA confirmed that the models developed were based on the latest scientific consensus and available data.

One of the major strengths of the framework is certainly represented by its adaptability and flexibility to test new microorganisms/genes and/or to change some attributes, steps of the value chain and/or to revise the functions currently describing the correlation between variables. The model framework was constructed with sufficient details such that new information obtained from whole genome sequencing or related to other influential variables can be integrated in future iterations as they become available. This is particularly important as the lack of relevant data on genotypic antimicrobial resistance was a critical challenge in the project.

Poor data availability for some estimated variables in each case studies was an important limitation of this project. These limitations reflect the fact that data currently available in the literature for the 3 case studies were often ambiguous, inconsistent between studies, or too sparse especially for a number of AMR-related parameters. However, if the uncertainty in estimated variables may lead to less robust model outputs, this did not represent a major obstacle towards the development of the modelling framework in itself as shown in this report. The values of these estimated variables could be easily updated

later on by future model users, as soon as better data become available. Future studies should focus on improving the amount of data available on these parameters to be able to obtain more accurate risk estimates particularly for antimicrobial resistant microorganisms.

The implementation of experts elicitation for the most uncertain parameters, in the short term, would help to overcome major data limitations. To this aim, the results of the correlation analysis can be used to help future model users to identify which variables have the highest influence on the model outcome and where to prioritize resources to collect or generate better data and thus obtain more reliable model outcomes.

4. Glossary

Abbreviation	Definition
AMR	Antimicrobial resistance
ARGs	Antimicrobial resistant genes
AMR1	*E. coli* and the ampC beta-lactamase gene *CMY-2*
AMR2	*Campylobacter* spp. and the mutated *GyrA gene*
EU	European Union
FSA	Food Standard Agency
OR	Odds Ratio
QRA	Quantitative Risk Analysis
UK	United Kingdom
WHO	World Health Organization
SD	Standard deviation
CFU	Colony-forming unit

5. Introduction

Antimicrobial resistance (AMR) is a major global public health challenge. AMR refers to microorganisms that become resistant to antimicrobial substances, such as antibiotics to which they were previously sensitive. The overuse of antibiotics has led to a dramatic increase of resistant patterns within the bacteria community jeopardizing veterinary and human medicine.

Unless urgent action is taken to reduce AMR globally, the number of deaths caused by AMR is predicted to increase to an estimated 10 million each year by 2050 (O'Neill 2014). Addressing the public health threat posed by AMR is a national strategic priority for the UK. It has led to the Government publishing both a 20-year vision of AMR ("UK 20-Year Vision for Antimicrobial Resistance" 2019) and a 5-year (2019 to 2024) AMR National Action Plan (NAP) (HM Government 2019), which sets out actions to slow the development and spread of AMR with a focus on reduction in the use of antimicrobials.

AMR is a complex issue driven by a variety of interconnected factors enabling microorganisms to survive antimicrobial treatments thus making such infections more difficult to treat. The food chain is one important transmission route of AMR bacteria to humans. Food contamination might occur during preharvest and/or postharvest stages, depending on the food type. For example, leafy greens might be contaminated with AMR bacteria at a pre-harvest stage through contaminated manure, soil or wildlife vectors and at post-harvest stage during food preparation. In the case of animal food products, meat has been identified as one of the main carriers of AMR bacteria. The contamination of meat might occur at the slaughterhouse in different processes through cross-contamination but also at consumer level due to inappropriate food handling.

Microbiological foodborne disease (FBD) in UK are responsible for both public health and financial burden on the society. According to a FSA project report published in 2020 (FSA 2020), in 2018 there were estimated to be 2.4 million FBD-related cases in the UK. Norovirus accounts for the highest number of cases at around 383,000, followed by Campylobacter and Clostridium perfringens with around 299,000 and 85,000 cases respectively. Listeria monocytogenes has the least number of estimated cases at 162 a

year, but has the highest proportion of fatalities (26 fatalities out of a total of 162 cases).. The current Foodborne Disease Strategy has been primarily focused on *Campylobacter spp* and *Listeria monocytogenes* to reduce the burden of disease in the country (FSA 2011; 2015). However, a new Foodborne Disease Strategy, is expected to be published soon.

One recent study identified *E. coli, Shigella* spp*., Salmonella enterica* and *Listeria monocytogenes* as the highest occurring AMR food-borne pathogens in the UK and chicken meat as the major meat carrier of AMR in the country (Yang et al. 2020). Importantly, a systematic review conducted in 2016 concluded that the data available on the AMR bacteria prevalence in food produced in the UK was limited (Willis et al. 2018).

AMR in food poses a health risk for UK consumers. The presence of antimicrobial resistant genes (ARGs) in food can amplify the burden of foodborne AMR in the UK population. Quantifying consumers' exposure to specific AMR bacteria and ARGs from chicken and lettuce can elucidate the relative importance of two different value chains on the AMR transmission.

Microbiological Risk Assessment team in FSA focuses on microbial risk in food, including that of antimicrobial resistance, and is required to provide microbial risk assessments with a quick turnaround. However due the complex nature of AMR, providing quantitative AMR risk assessments can be both time-consuming and labour-intensive.

To assist with the creation of quantitative risk assessments in the short timescales required, there is a need to develop easily adaptable 'off-the-shelf' modular farm-to-fork AMR templates for key products and production processes such as for AMR bacteria in poultry and fresh produce.

5.1 Project objectives

The principal objective of this project is the development of a stochastic and modular modelling framework to quantify consumer's exposure to AMR bacteria and ARGs that can be adapted to different microorganisms and ARGs in different value chains.

The specific objectives were:

1. Identification of the critical risk pathways for exposure to ARG through the food production chain of chicken meat and lettuce
2. Development of a modular off the shelf quantitative risk assessment model for exposure to ARG via chicken meat
3. Development of a modular off the shelf quantitative risk assessment model for exposure to ARG via lettuce
4. Development of user-friendly interface for the implementation of quantitative risk assessment models
5. Development of training and support material for FSA staff, including options for customization of models to other food products

5.2 Rationale of the experimental approach

To fulfil the principal objective, the project team agreed with FSA to develop the modelling framework using as case studies the two UK production systems, chicken and lettuce value chains. The framework is based on four modules including all critical production steps and intervention measures in the food chain (i.e., production, processing, post-processing and home-preparation).

The production chains were investigated by means of a literature review and a stakeholder elicitation workshop with the UK poultry and lettuce industry representatives. Representatives from the UK industry contributed to define the risk pathways of the models and to discuss the effectiveness of intervention measures influencing AMR and ARG in bacteria contamination in the food chain.

The model on the chicken production chain is based on an existing quantitative microbial risk assessment model (QMRA) model for *Campylobacter* spp. in broiler chicken developed by (WHO and FAO 2009) and adapted by (Collineau et al. 2020) which described a farm-to-fork QMRA of foodborne AMR, along the chicken production chain, to quantify the consumers' exposure to *Salmonella Heidelberg* resistant to third-generation cephalosporins. The model on the lettuce production chain is based on an existing QMRA model for *E. coli* in lettuce developed by Njage and Buys (2017) and

Pang et al. (2017). Other existing models were used to inform specific modules in both production chains. Both models were adapted to the specificities of UK industry through the inclusion of outputs from the literature review and inputs provided by relevant stakeholders of the UK industry gathered through the elicitation workshop.

To test the adaptability of the modelling framework, two combinations of microorganisms and ARGs (defined as AMR1 and AMR2 in this project and further described in section 5.3 of this report) were selected to test and validate the models. The first combination (AMR1) is represented by the microorganism *E. coli* and the ampC beta-lactamase gene *CMY-2*. This case study was tested for both chicken and lettuce. The second combination (AMR2) is represented by *Campylobacter jejuni* and the mutated *gyrA* gene. AMR-2 was tested only for the chicken value chain in order to test the ability of the developed modelling framework to be adapted to other pathogen and/or gene of interest. This approach helped identify the adjustments needed to ensure transferability to different microorganisms and value chains.

As part of the external quality assurance, an international advisory board, with expertise on quantitative food safety risk assessment, AMR and the UK value chains of chicken and lettuce, has assisted the project team throughout the project and actively contributed to identify critical aspects in the UK value chains and identifying critical hurdles of the model under real conditions.

5.3 Microorganisms

The critical risk pathways for AMR exposure were investigated for two microorganisms and resistance gene: the microorganism *Escherichia coli* (*E. coli*) and the ampC beta-lactamase gene CMY-2 (AMR1), and *Campylobacter* spp. and the mutated GyrA gene (AMR2).

5.3.1 *Escherichia coli* and AmpC β-lactamase gene CMY-2 (AMR 1)

E. coli belongs to the large Enterobacteriaceae family of gram-negative bacteria and is an important cause of intestinal and extraintestinal diseases in humans worldwide (CDC 2020). Although the majority of *E. coli* strains are harmless to humans, some of them such as *E. coli* O157 can cause severe disease (FSA 2018; WHO 2018).

E. coli is a ubiquitous bacteria, present in the microbiota of humans and warm-blooded animals (Miranda et al. 2008). The intestinal tract of chicken is usually colonized by *E. coli* during the first days of life (Ballou et al. 2016) and although the majority of *E. coli* strains are harmless, some of them can cause diseases in broilers (Mellata 2013). Poultry meat has the highest overall *E. coli* contamination levels and usually, the *E. coli* strains isolated from poultry shows higher multidrug resistant levels than in other meats (Manges and Johnson 2012). Faecal *E. coli* from poultry can be transferred to humans directly. The transmission of resistant clones and resistance plasmids of *E. coli* from poultry to poultry farmers has been described (van den Bogaard 2001).

E. coli is used as an indicator for faecal contamination of enteric pathogens in food and also as an indicator bacterium for AMR in food-producing animals. *E. coli* has shown ability to acquire AMR faster than other bacteria (Miranda et al. 2008). In the UK, *E. coli* imposes one of the least burden of food-borne diseases (Daniel et al. 2018).

E. coli was identified as a Hygiene Criterion at primary production of leafy greens and could be considered for validation and verification of Good Agricultural Practices (GAP) and Good Hygiene Practices (GHP). On the basis of this, growers should take appropriate corrective actions to improve the production processes. A Process Hygiene Criterion for *E. coli* in leafy green packaging plants or fresh cutting plants can give an indication of the degree to which collectively GAP, GHP, GMP or HACCP programs have been implemented (EFSA 2014).

The gene CMY-2 is the most common and well-documented AmpC β-lactamase in human and animal bacteria (Deng et al. 2015; Koga et al. 2019). CMY-2 encodes resistance to β-lactam antibiotics, including cephalosporins, one of the most clinically important medicines in human and veterinary medicine (Li et al. 2007). The simultaneous resistance pattern to amoxicillin-clavulanic acid, ceftiofur and cefoxitin is known as A2C

and is generally caused by the presence of CMY-2 gene (Caffrey et al. 2017). The gene CMY-2 is normally located in a plasmid, which facilitates its dissemination to other bacteria through horizontal gene transfer (Deng et al. 2015). Resistant *Enterobacteriaceae* are in the critical priority pathogen list of the WHO (WHO 2017).

Broilers and broiler meat may be highly contaminated with AmpC beta-lactamase producing *E. coli* and therefore are considered a source for human infection (Dierikx et al. 2013). Studies implemented in different European countries have shown an average proportion of about 42% (Minimum 12.2%, Maximum 89%) of CMY-2 in ESBL/AmpC *E. coli* positive samples (Ewers et al. 2012). In the UK, data on AmpC-producing *E. coli* is regularly collected in broilers at the slaughterhouse and retail level as part of the monitoring program (EC 2017). The latest results showed a prevalence of 6.1 % of AmpC-producing *E. coli* in broilers, with a decreasing trend (-70%) from 2016 to 2018 (EFSA/ECDC 2020).

5.3.2 Campylobacter spp and mutated gyrA gene

Campylobacter spp. are now the leading cause of zoonotic enteric infections in most developed and developing countries. *Campylobacter* spp. are one of the most prevalent food-borne pathogens in the UK (FSA 2015; 2011). Several reports provide in-depth information of the latest available knowledge on key characteristics of *Campylobacter* spp. (for example, resistance to high/low temperature). This information is thus not repeated in this report, which rather focuses on identifying the critical risk pathways for *Campylobacter* spp. exposure.

The mutated *gyrA* gene encodes resistance to fluoroquinolones, and fluoroquinolone-resistant *Campylobacter* spp. is on the high priority antibiotic list of the WHO (Jesse et al. 2006; Sproston, Wimalarathna, and Sheppard 2018; WHO 2017). The gene is generally located in the chromosome and the presence of a point mutation has been identified as the main mutation responsible for fluoroquinolone resistance in *Campylobacter* spp. in both *C. jejuni* and *C. coli*) (Bachoual et al. 2001; Jesse et al. 2006; EFSA 2020; Payot et al. 2006; Carattoli, Dionisi, and Luzzi 2002). Fluoroquinolone-resistant *Campylobacter* infections are however not more severe than antimicrobial susceptible infections (Wassenaar, Kist, and de Jong 2007).

Fluoroquinolone-resistant *Campylobacter* has been detected worldwide in chicken faecal samples and in retail chicken meat.

In the UK, the presence of fluoroquinolone resistant *C. jejuni* in chicken meat has shown an increase in the last few years from 21% in 2007-2008 to 49% in 2014-2015 (Sproston, Wimalarathna, and Sheppard 2018) with a widespread acquisition of antimicrobial resistance and with evidence for clonal expansion of resistant lineages in retail poultry (Wimalarathna et al. 2013).

6. Materials and Methods

6.1 Model development

The risk pathways of antimicrobial resistance (AMR) exposure from farm to consumer via chicken meat and lettuce used to develop the model structure were defined using as examples two microorganism and resistance genes: the microorganism *E. coli* and the ampC beta-lactamase gene *CMY-2,* and *Campylobacter* spp. and the mutated *GyrA* gene.

For both value chains, the definition of the risk pathways for AMR was based on:

- Literature review. Preference was given to publications on the poultry and lettuce value chains in the United Kingdom (UK) or European Union (EU), but data from other countries and/or food value chains has also been reviewed when deemed relevant. The focus was placed on the parameters that can be used to model the influence of production and processing steps on the abundance of bacteria and AMR genes in food.

- Stakeholder's consultation. Two parallel online workshops (i.e., one for each value chain) were organized with key representatives of the UK poultry and lettuce industries in order to discuss the results of the literature review and provide recommendation for the future modelling framework.

The main results of this first part of the work was that there is an extensive amount of data available on bacterial contamination for *E. coli* and *Campylobacter* spp. in meat and at various steps in the production chain but that information on the risk of transmission of AMR throughout the food chain remains however scarce. AMR information related to the production module could be found in the literature, but almost none could be identified in the other modules. The effect of various food processing steps on the risk of AMR remains largely unknown.

The results also highlighted the fact that the chicken meat and lettuce value chains in the UK can both fit in a simple common structure made of four modules (i.e., production module, processing module, post-processing module, and home preparation module). However, important differences between the two value chains can be observed and a specific model structure must be developed for each of them.

The full results of the literature review and stakeholder's consultation are presented in Appendix 1.

6.2 Modelling framework

6.2.1 Overview

The modelling framework is organized in 4 distinct modules that represent steps in the risk pathway:

- *Production module*: includes all the relevant on-farm practices having an influence on the probability of presence of bacteria carrying AMR genes in food.
- *Processing module*: includes all the food transformation processes from raw product to manufactured product including packaging and their associated probabilities of reducing or increasing bacteria load and AMR genes contamination in food.
- *Post-processing module*: focuses on transport and storage practices at retail having an influence on bacteria load and AMR genes contamination level
- *Home preparation module*: includes the key consumer behaviour (for example, washing lettuce or cooking meat) having an influence on the final AMR exposure which is a function of the prevalence and level of contamination of food units at the time of consumption.

Each module represents a part of the production chain and is connected to the others as shown in Figure 1. The prevalence and concentration of bacteria possessing AMR genes on a unit of interest (i.e., either birds, or lettuce) originating from positive and negative production units (i.e., either a poultry flock or production field) was followed from farm-to-fork. Cross contamination between positive and negative production units was assumed to occur only in the production and processing modules. To highlight this assumption and better visualize the steps where cross contamination can occur, Figure 1 presents separately the two different type of production units in the production and processing modules. The modelling framework ends with estimates of the probability of consumption and amount consumed of antimicrobial resistant bacteria via two routes: direct ingestion of contaminated product, and ingestion by cross-contamination (cf Figure 1).

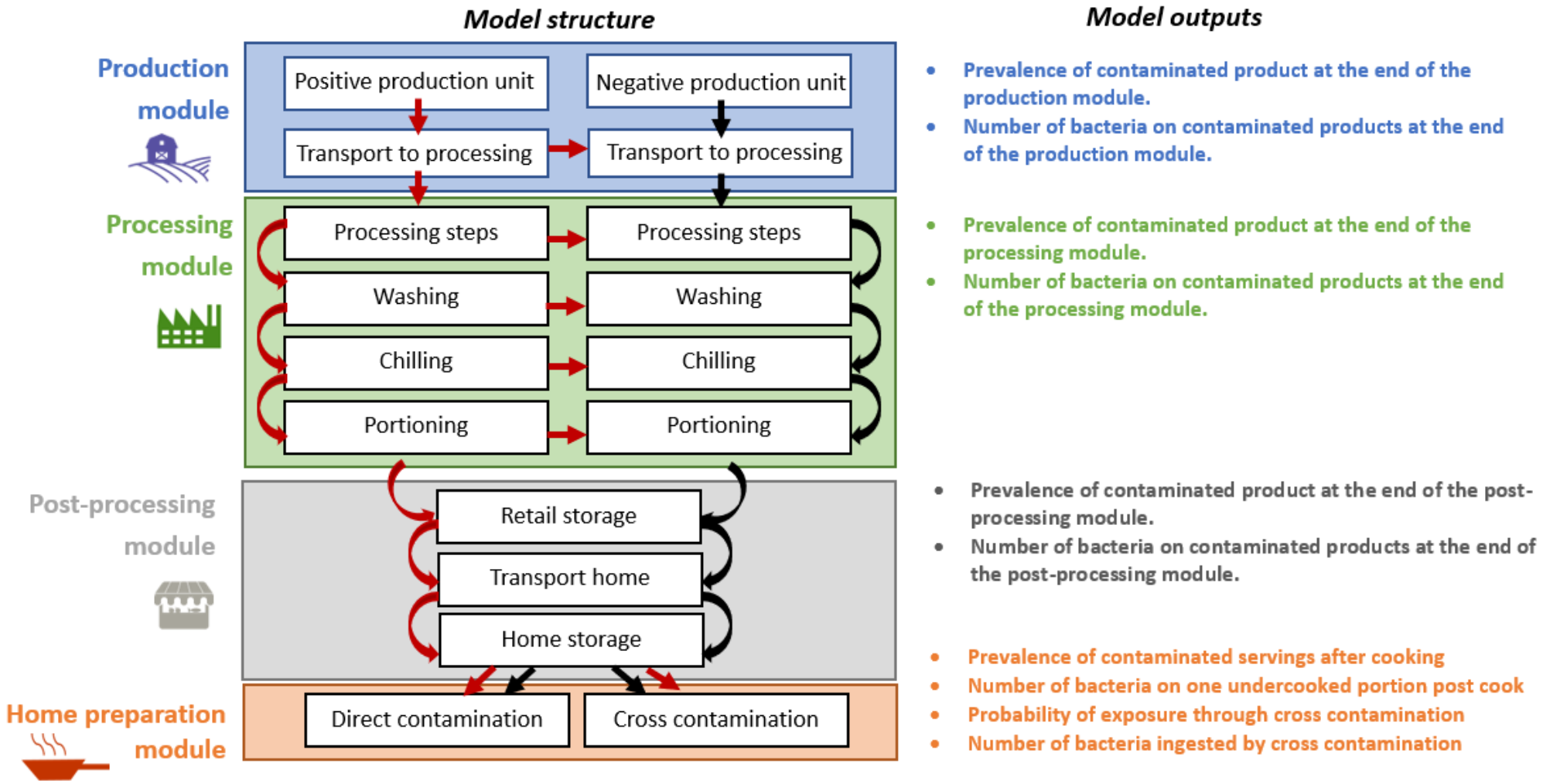


Figure 1: overall model structure and key model outputs for the chicken value chain. Red arrows = positive flocks, Black arrows = negative flocks. Horizontal red arrows = risk of cross-contamination between positive and negative flocks

Each module was made of four different types of variables: *selected variables*, *estimated variables, calculated variables,* and *output variables.* The definitions of each type of variables are provided in the sections below. Selected and estimated variables represent the model input variables.

The relationship between the different set of variables and the exact list of variables used in the model depend on the food production chain investigated. The numeric value of some of the variables used in the model depends on each specific case study investigated.

6.2.2 Variables

6.2.2.1 Naming convention

The following convention has been defined for variable names to improve model readability and future modifications:

- C = variables related to bacterial count data
- N = variables related to other count not related to bacteria count
- Prev = variables related to prevalence estimation
- P = variables related to probability distribution
- Prop = variables related to proportion
- F = variables related to increasing or decreasing factor. The sign used by the model user in the equation defines the direction of change
- B = variables with binary output
- T = variables related to temperature
- Time = variables related to time

All prevalence and probability distributions must be reported as proportion and not as percentage data.

In addition, calculations applied to both positive (“p”) and negative (“n”) production units are presented as single equations. Throughout this document, the infection status of a

unit by AMR-gene-carrying bacteria is represented by the subscript “i” which can take either of the values:

- “p”: originating from a positive production unit
- “n”: originating from a negative production unit

6.2.2.2 Selected variables

In this modelling framework, *selected variables* are variables defined by the model user before running the analysis. They are used to define a particular model scenario, including the value chain and the hazard risk pathway considered in the risk analysis (for example, food product, farm typology, microorganism and resistance gene investigated). Pre-defined categories for each selected variable are available in the modelling framework and are reported in Table 1.

Table 1: List of selected variables included in the modelling framework.

Type of variable	Selected variables description	Variable name	Pre-defined categories
General	Type of food product investigated in the risk analysis	*Product*	• Chicken • Lettuce
General	Packaging of the food product	*Pack_type*	• No packaging • MAP
General	Product sold portioned or not.	*Product_cut*	• Whole product (for example, whole carcass, whole lettuce) • Portion (for example, chicken breast, leafs)

Type of variable	Selected variables description	Variable name	Pre-defined categories
Variables only applicable if *Product* = *"Chicken"*	Product sold with or without skin.	*Meat_skin*	• Skin off • Skin on
Variables only applicable if *Product* = *"Chicken"*	Scalding type used at the slaughterhouse	*Scalding_type*	• Soft • Hard
Variables only applicable if *Product* = *"Lettuce"*	Prewashed salad or not	*Product_wash*	• Pre-washed • Not pre-washed

6.2.2.3 Estimated variables

Estimated variables are estimated based on the literature. They are often expressed as probability distributions. For example, the minimum growth temperature of a microorganism was estimated based on available published evidence for the microorganism investigated in the case studies, *E. coli* or *Campylobacter*. The estimated variables are the variables that the future model user will be able to easily adapt according to his/her needs and to the most recent knowledge available.

Detailed lists of the *estimated variables* used in each module for the chicken value chain are available in the following sections of this report. Pre-defined estimates of *estimated variables* have been defined for the case study investigated in this report.

Each *estimated variable* has one attribute named "Domain". The objective is to facilitate future model updates. Two categories are defined:

- “Bacteria” = the estimated variable is specific to the *microorganism or resistance gene* of interest (for example, prevalence of the pathogen, minimal growth temperature, or bacterial concentration in caeca content),
- “Other” = the estimated variable is not specific to the microorganism or resistance gene of interest. The variable is related to the *production chain or food product* of interest (for example, average size of carcasses, or average cooling temperature).

6.2.2.4 Calculated variables

Calculated variables are defined as variables calculated based on the value of the *selected* and *estimated variables* previously defined. For example, the number of bacteria on a portion of chicken meat after X days spent in a fridge at Y °C was calculated based on the estimated variable “minimum growth temperature”. Detailed lists of calculated variables used in each module for each value chain are available in the next sections the report.

6.2.2.5 Output variables

These variables are a special kind of calculated variables used to estimate the risk of AMR bacteria/gene exposure at the end of each module. They are the key variables used as results of the risk analysis. Their value is presented in terms of probability distribution, median and 95% prediction intervals. As key variables of interest, the output variables are also the target for the correlation analysis. A list of output variables is available in Table 2 and Figure 1.

6.2.3 Calculations

The stochastic model was built in R (R Development Core Team 2019) and uses several R packages to simulate probability distribution, compute and visualize the results. The list of packages and versions of packages used in the model and in the graphical interface are reported in the section describing the technical characteristics of the user interface.

The framework allows the model user to perform a number of Monte Carlo simulations of their choice. Briefly, Monte Carlo simulation randomly samples values from each *estimated variable* distribution and provides outputs as distributions for each parameter.

Table 2: List of output variables*. If i = n, the product comes from a negative production unit. If i = p, the product comes from a positive production unit

*Not all output variables apply to every production chain. For example, cooking in the home-preparation module, does not apply to the lettuce production chain

Module	Output variables description	Variable name
Production	Prevalence of unit of interest contaminated with AMR-gene-carrying bacteria at the end of the production module. In this module, a unit of interest can be a live bird or a lettuce.	*Prev_prod_i*
Production	Number of AMR-gene-carrying bacteria per unit of interest at the end of the production module. In this module a unit of interest can be a live bird or a lettuce.	*C_prod_i*
Processing	Prevalence of unit of interest contaminated with AMR-gene-carrying bacteria at the end of the processing module. In this module a unit of interest can be a whole carcass, a portioned chicken, a whole lettuce, or lettuces leaves.	*Prev_proc_i*
Processing	Number of AMR-gene-carrying bacteria per products at the end of the processing module. In this module a product can be a whole carcass, a portioned chicken, a whole lettuce, or lettuces leaves.	*C_proc_i*

Module	Output variables description	Variable name
Post processing	Prevalence of product contaminated with AMR-gene-carrying bacteria at the end of the post-processing module. In this module, the definition of a product is the same than in the processing module.	*Prev _pproc_i*
Post processing	Number of AMR-gene-carrying bacteria per products at the end of the post-processing module. In this module, the definition of a product is the same than in the processing module.	*C_pproc_i*
Home preparation	Prevalence of servings contaminated with AMR-gene-carrying bacteria after cooking	*Prev_home_cook_i*
Home preparation	Probability of exposure to AMR-gene-carrying bacteria through cross contamination	*P_home_cc_i*
Home preparation	Number of AMR-gene-carrying bacteria per product portion post cook	*C_home_cook_i*
Home preparation	Number of AMR-gene-carrying bacteria ingested by cross contamination	*C_home_cc_i*

6.3 Application of the modelling framework to the chicken meat production chain

This section of the report shows how the modelling framework can be used to investigate the risk of consumer exposure to antimicrobial resistance via the chicken meat production chain. The overall model structure is presented in Figure 2 and is based on an existing QMRA model for *Campylobacter* spp. in broiler chicken developed by (WHO and FAO 2009) and adapted by Collineau et al. (2020) for *Salmonella* Heidelberg to follow the population-level prevalence and individual bird level of

contamination throughout the model. In some cases, other existing models were used to inform specific equations as described in the following sections.

The details of the chicken model including list of estimated and calculated variables and correlations between variables are presented in Appendix 2.

6.4 Application of the modelling framework to the lettuce production chain

This section of the report shows how the modelling framework can be used to investigate the risk of consumer exposure to antimicrobial resistance genes via the lettuce production chain. The overall model structure is presented in Figure 3 and is based on an existing QMRA model for *E. coli* in lettuce developed by Njage and Buys (2017) and Pang et al. (2017). In some cases, other existing models were used to inform specific modules as described in the following sections.

The details of the lettuce model including list of estimated and calculated variables and correlations between variables are presented in Appendix 3.

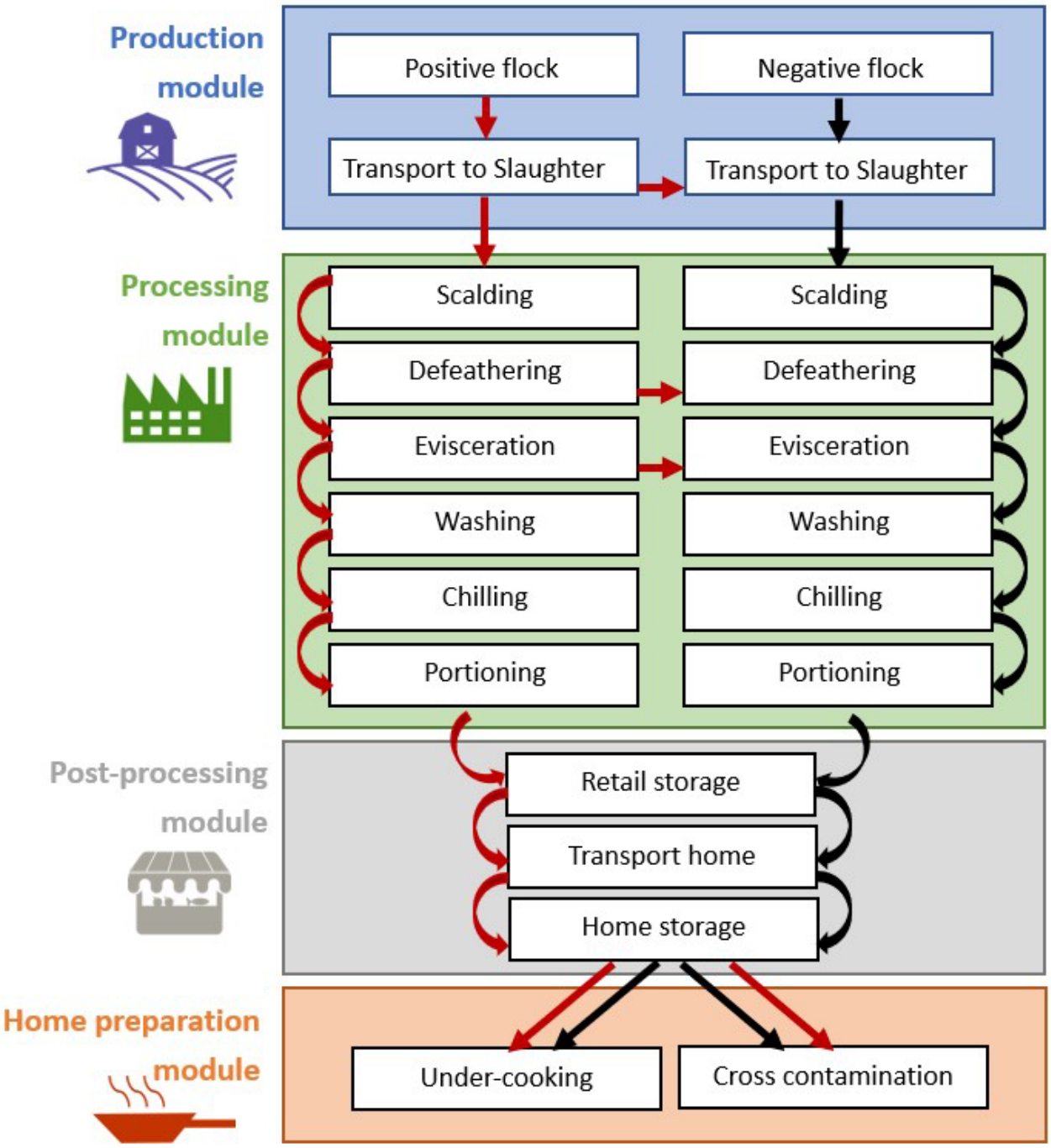


Figure 2: Flow diagram of the risk of exposure model based on (Collineau et al. 2020). Red arrows = positive flocks, black arrows = negative flocks

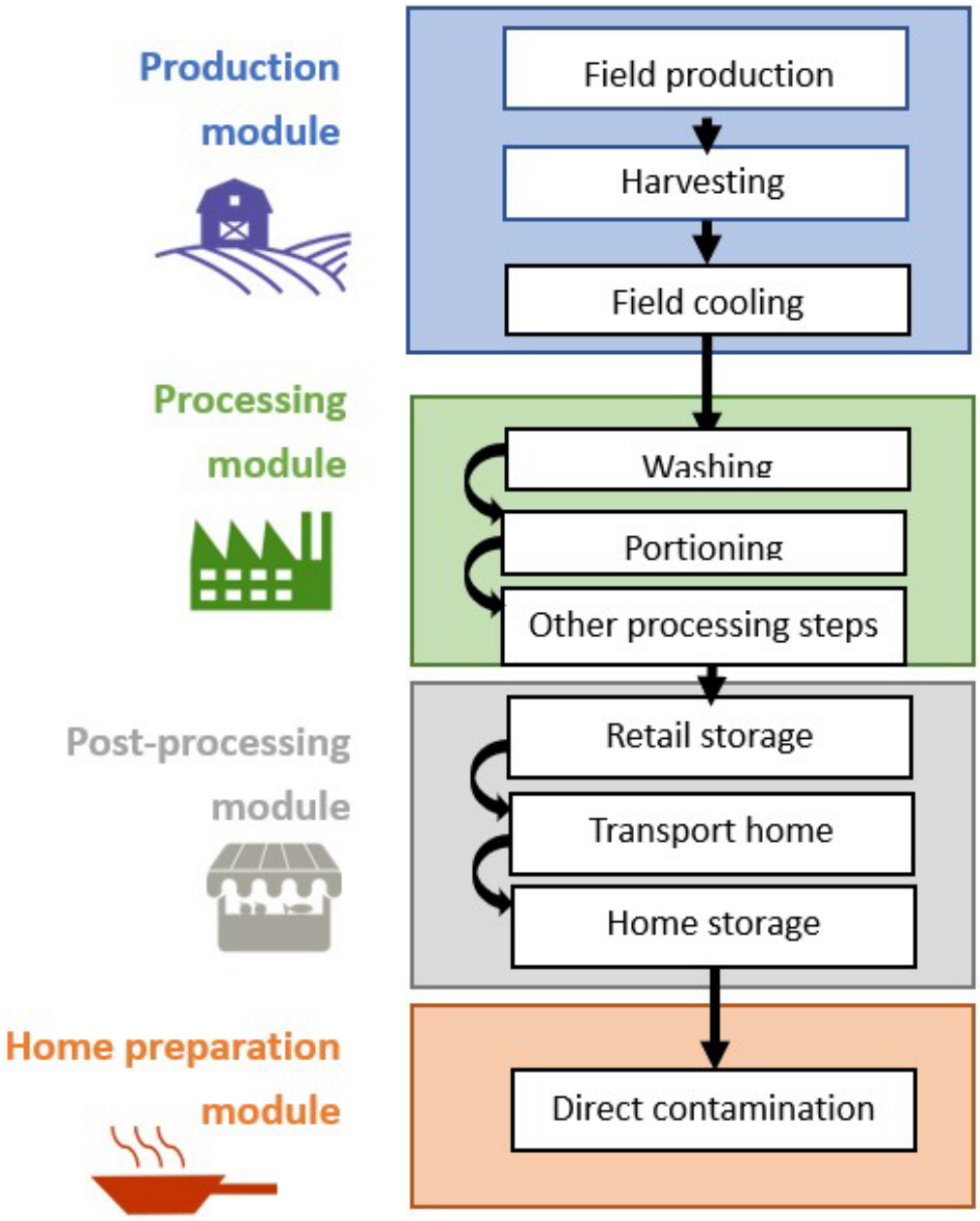


Figure 3: Flow diagram of the risk of exposure model based on (Njage and Buys 2017) (Pang et al. 2017)

6.5 Case studies

A case study was defined in this project by a combination of 3 elements: a type of food product, a microorganism, and a resistance gene. Three case studies were used as basis to develop and validate the modelling framework:

- The first case study focused on the microorganism "*E. coli"*, the resistance gene "ampC beta-lactamase gene *CMY-2",* and the food product "fresh skin off portioned chicken".
- The second case study presented in this report focused on the microorganism "*Campylobacter spp."*, the resistance gene "None*",* and the food product "fresh skin off portioned chicken". It was initially planned to investigate as a case study *Campylobacter spp.* carrying the mutated GyrA gene. However, based on the output of the literature review (see appendix 1) and the results of the first case study, the amount of data currently available on *Campylobacter spp.* carrying GyrA gene is not sufficient to properly validate the results of our study with other studies. To make sure that the proposed model provides results consistent with the current state of knowledge, it was decided together with the FSA to only investigate in this second case study the risk of bacteria exposure and not the risk of AMR gene exposure.
- The third case study looking at the risk of consumer exposure to "*E. coli* ", the resistance gene "None*",* and the food product *"*pre-washed outdoor grown bagged lettuce". For the same reasons as for the second case study, it was decided together with the FSA to only investigate in this third case study the risk of bacteria exposure and not the risk of AMR gene exposure.

Because of the lack of relevant data on genotypic antimicrobial resistance, two out of three of the cases studies were only based on phenotypic data (i.e., resistance gene "None").

6.6 Quality check

The modelling framework and the results of the three case studies were sent for external validation to the members of the advisory board (Dr Daniel Parker, Dr Lucie Collineau, Dr Monaghan), a project team member not closely involved in the design of the modelling framework (Prof Jaap Wagenaar), and FSA. Only minor comments and suggestions for modifications were submitted by all the reviewers. These comments were included in the version of the model presented in this report.

The results of the three case studies were also compared to the data available in the literature.

6.7 Correlation analysis

The modelling framework supports a global sensitivity analysis, or correlation analysis, to evaluate the impact of variability and uncertainty in the estimated variables on the uncertainty in the output variables.

The correlation between the values of the *estimated variable*s and the *outcome variables* was calculated using Spearman's rank correlation coefficients. Because this is a stochastic model, all the outcome variables are probability distributions.

7. Results

7.1 Chicken model

7.1.1 Case study 1: *E. coli* in chicken

7.1.1.1 Estimated variables

Input data for the model including quantitative information on the case study were gathered through existing literature using PubMed and Google Scholar. The literature review focused on the most recent and comprehensive studies performed in Europe and, when available, in the UK. However, when no data were available other publications on research studies performed in other regions have been considered. The value of the estimated variables used for this specific case study are reported in the Appendix 4.

7.1.1.2 Results of the risk assessment

The detailed results obtained for this case study including graphical representation of the outputs are reported in the Appendix 4 but a summary of the main findings is presented below and in Table 3.

Overall risk of AMR exposure

The overall risk of AMR exposure was estimated considering positive and negative flocks combined. The overall risk thus represents the average prevalence, and level of contamination, of a contaminated serving given the estimated proportion of positive and negative flocks in the overall population. These results shown an overall decrease of the prevalence of contaminated products and level of contamination per contaminated product throughout the value chain compared to the level of contamination at the production module. The median prevalence of contaminated serving after cooking equalled 1.5%. The probability of exposure through cross contamination was lower and equalled 0.00063%. The median number of AMR-gene-carrying bacteria per contaminated products was always low and below 6 CFU/item of interest. However, all the outcome variables presented highly skewed probability distributions (cf Appendix 4, Figure 1). As an example, the CFU of AMR-gene-carrying bacteria /contaminated bird arriving at the slaughterhouse (*C_prod*) varied from 4.4 to 3.06 E+08 CFU/carcass with a median value of 4.61 CFU/carcass.

Risk depending on the flock of origin

The risk of AMR exposure was estimated separately for positive and negative flocks as these populations present very different baseline values in terms of within-flock prevalence of contamination, and contamination load per bird. The proportion of positive *vs* negative flocks was defined by the between flock prevalence estimated for conventional farms (*Prev_Farm_type*). After 100 000 simulations, the model included 13 703 (13.7%) and 86 297 (86.3%) positives and negative flocks. For the negative flocks, the calculated median within-flock prevalence of contaminated birds at the end of the

production module was below 5%, while this prevalence was above 90% for the positive flocks. The prevalence of contaminated birds remained stable during the processing module for birds coming from negative flocks when this prevalence reached 100% (median) in positive flocks. In both cases, the prevalence of carcasses contaminated with AMR-gene-carrying bacteria drops after the post-processing module (i.e., median *Prev_pproc_n* = 3%, *Prev_pproc_p* = 63%). This result can be explained by the fact that modelling adjustments made to prevent products with very low contamination levels from being carried forward to the consumer stage of the model were only implemented in the post-processing and home-preparation modules. Carcasses with very low contaminated levels were thus counted as contaminated carcasses in the production and processing modules, which may have overestimated the prevalence of contaminated carcasses in these modules. The median prevalence of contaminated serving after cooking (i.e., direct ingestion of contaminated meat) equalled 1.2% when the meat came from a negative flock, and 25.2% when the meat came from a positive flock but was associated with very low median load of AMR-gene-carrying bacteria of this contaminated serving and equalled 0.0026 and 0.0029 CFU/piece of meat coming from negative or positive flocks respectively (cf Appendix 4 Figures 2 and 3). The median probability of exposure through cross contamination at the home-preparation module (i.e., NOT direct ingestion of contaminated meat) was below 0.01% for both positive and negative flocks. The median level of contamination with AMR-gene-carrying bacteria in case of exposure was also low and did not exceed 1 CFU/item of interest.

7.1.1.3 Correlation analysis

The full results of the correlation analysis are presented in the Appendix 4 and are not discussed in detail in this report. It should be however noted that the very large majority of the parameters were modelled as probability distributions to account for their uncertainty and variability. However few parameters used in the model were considered fixed when evidence found provided little doubt about their degree of variability (i.e., Minimum growth temperature- *T_growth_min* in the post-processing module**)** or when no information about variability and uncertainty could be found in the literature (for

example, probability of cross-contamination to occur during defeathering for birds from positive flocks). Depending on the parameter and in relation to the hazard under study, it might be necessary to build probability distributions of these parameters to account for uncertainty.

7.1.1.4 Comparison with the existing literature

At the time of writing, there is no published model investigating, at every step of the food production chain, the prevalence or level of bacterial contamination of chicken carcasses contaminated by *E. coli* carrying the ampC beta-lactamase gene *CMY-2.* Comparing our results with existing the scientific literature is thus only indirect.

Change of prevalence of contaminated carcasses along the food chain

Our results shown that prevalence of carcass contamination remains stable or slightly increases during the processing module. This result is consistent with the results obtained by Herman et al. (2003) who have shown that it is in general not possible for a slaughterhouse to decrease the prevalence of carcasses contaminated when status-positive animals were delivered. Supplementary contaminations can however occur during the processing module. In our study, the impact of cross contamination was mainly observed within positive flock at the scalding phase. This result is consistent with the fact that the probability of cross contamination was estimated as low in negative flocks (i.e., P_cross_df_n = Normal(0.02, 0.000557)) but relatively high for positive flocks (i.e., P_cross_df_p = 0.5).

The drop in prevalence of carcasses contaminated with AMR-gene-carrying bacteria observed after the post-processing module is due to the modelling adjustments made to prevent products with very low contamination levels (i.e., less than 1 CFU) from being carried forward to the consumer stage of the model. These adjustments were only implemented in the post-processing and home-preparation modules. Carcasses with very low contaminated levels were thus counted as contaminated carcasses in the production and processing modules, which may have overestimated the prevalence of contaminated carcasses in these modules.

Change of contamination load of contaminated carcasses along the food chain

Our results shown that the level of contamination decreases during the processing module which is consistent with the existing literature showing that slaughterhouses play a key role in the reduction of meat bacterial contamination (Belluco et al. 2016). The scalding phase appeared as the most important processing step to decrease bacteria contamination (*C_proc*), which is consistent with the results of Belluco et al. (2016).

Our results also indicated a slight increase in bacteria contamination during the post processing module. This is consistent with the fact that this where bacteria growth may occur if storage conditions are not appropriate. This result is supported in the results of the correlation analysis by the importance of variation in storage temperature at retail (*T_retail*) and fridge temperature (*T_fridge)* on the number of bacteria at the end of the post-processing module, *C_pproc*.

Table 3: Mean and median overall risk estimation per module for the first case study

Module	Output variables	Unit	Mean	Median
Production	*Prev_prod*	Prevalence of birds contaminated with AMR-gene-carrying bacteria arriving at the slaughterhouse	0.17	0.06
Production	*C_prod*	CFU of AMR-gene-carrying bacteria / bird arriving at the slaughterhouse	6.4E+06	4.6
Processing	*Prev_proc*	Prevalence of carcasses contaminated with AMR-gene-carrying bacteria	0.19	0.06
Processing	*C_proc*	CFU of AMR-gene-carrying bacteria / carcasses	6.6	0.0

Module	Output variables	Unit	Mean	Median
Post-processing	*Prev_pproc*	Prevalence of food item contaminated with AMR-gene-carrying bacteria	0.13	0.04
Post-processing	*C_pproc*	CFU of AMR-gene-carrying bacteria / food item	3.9E+03	1.0
Home preparation	*Prev_home_cook*	Prevalence of serving contaminated with AMR-gene-carrying bacteria after cooking	0.05	0.02
Home preparation	*C_home_cook*	CFU of AMR-gene-carrying bacteria ingested by food item	0.17	0.12
Home preparation	*P_home_cc*	Probability of exposure to AMR-gene-carrying bacteria through cross contamination	0.00	0.00
Home preparation	*C_home_cc*	CFU of AMR-gene-carrying bacteria ingested by cross contaminated food item	2.27	1.0

7.1.2 Case study 2: *Campylobacter* in chicken

7.1.2.1 Estimated variables

The second case study only differs from the first one in terms of microorganism and resistance gene. Therefore, only the *estimated variables* associated with the domain "Bacteria" differed between the two cases studies. Indeed, as a reminder, the *estimated variables* related to the domain "Bacteria" are specific to the **microorganism or resistance gene** of interest (for example, prevalence of the pathogen, minimal growth temperature, or bacterial concentration in caeca content), when the variables related to the domain "Other" are related to the **production chain or food product** of interest (for example, average size of carcasses, type of scalding technique, or average cooling temperature).

Input data for the model including quantitative information on the case study were gathered through existing literature using PubMed and Google Scholar. The literature review focused on the most recent studies performed in Europe and, when available, in the UK. However, when no data were available other publications on research studies performed in other regions have been considered. The value of the estimated variables used for this specific case study are reported in the Appendix 5.

7.1.2.2 Results of the risk assessment

The detailed results obtained for this case study including graphical representation of the outputs are reported in the Appendix 5 but a summary of the main findings is presented below and in Table 4.

Overall risk of AMR exposure

These results show an overall increase of the prevalence of contaminated products and level of contamination per contaminated product during the processing module. The median prevalence of contaminated serving after cooking equalled 20%. The median probability of exposure through cross contamination was lower and equalled 1.0E-04%. The median number of bacteria per contaminated products was always low and below 3 CFU/item of interest after 100 000 simulation runs.

Similar to the first case study, all the outcome variables presented highly skewed probability distributions. Four of the outcome variables (i.e., *Prev_prod, Prev_proc, Prev_pproc, Prev_home_cook*) also shown distinct peaks, which can be explained by the two different population considered together in this section (i.e., the positive and negative flocks) and the importance of cross contamination for *Campylobacter spp*.

Table 4: Mean and median overall risk estimation per module for the second case study

Module	Output variables	Unit	Mean	Median
Production	*Prev_prod*	Prevalence of birds contaminated with bacteria arriving at the slaughterhouse	0.28	0.12
Production	*C_prod*	CFU of bacteria /bird arriving at the slaughterhouse	2.4E+04	2.6
Processing	*Prev_proc*	Prevalence of carcasses contaminated with bacteria	0.68	0.68
Processing	*C_proc*	CFU of A bacteria / carcasses	7.5E+04	0
Post-processing	*Prev_pproc*	Prevalence of food item contaminated with bacteria	0.50	0.52
Post-processing	*C_pproc*	CFU of bacteria /food item	2.5E+04	1
Home preparation	*Prev_home_cook*	Prevalence of serving contaminated with bacteria after cooking	0.20	0.21
Home preparation	*C_home_cook*	CFU of bacteria ingested by contaminated food item	0.34	0.03
Home preparation	*P_home_cc*	Probability of exposure to bacteria through cross contamination	0.07	0.00
Home preparation	*C_home_cc*	CFU of bacteria ingested by cross contaminated food item	9.25	1.0

Risk depending on the flock of origin

The risk of bacteria exposure was estimated separately for positive and negative flocks as these populations present very different baseline values in terms of within-flock

prevalence of contamination, and contamination load per bird. The proportion of positive vs negative flocks was defined by the between flock prevalence estimated for conventional farms (Prev_Farm_type): 19 823 (19.8%) and 80 177 (80.2%) positives and negative flocks were included in the analysis, respectively.

For the negative flocks, the median within-flock prevalence of contaminated birds at the end of the production module was below 30% while this prevalence was above 95% for the positive flocks (see Appendix 5 Figure 2). This is in line with results from several studies that assumed that either none or all birds in a flock are infected with *Campylobacter* at arrival to the slaughterhouse (see for example (Rosenquist et al. 2003)). Indeed, it has been shown that the time from initial infection to a full-blown infection of all broilers in a flock occurs within a few days (Newell and Fearnley 2003; Hartnett et al. 2001; Katsma et al. 2007).

The prevalence of contaminated birds increased up to 60% during defeathering and evisceration for birds coming from negative flocks when this prevalence reached 100% (median) in positive flocks (see Appendix 5 Figures 2 and 3). This result is consistent with the fact that between flock cross contamination during these two processing steps was assumed to be high as confirmed by the literature accessed.

With regards to evisceration, in their study Berrang and Dickens (2000) confirmed that 86.7 % of birds sampled were Campylobacter positive when sampled post evisceration with an increase in number of contaminated birds compared to the previous step. While it is not possible to specifically impute the increase entirely to cross contamination between birds as contamination by viscera laceration can also occur, this is a plausible co-cause as reported in other papers (Hue et al. 2010).

In both cases, the prevalence of carcasses contaminated with bacteria drops after the post-processing module (i.e., median Prev_pproc_n = 39%, Prev_pproc_p = 63%). As with the first case study, this result can be explained by the fact that model adjustments made to prevent products with very low contamination levels (<1CFU/carcass) from being carried forward to the consumer stage of the model were only implemented in the post-processing and home-preparation modules. Carcasses with very low contaminated

levels were thus counted as contaminated carcasses in the production and processing modules, which overestimates the prevalence of truly contaminated carcasses in these modules.

The median prevalence of contaminated chicken servings after cooking equalled 17% when the meat came from a negative flock, and 25% when the meat came from a positive flock. The median load of bacteria of this contaminated serving was however low and was less than 1 CFU/piece of meat. The median probability of exposure through cross contamination was below 0.01% for both flocks. The median level of contamination with bacteria in case of exposure was also low and did not exceed 1 CFU/item of interest.

However, as indicated above, the probability distribution associated with the outcome are highlight skewed. When considering the 97.5% centile, the prevalence of contaminated serving increases to 40%. Similarly, the probability of cross-contamination reaches 65%. The level of contamination of these contaminated products remain however relatively low (i.e., 97.5% centile of C_home_cc = 74.4 CFU and C_home_cook = 3.1 CFU)

7.1.2.3 Correlation analysis

The full results of the correlation analysis are presented in the Appendix 5 and are not discussed in detail in this report.

7.1.2.4 Comparison with the existing literature

The major increase in prevalence of contaminated carcasses coming from negative flocks observed in our study during the processing module is consistent with results found in the literature (Allen et al. 2007; Dogan et al. 2019). Our estimated prevalence at the end of the processing module is however much higher than the one reported by these two studies (i.e., 60% in our study vs 30% in the observed data of (Allen et al. 2007) and in the model of (Dogan et al. 2019)). This difference can be explained by the fact that, in our model, 75% of carcasses were contaminated at very low level (cf Figure

17 and Figure 18). If we adjust the prevalence at the end of the processing module assuming that all carcasses with a contamination level below 1 CFU/carcass are not contaminated, the mean increased prevalence obtained with our model drops to 21.3%, which is then fully consistent with Allen et al. (2007) and Dogan et al. (2019).

The mean bacteria contamination at the end of the processing module (considering negative and positive flocks together) estimated by our model (i.e., 3.0E+04 CFU/carcass, or 3.4 log10 CFU/carcass), was also consistent with what was estimated by Allen et al. (2007) and Slader et al. (2002) on actual slaughterhouse data (2.5 and 1.1 log10CFU/carcass respectively), and Dogan et al. (2019) in a model (2.5 log10 CFU/carcass).

At the retailer level, the average prevalence of *Campylobacter spp.* contaminated chicken was estimated at 50%, which is consistent with the latest data available regarding the prevalence of contaminated fresh chicken in the UK (56%) at the industry level (Jorgensen et al. 2019).The percentage of highly contaminated products, product with a bacteria load above 1000 CFU/g, at the retailer level estimated in our study equalled 6.4% and was consistent with the percentage of highly contaminated products estimated by Jorgensen et al. (2019), 7%.

7.2 Lettuce model – case study 3

7.2.1 Estimated variables

Inputs data for the model included quantitative information on the case study were gathered through existing literature using PubMed and Google Scholar. The literature review focused on the most recent studies performed in Europe and, when available, in the UK. However, when no data were available other publications on research studies performed in other regions have been considered. The value of the estimated variables used for this specific case study are reported in the Appendix 6. It should be noted that the estimated variables not specific to any food product or microorganism (for example,

transport time, temperature of the fridge) were the same as those used in the chicken case studies.

7.2.2 Results of the risk assessment

The full results of the risk assessment are presented in Appendix 6 but a summary is presented below and in table 5.

Table 5: Mean and median overall risk estimation per module for the third case study

Module	Output variables	Unit	Mean	Median
Production	*Prev_prod*	Prevalence of lettuce contaminated with bacteria arriving at the processing plan	0.05	0.05
Production	*C_prod*	CFU of bacteria /lettuce arriving at the processing plan	0	0
Processing	*Prev_proc*	Prevalence of lettuce contaminated with bacteria	0.07	0.06
Processing	*C_proc*	CFU of A bacteria / lettuce	0	0
Post-processing	*Prev_pproc*	Prevalence of food item contaminated with bacteria	0.04	0.04
Post-processing	*C_pproc*	CFU of bacteria /food item	4.0E+04	1
Home preparation	*P_home_cook*	Probability of exposure to bacteria through direct contamination	0.04	0.04
Home preparation	*C_home_cook*	CFU of bacteria ingested by contaminated food item	3.7E+03	0.1

The result shows an overall slight increase of the prevalence of contaminated products and level of contamination per contaminated product during the processing module, in

response to exposure to the AMR gene. The median prevalence of contaminated servings equalled 4%. The median number of bacteria per serving was always low (< 1 CFU/g) but some lettuces end up being highly contaminated at the end of the post-processing module due to bacteria growth either at retail or at home as illustrated by the value of the mean (> 3.7E+05 CFU/g) and the results of the correlation analysis.

7.2.3 Correlation analysis

The full results of the correlation analysis are presented in the Appendix 6 and are not discussed in detail in this report.

7.2.4 Comparison with the existing literature

The results retrieved from the literature search were related to specific type of *E. coli* growth in different production systems (for example, *E. coli* O157:H7 in Australia (Bozkurt et al. 2021)) or *E. coli* carrying antimicrobial resistance gene (for example, ESBL/AmpC positive *E. coli* in South Africa (Njage and Buys 2017)) making the comparison with our own study challenging. In addition, validating the results of our model with the existing models was challenging because the quantitative risk assessments published on the lettuce sector do not model the dynamic of interim prevalence or contamination load along the different steps of the production chain. Instead, these assessments present only final estimates related to the expected number of illness (see for example Pang et al. (2017) or O'Flaherty et al. (2019)) making comparison with our own results impossible.

Sagoo et al. (2001) reported a prevalence of 0.5% of unsatisfactory uncooked ready-to-eat organic vegetables sampled at retail in the UK. In this study, 'unsatisfactory' means that *E. coli* count was above 10^2 CFU/g. In our study the prevalence of contaminated servings equalled 4% but included all products with a bacterial contamination load above 1 CFU/g. Focusing only on the servings contaminated at more than 10^2 CFU/g like Sagoo et al., we would obtain a median prevalence of 'unsatisfactory' servings of 0.3% in line with their study results. Most recent results (Williams and O'Brien 2019) suggest that 1.4% of lettuce might end up contaminated but without specifying the level

of contamination of the contaminated product making comparison with our results impossible.

7.3 Graphical user interface (GUI)

7.3.1 Technical characteristics

The Graphical user interface (GUI) developed for this project was developed as standalone App in R Shiny, https://shiny.rstudio.com/.

To be able to run the model and load the user interface, the App requires the following packages: "shiny", “shinydashboard", "shinycssloaders", "shinyWidgets", "reshape2", "tidyr", "ggplot2", "dplyr", "tidyverse", "plotly", "DT", "readxl", "ggrepel", "gridExtra", "ggfortify", "mc2d", "MCSim", "stats", "EnvStats", "extraDistr", "remotes", and "FAdist". All these packages are automatically installed and loaded when opening the Shiny App in R.

The GUI is available in the appendix 7 of this report. This folder “*QRA_shinyApp*” contains the Shiny App. This folder contains:

- A folder named “www”, which contains all the images and scripts used by the App
- 3 R scripts named “ui.R”, “server.R” and “global.R”. These scripts must be open in R studio to be able to load the Shiny App.

A detailed description of the structure of the App is available in the user manual provided as Appendix 8.

7.3.2 Using and modifying the GUI

A detailed description of how to run a model and upload data into the GUI is available in the user manual provided as Appendix 8. This document also provides guidance to future users on how to modify the App in order to either update an existing production chain (i.e., chicken or lettuce), or add a new production chain.

8. Discussion

8.1 Modelling framework

8.1.1 Strengths

8.1.1.1 Consistency with existing literature

Both models (for chicken and lettuce value chains) are based on pre-existing available models which were adapted and customized considering the project objectives and the focus on the three selected case studies. The validation process through comparison with available evidence and the internal and external peer reviewing process confirmed the robustness and quality of the overall modelling framework. The results of the internal model validations showed that the model outputs are consistent with the existing scientific literature. In addition, the results of the external model quality check done in parallel by external reviewers (i.e., Dr. Parker, Dr. Collineau, Prof. Wagenaar, and FSA) confirmed that the models developed were based on the latest scientific consensus and available data.

8.1.1.2 Adaptability and flexibility

One of the major strengths of the framework is certainly represented by its adaptability and flexibility to test new microorganisms/genes and/or to change some attributes, steps of the value chain and/or to revise the functions currently describing the correlation between variables.

The model framework structure was constructed in a way that new information obtained from whole genome sequencing or related to other influential variables can be integrated in future iterations as they become available. This is particularly important as, as described in the section below, the lack of relevant data on genotypic antimicrobial resistance was a critical challenge in the project. However as new evidence emerges on

AMR genes and their dynamic along the value chains, the model will be able to integrate the new knowledge.

The adaptability pertains also to the attribute, steps of the value chains and correlation and dependencies between variables. The models were developed with a focus on two very specific production chains in the UK where critical steps in the chains, risk management practices and risk factors were discussed and agreed with the stakeholders taking into consideration the selected case studies. Additional steps in the value chains or additional risk factors can be integrated in a relatively simple way based on the needs. Obviously, the integration of new steps in the value chains or new risk factor's parameters would require the acquisition of new input data for the additional parameterization of the model environment.

In the current model structure, most of the dependencies/correlation between variables are the same as those described by other authors (for example, Collineau et al 2020). The estimated variables of the model are those where no clear dependencies could be found in the literature. When dependencies were identified, variables were turned into calculated variables to take into account these dependencies in the model. However, as new evidence is generated suggesting additional correlations between variables might exist, then the model can be relatively easily updated if needed.

While an update of the modelling framework is certainly possible the level of complexities of this revision process may be different depending on the changes needed. This is further explained in Appendix 8.

8.1.1.3 Simple user interface

Another strength of the framework is represented by the GUI developed using the Shiny App. The GUI is very simple, user-friendly and allows users with limited knowledge in modelling and coding to easily run quantitative risk assessments and appreciate and interpret relatively easily the outputs from the models which are expressed in both tabular and graphical formats. The GUI provides clear instructions to users which are accompanied through the process with clear indications for each step.

8.1.2 Limitations

8.1.2.1 Availability of quality data

As with all stochastic modelling environments, the robustness of model outputs depends, among many other factors, on the availability of quality data. The lack of relevant data on genotypic antimicrobial resistance was a critical challenge in the project and, because of this, the models were developed mostly using phenotypic data.

Limitations regarding data availability for some *estimated variables* have been discussed in the previous sections. These limitations reflect the fact that data currently available in the literature are often ambiguous, inconsistent between studies, or too sparse especially for a number of AMR-related parameters. AMR-specific values could be entered in the modelling framework for every *estimated variable* as illustrated in the first case study in order to assess the risk of consumer exposure to specific AMR gene. However, the results of our literature reviews showed that, in practice, such data remains sparse. Similarly, other estimated variables not related to AMR parameters were also associated with high uncertainty because of the current lack of data in the literature. For example, the number of flocks transported before a given flock (*N_transp*), and the probability of cross-contamination occurrence *(P_h_wash*) because of poor kitchen hygiene were both highly influential for outputs of the model for the two case studies but the values of these two variables were also associated with large uncertainty.

Data availability is likely to be a major limitation for using the modelling framework to assess the risk of consumer exposure to specific AMR gene. However, if the uncertainty in *estimated variables* may lead to less robust model outputs, this did not represent a major obstacle towards the development of the modelling framework in itself as shown in this report. Indeed, the modelling approach used in this study require a large amount of input data but less than other modelling approaches (for example, Bayesian networks). It allows the model users to assess risk even in a context of data scarcity. In addition, because of the model flexibility, the values of the *estimated variables* could be easily updated later on by future model users, as soon as better data become available.

The implementation of experts' elicitation for the most uncertain parameters, in the short term, would help to overcome major data limitations. To this aim, the results of the correlation analysis can be used to help future model users to identify which variables have the highest influence on the model outcome and where to prioritize resources to collect or generate better data and thus obtain more reliable model outcomes. The results of the correlation analysis in the case studies already provide some indications in this sense. In addition, sections 8.2. and 8.3 below stressed areas for further research streams.

8.1.2.2 Bacteria load on a contaminated product homogeneously distributed

Another limitation is represented by the model inherent assumption that the bacteria load on a contaminated product (i.e., whole carcass) is homogeneously distributed across this product. The model results represent therefore an average level of meat contamination and the number of bacteria on a portion of meat is a proportion of the total amount of bacteria divided by the relative weight of the portion considered. However, this may not be the case (i.e., neck flap skin tends to have higher campylobacter numbers per cm than thigh skin as a result of the way birds are suspended during the slaughter process). Our choice to not include in the model a specific parameter related to "part of carcass" aimed to simplify the data collection process for future users as retrieving specific input data for different parts of carcasses can be very challenging. Further research on the bacteria load on contaminated products of different type and nature would be important. Once these data become available the decision to include a new parameter "part of carcass" could be re-considered. How to adjust the model in this sense is further explained in Appendix 8.

8.1.2.3 Handling new interventions

The current modelling framework allows to assess the effect of the most popular interventions used at different stages of the production chain to prevent bacterial contamination as agreed with the Industry stakeholders, project advisory panels and FSA experts. However, our modelling framework is not adapted to assess the effect of

every existing intervention implemented by stakeholders. It is not feasible to develop a model able to handle with sufficient precision all possible steps and interventions in the value chains. The current differences between systems and practices in place at production, processor and consumer level respectively and the inherent future developments are too vast to be accounted for in a general model framework that aims to generate meaningful outputs.

For some very specific intervention, the present model can still be used. Interventions which are essentially modifications of processes that are already represented in the model, such as rapid surface chilling, can be investigated by changing the parameters associated with the process ("chilling", in this case); Interventions which represent entirely new processes are likely to require model modification. On the other hand, this will not be possible for other very specific interventions which should be added in the model as a separated step and estimated variable. Model adaptation to new and specific interventions should be considered as part of future model development and might impair the sustainability of the current modelling framework in case of major changes of production practices. How to adjust the model in this sense is further explained in Appendix 8.

8.1.2.4 Adaptation to another microorganism

The results also show that a unique model structure can be used to model the risk associated with the two microorganisms of interest (i.e*., E. coli* and *Campylobacter* spp) for the chicken meat value chain. Most of the risk factors identified had an impact on both bacteria but some risk factors were specific to only one of them. This is clearly described in the review in Appendix 1. This result highlights the fact that the risk pathway proposed in Figure 2 is valid for *E. coli* and *Campylobacter* spp but might not be fully adapted for another bacteria. When a new bacteria is targeted, the models should be customized considering the hazard characteristics, hazard risk factors and effectiveness of intervention strategies for the specific hazards along value chains. In this case, new input data has to be generated.

8.1.3 Approach of sensitivity analysis

They are many approaches available to perform a sensitivity analysis in the context of risk assessment. The approach used in this modelling framework provides information about the impact of uncertainty and variability in estimated variables on uncertainty in the model outputs. The advantage of this approach is that it can be automatized and that it provides valuable information to decision makers to better manage risk. For examples:

- When an uncertain estimated variable has a large impact on the model outcome, it is recommended to gather better information on this specific variable to get more accurate model output

- When an estimated variable with important variability but low uncertainty has a large impact on the model outcome, new risk mitigation options targeting this specific variable might be implemented to better mitigate the risk.

Another approach of sensitivity analysis has been suggested by FSA during the project. This other approach aimed at investigating the percentage of change in the model outcome when the estimated variables change outside of the range of values already included in the case studies (for example, what would be the impact of a 10% change in disease prevalence on the final model outcome?). This approach provides different benefits to decision makers compared to the approach used in this study. Indeed, it allows decision makers to explore different scenario and identify the one with the maximum impact on risk reduction. The major drawback is that this approach cannot be easily automated. Indeed, percentage of change in estimated variables cannot be always easily defined when the estimated variables are probability distributions. In addition, the percentage of change, and thus the type of scenario tested must be carefully selected for each estimated variable. Indeed, the scenario tested must be relevant from a risk mitigation perspective.

Implementing this other approach of sensitivity analysis in the current modelling framework could be feasible but it would require significant changes to allow users to

define the scenario they want to explore for each estimated variable of interest. For illustration purpose, a brief comparison of the two approaches for the outcome variable *Prev_prod* and 4 selected estimated variables is presented and discussed in Appendix 9.

8.2 Chicken model

The full reports related to the chicken model (case studies 1 and 2) are also presented in Appendix 4 and 5.

The literature reviews showed different levels of data availability for the 2 case studies. Data gaps on estimated variables was a major issue with regards to the case study 1 (*E. Coli*) but less critical for case study 2 where more evidence was published and retrieved. However, in case study 2, the amount of data currently available on *Campylobacter* spp. carrying GyrA gene was not sufficient to properly validate the results of our study with other studies. To make sure that the proposed model provided results consistent with the current state of knowledge, this second case study focused on the risk of bacteria exposure and not the risk of AMR genes exposure.

In case study 1, lack of specific data in the production and processing modules did not allow to parametrize some of the estimated variables and further efforts should be dedicated to fill these gaps in knowledge. In the production module, for example, no specific data were found on the factor representing the impact of antimicrobial usage on between-flock prevalence of AMR bacteria (*F_AMU*) in conventional farms which would allow to parametrize the level of correlation between AM usage and prevalence of resistant *E. coli*. This is an important gap to be considered for further research. Simoneit et al. (2015) performed a literature review to assess the correlation between oral administration of antimicrobials and antimicrobial resistance in *E. coli* from chicken concluding that the searched papers provided indications of positive association between AMU and AMR but could not be proved with advanced statistical methods. This parameter can be adjusted if specific studies would generate the required data for the UK, a research stream that would be definitively worth fulfilling. In case study 2, this

parameter (*F_AMU*) was set to null because this case study was conducted at the bacteria level due to the lack of genetic data.

The implementation of appropriate biosecurity measures is a key strategy to reduce the general use of antibiotics at farm level (FAO 2019) and also to reduce the burden of resistant bacteria in farms (Furtula et al. 2010). With regards to case study 1, however, there are large gaps in the understanding of the most important risk factors and the most effective interventions to reduce the burden of *E. Coli* at farm level. More evidence was available for what concerns the case study 2.

Many different biosecurity measures can influence, with varying degree of effectiveness, the prevalence of *E. coli* and AMR level and therefore the integration of the different effects of different biosecurity measures in a unique parameter would not be an ideal solution. For the same reasons and the lack of clearly defined categories (i.e., poor, medium, high) describing the implementation of specific biosecurity measures, both the proportion of farms with poor biosecurity (*Prop_biosecurity*) and the factor representing the impact of poor biosecurity on contamination load (*F_biosecurity)* were not parameterized in this case study 1 model. These are important parameters and further efforts, and research should be dedicated to defining correct measures of the effect of relevant biosecurity measures.

During processing of broiler chickens, the level of bacteria contamination present on the broiler carcasses will fluctuate. With regards to the case study 1, the literature review highlighted data gaps or inconsistent results between studies on resistant *E. coli*. Some inconclusive or inconsistent results of studies regarding the main risk factors associated with the fluctuation of the *E. coli* concentration in the processing steps (Barco et al. 2014) could be due to the particular characteristics of these steps in the slaughterhouses and the implementation of the risk management practices (Pacholewicz et al. 2016). Major gaps in literature in this module hampered the selection of probability distributions of cross contamination of resistant *E. coli* in specific steps of processing. The probability of cross-contamination to occur during specific steps was based therefore on the author's estimate and (Collineau et al. 2020).

In case study 2, more consistent data were found. According to the report of Campylobacter risk assessment (WHO and FAO 2009) in broilers, the relative changes during the processes are similar in the various studies analysed. This despite the use of different methods for sampling and quantification and indicate therefore that the changes in concentrations of Campylobacter between processing steps in commercial broiler slaughter plants may be relatively uniform and consistent between studies.

The overall risk of AMR exposure was estimated considering positive and negative flocks combined. The overall risk thus represents the average prevalence, and level of contamination, of contaminated serving given the estimated proportion of positive and negative flocks in the overall population. When comparing the results between the 2 case studies, there are generally similar trends in the dynamics of the hazards along the value chains, but some differences do exist with regards the outputs which may be explained by the nature and characteristics of the different pathogens but also by the relative importance of cross contamination.

In case study 1, the outcome results showed an overall decrease of the prevalence of contaminated products and level of contamination per contaminated product throughout the value chain (see chapter 7.1.1.2) with a relatively small median prevalence of contaminated serving after cooking equalled 1.5%. The prevalence of carcass contamination remains stable or slightly increases during the processing module but the level of contamination decreases during the processing module. These findings are in line with the available literature. In case study 2, (see chapter 7.1.2.2) the results showed an overall increase of the prevalence of contaminated products and level of contamination per contaminated product during the processing module. The median prevalence of contaminated serving after cooking equalled 20%. Results are consistent with the literature which also confirmed the importance of cross contamination for Campylobacter spp. In both case studies all the outcome variables presented highly skewed probability distributions. Four of the outcome variables (i.e., *Prev_prod, Prev_proc, Prev_pproc, Prev_home_cook*) also shown distinct peaks, which can be explained by the two different population considered together in these figures (i.e., the

positive and negative flocks) and, for Campylobacter, the importance of cross-contamination.

The correlation analysis showed for both case studies that all within flock prevalence estimations were highly influenced by three estimated variables: the between farm prevalence (*Prev_farm_type),* the number of flocks transported before the current flock (*N_transp),* and the term for dampening the probability of carryover contamination from a positive flock transported prior to the current flock (*F_cross_trans)*. In terms of bacterial load contamination, many estimated variables have a large influence on the model. The influence of these parameters are discussed in the Appendices.

8.3 Lettuce model

The full report related to the lettuce model (case study 3) is presented in Appendix 6.

This case study is focused on the microorganism '*E. coli'*, the resistance gene 'ampC beta-lactamase gene *CMY-2',* and the food product 'outdoor grown pre-washed bagged salad'. Due to the lack of specific data on *E. coli* and AMR gene CMY-2 relevant to this assessment, in agreement with FSA, it was decided to focus the model on *E. coli* species (in general) only. Reasons for this food product choice included the higher susceptibility of microbial contamination of outdoor lettuce compared to indoor grown lettuce. Most of the lettuce grown in the UK is grown outdoors, however, about 20% is grown in glasshouses ("British Leafy Salad Association" 2021).

The challenge for ensuring safe produce is greatest for those vegetable products that are eaten uncooked, such as leafy salad vegetables. Even low levels of pathogens on these products could result in a considerable disease burden (J. M. Monaghan et al. 2017). Importantly, the microbial contamination that occur at field production might not be eliminated during further processing steps (Tyrrel, Knox, and Weatherhead 2006; Sapers 2001). Most of the factors affecting the risk of *E. coli* contamination in outdoor grown lettuce might also apply to other bacteria.

Similarly to the previous case studies, a few parameters were not parameterized due to lack of specific data or because they were not specifically relevant for *E. coli.* However, because they could be relevant for other hazards, for this specific case study, were set to no effect (1) or 0 depending on the parameter.

The results of the risk assessment show an overall slight increase of the prevalence of contaminated products and level of contamination per contaminated product during the processing module. The median prevalence of contaminated servings equalled 4%. The median number of bacteria per serving was always low (< 1 CFU/g) but some lettuces end up being highly contaminated at the end of the post-processing module due to bacteria growth either at retail or at home. As mentioned in chapter 7.2.1.4 validating the results of our model with the existing literature was challenging due to the lack of similar studies. However, the results of our risk assessment were in line with those of Sagoo et al. (2001).

With regards to the correlation analysis outputs, as expected, the prevalence of contaminated product throughout the production chain was mainly influenced by the baseline prevalence of contaminated lettuce (*Prev_base*). The probability of cross contamination occurring during the processing phase (*TR_overall*) and Farm practices (*Prop_biosecurity*) are also highly influential.

Considering the biosecurity measures adopted by producers, the UK fresh produce industry is characterized by very high production standards in response to the UK Food Safety Act (1990) (J. Monaghan, Thomas, and Goodburn 2008). Various factors are imputable for an increased risk of *E. coli* contamination including use of untreated manures and other animal wastes (see above), presence of wildlife, farmed animals and pests, worker health and hygiene practices. We could not find any data from the UK suitable to this case study related to the impact of poor biosecurity on contamination load (*F_biosecurity)* and further research on this aspect would be important. However, Liu *et al.* (2016) studied the impact of climate and management variables on the contamination of preharvest leafy greens with *E. coli*. An estimate from this study, referring to one management practice only, was used to parametrize (*F_biosecurity*) in

the current case study. Similarly, to the discussion points raised above for the chicken model, the integration of the different effects of different biosecurity measures in a unique parameter deserves some further consideration and dedicated research efforts.

8.4 Conclusion

This report presents a modelling framework and its application to two specific scenarios, the chicken meat and lettuce production chains, and to two specific case studies, risk of consumer exposure to *E. coli* carrying the ampC beta-lactamase gene *CMY-2* and to *Campylobacter spp*. via the consumption of fresh skin off portioned chicken or the consumption of outdoor grown pre-washed bagged lettuce respectively.

One of the major strengths of the framework is certainly represented by its adaptability and flexibility to test new microorganisms/genes and/or to change some attributes, steps of the value chain and/or to revise the functions currently describing the correlation between variables. This modelling framework is thus a powerful tool for decision maker to easily and quickly re-estimate risk as soon as new data become available and to test the effect different risk mitigation options.

The results of the internal model validations shown that the model outputs are consistent with the existing scientific literature. However, the lack of data to properly estimate some *estimated variables* and compare our results with was the main limitation of our study often impairing an in-depth model validation. These limitations reflect the fact that data currently available in the literature are often ambiguous or too sparce, particularly for a number of AMR-related parameters. Future studies should focus on improving the amount of data available on these parameters to be able to obtain more accurate risk estimates particularly for antimicrobial resistant microorganisms.

Several future model developments could be considered of interest to improve risk estimation. For example, model adaptation to new and specific interventions could be considered as part of future model development (for example, rapid surface chilling or sonastream). Future model development could also include the implementation of

additional approaches of correlation analysis in order to provide a better understanding of the risk associated with antimicrobial resistant microorganisms.

9. References

Allen, V. M., S. A. Bull, J. E. L. Corry, G. Domingue, F. Jørgensen, J. A. Frost, R. Whyte, A. Gonzalez, N. Elviss, and T. J. Humphrey. 2007. “Campylobacter Spp. Contamination of Chicken Carcasses during Processing in Relation to Flock Colonisation.” *International Journal of Food Microbiology* 113 (1): 54–61. https://doi.org/10.1016/j.ijfoodmicro.2006.07.011.

Bachoual, R., S. Ouabdesselam, F. Mory, C. Lascols, C.-J. Soussy, and J. Tankovic. 2001. “Single or Double Mutational Alterations of GyrA Associated with Fluoroquinolone Resistance in Campylobacter Jejuni and Campylobacter Coli.” *Microbial Drug Resistance* 7 (3): 257–61. https://doi.org/10.1089/10766290152652800.

Ballou, Anne L., Rizwana A. Ali, Mary A. Mendoza, J. C. Ellis, Hosni M. Hassan, W. J. Croom, and Matthew D. Koci. 2016. “Development of the Chick Microbiome: How Early Exposure Influences Future Microbial Diversity.” *Frontiers in Veterinary Science* 3. https://doi.org/10.3389/fvets.2016.00002.

Barco, Lisa, Simone Belluco, Anna Roccato, and Antonia Ricci. 2014. “Escherichia Coli and Enterobacteriaceae Counts on Poultry Carcasses along the Slaughter Processing Line, Factors Influencing the Counts and Relationship between Visual Faecal Contamination of Carcasses and Counts: A Review.” *EFSA Supporting Publications* 11 (8): 636E. https://doi.org/10.2903/sp.efsa.2014.EN-636.

Belluco, S., L. Barco, A. Roccato, and A. Ricci. 2016. “Escherichia Coli and Enterobacteriaceae Counts on Poultry Carcasses along the Slaughterline: A Systematic Review and Meta-Analysis.” *Food Control* 60 (February): 269–80. https://doi.org/10.1016/j.foodcont.2015.07.033.

Berrang, M. E., and J. A. Dickens. 2000. “Presence and Level of Campylobacter Spp. on Broiler Carcasses Throughout the Processing Plant.” *Journal of Applied Poultry Research* 9 (1): 43–47. https://doi.org/10.1093/japr/9.1.43.

Bogaard, A. E. van den. 2001. “Antibiotic Resistance of Faecal Escherichia Coli in Poultry, Poultry Farmers and Poultry Slaughterers.” *Journal of Antimicrobial Chemotherapy* 47 (6): 763–71. https://doi.org/10.1093/jac/47.6.763.

Bozkurt, Hayriye, Tina Bell, Floris van Ogtrop, Kim-Yen Phan-Thien, and Robyn McConchie. 2021. “Assessment of Microbial Risk during Australian Industrial Practices for Escherichia Coli O157:H7 in Fresh Cut-Cos Lettuce: A Stochastic Quantitative Approach.” *Food Microbiology* 95 (May): 103691. https://doi.org/10.1016/j.fm.2020.103691.

“British Leafy Salad Association.” 2021. 2021. https://www.britishleafysalads.co.uk/know/faq.shtml#:~:text=The%20UK%20season%20for%20wholehead,will%20take%20place%20around%20April.

Caffrey, Niamh, Omid Nekouei, Sheryl Gow, Agnes Agunos, and Sylvia Checkley. 2017. “Risk Factors Associated with the A2C Resistance Pattern among E. Coli Isolates from Broiler Flocks in Canada.” *Preventive Veterinary Medicine* 148 (December): 115–20. https://doi.org/10.1016/j.prevetmed.2017.11.001.

Carattoli, Alessandra, Anna Maria Dionisi, and Ida Luzzi. 2002. “Use of a LightCycler GyrA Mutation Assay for Identification of Ciprofloxacin-Resistant Campylobacter Coli.” *FEMS Microbiology Letters* 214 (1): 87–93. https://doi.org/10.1111/j.1574-6968.2002.tb11329.x.

CDC. 2020. “E.Coli (Escherichia Coli).” February 2020. https://www.cdc.gov/ecoli/index.html.

Collineau, Lucie, Brennan Chapman, Xu Bao, Branavan Sivapathasundaram, Carolee A. Carson, Aamir Fazil, Richard J. Reid-Smith, and Ben A. Smith. 2020. “A Farm-to-Fork Quantitative Risk Assessment Model for Salmonella Heidelberg Resistant to Third-Generation Cephalosporins in Broiler Chickens in Canada.” *International Journal of Food Microbiology* 330 (October): 108559. https://doi.org/10.1016/j.ijfoodmicro.2020.108559.

Daniel, Nicholas, Nuria Casadevall, Pei Sun, Daniel Sugden, and Vanna Aldin. 2018. "The Burden of Foodborne Disease in the UK 2018." FSA and the LSHTM. https://www.food.gov.uk/sites/default/files/media/document/the-burden-of-foodborne-disease-in-the-uk.pdf.

Deng, Hui, Hong-Bin Si, Shu-Yi Zeng, Jian Sun, Liang-Xing Fang, Run-Shi Yang, Ya-Hong Liu, and Xiao-Ping Liao. 2015. "Prevalence of Extended-Spectrum Cephalosporin-Resistant Escherichia Coli in a Farrowing Farm: ST1121 Clone Harboring IncHI2 Plasmid Contributes to the Dissemination of BlaCMY-2." *Frontiers in Microbiology* 6 (November). https://doi.org/10.3389/fmicb.2015.01210.

Dierikx, Cindy M., Jeanet A. van der Goot, Hilde E. Smith, Arie Kant, and Dik J. Mevius. 2013. "Presence of ESBL/AmpC -Producing Escherichia Coli in the Broiler Production Pyramid: A Descriptive Study." *PLoS ONE* 8 (11). https://doi.org/10.1371/journal.pone.0079005.

Dogan, Onay Burak, Jennifer Clarke, Fabio Mattos, and Bing Wang. 2019. "A Quantitative Microbial Risk Assessment Model of Campylobacter in Broiler Chickens: Evaluating Processing Interventions." *Food Control* 100 (June): 97–110. https://doi.org/10.1016/j.foodcont.2019.01.003.

EC. 2017. "Final Report of an Audit Carried out in the United Kingdom from 21 March 2017 to 31 March 2017 in Order to Evaluate the Monitoring and Reporting of Antimicrobial Resistance in Zoonotic and Commensal Bacteria in Certain Food-Producing Animal Population and Food." EC.

EFSA. 2014. "Scientific Opinion on the Risk Posed by Pathogens in Food of Non-animal Origin. Part 2 (Salmonella and Norovirus in Leafy Greens Eaten Raw as Salads)."

EFSA. 2020. "Update and Review of Control Options for Campylobacter in Broilers at Primary Production." *EFSA Journal* 18 (4): e06090. https://doi.org/10.2903/j.efsa.2020.6090.

EFSA/ECDC. 2020. "The European Union Summary Report on Antimicrobial Resistance in Zoonotic and Indicator Bacteria from Humans, Animals and Food

in 2017/2018." *EFSA Journal*, January. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6007.

Ewers, C., A. Bethe, T. Semmler, S. Guenther, and L.H. Wieler. 2012. "Extended-Spectrum β-Lactamase-Producing and AmpC-Producing Escherichia Coli from Livestock and Companion Animals, and Their Putative Impact on Public Health: A Global Perspective." *Clinical Microbiology and Infection* 18 (7): 646–55. https://doi.org/10.1111/j.1469-0691.2012.03850.x.

FAO. 2019. "Prudent and Efficient Use of Antimicrobials in Pigs and Poultry." Rome: FAO. https://doi.org/10.4060/CA6729EN.

FSA. 2011. "Foodborne Disease Strategy 2010-2015." https://acss.food.gov.uk/sites/default/files/multimedia/pdfs/fds2015.pdf.

FSA. 2015. "Food Standards Agency Strategic Plan 2015-20." https://www.food.gov.uk/sites/default/files/media/document/FSA-Strategic-plan-2015-2020.pdf.

FSA. 2018. "E.Coli. How E.Coli Spreads and What You Can Do to Prevent It Contaminating Your Food." January 2018. https://www.food.gov.uk/safety-hygiene/e-coli.

FSA. 2020. "The Burden of Foodborne Disease in the UK 2018."

Furtula, V., FOR EXAMPLE, Farrell, F. Diarrassouba, H. Rempel, J. Pritchard, and M.S. Diarra. 2010. "Veterinary Pharmaceuticals and Antibiotic Resistance of Escherichia Coli Isolates in Poultry Litter from Commercial Farms and Controlled Feeding Trials." *Poultry Science* 89 (1): 180–88. https://doi.org/10.3382/ps.2009-00198.

Hartnett, E., L. Kelly, D. Newell, M. Wooldridge, and G. Gettinby. 2001. "A Quantitative Risk Assessment for the Occurrence of Campylobacter in Chickens at the Point of Slaughter." *Epidemiology & Infection* 127 (2): 195–206. https://doi.org/10.1017/S0950268801005866.

Herman, L., M. Heyndrickx, K. Grijspeerdt, D. Vandekerchove, I. Rollier, and L. De Zutter. 2003. "Routes for Campylobacter Contamination of Poultry Meat: Epidemiological Study from Hatchery to Slaughterhouse." *Epidemiology & Infection* 131 (3): 1169–80. https://doi.org/10.1017/S0950268803001183.

HM Government. 2019. “Tackling Antimicrobial Resistance 2019-2024, The UK’s Five-Year National Action Plan.” Government of the United Kingdom.

Hue, Olivier, Sophie Le Bouquin, Marie-José Laisney, Virginie Allain, Françoise Lalande, Isabelle Petetin, Sandra Rouxel, et al. 2010. “Prevalence of and Risk Factors for Campylobacter Spp. Contamination of Broiler Chicken Carcasses at the Slaughterhouse.” *Food Microbiology* 27 (8): 992–99. https://doi.org/10.1016/j.fm.2010.06.004.

Jesse, T. W., M. D. Englen, L. G. Pittenger-Alley, and P. J. Fedorka-Cray. 2006. “Two Distinct Mutations in GyrA Lead to Ciprofloxacin and Nalidixic Acid Resistance in Campylobacter Coli and Campylobacter Jejuni Isolated from Chickens and Beef Cattle*.” *Journal of Applied Microbiology* 100 (4): 682–88. https://doi.org/10.1111/j.1365-2672.2005.02796.x.

Jorgensen, Frieda, Andre Charlett, Craig Swift, and Nicolae Corcionivoschi. 2019. “A Microbiological Survey of Campylobacter Contamination in Fresh Whole UK-Produced Chilled Chickens at Retail Sale.” Project FS102121. FSA.

Katsma, Wendelke E. A., Aline A. De Koeijer, Wilma F. Jacobs-Reitsma, Marie-Josée J. Mangen, and Jaap A. Wagenaar. 2007. “Assessing Interventions to Reduce the Risk of Campylobacter Prevalence in Broilers.” *Risk Analysis* 27 (4): 863–76. https://doi.org/10.1111/j.1539-6924.2007.00928.x.

Koga, Vanessa L., Renato P. Maluta, Wanderley D. da Silveira, Renan A. Ribeiro, Mariangela Hungria, Eliana C. Vespero, Gerson Nakazato, and Renata K. T. Kobayashi. 2019. “Characterization of CMY-2-Type Beta-Lactamase-Producing Escherichia Coli Isolated from Chicken Carcasses and Human Infection in a City of South Brazil.” *BMC Microbiology* 19 (1): 174. https://doi.org/10.1186/s12866-019-1550-3.

Li, Xian-Zhi, Manisha Mehrotra, Shiva Ghimire, and Lateef Adewoye. 2007. “Beta-Lactam Resistance and Beta-Lactamases in Bacteria of Animal Origin.” *Veterinary Microbiology* 121 (3–4): 197–214. https://doi.org/10.1016/j.vetmic.2007.01.015.

Liu, Cheng, Nynke Hofstra, and Eelco Franz. 2016. “Impacts of Climate and Management Variables on the Contamination of Preharvest Leafy Greens with

Escherichia Coli." *Journal of Food Protection* 79 (1): 17–29. https://doi.org/10.4315/0362-028X.JFP-15-255.

Manges, A. R., and J. R. Johnson. 2012. "Food-Borne Origins of Escherichia Coli Causing Extraintestinal Infections." *Clinical Infectious Diseases* 55 (5): 712–19. https://doi.org/10.1093/cid/cis502.

Mellata, Melha. 2013. "Human and Avian Extraintestinal Pathogenic *Escherichia Coli* : Infections, Zoonotic Risks, and Antibiotic Resistance Trends." *Foodborne Pathogens and Disease* 10 (11): 916–32. https://doi.org/10.1089/fpd.2013.1533.

Miranda, J.M., B.I. Vázquez, C.A. Fente, J. Barros-Velázquez, A. Cepeda, and C.M. Franco. 2008. "Evolution of Resistance in Poultry Intestinal Escherichia Coli During Three Commonly Used Antimicrobial Therapeutic Treatments in Poultry." *Poultry Science* 87 (8): 1643–48. https://doi.org/10.3382/ps.2007-00485.

Monaghan, J. M., J. C. Augustin, J. Bassett, R. Betts, B. Pourkomailian, and M. H. Zwietering. 2017. "Risk Assessment or Assessment of Risk? Developing an Evidence-Based Approach for Primary Producers of Leafy Vegetables To Assess and Manage Microbial Risks." *Journal of Food Protection* 80 (5): 725–33. https://doi.org/10.4315/0362-028X.JFP-16-237.

Monaghan, Jim, John I Thomas, and Kaarin Goodburn. 2008. "Food Standards Agency Project B17007. A Review of the Published Literature Describing Foodborne Illness Outbreaks Associated with Ready to Eat Fresh Produce and an Overview of Current UK Fresh Produce Farming Practices." Food Standard Agency.

Newell, D. G., and C. Fearnley. 2003. "Sources of Campylobacter Colonization in Broiler Chickens." *Applied and Environmental Microbiology* 69 (8): 4343–51. https://doi.org/10.1128/AEM.69.8.4343-4351.2003.

Njage, P.M.K., and E.M. Buys. 2017. "Quantitative Assessment of Human Exposure to Extended Spectrum and AmpC β-Lactamases Bearing E. Coli in Lettuce Attributable to Irrigation Water and Subsequent Horizontal Gene Transfer." *International Journal of Food Microbiology* 240 (January): 141–51. https://doi.org/10.1016/j.ijfoodmicro.2016.10.011.

O'Flaherty, E., A.G. Solimini, F. Pantanella, M. De Giusti, and E. Cummins. 2019. "Human Exposure to Antibiotic Resistant-Escherichia Coli through Irrigated

Lettuce." *Environment International* 122 (January): 270–80. https://doi.org/10.1016/j.envint.2018.11.022.

O'Neill, J. 2014. "Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations." London, UK: The Review on Antimicrobial Resistance.

Pacholewicz, Ewa, Arno Swart, Jaap A. Wagenaar, Len J. A. Lipman, and Arie H. Havelaar. 2016. "Explanatory Variables Associated with Campylobacter and Escherichia Coli Concentrations on Broiler Chicken Carcasses during Processing in Two Slaughterhouses." *Journal of Food Protection* 79 (12): 2038–47. https://doi.org/10.4315/0362-028X.JFP-16-064.

Pang, Hao, Elisabetta Lambertini, Robert L. Buchanan, Donald W. Schaffner, and Abani K. Pradhan. 2017. "Quantitative Microbial Risk Assessment for Escherichia Coli O157:H7 in Fresh-Cut Lettuce." *Journal of Food Protection* 80 (2): 302–11. https://doi.org/10.4315/0362-028X.JFP-16-246.

Payot, Sophie, Jean-Michel Bolla, Deborah Corcoran, Séamus Fanning, Francis Mégraud, and Qijing Zhang. 2006. "Mechanisms of Fluoroquinolone and Macrolide Resistance in Campylobacter Spp." *Microbes and Infection* 8 (7): 1967–71. https://doi.org/10.1016/j.micinf.2005.12.032.

Rosenquist, Hanne, Niels L Nielsen, Helle M Sommer, Birgit Nørrung, and Bjarke B Christensen. 2003. "Quantitative Risk Assessment of Human Campylobacteriosis Associated with Thermophilic Campylobacter Species in Chickens." *International Journal of Food Microbiology* 83 (1): 87–103. https://doi.org/10.1016/S0168-1605(02)00317-3.

Sagoo, S. K., C. L. Little, and R. T. Mitchell. 2001. "The Microbiological Examination of Ready-to-Eat Organic Vegetables from Retail Establishments in the United Kingdom." *Letters in Applied Microbiology* 33 (6): 434–39. https://doi.org/10.1046/j.1472-765X.2001.01026.x.

Sapers, Gerald M. 2001. "Efficacy of Washing and Sanitizing Methods for Disinfection of Fresh Fruit and Vegetable Products." *Food Technology and Biotechnology*, 7.

Simoneit, C., E. Burow, B.-A. Tenhagen, and A. Käsbohrer. 2015. "Oral Administration of Antimicrobials Increase Antimicrobial Resistance in E. Coli from Chicken – A

Systematic Review." *Preventive Veterinary Medicine* 118 (1): 1–7. https://doi.org/10.1016/j.prevetmed.2014.11.010.

Slader, J., G. Domingue, F. Jørgensen, K. McAlpine, R. J. Owen, F. J. Bolton, and T. J. Humphrey. 2002. "Impact of Transport Crate Reuse and of Catching and Processing on Campylobacter and Salmonella Contamination of Broiler Chickens." *Applied and Environmental Microbiology* 68 (2): 713–19. https://doi.org/10.1128/aem.68.2.713-719.2002.

Sproston, Emma L., Helen M. L. Wimalarathna, and Samuel K. Sheppard. 2018. "Trends in Fluoroquinolone Resistance in Campylobacter." *Microbial Genomics* 4 (8). https://doi.org/10.1099/mgen.0.000198.

Tyrrel, S. F., J. W. Knox, and E. K. Weatherhead. 2006. "Microbiological Water Quality Requirements for Salad Irrigation in the United Kingdom." *Journal of Food Protection* 69 (8): 2029–35. https://doi.org/10.4315/0362-028X-69.8.2029.

"UK 20-Year Vision for Antimicrobial Resistance." 2019. GOV.UK. 2019. https://www.gov.uk/government/publications/uk-20-year-vision-for-antimicrobial-resistance.

Wassenaar, Trudy M., Manfred Kist, and Anno de Jong. 2007. "Re-Analysis of the Risks Attributed to Ciprofloxacin-Resistant Campylobacter Jejuni Infections." *International Journal of Antimicrobial Agents* 30 (3): 195–201. https://doi.org/10.1016/j.ijantimicag.2007.01.019.

WHO. 2017. "Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, and Development of New Antibiotics." WHO. https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/.

WHO. 2018. "E.Coli." February 2018. https://www.who.int/news-room/fact-sheets/detail/e-coli.

WHO, and FAO, eds. 2009. *Risk Assessment of Campylobacter Spp. in Broiler Chickens. Technical Report*. Microbiological Risk Assessment Series 12. Geneva: World Health Organization : Food and Agriculture Organization of the United Nations.

Williams, S, and S.J O'Brien. 2019. "Assessing the Contribution Made by the Food Chain to the Burden of UK-Acquired Norovirus Infection." FSA. https://www.food.gov.uk/sites/default/files/media/document/assessing-the-contribution-made-by-the-food-chain-to-the-burden-of-uk-acquired-norovirus-infection.pdf.

Willis, C, F Jorgensen, N Elviss, S Cawthraw, L Randall, M Ellington, K Hopkins, C Swift, and N Woodford. 2018. "Surveillance Study of Antimicrobial Resistance in Bacteria Isolated from Chicken and Pork Sampled on Retail Sale in the United Kingdom." FS101196.

Wimalarathna, Helen ML, Judith F Richardson, Andy J Lawson, Richard Elson, Richard Meldrum, Christine L Little, Martin CJ Maiden, Noel D McCarthy, and Samuel K Sheppard. 2013. "Widespread Acquisition of Antimicrobial Resistance among Campylobacter Isolates from UK Retail Poultry and Evidence for Clonal Expansion of Resistant Lineages." *BMC Microbiology* 13 (July): 160. https://doi.org/10.1186/1471-2180-13-160.

Yang, Katherine, Annie Wang, Matthew Fu, Aaron Wang, Kevin Chen, Qian Jia, and Zuyi Huang. 2020. "Investigation of Incidents and Trends of Antimicrobial Resistance in Foodborne Pathogens in Eight Countries from Historical Sample Data." *International Journal of Environmental Research and Public Health* 17 (2): 472. https://doi.org/10.3390/ijerph17020472.

10. Appendix 1: critical risk pathways for antimicrobial resistance (AMR) exposure through the food production chain of chicken meat and lettuce in UK

10.1 Introduction

The purpose of this document is to define the risk pathways of AMR exposure from farm to consumer via chicken meat and lettuce using as examples two microorganism and resistance genes: the microorganism *E. coli* and the ampC beta-lactamase gene *CMY-2 (AMR1),* and *Campylobacter spp* and the mutated *GyrA gene (AMR2).* The results are represented by value chain: first the chicken meat, and then the lettuce.

For both value chains, the definition of the risk pathways for AMR was based on:

- Literature review. Preference was given to publications on the poultry and lettuce value chains in the United Kingdom (UK) or European Union (EU), but data from other countries and/or food value chains has also been reviewed when deemed relevant. The focus was placed on the parameters that can be used to model the influence of production and processing steps on the abundance of bacteria and AMR genes in food.
- Stakeholder's consultation. Two parallel online workshops (i.e., one for each value chain) were organized with key representatives of the UK poultry and lettuce industries in order to discuss the results of the literature review and provide recommendation for the future modelling framework.

The results of the literature review and stakeholder's consultation are presented below and organized in the four modules each representing different key intermediary steps in the food chain:

In this document a *hazard* is defined as a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. The *hazard* investigated in this report are the two selected microorganisms and their associated resistance gene. A *risk factor* is defined as a variable associated with an increased or decreased risk of *hazard* occurrence. A *risk pathway* lists all *risk factors* leading to the outcome of interest (i.e., consumer exposure to antimicrobial resistance). The *critical risk pathway* is the framework on which to base the future risk assessment modelling framework, including only the critical steps required for the risk to occur and presenting the underlying assumptions for excluding some steps.

10.2 Critical risk pathways for AMR exposure via chicken meat

With regards the chicken production chain, the critical risk pathways for AMR exposure were investigated for two microorganisms and resistance gene: the microorganism *E. coli* and the ampC beta-lactamase gene *CMY-2 (AMR1),* and *Campylobacter spp* and the mutated *GyrA gene (AMR2).*

In agreement with FSA, fresh portioned skin off chicken was selected as case study for the model development.

10.2.1 E. coli and the ampC beta-lactamase gene CMY-2 (AMR1)

A literature review was conducted using PubMed and Google Scholar. Search terms included: "chicken", "poultry", "broiler", "*E. coli*", and "*CMY-2 gene",* among others. The literature review focused on the most recent studies performed in the UK and Europe. However, when no data were available older publications performed in other context have been included. The inclusion of these publications, which may not represent the

current situation in the UK, have been highlighted the document whenever they were used.

It should be noted that the literature review primarily focused on AMR1. However, because it was often not possible to find information on AMR1, the literature search has been extended to risk factors having an influence on presence and abundance of *E. coli* in chicken meat.

The identified risk factors per module are reported below.

10.2.1.1 Production module

Risk factors associated with *E. coli* contamination in the production module are presented below and summarized in Table 1.

Table 6: Risk factors related to the production module and having an effect on E. coli and CMY-2

Risk factor	Effect	References
Season	No clear seasonal effect of *E. coli* contamination. However limited references were found.	(Hutchison et al. 2006; Lindblad et al. 2006)
On farm practices and farming typology	Differences in risk factors were found according to farming types including antibiotics use. Higher use of antibiotics, associated with a higher presence of resistant *E.coli*, has been observed in conventional farming than in organic and free-range farms. Environmental factors and density of animals may also play a role.	(Hussain et al. 2017; Musa et al. 2020).
Breeder flock	Contaminated breeder flocks increase the risk broiler contamination	(Poulsen et al. 2017)(Kemmett et al. 2014).
Feed	• The use of probiotics could reduce the presence of resistant *E. coli* • The absence of feed withdrawal from 8-12h before slaughter increases the risk of *E. coli* contamination of chicken carcass at the slaughterhouse	(EL-Sawah et al. 2018; Dame-Korevaar et al. 2020) (Musa et al. 2020; Warriss et al. 2004)
Antimicrobial usage	The use of antibiotics increases the risk of occurrence of resistant *E. coli*	(Roth et al. 2019; FAO 2019; EFSA/ECDC 2020)
Biosecurity practices	Biosecurity practices in farms reduces the risk of *E. coli* contamination in broilers	• (Mo et al. 2016; Furtula et al. 2010;

Risk factor	Effect	References
		Chinivasagam et al. 2009).
Thinning	No relevant data found for E. coli.	
Age and weight at slaughter	Age of chickens and weight seems to play a role in *E. coli* contamination at the slaughterhouse	(Dierikx et al. 2013; J. Northcutt et al. 2003; Cibin et al. 2014)
Transport at slaughter	Increased risk of *E. coli* contamination during transport at slaughter with contaminated crates	(Mollenkopf et al. 2018; R. J. Buhr et al. 2000; Furtula et al. 2010)

Season

Seasonal variation in the level of *E. coli* contamination of fresh chicken have been reported (Hutchison et al. 2006; Pointon et al. 2008). Results showed that the *Enterobacteriaceae* counts from chicken carcasses were significantly higher in summer than in winter in the UK (P=0.003)(Hutchison et al. 2006). Results from a study conducted by Lindblad et al (2006) in Sweden did not show any seasonal influence on the *E. coli* counts of chicken carcasses. According to the findings from the studies reviewed, there is no clear seasonality effect on the risk of *E. coli* contamination on chicken carcasses. In addition, no information on the effect of seasonality on resistant *E. coli* presence were found.

On farms practices and farming typology

Chicken meat samples tested at slaughterhouse level from free grazing broilers had shown to have higher levels of *E. coli* contamination (2.82 mean log 10 CFU/g, SD 0.08, $p<0.05$) than broiler carcasses from conventional farming (1.61 mean log 10 CFU/g, SD 1.22, $p<0.05$) (Voidarou et al. 2011). The higher levels of *E. coli* contamination had been related to a higher exposure to the outdoor environment (Voidarou et al. 2011). However, one study conducted by Davis et al (2018) compared the prevalence of *E. coli* in chicken meat samples at retail level from different type of farms (conventional farms, “raised without antibiotic” and organic farms).

Results showed that the prevalence of resistant *E. coli* in chicken meat samples was similar in the three types of farms and that the type of farms had little effect on the prevalence among resistant *E. coli* isolates. Results also showed that depending on the brand within each production category, the prevalence of resistant *E. coli* was different (G. S. Davis et al. 2018).

Chicken meat samples at the slaughterhouse from conventional broiler farms have shown to have a higher prevalence of multi drug resistant *E. coli* than chicken meat samples from free-range broiler farms and organic farms (Hussain et al. 2017; Musa et al. 2020). A study conducted by Hussain et al (2017) in commercial farms in India showed that the prevalence of multi-drug resistant *E. coli* in chicken meat samples (46%, 14/32) and faecal samples (40%, 15/39) from commercial farms was higher than the prevalence in chicken meat samples (15%, 2/13) and faecal samples (30% 11/36) from free range chicken (Hussain et al. 2017). Authors concluded that chicken raised under free-range conditions represent a lower risk of contamination with resistant *E. coli* than chicken raised in conventional farming and that this could be due to higher use of antibiotics in conventional farming (Hussain et al. 2017). Similar results were confirmed in European settings. Musa et al (2020) demonstrated that chicken samples from conventional farms in Italy had a higher prevalence of multi-resistance *E. coli* than the *E. coli* isolated from organic and antibiotic-free farms ($p<0.05$). Authors related this difference in the *E. coli* resistant counts between different farm types with the use of antibiotics in conventional farming while in organic farms there is no antibiotic treatment. In addition, an association was also found with the contact with potentially contaminated litter, which is lower in organic farms due to the availability of outdoor access. (Musa et al. 2020). Furthermore, contact between animals is more likely in conventional farms and this could play a role in the spread of resistant bacteria than direct contact to litter (Chuppava et al. 2019).

Based on the aforementioned findings, the use of antibiotic and other on-farm practices has been associated with the presence of resistant *E.coli* in chicken meat. These factors seem to be more predominant in some farm types than in others. Antibiotic use is more frequent in conventional farms than in organic or free-range farms.

Feed

The feed withdrawal prior slaughter has a role in reducing the faecal shedding during transportation and therefore, in reducing the contamination of resistant *E. coli* in chickens which, could play a role in the later contamination of chicken carcass during processing (Musa et al. 2020). General feed withdrawal prior slaughter on commercial broilers ranges from 8 to 12 hours (Bilgili 2002). After this period of time, the content of the gut became more fluid, which might leak during further processing steps and increase carcass contamination (Warriss et al. 2004). A study conducted by Northcutt et al (2003) showed that the length of feed withdrawal (0 to 12 hours) did not affect the *E. coli* counts recovered from whole carcass rinse of pre-eviscerated or eviscerated carcasses (J. Northcutt et al. 2003).

The use of probiotics could reduce the use of antibiotics in broilers by replacing antibiotics in the treatment of *E. coli* infection and by preventing the colonization of resistant *E. coli.* Chicken from 4 to 5 weeks are generally the most affected from *E. coli* infection. A study conducted by El-Sawah et al (2018) in Egypt showed that treating chicks with probiotics showed better results in controlling *E. coli* O157 infection than the combination of probiotics and antibiotics (EL-Sawah et al. 2018). Furthermore, Dame-Korevaar et al (2020), in an experimental study conducted in the Netherlands showed that a selection of pre- and probiotics led to a prevention of colonization with resistant E. coli in some broilers and in the remaining ones, a reduction in the colonization time of resistant *E. coli* (Dame-Korevaar et al. 2020).

Antibiotic usage

Antibiotics treatment can be administered in poultry farming through feed or drinking water to whole flocks (J. M. Miranda et al. 2008). The use of antibiotics in poultry is associated with the occurrence of AMR (FAO 2019). The use of betalactam antibiotics in broilers, such as penicillins, results in an increase of resistance rates in *E. coli* (Roth et al. 2019).

Callens et al (2018) showed moderate to strong correlation between the overall reduction of antibiotic use and reduction in resistance in commensal *E. coli* in broilers (Callens et al. 2018). In the Netherlands, the within-farm prevalence of ESBL/AmpC-producing E. coli in broilers decreased significantly from 66% in 2009 to 38% in 2016 in parallel with a large reduction on the use of antimicrobials in the same farms (RIVM 2017). In UK, the use of antibiotics in the poultry meat value chain was reduced by 71% between 2012 and 2016 (Parker 2018).

However, the epidemiology of AMR is complex and the level of resistance at farm level might be also influenced by factors other than the use of antibiotics (EFSA/ECDC 2020). Also, the reduction of just one class of antibiotic might not result in the reduction in the AMR levels for that antibiotic as resistance mechanisms can coexist in the same bacteria (EFSA/ECDC 2020). This has also been shown by Furtula et al (2010) in a study whose results did not show significant differences in the resistance levels in *E. coli* between the isolates from litter samples of broilers that used feeds with and without antibiotics (Furtula et al. 2010).

Based on the findings, the use antibiotics is correlated with the occurrence of resistant bacteria. The overall reduction of antibiotics is related to a reduction of resistant *E. coli*.

Breeder flock

E. coli can be transmitted from breeder flock to broiler flocks (Poulsen et al. 2017). Prior infections of the reproductive tract of breeders, egg hygiene and transportation all contribute to early colonization of the neonatal gut and to the contamination of the broiler flock (Kemmett et al. 2014).

Biosecurity practices

The implementation of appropriate biosecurity measures is a key strategy to reduce the general use of antibiotics at farm level (FAO 2019) and also to reduce the burden of resistant *E. coli* in farms (Furtula et al. 2010). High biosecurity levels at farm level have been associated in an study conducted in Norway with a reduction of the occurrence of

cephalosporin-resistant in broiler flocks (as long as there is no selection pressure from antimicrobial use) (Mo et al. 2016).

Cleaning and disinfection are critical management practices to reduce the burden of *E. coli.* However, findings from Dierikx et al (2013) showed that even after cleaning and disinfection, AmpC producing *E. coli* was still present in the poultry house and that after few production weeks, poultry feed became contaminated with AmpC producing *E. coli* (Dierikx et al. 2013). Regarding disinfectants agents, Caffrey et al (2017) identified that the use of hydrogen peroxide to disinfect water lines during the growing period of broilers was a risk factor for increasing A2C-resistant *E. coli*[1] in broilers (Caffrey et al. 2017).

The management of the chicken bedding is part of the biosecurity management on farms (Chinivasagam et al. 2016). *E. coli* is broadly spread in the chicken litter since the beginning of the production cycle (Chinivasagam et al. 2009; 2016). A study conducted in Australia showed that pilling of litter between farming cycle eliminates *E. coli* (Chinivasagam et al. 2009). In the UK, this is less relevant since litter is not commonly reused (D. Parker, personal communication).

Thinning

In the UK, thinning is a common practice. Independent processors may organize multiple depopulation cycles before finally emptying a shed. These practices have shown to be potentially risky resulting in the spreading of bacteria in poultry population. No published papers were found on the microbial risk on *E. coli* and thinning in European settings. All papers found are focused on *Campylobacter spp*. (see chapter 1.2.2.1.8 for further details).

Age and weight at slaughter

[1] The simultaneous resistant pattern to amoxicilin-clavulanic acid, ceftiofur and cefoxitin is known as A2C and is generally caused by the presence of CMY-2 gene

The duration of the production value chain and the age of bird at slaughter could be considered risk factors for the presence of *E. coli* at slaughterhouse level. A study conducted by Dierikx et al (2013) showed that AmpC producing *E. coli* was found at all levels in the broiler production pyramid and that the prevalence of AmpC-positive broilers in the farm increased within the first week from 0-24% to 96-100% and remained 100% until slaughter (independent of the use of antibiotics)(Dierikx et al. 2013).

Results from Northcutt et al (2003) showed that the *E. coli* counts of the chicken carcass samples significantly increase with the bird age ($P<0.05$)(J. Northcutt et al. 2003). A study conducted by Cibid et al (2014) in seven poultry slaughterhouses from Denmark and Italy showed that *E. coli* loads after evisceration and after chilling depended on the weight category of broilers. More specifically, the contamination of *E. coli* in poultry carcasses after evisceration and after chill was significantly lower in poultry from the 2-3kg category than in poultry from the categories <2kg and >3kg. Results also showed that there were no statistically significant differences on the *E. coli* loads in the aforementioned sampling steps between the weight categories <2kg and >3kg (Cibin et al. 2014).

Transport at slaughter

During transport to the slaughterhouse, broilers can shed *E. coli* as well as other pathogens through excreta in the crates. Broiler litter is a source of multiple antibiotic-resistant *E. coli* and therefore, it should be considered as a significant reservoir (Furtula et al. 2010; Ponce-Rivas, Muñoz-Márquez, and Khan 2012). AmpC beta-lactamase resistance *E. coli* has been found in samples of cage swabs (91.5%, 215/389) after the transportation of broilers (Mollenkopf et al. 2018). Also, contaminated crates during transportation could be a source of broiler exposure to *E. coli*, which increases the risk for *E. coli* contamination of broilers before slaughter (Mollenkopf et al. 2018).

Differences in flooring types may lead to different contamination levels of *E. coli* in broilers' feathers. Buhr et al (2000) showed that the *E. coli* counts from the feathered

rinses of broilers were 0.6 logs higher when they were transported on solid floor than on wire floor (R. J. Buhr et al. 2000).

Regarding cleaning procedures of the transport crate, a study conducted by Berrang et al (2005) assessed water spray and drying to reduce bacterial load on transport crates. Results demonstrated that the combination of water spray followed by a drying period of 24h period was effective in reducing the *E. coli* counts in the crates. However, results also showed that a drying period of 24h alone (without prior water spray) was also effective in reducing the *E. coli* counts (Berrang and Northcutt 2005).

10.2.1.2 Processing module

The poultry processing in large slaughterhouses is fast and greatly automated. The technological advances have helped to reduce contamination during processing, however, there are still chances for bacteria contamination and spread at the slaughterhouse (Althaus, Zweifel, and Stephan 2017).

The processing of chicken meat contributes to the transmission of resistant bacteria brought to the slaughter by colonized animals onto the meat product (Reich, Atanassova, and Klein 2013). Reich et al (2013) demonstrated that broilers shedding resistant *E. coli* through faeces led to a considerable proportion of chicken carcasses surface contaminated with resistant *E. coli* during slaughter (Reich, Atanassova, and Klein 2013). Althaus et al (2017) used *E. coli* and *Enterobacteriaceae* as indicators of faecal contamination on broilers. Results showed that 91% (409/450) and 93% (418/450) of the carcasses were contaminated with *E. coli* and *Enterobacteriaceae*, respectively (Althaus, Zweifel, and Stephan 2017).

Some inconclusive or inconsistent results of studies regarding the main risk factors associated with the fluctuation of the *E. coli* concentration in the processing steps (Barco et al. 2014) could be due to the particular characteristics of these steps in the slaughterhouses and the implementation of the risk management practices (Pacholewicz et al. 2016). Pacholewicz et al (2015) suggested that the critical processing steps should be validated within the same slaughterhouse using longitudinal

studies. They proposed *E. coli* as an indicator of hygiene in processing (Pacholewicz et al. 2015a).

Risk factors associated with *E. coli* contamination in the processing module are presented below and summarized in Table 2.

Table 7: Risk factors related to the processing module and having an effect on *E. coli* and *CMY-2 gene* contamination

Risk factor	Effect	References
Size of the slaughterhouse	No effect on the risk of *E. coli* contamination	(Barco et al. 2014)
Hygienic practices	Poor hygiene and disinfectant practices increases the risk of *E. coli* contamination during processing	(Adeyanju and Ishola 2014; Gregova et al. 2012)
Order at slaughter	No relevant data found	
Stunning and bleeding	No relevant data found. Minor cross-contamination might happen	(Heemskerk 2005)
Scalding	Increase risk of *E. coli* contamination on carcasses using one tank rather than two tanks	(Sales and Porto 2007; Cason, Hinton, and Ingram 2000; Althaus, Zweifel, and Stephan 2017)
Scalding	Interventions prior scalding such as brushing and the plugging of vents reduced the risk of *E. coli* contamination during scalding	(Pacholewicz et al. 2016; R. Buhr, Berrang, and Cason 2003)
Defeathering	Decrease risk of *E. coli* load in carcasses	(Althaus, Zweifel, and Stephan 2017; Pacholewicz et

Risk factor	Effect	References
		al. 2015a; Berrang and Bailey 2009)
Evisceration	Evisceration is associated with both higher *E. coli* load and higher risk of cross contamination.	(Althaus, Zweifel, and Stephan 2017; Barbut et al. 2009; Gill et al. 2006)
Washing	Reduction of the *E. coli* counts in poultry carcasses after washing	(Althaus, Zweifel, and Stephan 2017; J. K. Northcutt et al. 2003; Kemp et al. 2001)
Chilling	Reduction of E. coli growth and load after chilling	(Barco et al. 2014; Zhang et al. 2011; Huezo et al. 2007)
Cutting	No major effect on *E. coli* contamination	(Gill et al. 2006)
Skin removal	Removing the skin before processing has shown to reduce the E. coli levels in broiler carcasses. Limited effect of skin removal on the risk of *E. coli* contamination of chicken meat at retail level (differences also related to meat type)	(Berrang et al. 2002; Berrang, Ladely, and Buhr 2001; Cook et al. 2012)

Size of the slaughterhouse

Limited information were found on *E. coli* association with the size of the slaughterhouse, the level of mechanization and throughput. The size and the level of mechanization of the slaughterhouse have not been correlated to the counts of *E. coli* in broiler carcasses (Barco et al. 2014).

Hygienic practices in the slaughterhouse environment

Hygienic and disinfectant practices are essential to decrease the level of *E. coli* contamination during processing at the slaughterhouse. Chicken meat can be contaminated in the processing line with *E. coli* through contaminated equipment and lack of hygiene (Adeyanju and Ishola 2014).

Projahn et al. (2018) reviewed the results of investigations on the reduction of bacterial contaminants in the slaughterhouse environment. These investigations include sanitary treatment in slaughterhouses and the disinfection of conveyor belts and transport crates, the latter being critical concerning cross-contamination. From the results, the authors did not recommend peracetic acid as a sanitizing agent for cleaning slaughterhouse equipment. Hot water treatment does not result in significant reduction of *Enterobacteriaceae* whereas a mix of washing, soaking, and disinfectants or detergents proved to be more effective. Ultrasonic treatment of conveyor belts was more effective in combination with water temperatures around 60°C (Projahn et al. 2018).

Grenova et al (2012) isolated resistant *E. coli* from multiple parts of the slaughterhouse including portioning room, packaging room, evisceration room, killing room and shackling room (Gregova et al. 2012). Results from the same study showed that the *E. coli* isolates from the chicken meat at the slaughterhouse could be related with the circulation of environmental bacteria at the slaughterhouse (Gregova et al. 2012).

Order at slaughter

No data was found regarding the effect of the order at slaughter of broilers and *E. coli* risk of contamination at slaughter.

Stunning and bleeding

No studies or data was found on *E. coli* contamination in chicken meat during stunning and bleeding. However, the release of faeces by chicken during stunning and bleeding has been described (Heemskerk 2005). Few microbiological implications are expected from this step during processing.

Scalding

Scalding schemes adopting different temperature ranges are possible, a hard scalding that includes water temperature from 60-66°C during an immersion time of 45-90s and a soft scalding with water temperature ranging between 51-54°C with immersion times for 120 to 210s (Projahn et al. 2018). In Europe generally, the scalding constant temperature varies from 50 to 65°C with immersion times between 60 and 210s.

Depending on the final meat product (fresh or deep-frozen), the constant temperature during scalding will vary. In general, the scalding temperature for fresh chicken meat is a bit lower than for deep-frozen chicken meat.

There are also different scalding systems: single bath scalding tank, single bath with counterflow, multi bath scalding tanks and multi bath with counter flow (Löhren 2012). Scalding using one tank alone has been reported to be a niche of contamination, accumulating organic and microbial contamination from poultry (Sales and Porto 2007). The use of consecutive tanks during the scalding process aim to reduce the bacterial contamination of carcasses along the tanks (Cason, Hinton, and Ingram 2000,). According to Löhren (2012), the effect of scalding on the microbial burden in the poultry skin is disputable as studies have not shown strong evidence on the relation between the rate of contamination of the wastewater of the last scalding tank and the rate of contamination after defeathering (Löhren 2012). However, a literature review conducted by Barco et al (2014) confirmed that a decrease in the counts of *E. coli* of more than one log unit immediately after scalding in four out of the five considered studies. However, in two out of the five studies, chlorine was used in this step (Barco et al. 2014) and therefore, the decrease in *E. coli* contamination could be due to chlorine rather than the scalding process. In the EU, the use of chlorine is not allowed in higher concentrations than the ones used for potable water.

Althaus et al (2017) showed that the *E. coli* counts after scalding averaged out at 4.2 log CFU/g. In this study, the scalding step consisted of two tanks with different temperature and time intervals. The first tank with an average of 52.4°C and 120s and the second thank with an average of 52.5°C and 75s. The *E. coli* counts in the water samples of

the second scalding tank was significant lower ($p<0.05$) than in the first tank. This study did not take samples before scalding which could have been useful to assess the scalding process. (Althaus, Zweifel, and Stephan 2017).

Interventions prior scalding have been shown to be effective in reducing *E. coli* contamination in chicken carcasses. Studies reported that brushing as well as plugging and suturing the vents of broiler prior scalding reduce the contamination of *E. coli* in chicken carcass. Pacholewicz et al (2016) showed a significant decreased in *E. coli* on the brushed carcasses ($p<0.001$) (Pacholewicz et al. 2016). The *E. coli* concentrations on whole carcasses before scalding were reduced roughly by 0.3 log (Pacholewicz et al. 2016). Buhr et al (2003) demonstrated that plugging and suturing the vents of broilers before scalding is a preventive measure to reduce the contamination level of carcasses during picking. The reduction of *E. coli* contamination of chicken carcasses that had vents plugged and sutured was 1.7 $\log_{10}$ *E. coli* lower than those without the vents plugged (R. Buhr, Berrang, and Cason 2003). However, the adoption of this practice is demanding and difficult to implement in current high throughput slaughterhouses (D. Parker, personal communication)

Defeathering

Defeathering is generally associated with a decrease of the *E. coli* counts in poultry carcasses**.** Althaus et al (2017) showed that counts on *E. coli* in chicken carcasses were significantly reduced after defeathering (0.8 log CFU/g on average, $P <0.05$) most likely due to the physical removal of the feathers (Althaus, Zweifel, and Stephan 2017). Results from Pacholewicz et al (2015) also showed a significant reduction of *E. coli* concentration after defeathering in two different slaughterhouses. In one slaughterhouse, the *E. coli* concentration was significantly reduced by 1.26 log 10 ($p<0.01$) and in the other, by 0.44 log 10 ($p=0.01$) (Pacholewicz et al. 2015a). In another study, Berrang et al (2009) showed that after defeathering the *E. coli* loads decreased by 0.56 mean log CFU/ml rinse. However, in this study, the samples were taken after the application of a carcass spray washer with chlorine (mean concentration of 40 ppm) (Berrang and Bailey 2009).

In contrast with the aforementioned studies, Cason et al (2004) showed no significant difference ($p \leq 0.05$) in the *E. coli* counts between featherless and feathered broilers in rinse samples taken right after defeathering (Cason, Hinton, and Buhr 2004).

A study published by EFSA has reported that the type of defeathering method has been shown to significantly affect the level of *E. coli* contamination on poultry carcasses. Results showed that the *E. coli* loads were lower using a vertical or horizontal disk rather than using a combination of vertical, horizontal and counter-rotating (Barco et al. 2014).

Evisceration

Poultry meat can be contaminated with *E. coli* when, during processing, the intestines are damaged and the content accidentally spills in the exterior and interior of the carcass (Adeyanju and Ishola 2014; Russell 2003). The evisceration removal is generally an automated operation and the size of the birds are expected to be similar. The faecal contamination of the poultry carcasses can be visible and even more when during this automated process the intestines are damaged in series (Russell 2003)

Several studies showed that the evisceration process slightly increased the *E. coli* counts on average by 0.077 and 0.3 CFU/g)(Althaus, Zweifel, and Stephan 2017)(Barbut et al. 2009) (Gill et al. 2006).

Washing

In general, the washing step has been shown to reduce the *E. coli* counts in chicken carcasses. However, during washing, *E. coli* from contaminated chicken carcasses can be transferred to other chicken carcasses through water.

Results from Althaus et al (2017) has shown a significant even though slight reduction of the *E. coli* counts ($p<0.05$) after washing. Results from this study have shown almost unchanged *E. coli* counts after washing and in the chiller (Althaus, Zweifel, and Stephan 2017). Northcutt et al (2003) reported a non-significant decrease of the *E. coli* mean log 10 counts after washing in three different processing plants (J. K. Northcutt et

al. 2003). Furthermore, results from Kemp et al (2001) showed slightly significant reduction of the *E. coli* titers (log 10 CFU/ml) after washing (Kemp et al. 2001).

Chilling

E. Coli is a mesophile bacteria growing from 7-10 °C up to 50 °C with an optimum around 37 °C (Adams, Moss, and McClure 2018). *E. coli* can survive for long periods under refrigeration and frozen conditions storage but a temperature ≤7°C will limit its growth and the chilling process have largely shown a decrease in *E. coli* counts of poultry carcasses (Barco et al. 2014). The chilling process is divided in two main steps: pre-chilling and chilling, and often lasts from 25-96h before the cutting process (Nastasijević, Lakićević, and Petrović 2017).

Pre-chilling: The prechill step aims at reducing the carcass temperature quickly and therefore, to prevent the microbial growth. The water chilling process is more common in the USA while in Europe, air chilling has been more widely applied (Zhang et al. 2011). Poultry carcasses have to reach a temperature <4°C and it generally takes less than 2h (Nastasijević, Lakićević, and Petrović 2017).
Using chemicals on raw meat is discouraged in the EU as residues can persist in the surface of meat. Also, European consumers prefer food that has not been processed with additional additives (Christian James et al. 2007). The following options exist:

- *Spray chlorinated water*: The addition of chlorinated water to chill water has showed to significantly reduce the *E. coli* concentration(Zhang et al. 2011; R. J. Buhr et al. 2005). These studies are not reported here in detail as using chlorine concentrations higher than those used in potable water is not permitted in the EU.
- *Water chilling*/*immersion-chilled*: this process can include chlorinated water(Barco et al. 2014). The disadvantages of this method are the high amount of water required, the possible occurrence of cross-contamination and the increase of the poultry carcass weight by absorbing water (Zhang et al. 2011). Results from Buhr et al (2005) showed the *E. coli* concentration was reduced by log 0.43 cfu/100ml of carcass rinse ($p<0.05$) after immersion chilling with

chlorinated water (Buhr et al. 2005). A literature review conducted by Barco et al (2014) concluded that the use of chlorine in immersion chilling was not always associated with significant differences on the bacterial loads of chicken carcasses (Barco et al. 2014).

- *Air chilling*: Several studies have reported that air chilling has limited microbiological effect (C. James et al. 2006; González-Miret, Escudero-Gilete, and Heredia 2006). This has been observed in a study by Althaus et al (2017), which reported that the *E. coli* counts in the chicken carcass placed in the chiller using air chilling remained almost unchanged compared to counts after washing (Althaus, Zweifel, and Stephan 2017).

Results from Zhang et al (2011) showed major reductions of *E. coli* using immersion chilling with chlorinated water compared to air chilling, however, these differences were not statistically significant ($p>0.05$) (Zhang et al. 2011). A study conducted by Huezo et al (2007) compared the effectivity of air chilling and water chilling methods in the *E. coli* concentration on broiler carcasses. Results showed that both methods significantly reduced ($P< 0.05$) the *E. coli* concentration in broiler carcasses with no significant difference observed between them (Huezo et al. 2007). In this study, only potable water was used without chlorine (Huezo et al. 2007).

Chilling: Results from a study conducted by Boysen et al (2016) assessed the mean *E. coli* level ($\log_{10}$ CFU/g) after plucking, after evisceration and after chilling in three different slaughterhouses in Denmark. Results has shown that the *E. coli* levels after plucking and after evisceration were different in the three slaughterhouses and that the mean *E. coli* level ($\log_{10}$ CFU/g) in broiler carcasses after chilling was consistently highly reduced with comparable results independently of the contamination level after evisceration. In this study, the differences in the *E. coli* levels at different processing steps were suggested to be due to differences in the hygiene management in the processing steps.

In addition, *E. coli* was very likely inactivated under crust-freezing process (Christian James et al. 2007).

Cutting

The cutting and portioning process of the carcasses involves extra handling and exposure to more surfaces (for example, cutting boards, automatic deboning equipment etc.) and increasing therefore the risk for cross contamination if environmental parameters and hygiene practices are not fully applied. Temperature of the cutting room should be kept < 4°C at all times during cutting to minimize bacterial contamination (Nastasijević, Lakićević, and Petrović 2017).

The effects on the microbiological condition of product of carcass dressing, cooling, and portioning processes were studied on 25 randomly selected product units by Gill et al (2006). The numbers of bacteria on the final product after portioning were not substantially changed (Gill et al. 2006).

Skin removal

Results from Berrang et al (2002) showed that removing skin before processing reduces *E. coli* levels by 0.5 $\log_{10}$ CFU/carcass. In this study, the *E. coli* counts in the whole broiler carcass rinse with skin was 4.4 $\log_{10}$ CFU/carcass and without 3.9 $\log_{10}$ CFU/carcass (Berrang et al. 2002).

Berrang et al (2001) implemented a study to compare skin and the uncompromised underlying meat of broiler breasts, thighs, and drumsticks obtained from commercial processing facilities or retail outlets, for the presence of *Campylobacter, E. coli*, and other bacteria. In this study, the meat beneath the skin of dressed carcasses (bled and defeathered) of broilers had less bacterial contamination than the skin surface. *Campylobacter* and *E. Coli* results were similar; they were not recovered from breast meat samples, whereas 9 of 10 skin samples were positive with more than 100 cells/g. All 10 samples of thigh skin were contaminated with *E. coli* (2.3 log10 CFU/g), whereas only 1 of 10 thigh meat samples had measurable levels of *E. coli* (0.7 log10 CFU/g). Similarly, drumstick skin was much more contaminated than the underlying meat. The effect of evisceration in comparison to only dressed carcasses was also tested to account for the substantial additional risk associated to various processing steps before

chilling. In this case, and specifically for *E. Coli*, skin samples from the breast were more contaminated than breast meat. For thighs and drumsticks the presence or absence of skin did not affect *E. coli* average counts. Bacterial load were also counted from parts purchased at retail and then skinned in the laboratory. In this case, results showed that the products purchased at retail had overall lower levels of bacteria contamination and no significant difference was found between the different samples with and without skin (Berrang, Ladely, and Buhr 2001).

Similarly, results from Cook et al (2012) showed that there was no significant difference between the proportion of *E. coli* isolates from the skin-off chicken breasts samples (33%, 33/99) and from skin-on chicken breasts samples (41%, 77/187) purchased at retail. Also, the difference between skin on and off breast samples did not exceed 1 log. This study concluded that the risk for consumer exposure to *E. coli* from skin-off and off chicken breast samples is similar (Cook et al. 2012).

10.2.1.3 Post-processing module

Risk factors associated with *E. coli* contamination in the post-processing module are presented below and summarized in Table 3.

Table 8: Risk factors related to the post-processing module and having an effect on *E. coli* and *CMY-2 gene* contamination

Risk factor	Effect	References
Cool storage	Increased risk of *E. coli* growth when storage temperature is above 7°C	(WHO 2018; Adams, Moss, and McClure 2018; Kosmider et al. 2010; Nastasijević, Lakićević, and Petrović 2017)
Cool storage	Reduction of *E. coli* loads in chicken meat following crust-frozen and complete frozen	(Chaves et al. 2011)
Packaging	Using MAP with a higher content of CO_2 reduces the growth of *E. coli* in chicken	(Projahn et al. 2018).
Retailer	No relevant data found comparing levels of *E. coli* contamination on chicken meat in big or small business/retailers	
Transport to home and home storage	Increased risk of *E. coli* growth during thawing of chicken meat	(Ingham et al. 2005)

Cool storage

The management of the cold chain is crucial to maintain the freshness and safety of meat. The internal temperature of meat should be kept constantly < 7°C in the retail cabinet (Nastasijević, Lakićević, and Petrović 2017).

A study conducted by Brashears et al (1997) showed that chicken samples previously inoculated with *E. coli* did not show a decline in the *E. coli* load during storage at 5°C (Brashears, Reilly, and Gilliland 1998). Chaves et al (2011) demonstrated in an

experimental study that the presence or absence of skin in chicken meat was not a significant factor (P=0.01) in determining the survival of *E. coli* under crust frozen (-85°C for 20 minutes) or complete frozen (-85°C for 60 minutes) conditions. Also, freezing treatment was a significant factor in reducing the *E. coli* load from the surface of chicken meat. Results from the study also showed a reduction of the *E. coli* by 0.2 ±0.1 $\log_{10}$ CFU/ml of chicken meat rinse from crust-frozen or complete-frozen samples compared to unfrozen samples (Chaves et al. 2011). However, the fact that the *E. coli* load was not reduced more than 1 $\log_{10}$ CFU/ml during crust frozen and complete frozen treatments showed that none of these treatments would be useful in reducing the *E. coli* load of chicken samples that are already contaminated beforehand (Chaves et al. 2011).

Packaging

Different technologies exist to protect raw meat from recontamination and to prevent the growth of potential pathogenic bacteria. Projahn et al (2018) reports the results of tests of various combinations of gaseous substances and concentrations in modified atmosphere packaging (MAP) processes. Most of the tested MAP gases showed a reduction in the growth of *E. coli* or *Enterobacteriaceae* compared to the storage under air conditions. High portions of CO_2 in the gaseous mixtures was associated to better reduction of the growth of *E. coli* on chicken meat. However, none of the tested MAP processes led to a complete reduction of *E. coli* counts on chicken meat (Projahn et al. 2018).

As alternatives to MAP, active packaging has been developed where the packaging material is incorporated with different (reactive) substances strictly regulated in the EU to increase the shelf life of the meat(McMillin 2017). For instance, the incorporation of 3% carvacrol or 3% cinnamaldehyde into wrapping films reduced the amount of E. coli O157:H7 on chicken breast samples by up to 6.8 and 5.2 $\log_{10}$ CFU, respectively, after storage time of 72 h at 23◦C (Ravishankar et al. 2009). Other active packaging techniques using ovotransferrin or potassium sorbate are reported in (Seol et al. 2009)

Retailer

No studies were found regarding differences in *E. coli* contamination on chicken meat comparing size of business or retailers.

The presence of CMY-2-producing *E. coli* in retail chicken meat has been described in different countries, with evidence of contamination in the previous processing steps. A study conducted by Berg et al (2017) in Norway showed that 31% (124/406) of the samples taken of retail chicken meat had extended-spectrum cephalosporin-resistant E. coli and that a selection of these isolates (n=17) were screened and blaCMY-2 was detected in all of them (Berg et al. 2017). CMY-2 producing *E. coli* has been also isolated in 17 (85%) of chicken samples analysed from purchased local US supermarkets(Doi et al. 2010). A study conducted by Park et al (2012) in the US showed that 31.8% (7/22) chicken samples collected in local grocery stores were positive for CMY-2 producing *E. coli* (Y. S. Park et al. 2012). Koga et al (2019) in Brazil showed that all the isolates (n=8) AmpC-producing *E. coli* isolated from refrigerated chicken carcasses were positive to CMY-2 (Koga et al. 2019).

Transport to home and home storage

During transport from retail to home and also during home storage, *E. coli* can grow depending on the environmental and storage conditions.

E. coli generally grow at temperatures above 7°C (WHO 2018; Adams, Moss, and McClure 2018; Kosmider et al. 2010; Nastasijević, Lakićević, and Petrović 2017)**.** However, results from Jones et al (2004) indicated that *E. coli* growth dynamic are also influenced by temperature fluctuation (Jones, Gill, and McMullen 2004), a potential critical point during the transport from retail to home. The defrost/thawing of chicken meat may be an important risk factor for *E. coli* growth and further spread (Ingham et al. 2005). Therefore, these practices should be implemented carefully. It has been advised to defrost/thaw chicken meat under refrigeration temperatures or in cold water (USDA 2014) to limit its growth.

Results from an experimental study conducted by Ingham et al (2005) showed that defrosting a whole chicken at ≤30°C for ≤9h allowed the surface of the chicken meat to

reach 20°C leading to only a limited predicted growth of E. coli O157:H7. Authors concluded that this defrosting practice is a safe practice for big portions such as it is the whole chicken, while for smaller portions at higher temperatures and/or for longer times cannot be recommended (Ingham et al. 2005).

E. coli and resistant *E. coli* have been isolated from the liquid collected from defrosted chicken carcass. Results from a study conducted by Caudry et al (1979) showed that more than 30% of the *E. coli* isolated from the liquid collected from defrosted chicken carcass, could still transfer one of their resistant determinants. These findings therefore suggest that handling chicken thaw (liquid) is a potential health hazard as resistant *E. coli* from chicken can harbour a broad range of resistant determinants that are clinically relevant in humans (Caudry and Stanisich 1979).

10.2.1.4 Home-preparation module

Risk factors associated with *E. coli* contamination in the home-preparation module are presented below and summarized in Table 4.

Table 9: Risk factors related to the Home-preparation module and having an effect on *E. coli* and *CMY-2 gene* contamination

Risk factor	Effect	References
Kitchen hygiene	Low hygiene practices during chicken preparation increases the risk of cross-contamination of *E. coli*	(Kosmider et al. 2010; Warren et al. 2008)
Cooking temperature	*E. coli* O157:H7 is inactivated at 65°C in 2.6 min using conventional heating and at 73.4°C after 35s using microwave heating	(Apostolou et al. 2005; Juneja 1997)

Kitchen hygiene

During the preparation of chicken meat, colonization of humans by resistant *E. coli* from chicken meat has been demonstrated (Warren et al. 2008; van den Bogaard 2001). The

occurrence of cross-contamination of *E. coli* due to low hygiene practices during the preparation of chicken meat has been considered highly relevant by experts (Kosmider et al. 2010).

Cooking temperature

The inactivation or elimination of *E. coli* during cooking chicken meat depends on the temperature and time. The Advisory Committee on the Microbiological Safety of Food (ACMSF) in the UK recommend to heat for 2 min at 70°C meat products to reduce at least 6 $\log_{10}$ E. coli O157:H7(ACMSF 2007).

A study using conventional heating demonstrated that internal temperature at 65°C in chicken meat inactivates *E. coli* O157:H7 in 2.6 min (Juneja 1997). Also, a study conducted by Apostolou et al (2005) assessed the effect of using domestic microwave ovens at full power on *E. coli* O157:H7 on chicken breasts and fresh whole chicken. Results of the chicken breast portions showed that after 30s of microwave heating and reaching surface temperature of 69.8°C, 83 CFU/g *E. coli* O157:H7 was detected. Also, *E. coli* O157:H7 was eliminated after 35s of microwave exposure at 73.7°C. This study also showed that after 15s of microwave heating, chicken looked cooked but the surface temperature was around 49.2°C and *E. coli* O157:H7 was present (Apostolou et al. 2005).

10.2.2 Campylobacter spp and the mutated GyrA gene (AMR2)

A literature review was conducted using PubMed and Google Scholar. Search terms included: “chicken”, “poultry”, “campylobacter”, and “*GyrA gene”.* The literature review focused on the most recent studies performed in the UK and Europe. However, when no data were available older publications performed in other contexts have been included. The inclusion of these publications, which may not represent the current situation in the UK, have been highlighted the document whenever they were used.

It should be noted that the literature review primarily focused on AMR2. However, because it was often not possible to find information on AMR2, the literature search has

been extended to risk factors having an influence on presence and abundance of *Campylobacter spp* in chicken meat.

The risk factors identified per module are presented below.

10.2.2.4 Production module

On-farm practices have been identified as the highest risk for occurrence of antimicrobial resistance. The role of antimicrobial usage is well established, but there are other factors, which have an impact as well. At the time of writing, the amount of information related to these other factors is however very scarce and further studies are needed to characterize the contribution of specific practices within the various management systems on the occurrence of antimicrobial resistance (Murphy et al. 2018).

Extensive information can be however found on the risk of carcass contamination with *Campylobacter spp.* The risk of carcass contamination is more strongly influenced by on-farm production practices compared with slaughterhouse activities with on-farm factors being 3.5 times more important than processing plant factors in the model developed by Hutchison et al. (2017; 2016). The production module is thus a critical module for assessing *Campylobacter spp* contamination.

Several studies assumed that either none or all birds in a flock are infected with Campylobacter at arrival to the slaughterhouse (see for example (Rosenquist et al. 2003)). This assumption can be made since it has been shown that the time from initial infection to a full-blown infection of all broilers in a flock occurs within a few days (Newell and Fearnley 2003; Hartnett et al. 2001; Katsma et al. 2007). In addition, Allen *et al*. (2007) show that even if carcasses come from partially colonised flock (≤30% of caeca campylobacter-positive), 90 to 100% of carcasses end up being contaminated with Campylobacter. The factors affecting the risk of *Campylobacter spp* contamination and associated with the production module should thus not be interpreted in terms of rate of *Campylobacter spp* contamination but only in terms of presence or absence of

Campylobacter spp colonisation. These factors are presented below and summarized in Table 5.

Season

Seasonal variation in the level of Campylobacter contamination of fresh chicken are reported by many authors (Meldrum, Tucker, and Edwards 2004; Newell et al. 2011; Jorgensen et al. 2011), with a peak in June and the lowest positive rates in January, March, and December. The reason of the seasonality of Campylobacter infections in poultry is still unknown but it indicates that the relative importance of potential reservoirs and transmission routes can change over the course of the year. No information were found regarding the effect of season on Campylobacter resistance.

Farming typology

The production system seems to have an influence on the risk of exposure to *Campylobacter spp.* Studies conducted in Denmark show an infestation of 100% of organic broiler flocks, from 36.7% of conventional broiler flocks and from 49.2% of extensive indoor broiler flocks suggesting that organic broiler flocks constitute a strong potential for introduction of *Campylobacter spp* (Heuer et al. 2001). The highest risk associated with organic farms has been also highlighted by Rosenquiest *et al.* (2013) who show that, in Denmark, the yearly mean prevalence being 54.2% (CI: 40.9–67.5) for organic and 19.7% (CI: 14.8–24.7) for conventional carcasses with obvious differences in all quarters of the year. The risk for consumer has been estimated at 1.7 times higher with organic carcasses compared to conventional carcasses. No similar study was found for the UK context. Based on data provided by the British poultry council (BPC) free-range chicken accounts for 5% and organic 1% of UK chicken production. The remaining 94% comes from intensively reared birds.

Level of ciprofloxacin-resistance of *Campylobacter spp* found in chicken meat originated from different production system in the UK has been estimated by Soonthornchaikul et al. (2006). All of the isolates belonging to the organically-reared group showed to be

susceptible to ciprofloxacin, whereas the isolates from intensively reared chickens showed different resistances to this antibiotic (from 8.7% to 26.7%). In addition, in Portugal, Fraqueza et al. (2014) showed that *Campylobacter spp* isolates from extensive indoor chicken were significantly less resistant (from 58 to 77%) than those from organic (from 91 to 97%) and intensive production (from 95 to 96%).

Antimicrobial usage

Fluoroquinolone treatment has been associated with an increased proportion of quinolone-resistant strains. In a study conducted in the UK (Griggs et al. 2005), the majority of the fluroquinolone-resistant isolates collected after treatment, whether they were *C. jejuni* or *C. coli,* had a mutation in *gyrA*.

Table 10: Risk factors related to the production module and having an effect on *Campylobacter spp* and *GyrA gene* contamination

Risk factor	Effect	References
Season	Increased risk of Campylobacter contamination during summer	(Meldrum, Tucker, and Edwards 2004; Newell et al. 2011; Jorgensen et al. 2011)
Farming typology	Increased risk of Campylobacter contamination for organic broiler flocks compared to conventional broiler flocks. Lower risk of ciprofloxacin-resistance for organic broiler flocks compared to conventional broiler flocks.	(Heuer et al. 2001) (Rosenquist et al. 2013) (Soonthornchaikul et al. 2006) (Fraqueza et al. 2014)
Antimicrobial usage	Fluoroquinolone treatment has been associated with an	(Griggs et al. 2005)

Risk factor	Effect	References
	increased proportion of quinolone-resistant strains.	
Breeder flock	None	(Keener et al. 2004; Newell et al. 2011)
Feed and other farm inputs	The absence of feed withdrawal from 8-12h before slaughter increases the risk of Campylobacter contamination of chicken carcass at the slaughterhouse.	(Keener et al. 2004; Newell et al. 2011)
Housing system	None	(Hutchison et al. 2017)(Newell et al. 2011)
Biosecurity practices	Increased risk of Campylobacter contamination with low biosecurity practices	(Hutchison et al. 2017)(Georgiev, Beauvais, and Guitian 2017)(Gibbens et al. 2001) (Herman et al. 2003)(Hutchison et al. 2017; Ellis-Iversen et al. 2012)(Newell et al. 2011)(Jonsson et al. 2012)(Sommer et al. 2013) (Wedderkopp et al. 2001)(Evans and Sayers 2000)
Thinning	Increased risk of Campylobacter contamination with thinning	(Hald, Rattenborg, and Madsen 2001; Georgiev, Beauvais, and Guitian 2017; Hue et al. 2010)
Age at slaughter	None	(Hutchison et al. 2017) (Herman et al. 2003) (Evans and Sayers 2000) (Allen et al. 2008)
Gender	None	(Hutchison et al. 2017)

Risk factor	Effect	References
Transport at slaughter	Increased risk of Campylobacter contamination during transport at slaughter due to contaminated crates	(Hastings et al. 2011; Ridley et al. 2011; Slader et al. 2002)

Breeder flock

Egg transmission of *Campylobacter spp* from the breeder flock has not been recognized as a source of risk of contamination because of the inability to culture *Campylobacter spp* from hatchery samples or from newly hatched chicks (Keener et al. 2004; Newell et al. 2011). This risk factor can thus be excluded from the rest of the analysis.

Feed and other farm inputs

Feed has not been implicated in the spread of *Campylobacter spp* because it is too dry to favor survival (Keener et al. 2004; Newell et al. 2011). In general, the risk of passive carriage of *Campylobacter spp* into the farm by commodities such as feed, litter, and air appear minimal. These risk factors can thus be excluded from the rest of the analysis. Feed withdrawal time represents the total time that birds are deprived of food before slaughter and has an effect on risk of carcass contamination (Rasschaert et al. 2020). The optimal feed withdrawal time is a window of 8-12 hours. Insufficient feed withdrawal time results in intestines still partially filled with feed and feces and increased risk of carcass contamination. Long feed withdrawal time results in decreased intestinal strength, which may also lead to carcass contamination during slaughter.

Housing system

The age of the house has been reported as a risk factor for *Campylobacter spp* contamination because of the potential poor integrity of older constructions (see for example (Chowdhury et al. 2012)) but results are inconsistent and other studies actually reported no statistically significant difference between the prevalence of colonized flocks and the age of the houses (Newell et al. 2011). The material used to construct houses

has been recently significantly associated with the numbers of *Campylobacter spp* in litter (Hutchison et al. 2017). The authors hypothesized that steel frames are generally stronger than the equivalent timber ones and are thus used to construct larger sheds than timber-framed ones. However, larger sheds can hold a larger number of birds, and so the protective effect of wood framing may simply be a proxy for the number of birds placed and the number of depopulations, stress events and exposure to catchers required to clear the shed. Housing system is thus probably more a real risk factor for *Campylobacter spp* contamination but rather a factor associated with other true risk factors such as poor biosecurity and higher stress. This risk factor can thus be excluded from the rest of the analysis.

Biosecurity practices

Enhanced biosecurity practices decreased the proportion of highly colonized batches from 72.9% to 41.7% (measured at thinning) (Georgiev, Beauvais, and Guitian 2017). Another study performed at 42 days of age reported similar results with a reduction from 71% to 38% (Gibbens et al. 2001). These results are consistent with the results of (Herman et al. 2003) showing that farms with *Campylobacter*-positive broilers were characterized by the circulation of *Campylobacter spp* in the environment (puddles, dung hill) and on the footwear of the farmer.

Farm worker hygiene is thus a key important factor in reducing the risk of *Campylobacter spp* colonization (Newell et al. 2011). For example increasing the frequency of dipping boots in disinfectant was significantly correlated with lowered numbers of *Campylobacter spp* in house litter (Hutchison et al. 2017; Ellis-Iversen et al. 2012). However, it should be noted that to have a protective effect, boot dip should be changed at least twice weekly, otherwise insufficient or old active disinfectant can act as a reservoir for *Campylobacter spp*. The relative efficacy of house specific boots compared with boot dips remains unclear from available intervention studies (Newell et al. 2011).

The strict use of a hygiene barrier can reduce the risk of flock infection by about 50% (Newell et al. 2011). For example, if the distance between the stacked used litter and the poultry house is less than 200 meters, then the risk of flock infection may increase 5-fold or more (Newell et al. 2011). In addition, as shown by Hutchison et al. (2017) and Ellis-Iversen et al. (2012) presence of animals such as cattle, dogs, wildlife and rodents were significantly associated with positive flocks. Other studies have reported that farms with nonpoultry livestock in close proximity (<2 km, (Jonsson et al. 2012)) and the density of nonpoultry farming operations near to broiler farms (Sommer et al. 2013) are also risk factors for *Campylobacter spp* colonization.

Intensive cleaning and disinfection of facilities between flocks appeared to have limited effectiveness in preventing cross-contamination in one study from Wedderkopp et al. (2001), while no *Campylobacter spp* was detected after cleaning and disinfection in another study from Evans and Sayers (2000).

Thinning

In the UK, independent processors will undertake multiple partial depopulations before finally emptying a shed. As example the thinning practice was observed in 90% of batches included in a recent study conducted in the UK (Georgiev, Beauvais, and Guitian 2017). In particular, independent farms with very large sheds, such as those containing more than 50 000 birds; might partially depopulate the sheds before final clearance (Hutchison et al. 2017). Current UK practices is to implement one single partial depopulation before final clearance. The employees undertaking catching are a risk factor for *Campylobacter spp* colonization by birds in a house (Hue et al. 2010; Allen et al. 2008). Thus, if catching occurs in large sheds many times before some birds are caught, then there is an increasing likelihood the remaining birds will become colonized with *Campylobacter spp* as shown by many studies (Hald, Rattenborg, and Madsen 2001; Georgiev, Beauvais, and Guitian 2017; Hue et al. 2010). For example, (Georgiev, Beauvais, and Guitian 2017) show that where only 42% of batches raised under enhanced biosecurity were colonized at thinning, this proportion increased to 65% at final depopulation. (Hue et al. 2010) also show that previous thinning of the

flocks increases the risk of *Campylobacter spp* contamination of the flock at the slaughterhouse (OR 3.3).

Age and weight at slaughter

The proportion of flocks infected at slaughter increased with the age at slaughter, from 50% when slaughtered at 28-35 days to 97% when slaughtered at 50 days of older (Evans and Sayers 2000). In general, the infection of broiler flocks increased continuously during the rearing time (Herman et al. 2003) and (Hutchison et al. 2017) estimated that for each day a bird was farmed there was a mean increase in log10 *Campylobacter spp* numbers of 0-331 CFU/g litter. Allen et al. (2008) however reported that campylobacters were isolated from chicken catchers, their clothing, vehicles and equipment immediately after arrival on farms. The isolations were from different sets of catchers working for a variety of UK processors in the mid-2000s, which was compelling evidence that in the United Kingdom, historically at least, the breaking of biosecurity was credibly implicated with *Campylobacter spp* colonization and bird age.

Gender

Gender of the birds slaughtered has been reported as a potential risk factor for carcass contamination with *Campylobacter spp*. However, Hutchison et al. (2017) show that there was no significant gender and age interaction influence on the change in Campylobacter numbers between the different gender categories. This can be explained by the fact that it is common in the United Kingdom for the lighter female birds to be cleared from houses first, with the males allowed to grow on to a greater weight. In the statistical model developed by Hutchison et al. (2017), it was determined that although females were cleared in preference to males for roughly half of the time, there were also some processors that would harvest males first if they reached a set target weight before the females, thereby potentially masking any effect for age by gender. This risk factor can thus be excluded from the rest of the analysis.

Transport at slaughter

Several studies have pointed out the role of crates used to transport live poultry to slaughterhouse in *Campylobacter spp* contamination despite periodic sanitization (Hastings et al. 2011; Ridley et al. 2011; Slader et al. 2002). *Campylobacter spp* on crates survived for at least 3 h after sanitization, a period of time equivalent to the journey from the processing plant to the majority of farms in the catchment, showing the potential for involvement of crates in transmission. Hasting et al. (2011) reported that the inclusion of a silver ion biocide in poultry transportation crates to levels demonstrating acceptable antibacterial activity in vitro reduces the level of bacterial contamination during normal crate use compared to standard crates.

10.2.2.2 Processing module

In terms of antimicrobial-resistance, it is plausible that abattoir interventions may have varying effects but no data are currently available and further study are needed to understand the impact of interventions at abattoir on the occurrence of antimicrobial-resistant bacteria (Murphy et al. 2018). A significant correlation however exits between the bacterial contamination of the broilers during rearing and the carcasses after processing and Herman et al. (2003) have shown that it is in general not possible for a slaughterhouse to avoid contamination of carcasses when status-positive animals were delivered. Supplementary contamination can however occur during slaughtering (Herman et al. 2003; Allen et al. 2007; Colles et al. 2010; Hastings et al. 2011) and Allen et al. (2007) show that even if carcasses come from negative flocks, 30% of carcasses end up being contaminated with *Campylobacter spp*. *Campylobacter spp* have been indeed isolated from both the air and equipment and machinery in slaughterhouses (Berndtson, Danielsson-Tham, and Engvall 1996; Allen et al. 2007) and are able to survive overnight on food processing surfaces after cleaning and disinfection procedures have been completed (Peyrat et al. 2008). However, there is a considerable differential in the ability of campylobacter strains to survive different environmental stresses and Newell et al. (2001) show that, while some subtypes survived all the processing stages, others apparently survived only to the chilling stage.

If avoiding contamination at the slaughterhouse is in general not possible, Pacholewicz et al. (2015b) and Dogan et al. (2019) have shown that slaughterhouse processing can significantly reduce Campylobacter concentration but that there are variability in concentration between slaughterhouses depending on the degree of control of each step of the process. The authors also reported variability between batches. The model developed by Hutchison et al. (2016) have identified the chilling, washing and de-feathering process stages as being statistically-significantly correlated with the numbers of bacteria on carcasses. Additional risk factors have been however identified (for example, evisceration) and are presented below and summarized in Table 6.

Size of the slaughterhouse

No information have been found regarding the risk of *Campylobacter spp* contamination associated with the size of the slaughterhouse.

Hygienic practices in the slaughterhouse environment

The use of insufficiently cleaned and disinfected crates may have a major impact on the *Campylobacter spp* contamination (Rasschaert et al. 2020). This result is consistent with the fact that *Campylobacter spp* have been indeed isolated from both the air and equipment and machinery in slaughterhouses (Berndtson, Danielsson-Tham, and Engvall 1996; Allen et al. 2007) and are able to survive overnight on food processing surfaces after cleaning and disinfection procedures have been completed (Peyrat et al. 2008).

Order at slaughter

Order at slaughter has been identified as a risk factor for contamination (OR=3.5) (Hue et al. 2010) and some study suggest that slaughtering of *Campylobacter* -negative flocks at the beginning of the day may reduce carcass contamination (Newell et al. 2001). However, Rosenquist et al. (2003) show limited effect of this approach for reduction of contamination.

Stunning and bleeding

This step of the process is rarely integrated in models looking at risk of *Campylobacter spp* contamination because it is considered as having few microbiological implication (see for examples (M. J. Nauta, Jacobs-Reitsma, and Havelaar 2007; Rosenquist et al. 2003; Havelaar and Evers n.d.; World Health Organization and Food and Agriculture Organization of the United Nations 2009; Chapman et al. 2016)).

Scalding

The scalding procedure is used to open the feather follicles to facilitate the removal of feathers. At this stage a proportion of *Campylobacter spp* is washed off the carcass, resulting in lower concentration of bacteria but contaminated scald water and subsequent cross-contamination to the next carcasses in line (Havelaar and Evers n.d.; Keener et al. 2004). A decrease in *Campylobacter spp* concentration of between 20 to 40% is reported after scalding by several authors with minimum decrease of 1.3 cfu/g, and maximum decrease of 2.9 cfu/mL (Pacholewicz et al. 2015b; Guerin et al. 2010). Plugging the cloacae with tampons and sutured can however reduce the risk of cross-contamination (Berrang et al. 2001).

Defeathering

During defeathering a proportion of organisms is washed off or removed with the feathers, but a number of organisms is also added via cross-contamination. Inadequate plucking is associated with both higher bacteria load and higher risk of cross contamination (Hutchison et al. 2017; Pacholewicz et al. 2015b; Guerin et al. 2010)(Allen et al. 2007; 2003). Studies reported an increase of *Campylobacter spp* prevalence of between 10 and 72% after defeathering (Guerin et al. 2010). In addition, 1 of 120 broiler breast skin samples was positive for *Campylobacter spp* before defeathering, while 95 to 120 of the samples were positive after defeathering in a study conducted by Berrang et al. (2001).

Table 11: Risk factors related to the processing module and having an effect on *Campylobacter spp* and *GyrA gene* contamination

Risk factor	Effect	References
Size of the slaughterhouse	No relevant data found	
Order at slaughter	Increased risk of cross-contamination if Campylobacter-positive flocks are slaughtered at the beginning of the day	(Hue et al. 2010)(Newell et al. 2001) (Rosenquist et al. 2003)
Stunning and bleeding	Considered as having few microbiological implication	(M. J. Nauta, Jacobs-Reitsma, and Havelaar 2007; Rosenquist et al. 2003; Havelaar and Evers n.d.; World Health Organization and Food and Agriculture Organization of the United Nations 2009)(Chapman et al. 2016)
Scalding	Decrease Campylobacter concentration but can increase cross-contamination via scald water.	(Havelaar and Evers n.d.; Keener et al. 2004) (Pacholewicz et al. 2015b; Guerin et al. 2010)(Berrang et al. 2001)
Defeathering	Inadequate plucking is associated with both higher Campylobacter load and higher risk of cross contamination	(Hutchison et al. 2017; Pacholewicz et al. 2015b; Guerin et al. 2010)(Allen et al. 2007; 2003)(Berrang et al. 2001).
Evisceration	Evisceration is associated with both higher Campylobacter load	(Rosenquist et al. 2006; Guerin et al. 2010; Pacholewicz et al.

Risk factor	Effect	References
	and higher risk of cross contamination. Some studies reported a reduction in Campylobacter load.	2015b; Hue et al. 2010; Huang et al. 2017; Berrang, Buhr, and Cason 2000) (Lu et al. 2018)(Allen et al. 2007)
Washing	Mainly decrease Campylobacter load but inconsistent results have been reported regarding Campylobacter prevalence	(Guerin et al. 2010; Havelaar and Evers n.d.), (Lu et al. 2018)
Pre-chilling treatment	Effect depend on the type of pre-chilling process implemented and how this process is implemented. Mainly decrease Campylobacter load but can also favour cross-contamination.	(Hutchison et al. 2017)(Huang et al. 2017) (Allen et al. 2008)(Y. Li, Yang, and Swem 2002)(Rosenquist et al. 2003)(Rosenquist et al. 2006; Pacholewicz et al. 2015b).(Guerin et al. 2010)(Allen et al. 2007)
Chilling	Chilled storage (at 4°C) does not seem to affect the concentration of Campylobacter considerably but when it does, it tends to decrease the concentration of bacteria especially when lower temperature are used. No significant difference between longer and shorter chilling process has been identified	(Hutchison et al. 2017)(Huang et al. 2017) (Allen et al. 2008)(Y. Li, Yang, and Swem 2002)(Rosenquist et al. 2003)(Rosenquist et al. 2006; Pacholewicz et al. 2015b).(Guerin et al. 2010)(Allen et al. 2007)

Risk factor	Effect	References
Cutting	Only scarce data available but prevalence might be higher in whole chicken compared to portions	(M. J. Nauta, Jacobs-Reitsma, and Havelaar 2007)(Food Standard Agency 2003).
Skin removal	Removal of skin before processing reduces Campylobacter load and reduce Campylobacter prevalence but differences reported are minor.	(Berrang et al. 2002; M. J. Nauta, Jacobs-Reitsma, and Havelaar 2007)(Scherer et al. 2006) (Sampers et al. 2008)(Food Standard Agency 2003)(M. A. Davis and Conner 2007).

Evisceration

The evisceration process involves removal of the feet, head and viscera of the birds, and the harvesting of edible offal. Several studies found in the literature (Rosenquist et al. 2006; Guerin et al. 2010; Pacholewicz et al. 2015b; Hue et al. 2010; Huang et al. 2017; Berrang, Buhr, and Cason 2000) indicated that both the *Campylobacter spp* positive rate and concentration in the process of evisceration was increased greatly possibly due to intestinal content leakage.

The process can be done either manually or mechanically. Manual evisceration can introduce human-borne contamination to the production line, while poorly calibrated machinery can also perforate the intestinal lining, leading to the spread of luminal contents (Lu et al. 2018). Increase of concentration of 0.5 log10 cfu/g have been reported (Rosenquist et al. 2006) but other studies reported decrease of concentration after evisceration. For example, in UK, Allen et al. (2007) shown a reduction in the numbers of *Campylobacter spp* on carcasses after evisceration process in 7 out of 10 flocks (compared with after plucking) and Guerrin et al. (2010) reported a decrease of 0.3 cfu/g in the *Campylobacter spp* concentration.

In terms of increase prevalence, Hue et al (2010) reported an OR of 2.6, and (Guerin et al. 2010) an increase of 15% in *Campylobacter spp* prevalence. Hue et al (2010) indicated that a temperature of the evisceration room above 15°C increases the risk of contamination (OR=3.1).

Washing

The efficacy of carcass washing depends on a number of factors, including the number and type of washers, water pressure, nozzle arrangement, flow rate, line speed, water temperature, presence of sanitizing agents such as chlorine, and the use of surfactants (Lu et al. 2018). Washing seems mainly to decrease *Campylobacter spp* concentration from between 0.3 cfu/mL to 1.1 cfu/mL (Guerin et al. 2010; Havelaar and Evers n.d.). The impact of washing on Campylobacter contamination is however hard to assess and studies reported inconsistent results ranging from a 23% decrease to a 13.3% increase of *Campylobacter spp* prevalence (Guerin et al. 2010).

Cutting

At the cutting stage chilled carcasses are deboned and portioned. Count data on the effects of cutting on the numbers of *Campylobacter spp* on the chicken products remain scarce (M. J. Nauta, Jacobs-Reitsma, and Havelaar 2007). The process can be done either manually or mechanically. In the UK, samples taken at a retail storage facility indicated that prevalence of *Campylobacter spp* was 57% and 46% in whole chickens and portions respectively (Food Standard Agency 2003).

Skin removal

Beerang et al. (2002) show that removal of skin before processing reduces *Campylobacter spp* levels by 0.7 log10 CFU/carcass. However, in this study the level on the meat was only about 1 log lower than on the skin, which suggests substantial cross-contamination between skin and muscle due to damaged skins (Berrang et al. 2002; M. J. Nauta, Jacobs-Reitsma, and Havelaar 2007). (Scherer et al. 2006) estimated the

concentration of *Campylobacter spp* on the surface of positive chicken legs being a median of 2.4 log CFU/g of skin, when concentration in the muscle gave results mainly under the detection limit of the most-probable-number method (0.3 MPN Campylobacter per g). External contamination was thus significantly higher than internal. This results is consistent with a study conducted in Belgium showing that the presence of skin significantly increase the probability of being positive for *Campylobacter spp* (Sampers et al. 2008). In the UK, no significant difference in contamination frequency between wrapped and unwrapped chickens has been identified so far (Food Standard Agency 2003). Survival of *Campylobacter spp* appears also similar between skin and meat (M. A. Davis and Conner 2007).

Pre-chilling treatment

Pre-chilling interventions aimed at reducing the degree of cross-contamination of carcasses during slaughter and include heating carcasses' surfaces by steam or hot water, acid sprays, irradiation, or the use of chlorinated water:

- *Chlorinated water:* The effect of processing using chlorinated water on numbers of *Campylobacter spp* on carcasses is unclear (Y. Li, Yang, and Swem 2002; Mead, Hudson, and Hinton 1995). These studies are not reported here in detail as using chlorine concentrations higher than those used in potable water is not permitted in the EU.
- *Air chilling:* Air chilling including carcass wash prior to the chilling operation caused significant reduction of *Campylobacter spp* 0.83 log10 cfu/g (Rosenquist et al. 2006). Air chilling is almost-exclusively used in broiler processing in the United Kingdom and there are reports that chilling can reduce the numbers of *Campylobacter spp* measured from carcasses (Hutchison et al. 2017). However, although the observed reductions were significant ($P < 0.001$), they were quite small and in only three of the positive batches was the reduction greater than one log. The effect of air chilling on carcass contamination was highly variable between different batches and plants. Cross-contamination between carcasses in the chiller was identified by other authors

(Huang et al. 2017) and was suspected to be one of the reasons for the variable results, although the mechanism of spread is not known.

- *Water chilling:* Water chilling including carcass wash prior to the chilling operation caused significant reduction of *Campylobacter spp* 0.97 log10 cfu/g (Rosenquist et al. 2006). Chilling with added water sprays, which is commonly observed in British plants, can increase bacterial counts from the cavity of the carcass, especially for *Pseudomonas spp* (Allen et al. 2008). The use of water to aid chilling meant that some parts of the carcass were likely to retain enough moisture during storage to allow for survival of *Campylobacter spp* and also to withstand the drying process that occurs at the same time as chilling. Carcasses treated using high-temperature water spray using an inside-outside bird washer show however a lower number of *Campylobacter spp*: 1.28 $\log_{10}$ cfu per carcass at 55°C and 1.43 $\log_{10}$ cfu at 60°C (the highest temperature which may be used before the colour of the skin changes significantly) (Y. Li, Yang, and Swem 2002).
- *Rapide surface cooling*: The technique involves the rapid chilling of the surface of poultry using cooled liquid nitrogen vapour delivered at -196°C. It does not frozen the flesh and caused average reductions in the numbers of *Campylobacter* of between 0.9 and 1.5 log10 cfu/g when tested the day after treatment and between 0.9 and 1.3 log10 cfu/g when tested a further six days later (Burfoot et al. 2016).
- *Ultrasound treatment:* Sonostream® is a recently developed method of food surface decontamination, which employs steam and ultrasound for effective heat transfer and short treatment times, resulting in significant reduction in surface bacteria. The results of Harsen and Larsen (2007) showed an average reduction of 2.51 log10 units (CFU/ml) and no visual changes of the chicken carcasses.

Chilling

Chilled storage (at 4°C) does not seem to affect the concentration of *Campylobacter spp* considerably but when it does, it tends to decrease the concentration of bacteria (Rosenquist et al. 2003)(Rosenquist et al. 2006; Pacholewicz et al. 2015b). For example, Guerrin et al. (2010) reported decrease of *Campylobacter spp* concentration

after chilling ranging between 0.4 cfu/mL, to 2.9 cfu/mL. It should be however noted that no reduction where observed in the plant with manual re-hanging and water sprays in the first section of the chiller. No significant difference between longer and shorter chilling process has been identified (Allen et al. 2007) but lower post-chilling carcass temperature has been associated with fewer *Campylobacter spp* (Hutchison et al. 2017).

In terms of *Campylobacter spp* prevalence, 100% decrease to 26.6% increase have been reported in the literature (Guerin et al. 2010).

10.2.2.3 Post-processing module

Risk factors associated with *Campylobacter spp* contamination in the post-processing module are presented below and summarized in Table 7.

Table 12: Risk factors related to the post-processing module and having an effect on *Campylobacter spp* and *GyrA gene* contamination

Risk factor	Effect	References
Cool storage	Crust and flash freezing decrease the concentration of Campylobacter	(Food Standard Agency 2003). (Rosenquist et al. 2003)(Rosenquist et al. 2006; Pacholewicz et al. 2015b)(Huang et al. 2017; Rosenquist et al. 2003; 2006)(D. Harrison et al. 2013)(Haughton et al. 2012).
Retail storage and packaging	No major difference highlighted in the literature, but studies reported that at the large retailers, the risk of contamination is probably lower than at small scale butcheries	(Meldrum, Tucker, and Edwards 2004) (De Boeck et al. 2016)(Food Standard Agency 2003).

Risk factor	Effect	References
Transport to home and home storage	Thawing of chicken product at ambient temperature for extensive period might increase Campylobacter load.	(Keener et al. 2004) (Rosenquist et al. 2003)

Cool storage

The chicken can be stored either fresh or frozen. In 2003, fresh poultry accounts for 66 per cent of retail sales, while frozen and cooked poultry have increased in their popularity. For example, over the period of 1990–2000, the sales of uncooked poultry decreased by 3 per cent and the sales of cooked poultry increased by 160 per cent. While fresh chicken is preferred for household consumption, frozen meat is used widely in the food services and food processing industry. On average in 2000's, after slaughter, 92% of chicken meat is chilled and/or frozen, and 8% is cooked (Yakovleva and Flynn 2004).

In the UK, the frequency of *Campylobacter spp* contamination of fresh chicken (56%) has been estimated higher than for frozen chicken (31%) (Food Standard Agency 2003). As discussed above, chilled storage (at 4°C) does not seem to affect the concentration of *Campylobacter spp* considerably but when it does, it tends to decrease the concentration of bacteria (Rosenquist et al. 2003)(Rosenquist et al. 2006; Pacholewicz et al. 2015b).

Flash freezing decreases the concentration of *Campylobacter spp* and seems to play a role on bacteria growth inhibition. (Huang et al. 2017; Rosenquist et al. 2003; 2006) report a reduction of 1.38 log10 cfu/g on average after freezing operation. Harrison et al. (2013) determined the numbers of campylobacters on the livers were immediately before and after a 24-h or 7-days freeze treatment and daily during 3 days post-thaw refrigerated storage. The results shown that freezing for 24 h at -25°C can reduce numbers of *Campylobacter spp* by up to 2 log10 CFU g(-1). Freezing the livers for 24 h at -25°C, thawing overnight in a fridge set to 4°C and refreezing for another 24 h at -

25°C reduced the numbers of bacteria by up to three logs. Reduction in the numbers of organisms was significantly greater following a second freeze treatment compared with a single freeze treatment. Crust freezing can also reduce the levels of *C. jejuni* by between 0.5 and 1.5 log10 CFU/g with minimal impacts on the colour of treated skin (Haughton et al. 2012).

The storage time can decrease *Campylobacter spp* concentration when the meat is frozen (Georgsson et al. 2006), but has no influence on fresh meat (Pintar et al. 2007).

Packaging

Modified atmosphere packaging can help controlling microbial growth as shown by Boysen et al. (2007) when working on *C. jejuni*: the strains survived significantly longer when exposed to 100% N2 and 70/30% N2/CO2 compared to an oxygen-containing gas mixture (i.e., 70/30% O2/CO2). For the two anaerobic gas mixtures, the reductions only reached 0.3–0.8 log10 CFU/mL while, in the presence of oxygen, the numbers of *C. jejuni* were reduced by a minimum of 4.6 log10 CFU/mL over 21 days. When inoculated onto chicken fillets, the *C. jejuni* strains also died significantly faster in the oxygen-containing gas mixture, 70/30% O2/CO2, reaching reductions of 2.0–2.6 log10 CFU/g after 8 days. In the gas mixture without oxygen (70/30% N2/CO2), no reductions were observed.

Retailer

The British chicken supply chain relies on several main distribution outlets: 71 per cent of chicken is sold through supermarket chains, 15 per cent through food services and 14 per cent through other independent retailers according to the British Poultry Council. These different type of distribution outlets can in theory be associated with different risk of *Campylobacter spp* contamination but so far no differences were found between samples taken from retailers or butchers (Meldrum, Tucker, and Edwards 2004). A study conducted in Belgium shown that the risk of microbial pathogens contamination at retail greatly varies depending on the scale and the scope of the business: on the one hand, at the large retailers, the risk of contamination is probably lower than at small

scale butcheries, however its possible effects can reach a much higher number of consumers (De Boeck et al. 2016). Average contamination level in retail chicken products in the UK was estimated at 50% in 2001 but, as indicated above, differences were identified between fresh and frozen chicken (56% versus 31%)(Food Standard Agency 2003).

Transport to home and home storage

Thawing of poultry products at ambient room temperature for extensive periods is not recommended and (Keener et al. 2004) reported that *C. jejuni* cells could replicate at room temperatures and under refrigeration at 4 °C. This step of the process if usually ignored in QRA (see for example (Rosenquist et al. 2003)).

10.2.2.4 Home preparation module

The transfer of *Campylobacter spp* from a *Campylobacter spp* contaminated chicken to the consumer may occur through several contamination routes and contribute significantly to the risk of *Campylobacter spp* infection (Rosenquist et al. 2003; Mylius, Nauta, and Havelaar 2007). Humans may become infected by direct contact, i.e. by licking fingers that have been in contact with a chicken or packaging (i.e., *Campylobacter* spp has been also isolated from the outside and inside of the packaging (Jørgensen et al. 2002; W. A. Harrison et al. 2001)) or, indirectly, by consuming an undercooked chicken meal or a food item, for example, salad or prepared chicken, which has been cross-contaminated during handling or preparation of a raw chicken. It is not known to which extend each of these processes contributes to the overall transfer of *Campylobacter spp* from chickens to consumers. The most important steps are described below and summarized in Table 8.

Table 13: Risk factors related to the home preparation module and having an effect on *Campylobacter spp* and *GyrA gene* contamination

Risk factor	Effect	References
Kitchen hygiene	Low kitchen hygiene is associated with higher risk of Campylobacter cross-contamination	(Yang et al. 1998)(Mylius, Nauta, and Havelaar 2007)(Mylius, Nauta, and Havelaar 2007).
Cooking temperature	Cooking temperature below 75°C increase the risk of Campylobacter contamination	(Whyte, Hudson, and Graham 2006) (Dogan et al. 2019)

Kitchen hygiene

Several studies have shown that the extent of kitchen hygiene (safe/unsafe food handling) depends on age and sex (see for example (Yang et al. 1998) looking at consumers behaviour in the US). Kitchen hygiene has an impact on risk of cross-contamination via, for examples, unwashed cutting boards, hands or knife. Variation in whether the cutting board is washed in between the preparation of chicken meat and raw food items is more important to cross-contamination than whether the cook washed his or her hands in between these actions (Mylius, Nauta, and Havelaar 2007). It should be however noted that washing the cutting board has been estimated as being already at a very high level of compliance in a study conducted in the Netherlands (Mylius, Nauta, and Havelaar 2007).

Several factors may influence the number of *Campylobacter spp* transferred from a raw chicken to a cutting board and further to a prepared meal. Such factors include (Zhao et al. 1998): the amount of drip fluid, the contact area between the raw chicken and the cutting board, the time lag between placing the raw chicken and the prepared chicken on the cutting board, etc. Dawkins et al. (1984) examined work surfaces, sinks, and floors of areas where fresh and frozen chicken had been processed. Cleaning with

detergent and hot water (or steam) and drying was sufficient to remove *C. jejuni* from the environment. They reported that drying surfaces after washing was an important factor in controlling persistence in the environment. In previous study, modellers have only included some but not all of these potential risks (see for example (Rosenquist et al. 2003)).

Cooking process

Campylobacter spp is rather temperature sensitive and using pan-frying, a total duration of 5 minutes is enough to inactivate naturally occurring *Campylobacter spp* in chicken livers (Whyte, Hudson, and Graham 2006). This included 2-3 minutes to reach an internal temper of 70-80°C and maintaining this temperature for 2–3 min. Many health authorities recommend thawing poultry rapidly and cooking it thoroughly to an internal end point temperature of 75 °C. Dogan et al. (2019) have identified the cooking temperature as the leading factor in preventing the occurrence of campylobacteriosis among broiler chicken consumers.

10.2.3 Stakeholders consultation

An online workshop was organized on September 17th from 9 to 12 a.m. with stakeholders from UK chicken industry. Invitations were sent to 46 potential participants, and 11 of them attended the workshop: Daniel Parker (Slatehall), Steve Moore (Avara Foods), Allan Ball (Slatehall), Sara Perez (Poultry Health services), Peter O'Kane (Slatehall), Marie Burnett (British Poultry Council), Laura Higham (FAI farm), Keith Warner (Avara Foods), Daniel Dring (P.D. Hook), Lulia Gherman (FSA), and Anthony Wilson (FSA).

The results of the literature review were presented and discussed with the participants. Online questionnaires were used during the workshop to support the discussion and collect detailed information related to key risk factors identified during the literature review. The material used during the workshop, the recordings, and the minutes are attached to this report.

The key output of the workshop indicated that additional risk factors should be maybe included and/or that additional information should be at least added on:

- Stocking density
- Stress
- More information needed on the difference between organic vs conventional production and on the effect of the age of the birds on the risk of carcass contamination
- More information needed on effect of the breeder flock especially regarding the risk of *E. coli* contamination.

For sake of simplicity, missing information related to difference between production type, age of birds, and effect of breeder flock on the risk of *E. coli* contamination were directly added in literature review available above. However, no specific evidence related to the effect of stress or stocking density on the risk of *Campylobacter spp* or *E. coli* were found in the literature. These two factors are usually considered having an

influence on chicken health, welfare and production performance (see for example (Estevez 2007; Dozier et al. 2006)).

Based on stakeholder feedback, the following risk factors should be removed from the modelling framework:

- No plugging of cloacae before evisceration in the UK
- Only mechanical evisceration for chicken
- No pre-chilling processing using water for chicken in the UK, only air pre-chilling
- Only water allowed to wash the chicken carcasses

Some quantitative information about specific processing steps were collected during the workshop but information were missing due to the background of the participants. It was agreed that the project team will send an updated version of the questionnaire to other stakeholders from the processing and post-processing module to collect the missing information. This new activity is directly linked to the definition of value of the model parameters and is thus planned to be part of the second deliverable of this project.

Two important concerns were raised by the participants:

- The project team might need to invest more time to collect all the data needed to have a complete overview of the UK industry. The project team and FSA highlighted the fact that the objective of the project is not to collect data on every practice of the UK industry but rather to collect realistic data to test the modelling framework and identify critical gaps in the literature in order to make recommendations for futures studies. The model is intended to be flexible enough to allow FSA to modify it later on with new input values if needed.

- Given the absence of data on antimicrobial resistance but the large amount of information available on bacterial contamination, the project team should be careful when presenting the model outputs. Model limitations on this specific question should be clearly highlighted.

10.2.4 Critical risk pathway

A large majority of the risk factors identified for AMR1 were also valid for AMR2 allowing to build a unique critical risk pathway of consumer exposure for the two selected microorganisms and resistance genes. When a risk factor was identified for only AMR1 or AMR2, it has been kept in the critical risk pathway. In the future model, it will be possible to exclude the risk factor associated with, for example, only AMR1 but not AMR2. All the risk factors where no information could be found in the literature (for example, stress, housing system) were not included in the final proposed critical risk pathway but their inclusion needs to be further discussed with FSA.

The proposed critical risk pathway is presented in Figure 1.

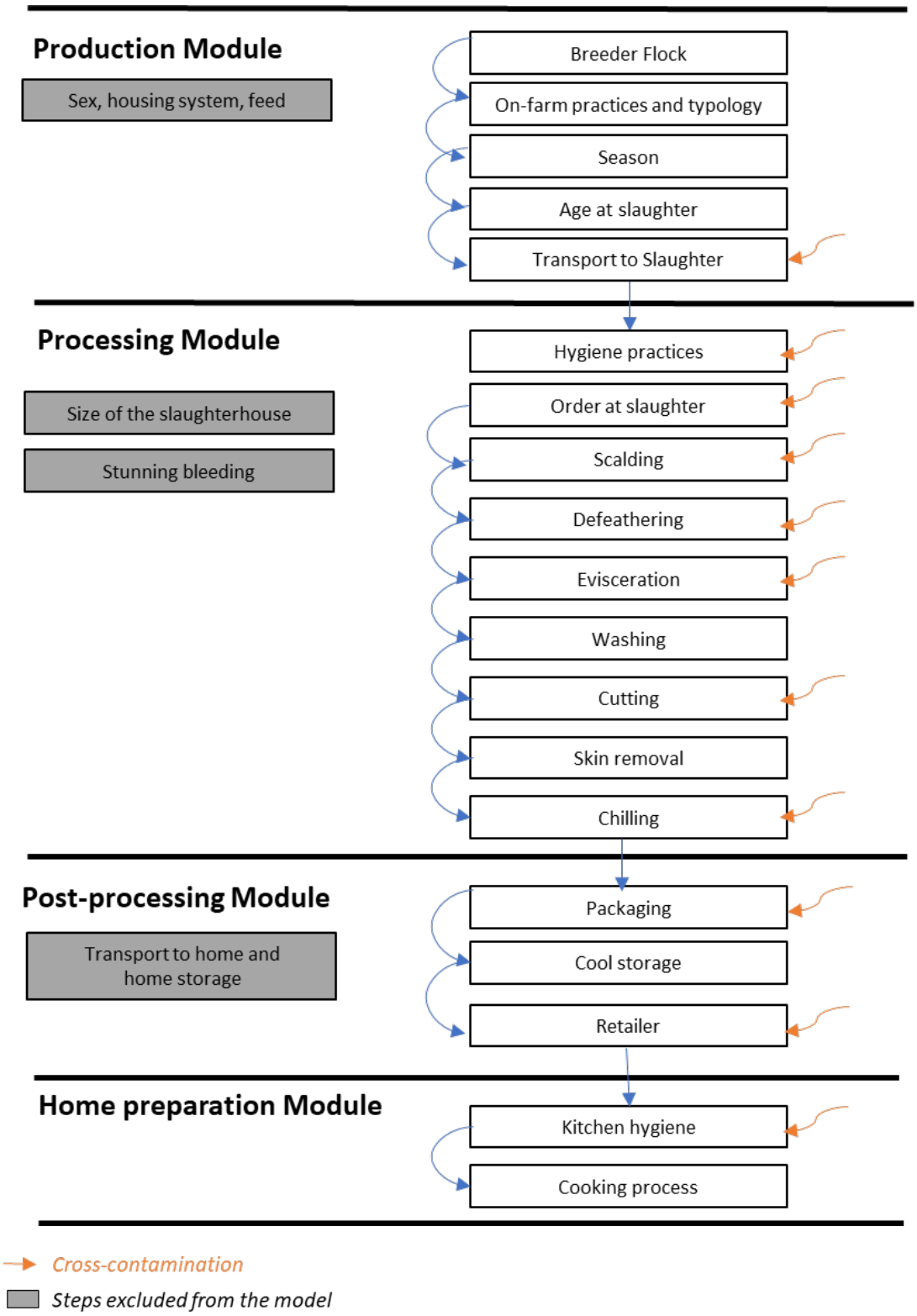

Production Module
Sex, housing system, feed
Breeder Flock
On-farm practices and typology
Season
Age at slaughter
Transport to Slaughter
Processing Module
Size of the slaughterhouse
Stunning bleeding
Hygiene practices
Order at slaughter
Scalding
Defeathering
Evisceration
Washing
Cutting
Skin removal
Chilling
Post-processing Module
Transport to home and home storage
Packaging
Cool storage
Retailer
Home preparation Module
Kitchen hygiene
Cooking process
Cross-contamination
Steps excluded from the model

Figure 4: Value chain of portioned skin-off fresh chicken in the UK and critical risk factors associated with AMR1 and AMR2 consumer exposure.

10.3 Critical risk pathways for AMR exposure via lettuce

For the lettuce production chain, the critical risk pathways for AMR exposure were established for only one microorganism and one resistance gene: the microorganism *E. coli* and the ampC beta-lactamase gene *CMY-2.*

Furthermore, after discussing the different available products of lettuce in the UK market, it has been decided with FSA to use outdoor grown pre-washed bagged lettuce for the project development. Reasons for this choice included the higher susceptibility of microbial contamination of outdoor lettuce compared to indoor grown lettuce as the latter is more protected from the outside environment (Holvoet et al. 2015) and also, the increased sale of pre-cut and pre-washed bags of salad in the UK (Sheane, McCosker, and Lillywhite 2017).

A literature review was carried out with PubMed and Google Scholar search tools. The search combined terms included: lettuce, salad, leafy greens, vegetables, bagged salad, ready-to-eat, *Escherichia coli*, CMY-2, bla_{CMY-2}, ampC beta-lactamase CMY-2 gene, outbreak, contamination, risk-assessment, production, processing, storage, home preparation, among others.

The findings highlighting the steps that have an influence on abundance of *E. coli* in outdoor grown pre-washed bagged lettuce are presented below. When it was available, information on the CM2-gene was also included.

The risk factors identified per module are reported below.

10.3.2 Production module

The microbial contamination that occur at field production might not be eliminated during further processing steps(Tyrrel, Knox, and Weatherhead 2006; Sapers 2001). This is the case of Shiga toxin-producing *E. coli* (STEC) in leafy green vegetables,

whose presence might not be removed in later steps but also increased under certain conditions (Codex Alimentarius Commission 2019). Furthermore, ready-to-eat crops with a short growing season such as salads are especially vulnerable to microbial contamination (FSA 2009). Therefore, it is essential to identify sources of contamination at production level in order to minimize the microbial contamination of fresh products (Codex Alimentarius Commission 2019; J. M. Monaghan and Hutchison 2012).

Many growers of fresh produce in the UK are required by their customers to apply strict standards of production to reduce the risks of microbial contamination (Finch, Samuel, and Lane 2014). The most applied scheme in the UK is the Red Tractor Fresh Produce (RTFP) Scheme, which include general standards for fresh produce (Red Tractor Certified Standards for farms 2019) and specifically for outdoor lettuce (Red Tractor Assurance for Farms 2017).

The majority of the factors affecting the risk of *E. coli* contamination in outdoor grown lettuce might also apply to other bacteria and are presented in Table 9.

Table 14: Risk factors that have an effect in the burden on *E. coli* in the production module.

Risk factor	Effect	References
Irrigation water	Contaminated water increases the risk of *E. coli* contamination of lettuce.	(Söderstöm, Lindberg, and Anderson 2005; Holvoet et al. 2013; Njage and Buys 2015; Jay et al. 2007; Gelting 2006; Tyrrel, Knox, and Weatherhead 2006)
Animal manure or organic fertilizer	Increased risk of *E. coli* contamination of crops with untreated manure	(Valcour et al. 2002; A. N. Jensen et al. 2013; Islam et al. 2004; Solomon, Yaron, and Matthews 2002)

Risk factor	Effect	References
Wildlife animals and pests	Increased risk of *E. coli* contamination of crops with presence of wildlife animals and pests	(Gelting 2006; Luna-Guevara et al. 2019; Holvoet et al. 2015)
Harvest season	Increased contamination risk of *E. coli* in lettuce in autumn compared to spring	(M. Oliveira et al. 2012; Ailes et al. 2008)
Age of lettuce leaves	Contaminated inner lettuce leaves with *E. coli* at pre-harvest stage may be a risk factor for post-harvest contamination	(Brandl and Amundson 2008).
Worker health and hygienic practices	Increase microbial contamination risk through fecal-oral transmission and cross-contamination when adequate hygienic, disinfectant practices are not properly performed and sick workers handle lettuce	(Codex Alimentarius Commission 2019; Suslow et al. 2003; McEvoy et al. 2009).
Cooling temperature	Risk of *E. coli* when temperature exceeds 7°C	(WHO 2018; Adams, Moss, and McClure 2018)
Cooling method	Hydrocooling might increase the risk of contamination and spread of pathogens	(Gil et al. 2015)

Irrigation water

Irrigation water is considered the major risk factor of microbial contamination of crops (Gil et al. 2015). Poor quality water sources might be contaminated with faecal bacteria that can be latter transferred to crops through irrigation (Tyrrel, Knox, and Weatherhead 2006).

Sources of irrigation water include surface water such as rivers and reservoirs (Uyttendaele et al. 2015) . Rivers are the main source for crops irrigation but also collectors of urban wastewater, which might contain faecal matter (Tyrrel, Knox, and Weatherhead 2006). Other sources of irrigation water include groundwater (wells, boreholes), rainwater for irrigation and tap water (to a lesser extent) (Uyttendaele et al. 2015).

Formerly in the UK, untreated irrigation water used in crops and it was generally extracted from rivers and other surface waters (Tyrrel, Knox, and Weatherhead 2006). Nowadays, the majority of commercial scale lettuce growers in the UK treat irrigation water through, for example, UV units (J. Monaghan, personal communication). The Department for Environment Food & Rural Affairs (DEFRA) published farming rules for water, including the management of manure and soil (DEFRA 2017). The compliance of UK commercial growers with the RTFP is aligned with the Guidance in Annex 2 of the EC 2017/c 163/01[2] guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (J. Monaghan, personal communication). Concerns have been raised regarding small growers that reach the market with limited enforcement on the aforementioned requirements (J. Monaghan, Thomas, and Goodburn 2008).

The use of irrigated water from a small stream was described as a potential cause for water contamination linked to an outbreak of enterohemorrhagic *E. coli* (EHEC) O157:H7 in iceberg lettuce in Sweden (Söderstöm, Lindberg, and Anderson 2005). Furthermore, a study conducted by Tamtam et al (2011) in France showed that the use of wastewater for irrigation can contain antibiotics residues, which can remain in the soil from a few days to several months and be absorbed by crops or filter into groundwater (Tamtam et al. 2011).

[2] Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (EC 2017/C 163/01).

The presence of resistant *E. coli* and CMY2- producing *E. coli* has been also described in irrigation water and lettuce. A study carried out by Holvoet et al (2013) in Belgium that tested for AMR *E. coli* in irrigation water used for lettuce crops, found that, 4.3 % (7/161) of the *E. coli* isolates from irrigation water were ampicillin resistant (B-lactam antibiotic)(Holvoet et al. 2013, 20). Also, in South Africa, Njage and Buys (2014) showed that the prevalence of CMY-2 in *E. coli* isolates from a river was 43% (6/14) and that the prevalence of the same gene and bacteria in lettuce irrigated with water of that river was 30% (3/10)(Njage and Buys 2015).

Irrigation water could be also contaminated by the close presence of livestock through surface runoff from grazing areas into cultivated fields especially during intensive rain (Fairbrother and Nadeau 2006; Luna-Guevara et al. 2019). This has been described in a study conducted by Jay et al (2007) in the US, in which the presence of feral swine close to spinach fields was described as a very likely source of contamination of irrigation water with *E. coli* O57:H7 (Jay et al. 2007). Also, an *E. coli* O157:H7 outbreak in the US related to bagged spinach was associated with water contaminated through grazing cattle or wildlife activity (Gelting 2006). Potential factors described for the contamination of water included surface runoff from grazing areas into cultivated field, drilled irrigation wells, groundwater contamination and the direct use of untreated surface water for irrigation (Gelting 2006).

The main irrigation methods for crops include surface furrow, surface and subsurface drip and overhead sprinkler. Fonseca et al (2011) showed in an experiment conducted in the US, that sprinkle irrigation increased the risk of lettuce contamination with *E. coli* compared to surface furrow and subsurface drip methods(Fonseca et al. 2011) . Also, the size of irrigation droplets have shown to influence the splash from soil, which might lead to contamination(J. M. Monaghan and Hutchison 2012) In the UK, surface furrow irrigation method is not applied (J, Monaghan, personal communication).

The parameters that might impact the risk of STEC contamination of fresh leafy greens include the type of irrigation, the source of water, the contact of edible parts with

irrigation water and the presence of the bacteria in irrigation water (Codex Alimentarius Commission 2019).

Animal manure or organic fertilizers

The FSA provide guidelines on the management of manure for ready-to-eat-crops (FSA 2009). These guidelines highlight the importance of handling manure and the length of the time manure is stored for the survival of microorganisms (FSA 2009).

Animal manure can be used as a fertilizer in crops (Bicudo and Goyal 2003). *E. coli* is part of the gut flora of many animal species and therefore, animal manure can be a source of contamination of *E. coli* for soil and crops (Bicudo and Goyal 2003; Smet et al. 2008). The use of manure in crops by solid and liquid spread has been found to be associated with human STEC (Valcour et al. 2002). However, no information was provided in this study regarding the treatment of the manure.

The transference of *E. coli* from untreated animal slurry fertilizer to lettuce was studied in Denmark by Jensen et al (2013). The results of this study showed that the use of contaminated animal slurry with *E. coli* in lettuce seedlings led to a contamination between 36 to 54% of the lettuce samples. This contamination was suggested to have happened through the roots and to the surface of the lettuce leaves, which could have occurred through the splash of the rain or irrigation (Jensen et al. 2013).

Islam et al (2004) showed in a study conducted in the US, that lettuce and parsley grown in soil that contains manure contaminated with *E. coli* O157:H7 can become contaminated (Islam et al. 2004). Solomon et al (2002) demonstrated through an experiment carried out in the US that *E. coli* O157:H7 from manure contaminated with high a concentration of E.coli (10^{6-7} CFU/g) can be absorbed to lettuce plant tissue leading to inefficiencies in the elimination of this bacteria through further surface sanitation processes (Solomon, Yaron, and Matthews 2002).

The common treatments of animal manure implemented before land application are:

- *Stacked manure and slurry* with a duration at least of 6 months prior to spreading (FSA 2009).
    - For stacking of manure, it is recommended at least 8 weeks to reduce AMR (VMD/FSA/APHA 2016)
    - For storage of slurry, it is recommended at least 3 months to reduce AMR (VMD/FSA/APHA 2016)

The FSA recommends batch storage before composting and lime treatment of slurry (FSA 2009).

- *Composting:* Generates temperatures from 55 to 65°C under proper conditions(Mukherjee, Speh, and Diez-Gonzalez 2007). During the process, the temperature should be monitored and it should last at least 3 months (FSA 2009) The time-temperature regimes to eliminate *E. coli* are fairly variable (Cempirkova and Soch 2007). The inactivation of *E. coli* in animal manure can be influenced by other factors besides temperature and time such as ammonia, moisture and feedstock characteristics (Turner 2002).
- *Lime treatment of slurry*. Many lime products are used as chemical compounds (Cempirkova and Soch 2007) Adding lime to slurry raises the pH to 12 for at least 2h (FSA 2009). The alkaline conditions inhibit many intestinal bacteria (Cempirkova and Soch 2007).

In the UK, the use of untreated manure and slurry is discouraged in the production of ready to eat crops within 12 months of harvest and less than 6 months before planting (VMD/FSA/APHA 2016). Similarly, livestock grazing is discourage in fields for the production of ready to eat crops within 12 months of harvest and less than 6 months before planting (VMD/FSA/APHA 2016).

Wildlife animals and pests

The presence of wildlife and pests represent a potential source of *E. coli* in field crops. The faeces of wild animals may be a source as well as flies and other insects of fresh products (Luna-Guevara et al. 2019). A study carried out in the UK found out that 2.9%

of bacterial isolates from faecal samples of wild birds were verocytotoxin-producing *E. coli* O157:H7 (Wallace, Cheasty, and Jones 1997). The risks posed by livestock, wildlife and pests for microbial contamination of lettuce crops depends on the prevalence, burden of pathogens carried by the hosts and also the interaction with the production field (Holvoet et al. 2015).

Harvest season

Some studies conducted under outdoor conditions, have shown than seasonality affects the survival of *E. coli* in vegetables. A study conducted by Oliveira et al (2012) in Spain, showed that the *E. coli* O157:H7 counts in lettuce leaves was higher in autumn than in spring. In autumn, mean counts of *E. coli* were 3.91 log CFU/g in the outer lettuce leaves and 2.98 log CFU/g in the inner lettuce leaves, while in spring, the *E. coli* mean counts were 1.15 log CFU/g for the outer lettuce leaves and 0.94 log CFU/g for the inner leaves (M. Oliveira et al. 2012). The differences in temperature and humidity between and autumn and summer were mentioned as possible factors influencing *E. coli* presence, as well as other factors such as solar radiation and soil composition. The average temperature and humidity of the region in autumn in which the study was conducted was 10°C and 82%, respectively. In spring the average temperature was 17°C with a humidity of 62%. Ailes et al (2008) showed similar seasonal trends in coriander and parsley in an study conducted in the US (Ailes et al. 2008).

Age of lettuce leaves

A study carried out by Brandl and Amundson (2008) in the US suggested that the age of the lettuce leaves plays a role in the multiplication of *E. coli* O157:H7 under conditions of warm temperature and the presence of free water on the leaves. Results from this study showed that the population size of *E. coli* O157:H7 was 10 fold higher on young (inner) leaves of lettuce than on middle leaves harvested from mature lettuce heads. The study suggested that this difference could be due to the higher content of nitrogen and carbon in the young leaves and it concludes that young lettuce leaves might be associated to a higher contamination risk of *E. coli* O157:H7 (Brandl and Amundson 2008). These findings suggest that if young leaves get contaminated at pre-harvest

stage, they could be a risk factor for post-harvest contamination (Brandl and Amundson 2008).

Worker health and hygienic practices

Workers on the field can transfer microorganisms to fresh leafy vegetables by direct contact (EFSA 2014, 201) Adequate hygienic practices of workers as well as appropriate sanitary facilities during harvest, sorting and packaging are essential to minimize the risk of contamination of leafy greens. This includes adequate hygiene, hand washing and drying, and if necessary, the use of gloves(Suslow et al. 2003).

In order to avoid potential contamination with STEC, workers that suffer from disease caused by this pathogen should not handle leafy vegetables and should not access the harvest site (Codex Alimentarius Commission 2019). Furthermore, knives and cutting edges used to trim lettuce as well as containers used for transportation should be cleaned and disinfected to avoid cross-contamination (Codex Alimentarius Commission 2019). A study conducted by McEvoy et al (2008) in the US showed that a single contaminated coring knife with *E. coli* O157:H7 could contaminate at least nineteen lettuce heads (McEvoy et al. 2009).

Cooling temperature at storage

Leafy greens should be cooled promptly (less than 90 minutes) after harvest (Gil et al. 2015). The cooling temperature should be lower than 7°C to limit *E. coli* growth (WHO 2018; Adams, Moss, and McClure 2018). In the UK, lettuce is cooled down to 4°C after harvest and stored with ca. 100% relative humidity to prevent dehydration (Terry et al. 2011).

The most commonly used cooling systems in leafy greens include forced air, hydrocooled and vacuum-cooled. In hydrocooling and vacuum-cooling, the water used should be disinfected. A literature review conducted by Gil et al (2015) showed that hydrocooling might pose a risk for contamination with pathogens and their spread (Gil et al. 2015).

10.3.3 Processing module

During processing, there is no step that completely eliminates pathogens from fresh-cut products. Instead, a combination of measures creating suboptimal growth conditions is applied to prevent the growth of pathogens (Oliveira and Oliveira 2019; Sapers 2001).

Fresh-cut vegetables, such as bagged lettuce, should be processed under food safety and quality management systems to ensure safety and quality of the food product (Varzakas and Arvanitoyannis 2008). The implementation of Good Management Practices (GMP) and Good Hygiene Practices (GHP) with Standard Operating Procedures are pre-requisites for a Hazard Analysis and Critical Control Points (HACCP) in all processing steps ("Microbiological Hazards in Fresh Leafy Vegetables and Herbs: Meeting Report" 2008). The Critical Control Points (CCP) of ready to eat vegetables, such as bagged lettuce, include the receiving step, storage, first washing and disinfection, packaging and, storage and distribution steps (Varzakas and Arvanitoyannis 2008).

The main factors associated with the risk of AMR1 occurrence in the processing module are summarized in Table 10 and detailed below.

Table 15: Risk factors that have an effect in the burden on *E. coli* in the processing module.

Risk factor	Effect	References
Storage temperature	Increase risk of *E. coli* growth at temperature higher than 7°C	(WHO 2018; Adams, Moss, and McClure 2018)
Washing method	Reduction of *E. coli* burden after washing with chlorinated water	(Beuchat and Ryu 1997; C.-M. Park et al. 2001)
Modified atmosphere packaging	No clear effect of the combination of gases in the *E. coli* growth	(Francis and O'Beirne 2001; Abdul-Raouf, Beuchat, and Ammar 1993)

Risk factor	Effect	References
Hygienic practices	Increase risk of microbial contamination when proper hygienic practices are not conducted	("Microbiological Hazards in Fresh Leafy Vegetables and Herbs: Meeting Report" 2008; Duffy et al. 2005)
Cold storage and transport to retail	Increase risk of *E. coli* growth at temperature higher than 7°C	(WHO 2018; Adams, Moss, and McClure 2018)

Pre-processing storage temperature

The temperature of lettuce when it is received in the processing plant should be lower than 5°C (Varzakas and Arvanitoyannis 2008). Temperatures exceeding 7°C allow the growth of *E. coli* (WHO 2018; Adams, Moss, and McClure 2018). During the storage stage, the temperature should be monitored (Varzakas and Arvanitoyannis 2008).

Luo et al (2010) showed in a study conducted in the US, that the storage temperature of packaged fresh-cut iceberg and romaine lettuce at 5°C allowed *E. coli* O157:H7 to survive but limited its growth. They also showed that the storage of lettuce at 12°C for 3 days facilitates the growth of *E. coli* O157:H7 more than 2 log CHU/g (Luo, He, and McEvoy 2010). Thus, the maintenance of fresh cut lettuce at a lower temperature of 7°C is essential to reduce food safety risks.

Shredding, cutting, or chopping

Lettuce damaged through mechanical bruising during harvesting and processing can significantly increase the multiplication of *E. coli* O57:H7. Brand et al (2008) tested the effect of leaf damage on the growth of *E. coli* O157:H7 in a short period of time. Results of this study conducted in the US, showed that the abundance of *E. coli* O157:H7 increased 4-4.5 and 11 fold on lettuce leaves that had been mechanically bruised, cut into large pieces and shredded in several pieces. However, the abundance of *E. coli* O157:H7 only had increased two fold on the lettuce leaves that were left undamaged

after harvest. The authors suggested that the growth of *E. coli* could be related with the release of latex by the leaves (Brandl 2008).

Washing

Washing with water during the processing of bagged lettuce is a common practice to remove soil and gross debris. The first washing and disinfection step is generally done with water with added biocides (chlorine, citric acid-ascorbic acid). The temperature of water should be 1-4°C and the concentration of the disinfectant at 100 ppm Cl_2 (Varzakas and Arvanitoyannis 2008). In the second wash, just clean water is normally used (Varzakas and Arvanitoyannis 2008). The use of chlorine in wash water for ready to eat leafy salads is allowed in the UK <100 ppm total)(ACMSF, 2008)

A study conducted by Miranda et al (2016) in Italy, showed that under experimental conditions, using chlorination to disinfect surface water was the fastest process to reach a total inactivation of resistant *E. coli* compared to an advanced oxidation processes (Miranda et al. 2016).

Washing with chlorinated water reduces the bacteria population on vegetables but cannot guarantee the complete elimination of the pathogens (Beuchat and Ryu 1997). The effectivity of chlorine relies on the amount of free available chlorine in the water that comes in touch with the microbial cells (Beuchat and Ryu 1997). Results from a study conducted by Beuchat and Ryu (1997) in the US showed that compared to a water wash only, the abundance of *E. coli* O157:H7 was reduced by 2.41 $\log_{10}$ CFU per lettuce leaf when a 3 min chlorinated water treatment (45 ppm residual chlorine) was used (Park et al. 2001).

Based on the results found, chlorination can inactivate *E. coli* in water (Miranda et al. 2016) and washing with chlorinated water lettuce reduces the abundance of *E. coli* but might not completely eliminate it (Beuchat and Ryu 1997; Park et al. 2001)

Packaging

Modified atmosphere packaging (MAP) extends the shelf life of bagged salad and, at the same time, protects from microbial contamination. A regular equilibrium modified atmosphere (EMA) of a packet of salad contains 5% oxygen, 15% CO_2 and 80% N_2. This mix of gases can extend the shelf life of salad up to 8 days (DTU, 2008).

The reduction of temperature and the concentration of atmospheric oxygen reduces the respiration rate of lettuce. Takeuchi et al (2001) showed that respiration rate of lettuce has no effect in the attachment and penetration of *E. coli* O157 in lettuce (Takeuchi, Hassan, and Frank 2001). This study conducted in the US, also showed that under 21% oxygen, cells of *E. coli* O157:H7 penetrated more into lettuce at 4°C than at 10°C, 22°C and 37°C. Furthermore, the degree of penetration of *E. coli* O157:H7 into lettuce tissue at 4 or 22°C was shown to be higher under 21% oxygen than under 2.7% oxygen (Takeuchi, Hassan, and Frank 2001). This effect decreases the chances of removal of bacteria through successive washing of packaged bagged lettuce containing 21% oxygen. However, this study was not done under MAP conditions, it only considered different gas concentration of oxygen and not a combination of different gasses (Takeuchi, Hassan, and Frank 2001).

Francis et al (2001) demonstrated in a study in Ireland that package atmosphere of iceberg lettuce containing 9-12% CO_2 and 2-4% O_2 did not show inhibitory effect on the *E. coli* O157:H7 growth on shredded lettuce, compared to growth in air. They also showed that packaging atmosphere including 3% O2 and 97% N2 did not have any effect on the growth of *E. coli* O157:H7 (Francis and O'Beirne 2001).

Abdul-Raouf et al (1993) investigated the survival and growth of *E. coli* O157:H7 on lettuce leaves under the effects of MAP, storage temperature and time. Results from this study conducted in Egypt showed that, the numbers of *E. coli* O157:H7 on shredded lettuce at 5°C was reduced during a storage period of 14 days and that *E. coli* O157.H7 increased on lettuce stored at 12°C and 21°C (Abdul-Raouf, Beuchat, and Ammar 1993)

Based on the studies found, the combination of gasses of the MAP of bagged lettuce seems to have no inhibitory effect on the growth of *E. coli* (Takeuchi, Hassan, and

Frank 2001; Francis and O'Beirne 2001). Storing MAP bagged salad at temperature lower than 7°C reduces the growth of *E. coli*(Abdul-Raouf, Beuchat, and Ammar 1993)

Hygiene practices

Adequate sanitation of all processing equipment is necessary to prevent contamination during processing (EFSA 2014). Equipment such as knives, blades, and other food contact surface should be properly disinfected(WHO/FAO 2008) . Cutting boards of lettuce, for example, have been shown to be a place where pathogens can remain and therefore require rigorous sanitation (Varzakas and Arvanitoyannis 2008). Contaminated water baths or dump tanks used by packers have been also described as potential sources of contamination for *E. coli* in a study conducted in the US (Duffy et al. 2005).

Cold storage and transport to retail

Bagged lettuce is generally stored in refrigerators (4-6°C) until it is transferred to trucks, which should also have the same temperature (Varzakas and Arvanitoyannis 2008). Temperature should be monitored during storage and transportation (WHO/FAO 2008) as temperature lower than 7°C limit the growth of *E. coli* (Luo, He, and McEvoy 2010; WHO 2018; Adams, Moss, and McClure 2018).

Transportation to retail might vary depending on the geographic distances between the production warehouse to the supermarket or distribution centre. No study was found comparing the time-interval of transportation and *E. coli* growth in bagged salad.

10.3.4 Post-processing module

The only risk factor associated with the post-processing module was cold storage at retail. Pre-packed bagged salad has a storage life about 7-10 days under refrigeration temperatures ≤ 5°C (Tsironi et al. 2017). Temperature should be lower than 7°C to limit the growth of E. coli (WHO 2018; Adams, Moss, and McClure 2018).

The presence of *E. coli* in MAP bagged salad within the self-life time has been described. Oliveira et al (2011) showed in a study conducted in Brazil that 19.2% (5/26) of the lettuce samples from bagged salad under MAP within the shelf life of up to 8 days at retail were positive to *E. coli*. (M. A. de Oliveira et al. 2011).However, no information was provided regarding the interval of time during transportation from the supermarket to the laboratory and storage conditions until the analyses was conducted.

The only risk factor associated with the risk of AMR1 occurrence in the post-processing module is summarized in Table 11 and detailed below.

Table 16: Risk factors related to the post-processing module and having an effect on *E. coli*

Risk factor	Effect	References
Cold storage at retail	Increase risk of *E. coli* growth at temperature higher than 7°C	(WHO 2018; Adams, Moss, and McClure 2018)

10.3.5 Home preparation module

The main risk factors associated with the risk of AMR1 occurrence in the home-preparation module are summarized in Table 12 and detailed below.

Table 17: Risk factors that have an effect in the burden on *E. coli* in the post-processing module.

Risk factor	Effect	References
Cold storage at home	Increase risk of *E. coli* growth at temperatures higher than 7°C during refrigeration	(WHO 2018; Adams, Moss, and McClure 2018)
Washing method	Increase risk of *E. coli* spread when washing is not done appropriately	(Uhlig et al. 2017; D. A. Jensen et al. 2015)

Risk factor	Effect	References
Hygienic practices	Increase risk of *E. coli* contamination with poor hygiene and disinfection practices	(WHO 2006; Lynch, Tauxe, and Hedberg 2009)

Cold storage at home

To limit the growth of *E. coli*, cold temperature should be lower than 7°C(WHO 2018; Adams, Moss, and McClure 2018). The mean temperature of European domestic refrigerators is 6.64°C, showing variations between Northern and Southern European countries (Nauta 2003).

Washing method

Bagged salads should include information on the labelling to inform consumers on how to safely handle leafy greens (EFSA 2014). A group of experts concluded that bagged salads that are labelled as “washed” or “ready-to-eat” do not require further washing steps at home unless it is specified in the label. They stated that further washing steps done by consumers could lead to cross-contamination during washing by consumers or also through direct contact with contaminated surfaces (Palumbo et al. 2007).

Washing lettuce leaves with tap water (8L/min) during 20s has been showed to significantly reduced *E. coli* contamination. A study conducted by Uhlig et al (2017) in Sweden simulated household washing methods. They compared the *E. coli* contamination after several times of washed of ready-to-eat mixed salad with tap water at different rates. Results showed that the *E. coli* counts were significantly reduced from 5.7 to 5.2 $\log_{10}$ CFU/g in ready-to-eat salad after the first wash with tap water (8L/min) of 20s compared to unwashed salad. These findings also showed that ready-to eat salad still contained amounts of viable bacteria (Uhlig et al. 2017).

Washing cut salad in a container spread *E. coli* when some of the leaves are already contaminated (Jensen et al. 2015). Jensen et al (2015) conducted a study in the US, in

which the effectiveness of washing lettuce with tap water in a bowl during different periods of time was assessed. Results from the study showed that when a single lettuce leaf is contaminated with *E. coli* O157:H7 and washed with other uncontaminated leaves in a bowl of water during 30s, 1min, 2 min and 5min, the initial concentration of *E. coli* O157:H7 is diluted and spread in all the lettuce leaves (Jensen et al. 2015).

Hygienic practices at home

Appropriate hygienic practices by consumers such as hand washing and disinfection of the kitchen equipment as well as keeping separately raw meat and raw vegetables is essential to reduce the risk of *E. coli* contamination (WHO 2006; Lynch, Tauxe, and Hedberg 2009)

10.3.6 Stakeholders consultation

An online workshop was organized on the 17th of September 2020 from 9 to 12 am (UK time) with stakeholders from the UK lettuce industry. Invitations were sent to 22 people out of which 11 confirmed their participation.
The name and affiliation of participants include: Crawford Comrie (Kettle Produce), Jim Monaghan (Harper Adams University), Karin Goodburn (Chilled Food Association), Darren Gedge (G's Fresh), Caroline Floyd (Bakkavor Foods), Anthony Oakes (Agrial Fresh), Paul Cook (FSA), Erin Lewis (FSA), Sue Feuerhelm (Bakkavor), Siân Thomas (Fresh Produce Consortium), Liz Finch (Jepco).

Key findings of the literature review on risk factors and the value chain of outdoor grown bagged salad were presented during the first part of the workshop. Results were discussed in a plenary session with the stakeholders. During the break, a questionnaire on the risk management strategies was shared with the workshop participants and in the second part of the workshop, results of the questionnaire were plenary discussed. The minutes of the workshop are attached to this report.

The key outputs of the workshop included the identification of additional risk factors, additional steps in the value chain and risk management strategies.

The additional risk factors and value chain steps identified during the workshop are presented here below and grouped per module (more details are available in the minutes of the workshop):

- Risk factors:
    - Production module: flooding events, location of the growing site and of water sources and the presence of animals. These three risk factors are normally considered within the pre-planting risk assessment.
- Steps in the value chain:
    - Production module: pre-seed treatment, pre-planting risk assessment, propagation step, pre-harvest risk assessment and field cooling
    - Processing module: vacuum/air cooling
    - Post-processing module: food services (restaurants/catering)
    - Home preparation module: transport from retail to consumers' home

Some quantitative information was collected through the questionnaires. These include, for example, the temperature during transportation and cool storage.

The main discussion points on risk management strategies include:

- The inclusion of treatment of irrigation water
- The exclusion in the model of risk management practices that are considered within the risk-assessment prior to planting. These include the application of wildlife fencing, source of water and close proximity with livestock grazing fields
- Modified Atmosphere Package is a common packaging technique (mainly for iceberg lettuce), used mostly to maintain product quality (preventing oxidative pinking and browning). The MAP has a fairly consistent gas composition in the industry.

The steps in the value chain as well as the risk factors might vary depending on the varieties of leaf green lettuce. More than 20 lettuce varieties are produced in the UK,

depending on factors such as the geographical location and the period of the year. Participants stressed that baby leaf lettuce is a higher risk product than romaine/iceberg lettuce due to the harvest method and the rapid growing time. The information gathered in the literature review and during the workshop did not consider this huge variety and the focus (agreed with FSA) was on outdoor grown pre-washed bagged lettuce only. For consideration on a specific lettuce variety, further information will have to be requested from stakeholders.

10.3.7 Summary Critical risk pathway

The following figure summarises the steps in the value chain and risk pathways of outdoor grown bagged salad for AMR1. A pre-production module including “pre-seed treatment”, “pre-planting risk assessment”, “propagation step” and “pre-harvest risk assessment” was suggested by the workshop participants. However, the further addition of this module in the value chain is out of the scope of this project. FSA should consider its integration in future projects.

Based on feedback received from the workshop participants we included in the current value chain two additional steps: “field cooling” in the production module and “vacuum/air cooling” in the processing module. The level of data availability related to these 2 steps is not clear and no specific information were gathered during the workshop. Therefore, the inclusion of these two steps in the final value chain (and model) needs to be agreed with FSA as it is likely that additional efforts to collect evidence should be dedicated to integrate them in the model.

“Transport from retail to home” was also mentioned during the workshop but due to the lack of data availability on AMR1 in outdoor grown bagged salad, this step was excluded from the value chain.

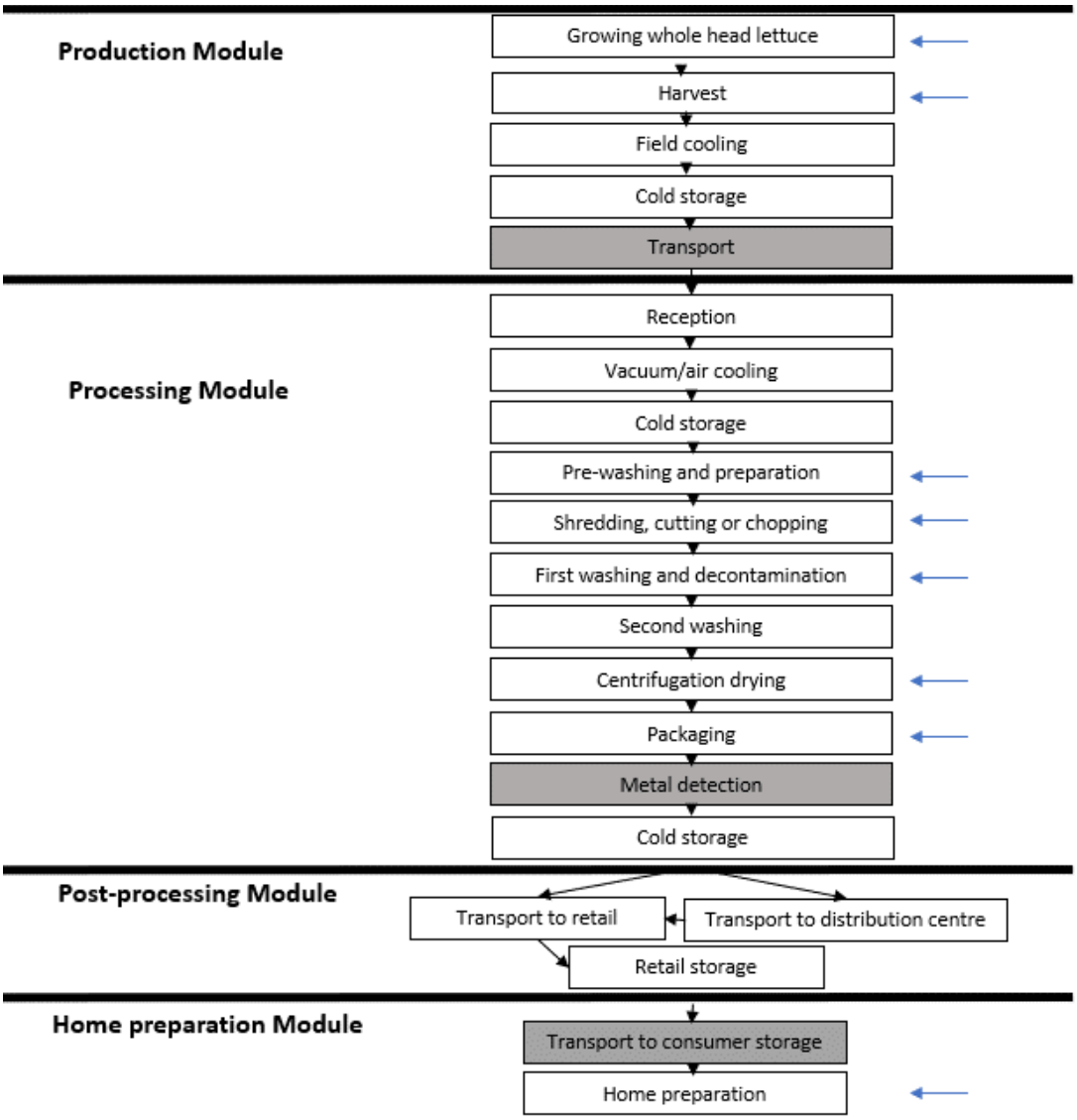


Figure 5: Value chain of outdoor grown bagged salad was presented during the workshop. Steps of the value chain in grey are excluded from the model.

10.4 Conclusion

The literature review and stakeholder's consultation conducted in this first part of the project highlighted the extensive amount of data available on bacterial contamination for *E. coli* and *Campylobacter spp*. Information on the risk of transmission of antimicrobial resistance throughout the food chain remain however scarce: Information related to the production module could be found in the literature, but almost none could be identified

in the other modules. The effect of various food processing steps on the risk of antimicrobial resistance remains largely unknown. This gap in the literature was expected and will impair our ability to properly assess the risk of consumer exposure to antimicrobial resistance. Future studies should investigate whether *E. coli* and *Campylobacter spp* with resistance genes might be more (or less) susceptible to be transmitted in chicken meat and lettuce. The lack of data is however not a critical issue for this project as the modelling framework that we will developed should be able to include new data (for example, data related to AMR) as soon as they become available.

Other critical gaps have been identified in the literature in particular regarding the effect of transport (from processing to post-processing sector or transport home) of bacterial growth. Similarly, no data related to the effect of stress or stocking density on *E. coli* or *Campylobacter* contamination in chicken meat were found in the literature. Future studies should be conducted to better understand the role of these factors on the risk of bacterial and thus AMR chicken meat contamination. The cost-benefit of adding in the future model steps where no data at all is currently available should be discussed with FSA.

Our results show that a unique model structure can be used to model the risk associated with two microorganisms of interest (i.e., *E. coli* and *Campylobacter spp)* for the chicken meat value chain. Most of the risk factors identified had an impact on both bacteria but some risk factors were specific to only one of them. This result highlights the fact that the risk pathway proposed in Figure 1 is valid for *E. coli* and *Campylobacter spp* but might not be fully adapted for another bacteria.

Our report also highlighted the fact that the chicken meat and lettuce value chains in the UK can both fit in a simple common structure made of four modules (i.e., production module, processing module, post-processing module, and home preparation module). However, important difference between the two value chains can be observed and specific model structure should be developed for each of them. For example, the risk factor “packaging” occurred in the post-processing module in the chicken value chain when it occurs mainly in the processing module in the lettuce value chain. Such

differences were expected given the differences of between these two types of food products.

10.5 References

Abdul-Raouf, U M, L R Beuchat, and M S Ammar. 1993. “Survival and Growth of Escherichia Coli O157:H7 on Salad Vegetables.” *Applied and Environmental Microbiology* 59 (7): 1999–2006. https://doi.org/10.1128/aem.59.7.1999-2006.1993

ACMSF. 2007. “Ad Hoc Group on Safe Cooking of Burgers.” https://acmsf.food.gov.uk/sites/default/files/multimedia/pdfs/acmsfburgers0807.pdf

Adams, Martin R, Maurice O Moss, and Peter McClure. 2018. Food Microbiology. 4th ed. Cambridge: Royal Society of Chemistry.

Adeyanju, Gladys Taiwo, and Olayinka Ishola. 2014. “Salmonella and Escherichia Coli Contamination of Poultry Meat from a Processing Plant and Retail Markets in Ibadan, Oyo State, Nigeria.” SpringerPlus 3 (1): 139. https://doi.org/10.1186/2193-1801-3-139

Ailes, Elizabeth C., Juan S. Leon, Lee-Ann Jaykus, Lynette M. Johnston, Haley A. Clayton, Sarah Blanding, David G. Kleinbaum, Lorraine C. Backer, and Christine L. Moe. 2008. “Microbial Concentrations on Fresh Produce Are Affected by Postharvest Processing, Importation, and Season.” Journal of Food Protection 71 (12): 2389–97. https://doi.org/10.4315/0362-028X-71.12.2389.

Allen, V. M., S. A. Bull, J. E. L. Corry, G. Domingue, F. Jørgensen, J. A. Frost, R. Whyte, A. Gonzalez, N. Elviss, and T. J. Humphrey. 2007. “Campylobacter Spp. Contamination of Chicken Carcasses during Processing in Relation to Flock Colonisation.” International Journal of Food Microbiology 113 (1): 54–61. https://doi.org/10.1016/j.ijfoodmicro.2006.07.011.

Allen, V. M., M. H. Hinton, D. B. Tinker, C. Gibson, G. C. Mead, and C. M. Wathes. 2003. “Microbial Cross-Contamination by Airborne Dispersion and Contagion

during Defeathering of Poultry." British Poultry Science 44 (4): 567–76. https://doi.org/10.1080/00071660310001616183.

Allen, V. M., H. Weaver, A. M. Ridley, J. A. Harris, M. Sharma, J. Emery, N. Sparks, M. Lewis, and S. Edge. 2008. "Sources and Spread of Thermophilic Campylobacter Spp. during Partial Depopulation of Broiler Chicken Flocks." Journal of Food Protection 71 (2): 264–70. https://doi.org/10.4315/0362-028X-71.2.264.

Althaus, Denise, Claudio Zweifel, and Roger Stephan. 2017. "Analysis of a Poultry Slaughter Process: Influence of Process Stages on the Microbiological Contamination of Broiler Carcasses." Italian Journal of Food Safety 6 (4). https://doi.org/10.4081/ijfs.2017.7097.

Apostolou, I., C. Papadopoulou, S. Levidiotou, and K. Ioannides. 2005. "The Effect of Short-Time Microwave Exposures on Escherichia Coli O157:H7 Inoculated onto Chicken Meat Portions and Whole Chickens." International Journal of Food Microbiology 101 (1): 105–10. https://doi.org/10.1016/j.ijfoodmicro.2004.10.043.

Bachoual, R., S. Ouabdesselam, F. Mory, C. Lascols, C.-J. Soussy, and J. Tankovic. 2001. "Single or Double Mutational Alterations of GyrA Associated with Fluoroquinolone Resistance in Campylobacter Jejuni and Campylobacter Coli." Microbial Drug Resistance 7 (3): 257–61. https://doi.org/10.1089/10766290152652800.

Ballou, Anne L., Rizwana A. Ali, Mary A. Mendoza, J. C. Ellis, Hosni M. Hassan, W. J. Croom, and Matthew D. Koci. 2016. "Development of the Chick Microbiome: How Early Exposure Influences Future Microbial Diversity." Frontiers in Veterinary Science 3. https://doi.org/10.3389/fvets.2016.00002.

Barbut, S., L.F. Moza, F. Nattress, B. Dilts, and C.O. Gill. 2009. "The Microbiological Conditions of Air- or Water-Chilled Carcasses Produced at the Same Poultry Packing Plant." Journal of Applied Poultry Research 18 (3): 501–7. https://doi.org/10.3382/japr.2008-00131.

Barco, Lisa, Simone Belluco, Anna Roccato, and Antonia Ricci. 2014. "Escherichia Coli and Enterobacteriaceae Counts on Poultry Carcasses along the Slaughter Processing Line, Factors Influencing the Counts and Relationship between Visual Faecal Contamination of Carcasses and Counts: A Review." EFSA

Supporting Publications 11 (8): 636E. https://doi.org/10.2903/sp.efsa.2014.EN-636.

Berg, E.S., A.L. Wester, J. Ahrenfeldt, S.S. Mo, J.S. Slettemeås, M. Steinbakk, Ø. Samuelsen, et al. 2017. “Norwegian Patients and Retail Chicken Meat Share Cephalosporin-Resistant Escherichia Coli and IncK/ Bla CMY-2 Resistance Plasmids.” Clinical Microbiology and Infection 23 (6): 407.e9-407.e15. https://doi.org/10.1016/j.cmi.2016.12.035.

Berndtson, E., M. -L. Danielsson-Tham, and A. Engvall. 1996. “Campylobacter Incidence on a Chicken Farm and the Spread of Campylobacter during the Slaughter Process.” International Journal of Food Microbiology 32 (1): 35–47. https://doi.org/10.1016/0168-1605(96)01102-6.

Berrang, M. E., R. J. Buhr, and J. A. Cason. 2000. “Campylobacter Recovery from External and Internal Organs of Commercial Broiler Carcass Prior to Scalding.” Poultry Science 79 (2): 286–90. https://doi.org/10.1093/ps/79.2.286.

Berrang, M. E., R. J. Buhr, J. A. Cason, and J. A. Dickens. 2001. “Broiler Carcass Contamination with Campylobacter from Feces during Defeathering.” Journal of Food Protection 64 (12): 2063–66. https://doi.org/10.4315/0362-028X-64.12.2063.

——. 2002. “Microbiological Consequences of Skin Removal Prior to Evisceration of Broiler Carcasses.” Poultry Science 81 (1): 134–38. https://doi.org/10.1093/ps/81.1.134.

Berrang, M. E., S. R. Ladely, and R. J. Buhr. 2001. “Presence and Level of Campylobacter, Coliforms, Escherichia Coli, and Total Aerobic Bacteria Recovered from Broiler Parts with and without Skin.” Journal of Food Protection 64 (2): 184–88. https://doi.org/10.4315/0362-028X-64.2.184.

Berrang, M.E., and J.S. Bailey. 2009. “On-Line Brush and Spray Washers to Lower Numbers of Campylobacter and Escherichia Coli and Presence of Salmonella on Broiler Carcasses during Processing.” Journal of Applied Poultry Research 18 (1): 74–78. https://doi.org/10.3382/japr.2008-00067.

Berrang, M.E., and J.K. Northcutt. 2005. “Use of Water Spray and Extended Drying Time to Lower Bacterial Numbers on Soiled Flooring from Broiler Transport

Coops.” Poultry Science 84 (11): 1797–1801. https://doi.org/10.1093/ps/84.11.1797.

Beuchat, L. R., and J. H. Ryu. 1997. “Produce Handling and Processing Practices.” Emerging Infectious Diseases 3 (4): 459–65. https://doi.org/10.3201/eid0304.970407

Bicudo, J. R., and S. M. Goyal. 2003. “Pathogens and Manure Management Systems: A Review.” Environmental Technology 24 (1): 115–30. https://doi.org/10.1080/09593330309385542.

Bilgili, S.F. 2002. “Slaughter Quality as Influenced by Feed Withdrawal.” World’s Poultry Science Journal 58 (2): 123–30. https://doi.org/10.1079/WPS20020012.

Bogaard, A. E. van den. 2001. “Antibiotic Resistance of Faecal Escherichia Coli in Poultry, Poultry Farmers and Poultry Slaughterers.” Journal of Antimicrobial Chemotherapy 47 (6): 763–71. https://doi.org/10.1093/jac/47.6.763.

Boysen, Louise, Susanne Knøchel, and Hanne Rosenquist. 2007. “Survival of Campylobacter Jejuni in Different Gas Mixtures.” FEMS Microbiology Letters 266 (2): 152–57. https://doi.org/10.1111/j.1574-6968.2006.00525.x.

Brandl, M. T. 2008. “Plant Lesions Promote the Rapid Multiplication of Escherichia Coli O157:H7 on Postharvest Lettuce.” Applied and Environmental Microbiology 74 (17): 5285–89. https://doi.org/10.1128/AEM.01073-08.

Brandl, M. T., and R. Amundson. 2008. “Leaf Age as a Risk Factor in Contamination of Lettuce with Escherichia Coli O157:H7 and Salmonella Enterica.” Applied and Environmental Microbiology 74 (8): 2298–2306. https://doi.org/10.1128/AEM.02459-07.

Brashears, Mindy M, Siobhan S Reilly, and Stanley E Gilliland. 1998. “Antagonistic Action of Cells of Lactobacillus Lactis toward Escherichia Coli 0157:H7 on Re’frigerated Raw Chicken Meatt.” J. Food Prot. 61 (2): 166–70. https://doi.org/10.4315/0362-028X-61.2.166

Buhr, Rj, Me Berrang, and Ja Cason. 2003. “Bacterial Recovery from Breast Skin of Genetically Feathered and Featherless Broiler Carcasses Immediately Following Scalding and Picking.” Poultry Science 82 (10): 1641–47. https://doi.org/10.1093/ps/82.10.1641.

Buhr, R.J., D.V. Bourassa, J.K. Northcutt, A. Hinton, K.D. Ingram, and J.A. Cason. 2005. “Bacteria Recovery from Genetically Feathered and Featherless Broiler Carcasses after Immersion Chilling.” Poultry Science 84 (9): 1499–1504. https://doi.org/10.1093/ps/84.9.1499.

Buhr, R.J., J.A. Cason, J.A. Dickens, A. Hinton, and K.D. Ingram. 2000. “Influence of Flooring Type during Transport and Holding on Bacteria Recovery from Broiler Carcass Rinses before and after Defeathering.” Poultry Science 79 (3): 436–41. https://doi.org/10.1093/ps/79.3.436.

Burfoot, Dean, Jeremy Hall, Keith Nicholson, Kirsty Holmes, Cedric Hanson, Simon Handley, and Elizabeth Mulvey. 2016. “Effect of Rapid Surface Cooling on Campylobacter Numbers on Poultry Carcasses.” Food Control 70 (December): 293–301. https://doi.org/10.1016/j.foodcont.2016.05.041.

Caffrey, Niamh, Omid Nekouei, Sheryl Gow, Agnes Agunos, and Sylvia Checkley. 2017. “Risk Factors Associated with the A2C Resistance Pattern among E. Coli Isolates from Broiler Flocks in Canada.” Preventive Veterinary Medicine 148 (December): 115–20. https://doi.org/10.1016/j.prevetmed.2017.11.001.

Callens, Bénédicte, Mickaël Cargnel, Steven Sarrazin, Jeroen Dewulf, Bart Hoet, Katie Vermeersch, Pierre Wattiau, and Sarah Welby. 2018. “Associations between a Decreased Veterinary Antimicrobial Use and Resistance in Commensal Escherichia Coli from Belgian Livestock Species (2011–2015).” Preventive Veterinary Medicine 157 (September): 50–58. https://doi.org/10.1016/j.prevetmed.2017.10.013.

Carattoli, Alessandra, Anna Maria Dionisi, and Ida Luzzi. 2002. “Use of a LightCycler GyrA Mutation Assay for Identification of Ciprofloxacin-Resistant Campylobacter Coli.” FEMS Microbiology Letters 214 (1): 87–93. https://doi.org/10.1111/j.1574-6968.2002.tb11329.x.

Cason, J. A., A. Hinton, and K. D. Ingram. 2000. “Coliform, Escherichia Coli, and Salmonellae Concentrations in a Multiple-Tank, Counterflow Poultry Scalder.” Journal of Food Protection 63 (9): 1184–88. https://doi.org/10.4315/0362-028X-63.9.1184.

Cason, J.A., A. Hinton, and R.J. Buhr. 2004. “Impact of Feathers and Feather Follicles on Broiler Carcass Bacteria.” Poultry Science 83 (8): 1452–55. https://doi.org/10.1093/ps/83.8.1452.

Caudry, S D, and V A Stanisich. 1979. “Incidence of Antibiotic-Resistant Escherichia Coli Associated with Frozen Chicken Carcasses and Characterization of Conjugative R Plasmids Derived from Such Strains.” Antimicrobial Agents and Chemotherapy 16 (6): 701–9. https://doi.org/10.1128/AAC.16.6.701.

CDC. 2020. “E.Coli (Escherichia Coli).” February 2020. https://www.cdc.gov/ecoli/index.html.

Cempirkova, R, and M Soch. 2007. “The Analysis of Real Microbiological Risks for Dissociated Slurry.” Agricultura Tropica et Subtropica 40 (4). https://pdfs.semanticscholar.org/146e/bcfdb56c126aa6d2136f8408cc9efb51ec90.pdf.

Chapman, Brennan, Ainsley Otten, Aamir Fazil, Natalie Ernst, and Ben A. Smith. 2016. “A Review of Quantitative Microbial Risk Assessment and Consumer Process Models for Campylobacter in Broiler Chickens.” Microbial Risk Analysis, SI\: Campylobacter, 2–3 (June): 3–15. https://doi.org/10.1016/j.mran.2016.07.001.

Chaves, B.D., I.Y. Han, P.L. Dawson, and J.K. Northcutt. 2011. “Survival of Artificially Inoculated Escherichia Coli and Salmonella Typhimurium on the Surface of Raw Poultry Products Subjected to Crust Freezing.” Poultry Science 90 (12): 2874–78. https://doi.org/10.3382/ps.2011-01640.

Chinivasagam, H. N., T. Tran, L. Maddock, A. Gale, and P. J. Blackall. 2009. “Mechanically Ventilated Broiler Sheds: A Possible Source of Aerosolized Salmonella, Campylobacter, and Escherichia Coli.” Applied and Environmental Microbiology 75 (23): 7417–25. https://doi.org/10.1128/AEM.01380-09.

Chinivasagam, H.N., W. Estella, H. Rodrigues, D.G. Mayer, C. Weyand, T. Tran, A. Onysk, and I. Diallo. 2016. “On-Farm Campylobacter and Escherichia Coli in Commercial Broiler Chickens: Re-Used Bedding Does Not Influence Campylobacter Emergence and Levels across Sequential Farming Cycles.” Poultry Science 95 (5): 1105–15. https://doi.org/10.3382/ps/pew003.

Chowdhury, S., M. Sandberg, G. E. Themudo, and A. K. Ersbøll. 2012. “Risk Factors for Campylobacter Infection in Danish Broiler Chickens.” Poultry Science 91 (10): 2701–9. https://doi.org/10.3382/ps.2012-02412.

Chuppava, Bussarakam, Birgit Keller, Amr Abd El-Wahab, Christian Sürie, and Christian Visscher. 2019. “Resistance Reservoirs and Multi-Drug Resistance of Commensal Escherichia Coli From Excreta and Manure Isolated in Broiler Houses With Different Flooring Designs.” Frontiers in Microbiology 10 (November). https://doi.org/10.3389/fmicb.2019.02633.

Cibin, V, M Mancin, K Pedersen, F Barrucci, S Belluco, A Roccato, F Cocola, et al. 2014. “Usefulness of Escherichia Coli and Enterobacteriaceae as Process Hygiene Criteria in Poultry: Experimental Study.” EFSA Supporting Publications, 121. https://doi.org/10.2903/sp.efsa.2014.EN-635

Codex Alimentarius Commission. 2019. “Proposed Draft Guideliens for the Control of Shiga Toxi-Producing Escherichia Coli (STEC) in Beef, Leafy Greens, Raw Milk and Cheese Produced from Raw Milk, and Sprouts.” http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-712-51%252FWD%252Ffh51_08e.pdf.

Colles, Frances M., Noel D. McCarthy, Samuel K. Sheppard, Ruth Layton, and Martin C. J. Maiden. 2010. “Comparison of Campylobacter Populations Isolated from a Free-Range Broiler Flock before and after Slaughter.” International Journal of Food Microbiology 137 (2): 259–64. https://doi.org/10.1016/j.ijfoodmicro.2009.12.021.

Cook, Angela, Joseph Odumeru, Susan Lee, and Frank Pollari. 2012. “Campylobacter, Salmonella, Listeria Monocytogenes, Verotoxigenic Escherichia Coli, and Escherichia Coli Prevalence, Enumeration, and Subtypes on Retail Chicken Breasts with and without Skin.” Journal of Food Protection 75 (1): 34–40. https://doi.org/10.4315/0362-028X.JFP-11-206.

Dame-Korevaar, Anita, Egil A.J. Fischer, Jeanet van der Goot, Francisca Velkers, Daniela Ceccarelli, Dik Mevius, and Arjan Stegeman. 2020. “Early Life Supply of Competitive Exclusion Products Reduces Colonization of Extended Spectrum

Beta-Lactamase-Producing Escherichia Coli in Broilers.” Poultry Science 99 (8): 4052–64. https://doi.org/10.1016/j.psj.2020.04.025.

Daniel, Nicholas, Nuria Casadevall, Pei Sun, Daniel Sugden, and Vanna Aldin. 2018. “The Burden of Foodborne Disease in the UK 2018.” FSA and the LSHTM. https://www.food.gov.uk/sites/default/files/media/document/the-burden-of-foodborne-disease-in-the-uk.pdf.

Davis, Gregg S., Kara Waits, Lora Nordstrom, Heidi Grande, Brett Weaver, Katerina Papp, Joseph Horwinski, et al. 2018. “Antibiotic-Resistant Escherichia Coli from Retail Poultry Meat with Different Antibiotic Use Claims.” BMC Microbiology 18 (1): 174. https://doi.org/10.1186/s12866-018-1322-5.

Davis, M. A., and D. E. Conner. 2007. “Survival of Campylobacter Jejuni on Poultry Skin and Meat at Varying Temperatures.” Poultry Science 86 (4): 765–67. https://doi.org/10.1093/ps/86.4.765.

Dawkins, H. C., F. J. Bolton, and D. N. Hutchinson. 1984. “A Study of the Spread of Campylobacter Jejuni in Four Large Kitchens.” The Journal of Hygiene 92 (3): 357–64. https://doi.org/10.1017/S0022172400064573

De Boeck, E., L. Jacxsens, M. Bollaerts, M. Uyttendaele, and P. Vlerick. 2016. “Interplay between Food Safety Climate, Food Safety Management System and Microbiological Hygiene in Farm Butcheries and Affiliated Butcher Shops.” Food Control 65 (July): 78–91. https://doi.org/10.1016/j.foodcont.2016.01.014.

DEFRA. 2017. “Farming Rules for Water-Getting Full Value from Fertilisers and Soil. Policy Paper.” Department fro Environment Food & Rural Affairs. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/695598/farming-rules-for-water-policy-paper-v2.pdf.

Deng, Hui, Hong-Bin Si, Shu-Yi Zeng, Jian Sun, Liang-Xing Fang, Run-Shi Yang, Ya-Hong Liu, and Xiao-Ping Liao. 2015. “Prevalence of Extended-Spectrum Cephalosporin-Resistant Escherichia Coli in a Farrowing Farm: ST1121 Clone Harboring IncHI2 Plasmid Contributes to the Dissemination of BlaCMY-2.” Frontiers in Microbiology 6 (November). https://doi.org/10.3389/fmicb.2015.01210.

Dierikx, Cindy M., Jeanet A. van der Goot, Hilde E. Smith, Arie Kant, and Dik J. Mevius. 2013. “Presence of ESBL/AmpC -Producing Escherichia Coli in the Broiler Production Pyramid: A Descriptive Study.” PLoS ONE 8 (11). https://doi.org/10.1371/journal.pone.0079005.

Dogan, Onay Burak, Jennifer Clarke, Fabio Mattos, and Bing Wang. 2019. “A Quantitative Microbial Risk Assessment Model of Campylobacter in Broiler Chickens: Evaluating Processing Interventions.” Food Control 100 (June): 97–110. https://doi.org/10.1016/j.foodcont.2019.01.003.

Doi, Y., D.L. Paterson, P. Egea, A. Pascual, L. López-Cerero, M.D. Navarro, J.M. Adams-Haduch, Z.A. Qureshi, H.E. Sidjabat, and J. Rodríguez-Baño. 2010. “Extended-Spectrum and CMY-Type b-Lactamase-Producing Escherichia Coli in Clinical Samples and Retail Meat from Pittsburgh, USA and Seville, Spain.” Clinical Microbiology and Infection 16 (1): 33–38. https://doi.org/10.1111/j.1469-0691.2009.03001.x.

Dozier, W. A., J. P. Thaxton, J. L. Purswell, H. A. Olanrewaju, S. L. Branton, and WB Roush. 2006. “Stocking Density Effects on Male Broilers Grown to 1.8 Kilograms of Body Weight1.” Poultry Science 85 (2): 344–51. https://doi.org/10.1093/ps/85.2.344.

Duffy, E. A., L. M. Lucia, J. M. Kells, A. Castillo, S. D. Pillai, and G. R. Acuff. 2005. “Concentrations of Escherichia Coli and Genetic Diversity and Antibiotic Resistance Profiling of Salmonella Isolated from Irrigation Water, Packing Shed Equipment, and Fresh Produce in Texas.” Journal of Food Protection 68 (1): 70–79. https://doi.org/10.4315/0362-028X-68.1.70.

EC. 2017. “Final Report of an Audit Carried out in the United Kingdom from 21 March 2017 to 31 March 2017 in Order to Evaluate the Monitoring and Reporting of Antimicrobial Resistance in Zoonotic and Commensal Bacteria in Certain Food-Producing Animal Population and Food.” EC.

ECDC, EFSA, EMA. 2017. “ECDC. EFSA and EMA Joint Scientific Opinion on a List of Outcome Indicators as Regards Surveillance of Antimicrobial Resistance and Antimicrobial Consumption in Humans and Food-Producing Animals.” https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.5017.

EFSA. 2014. “Scientific Opinion on the Risk Posed by Pathogens in Food of Non-animal Origin. Part 2 (Salmonella and Norovirus in Leafy Greens Eaten Raw as Salads).”

———. 2020. “Update and Review of Control Options for Campylobacter in Broilers at Primary Production.” EFSA Journal 18 (4): e06090. https://doi.org/10.2903/j.efsa.2020.6090.

EFSA/ECDC. 2020. “The European Union Summary Report on Antimicrobial Resistance in Zoonotic and Indicator Bacteria from Humans, Animals and Food in 2017/2018.” EFSA Journal, January. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6007.

Ellis-Iversen, J., A. Ridley, V. Morris, A. Sowa, J. Harris, R. Atterbury, N. Sparks, and V. Allen. 2012. “Persistent Environmental Reservoirs on Farms as Risk Factors for Campylobacter in Commercial Poultry.” Epidemiology and Infection 140 (5): 916–24. https://doi.org/10.1017/S095026881100118X.

EL-Sawah, Azza A., AL Hussien M. Dahshan, El-Shaymaa El-Nahass, and A.I. Abd El-Mawgoud. 2018. “Pathogenicity of Escherichia Coli O157 in Commercial Broiler Chickens.” Beni-Suef University Journal of Basic and Applied Sciences 7 (4): 620–25. https://doi.org/10.1016/j.bjbas.2018.07.005.

Estevez, I. 2007. “Density Allowances for Broilers: Where to Set the Limits?” Poultry Science 86 (6): 1265–72. https://doi.org/10.1093/ps/86.6.1265.

Evans, S. J, and A. R Sayers. 2000. “A Longitudinal Study of Campylobacter Infection of Broiler Flocks in Great Britain.” Preventive Veterinary Medicine 46 (3): 209–23. https://doi.org/10.1016/S0167-5877(00)00143-4.

Ewers, C., A. Bethe, T. Semmler, S. Guenther, and L.H. Wieler. 2012. “Extended-Spectrum β-Lactamase-Producing and AmpC-Producing Escherichia Coli from Livestock and Companion Animals, and Their Putative Impact on Public Health: A Global Perspective.” Clinical Microbiology and Infection 18 (7): 646–55. https://doi.org/10.1111/j.1469-0691.2012.03850.x.

Fairbrother, J M, and É Nadeau. 2006. “Escherichia Coli: On-Farm Contamination of Animals.” Revue Scientifique et Technique (International Office of Epizootics) 25 (2): 555–69. https://doi.org/10.20506/rst.25.2.1682

FAO. 2019. “Prudent and Efficient Use of Antimicrobials in Pigs and Poultry.” Rome: FAO. https://doi.org/10.4060/CA6729EN.

Finch, H. J. S., A. M. Samuel, and G. P. F. Lane. 2014. “Chapter 17- Fresh Produce Crops.” In Lockhart & Wiseman’s Crop Husbandry Including Grassland, edited by H. J. S. Finch, A. M. Samuel, and G. P. F. Lane, Ninth, 396–430. Woodhead Publishing. https://doi.org/10.1533/9781782423928.3.396.

Fonseca, J. M., S. D. Fallon, C. A. Sanchez, and K. D. Nolte. 2011. “Escherichia Coli Survival in Lettuce Fields Following Its Introduction through Different Irrigation Systems.” Journal of Applied Microbiology 110 (4): 893–902. https://doi.org/10.1111/j.1365-2672.2011.04942.x.

Food Standard Agency. 2003. “UK Wide Survey of Salmonella and Campylobacter Contamination of Fresh and Frozen Chicken on Retail Sale.” Food Standard Agency. https://www.food.gov.uk/print/pdf/node/767.

Francis, G A, and D O’Beirne. 2001. “Effects of Vegetable Type, Package Atmosphere and Storage Temperature on Growth and Survival of Escherichia Coli O157:H7 and Listeria Monocytogenes.” Journal of Industrial Microbiology and Biotechnology 27 (2): 111–16. https://doi.org/10.1038/sj.jim.7000094.

Fraqueza, M.J., A. Martins, A.C. Borges, M.H. Fernandes, M.J. Fernandes, Y. Vaz, R.J.B. Bessa, and A.S. Barreto. 2014. “Antimicrobial Resistance among Campylobacter Spp. Strains Isolated from Different Poultry Production Systems at Slaughterhouse Level.” Poultry Science 93 (6): 1578–86. https://doi.org/10.3382/ps.2013-03729.

“Fresh Produce: Agency Advice on Re-Washing Ready to Eat Leafy Salads.” 2008. https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/committee/acm891revised.pdf.

FSA. 2009. “Managing Farm Manures for Food Safety. Guidelines for Growers to Reduce the Risks of Microbiological Contamination of Ready-to-Eat Crops.” Food Standard Agency. https://www.food.gov.uk/sites/default/files/media/document/manuresguidance%20(1).pdf.

———. 2011. “Foodborne Disease Strategy 2010-2015.” https://acss.food.gov.uk/sites/default/files/multimedia/pdfs/fds2015.pdf.

———. 2015. “Food Standards Agency Strategic Plan 2015-20.” https://www.food.gov.uk/sites/default/files/media/document/FSA-Strategic-plan-2015-2020.pdf.

———. 2018. “E.Coli. How E.Coli Spreads and What You Can Do to Prevent It Contaminating Your Food.” January 2018. https://www.food.gov.uk/safety-hygiene/e-coli.

Furtula, V., FOR EXAMPLE, Farrell, F. Diarrassouba, H. Rempel, J. Pritchard, and M.S. Diarra. 2010. “Veterinary Pharmaceuticals and Antibiotic Resistance of Escherichia Coli Isolates in Poultry Litter from Commercial Farms and Controlled Feeding Trials.” Poultry Science 89 (1): 180–88. https://doi.org/10.3382/ps.2009-00198.

Gelting, Rick. 2006. “Investigation_of_an_E_Coli_Outbreak_Associated_with_Dole_Pre-Packaged_Spinach.” CDC. https://www.cdc.gov/nceh/ehs/docs/Investigation_of_an_E_Coli_Outbreak_Associated_with_Dole_Pre-Packaged_Spinach.pdf.

Georgiev, M., W. Beauvais, and J. Guitian. 2017. “Effect of Enhanced Biosecurity and Selected On-Farm Factors on Campylobacter Colonization of Chicken Broilers.” Epidemiology & Infection 145 (3): 553–67. https://doi.org/10.1017/S095026881600251X.

Georgsson, Franklín, Ásmundur E. Þorkelsson, Margrét Geirsdóttir, Jarle Reiersen, and Norman J. Stern. 2006. “The Influence of Freezing and Duration of Storage on Campylobacter and Indicator Bacteria in Broiler Carcasses.” Food Microbiology 23 (7): 677–83. https://doi.org/10.1016/j.fm.2005.10.003.

Gibbens, J. C, S. J. S Pascoe, S. J Evans, R. H Davies, and A. R Sayers. 2001. “A Trial of Biosecurity as a Means to Control Campylobacter Infection of Broiler Chickens.” Preventive Veterinary Medicine 48 (2): 85–99. https://doi.org/10.1016/S0167-5877(00)00189-6.

Gil, Maria I., Maria V. Selma, Trevor Suslow, Liesbeth Jacxsens, Mieke Uyttendaele, and Ana Allende. 2015. “Pre- and Postharvest Preventive Measures and Intervention Strategies to Control Microbial Food Safety Hazards of Fresh Leafy Vegetables.” Critical Reviews in Food Science and Nutrition 55 (4): 453–68. https://doi.org/10.1080/10408398.2012.657808.

Gill, C, L Moza, M Badoni, and S Barbut. 2006. “The Effects on the Microbiological Condition of Product of Carcass Dressing, Cooling, and Portioning Processes at a Poultry Packing Plant.” International Journal of Food Microbiology 110 (2): 187–93. https://doi.org/10.1016/j.ijfoodmicro.2006.04.020.

González-Miret, M.L., M.L. Escudero-Gilete, and F.J. Heredia. 2006. “The Establishment of Critical Control Points at the Washing and Air Chilling Stages in Poultry Meat Production Using Multivariate Statistics.” Food Control 17 (12): 935–41. https://doi.org/10.1016/j.foodcont.2005.06.012.

Gregova, Gabriela, Marta Kmetova, Vladimír Kmet, Jan Venglovsky, and Alexander Feher. 2012. “Antibiotic Resistance of Escherichia Coli Isolated from a Poultry Slaughterhouse.” Annals of Agricultural and Environmental Medicine 19 (1): 75–77.

Griggs, Deborah J., Maggie M. Johnson, Jennifer A. Frost, Tom Humphrey, Frieda Jørgensen, and Laura J. V. Piddock. 2005. “Incidence and Mechanism of Ciprofloxacin Resistance in Campylobacter Spp. Isolated from Commercial Poultry Flocks in the United Kingdom before, during, and after Fluoroquinolone Treatment.” Antimicrobial Agents and Chemotherapy 49 (2): 699–707. https://doi.org/10.1128/AAC.49.2.699-707.2005.

Guerin, M. T., C. Sir, J. M. Sargeant, L. Waddell, A. M. O’Connor, R. W. Wills, R. H. Bailey, and J. A. Byrd. 2010. “The Change in Prevalence of Campylobacter on Chicken Carcasses during Processing: A Systematic Review.” Poultry Science 89 (5): 1070–84. https://doi.org/10.3382/ps.2009-00213.

“Guide Packaging of Fresh Fruit and Vegetables.” 2008. Danish Technological Institute. https://www.modifiedatmospherepackaging.com/~/media/Modifiedatmospherepackaging/Pictures/Guide%20%20%20Packaging%20of%20Fresh%20Fruit%20and%20Vegetables%20%20%20PDF%20file.ashx.

Hald, B., E. Rattenborg, and M. Madsen. 2001. “Role of Batch Depletion of Broiler Houses on the Occurrence of Campylobacter Spp. in Chicken Flocks.” Letters in Applied Microbiology 32 (4): 253–56. https://doi.org/10.1046/j.1472-765x.2001.00896.x.

Hansen, D, and B S Larsen. 2007. “Reduction of Campylobacter on Chicken Carcasses by SonoSteam® Treatment,” 7.

Harrison, D., J. E. L. Corry, M. A. Tchórzewska, V. K. Morris, and M. L. Hutchison. 2013. “Freezing as an Intervention to Reduce the Numbers of Campylobacters Isolated from Chicken Livers.” Letters in Applied Microbiology 57 (3): 206–13. https://doi.org/10.1111/lam.12098.

Harrison, W. A., C. J. Griffith, D. Tennant, and A. C. Peters. 2001. “Incidence of Campylobacter and Salmonella Isolated from Retail Chicken and Associated Packaging in South Wales.” Letters in Applied Microbiology 33 (6): 450–54. https://doi.org/10.1046/j.1472-765X.2001.01031.x.

Hartnett, E., L. Kelly, D. Newell, M. Wooldridge, and G. Gettinby. 2001. “A Quantitative Risk Assessment for the Occurrence of Campylobacter in Chickens at the Point of Slaughter.” Epidemiology & Infection 127 (2): 195–206. https://doi.org/10.1017/S0950268801005866.

Hastings, R., F. M. Colles, N. D. McCarthy, M. C. J. Maiden, and S. K. Sheppard. 2011. “Campylobacter Genotypes from Poultry Transportation Crates Indicate a Source of Contamination and Transmission.” Journal of Applied Microbiology 110 (1): 266–76. https://doi.org/10.1111/j.1365-2672.2010.04883.x.

Haughton, Pippa N., James Lyng, Denis Cronin, Seamus Fanning, and Paul Whyte. 2012. “Effect of Crust Freezing Applied Alone and in Combination with Ultraviolet Light on the Survival of Campylobacter on Raw Chicken.” Food Microbiology 32 (1): 147–51. https://doi.org/10.1016/j.fm.2012.05.004.

Havelaar, Arie, and Eric Evers. n.d. “Risk Assessment of Campylobacter in the Netherlands via Broiler Meat and Other Routes.” Accessed August 17, 2020. https://www.academia.edu/19153192/Risk_assessment_of_Campylobacter_in_the_Netherlands_via_broiler_meat_and_other_routes.

Heemskerk, W.J.C. 2005. “Preventive Strategies during Slaughter of Poultry, to Improve Food Safety.” In . Doorwerth, The Netherlands. https://www.cabi.org/Uploads/animal-science/worlds-poultry-science-association/WPSA-the-netherlands-2005/119.pdf.

Herman, L., M. Heyndrickx, K. Grijspeerdt, D. Vandekerchove, I. Rollier, and L. De Zutter. 2003. “Routes for Campylobacter Contamination of Poultry Meat: Epidemiological Study from Hatchery to Slaughterhouse.” Epidemiology & Infection 131 (3): 1169–80. https://doi.org/10.1017/S0950268803001183.

Heuer, O. E., K. Pedersen, J. S. Andersen, and M. Madsen. 2001. “Prevalence and Antimicrobial Susceptibility of Thermophilic Campylobacter in Organic and Conventional Broiler Flocks.” Letters in Applied Microbiology 33 (4): 269–74. https://doi.org/10.1046/j.1472-765X.2001.00994.x.

Holvoet, Kevin, Imca Sampers, Benedicte Callens, Jeroen Dewulf, and Mieke Uyttendaele. 2013. “Moderate Prevalence of Antimicrobial Resistance in Escherichia Coli Isolates from Lettuce, Irrigation Water, and Soil.” Applied and Environmental Microbiology 79 (21): 6677–83. https://doi.org/10.1128/AEM.01995-13.

Holvoet, Kevin, Imca Sampers, Marleen Seynnaeve, Liesbeth Jacxsens, and Mieke Uyttendaele. 2015. “Agricultural and Management Practices and Bacterial Contamination in Greenhouse versus Open Field Lettuce Production.” International Journal of Environmental Research and Public Health 12 (1): 32–63. https://doi.org/10.3390/ijerph120100032.

Huang, Jinlin, Xiaoqi Zang, Weihua Zhai, Chunai Guan, Tianyao Lei, and Xinan Jiao. 2017. “Quantitative Analysis of Campylobacter Spp. Contamination in Chicken Slaughtering Lines by ‘Label Tracking Method’ in Eastern China.” Food Control 80 (October): 67–73. https://doi.org/10.1016/j.foodcont.2017.03.052.

Hue, Olivier, Sophie Le Bouquin, Marie-José Laisney, Virginie Allain, Françoise Lalande, Isabelle Petetin, Sandra Rouxel, et al. 2010. “Prevalence of and Risk Factors for Campylobacter Spp. Contamination of Broiler Chicken Carcasses at the Slaughterhouse.” Food Microbiology 27 (8): 992–99. https://doi.org/10.1016/j.fm.2010.06.004.

Huezo, R., J. K. Northcutt, D. P. Smith, D. L. Fletcher, and K. D. Ingram. 2007. “Effect of Dry Air or Immersion Chilling on Recovery of Bacteria from Broiler Carcasses.” Journal of Food Protection 70 (8): 1829–34. https://doi.org/10.4315/0362-028X-70.8.1829.

Hussain, Arif, Sabiha Shaik, Amit Ranjan, Nishant Nandanwar, Sumeet K. Tiwari, Mohammad Majid, Ramani Baddam, et al. 2017. “Risk of Transmission of Antimicrobial Resistant Escherichia Coli from Commercial Broiler and Free-Range Retail Chicken in India.” Frontiers in Microbiology 8 (November). https://doi.org/10.3389/fmicb.2017.02120.

Hutchison, M. L., M. J. Taylor, M. A. Tchòrzewska, G. Ford, R. H. Madden, and T. G. Knowles. 2017. “Modelling-Based Identification of Factors Influencing Campylobacters in Chicken Broiler Houses and on Carcasses Sampled after Processing and Chilling.” Journal of Applied Microbiology 122 (5): 1389–1401. https://doi.org/10.1111/jam.13434.

Hutchison, M. L., M. A. Tchòrzewska, D. Harrison, J. E. L. Corry, M. J. Taylor, R. H. Madden, V. Allen, and T. G. Knowles. 2016. “Monitoring of Campylobacters in UK Poultry Slaughter Batches and Carcasses and the Collection of Information from Primary Production and Processing for Risk Factor Elucidation.” https://www.food.gov.uk/sites/default/files/media/document/fs241051afinalreport.pdf.

Hutchison, M. L., L. D. Walters, G. C. Mead, M. Howell, and V. M. Allen. 2006. “An Assessment of Sampling Methods and Microbiological Hygiene Indicators for Process Verification in Poultry Slaughterhouses.” Journal of Food Protection 69 (1): 145–53. https://doi.org/10.4315/0362-028X-69.1.145.

Ingham, Steven C., Rishi K. Wadhera, Melody A. Fanslau, and Dennis R. Buege. 2005. “Growth of Salmonella Serovars, Escherichia Coli O157:H7, and Staphylococcus Aureus during Thawing of Whole Chicken and Retail Ground Beef Portions at 22 and 30°C.” Journal of Food Protection 68 (7): 1457–61. https://doi.org/10.4315/0362-028X-68.7.1457.

Islam, Mahbub, Michael P. Doyle, Sharad C. Phatak, Patricia Millner, and Xiuping Jiang. 2004. “Persistence of Enterohemorrhagic Escherichia Coli O157:H7 in Soil and

on Leaf Lettuce and Parsley Grown in Fields Treated with Contaminated Manure Composts or Irrigation Water." Journal of Food Protection 67 (7): 1365–70. https://doi.org/10.4315/0362-028X-67.7.1365.

James, C., C. Vincent, T.I. de Andrade Lima, and S.J. James. 2006. "The Primary Chilling of Poultry Carcasses—a Review." International Journal of Refrigeration 29 (6): 847–62. https://doi.org/10.1016/j.ijrefrig.2005.08.003.

James, Christian, Stephen J. James, Neil Hannay, Graham Purnell, Catia Barbedo-Pinto, Hilmi Yaman, Marlene Araujo, et al. 2007. "Decontamination of Poultry Carcasses Using Steam or Hot Water in Combination with Rapid Cooling, Chilling or Freezing of Carcass Surfaces." International Journal of Food Microbiology 114 (2): 195–203. https://doi.org/10.1016/j.ijfoodmicro.2006.09.019.

Jay, Michele T., Michael Cooley, Diana Carychao, Gerald W. Wiscomb, Richard A. Sweitzer, Leta Crawford-Miksza, Jeff A. Farrar, et al. 2007. "Escherichia Coli O157:H7 in Feral Swine near Spinach Fields and Cattle, Central California Coast." Emerging Infectious Diseases 13 (12): 1908–11. https://doi.org/10.3201/eid1312.070763.

Jensen, A. N., C. Storm, A. Forslund, D. L. Baggesen, and A. Dalsgaard. 2013. "Escherichia Coli Contamination of Lettuce Grown in Soils Amended with Animal Slurry." Journal of Food Protection 76 (7): 1137–44. https://doi.org/10.4315/0362-028X.JFP-13-011.

Jensen, Dane A., Loretta M. Friedrich, Linda J. Harris, Michelle D. Danyluk, and Donald W. Schaffner. 2015. "Cross Contamination of Escherichia Coli O157:H7 between Lettuce and Wash Water during Home-Scale Washing." Food Microbiology 46 (April): 428–33. https://doi.org/10.1016/j.fm.2014.08.025.

Jesse, T. W., M. D. Englen, L. G. Pittenger-Alley, and P. J. Fedorka-Cray. 2006. "Two Distinct Mutations in GyrA Lead to Ciprofloxacin and Nalidixic Acid Resistance in Campylobacter Coli and Campylobacter Jejuni Isolated from Chickens and Beef Cattle*." Journal of Applied Microbiology 100 (4): 682–88. https://doi.org/10.1111/j.1365-2672.2005.02796.x.

Jones, T., C.O. Gill, and L.M. McMullen. 2004. "The Behaviour of Log Phase Escherichia Coli at Temperatures That Fluctuate about the Minimum for Growth."

Letters in Applied Microbiology 39 (3): 296–300. https://doi.org/10.1111/j.1472-765X.2004.01593.x.

Jonsson, Malin E., Mariann Chriél, Madelaine Norström, and Merete Hofshagen. 2012. “Effect of Climate and Farm Environment on Campylobacter Spp. Colonisation in Norwegian Broiler Flocks.” Preventive Veterinary Medicine 107 (1): 95–104. https://doi.org/10.1016/j.prevetmed.2012.05.002.

Jørgensen, F, R Bailey, S Williams, P Henderson, D. R. A Wareing, F. J Bolton, J. A Frost, L Ward, and T. J Humphrey. 2002. “Prevalence and Numbers of Salmonella and Campylobacter Spp. on Raw, Whole Chickens in Relation to Sampling Methods.” International Journal of Food Microbiology 76 (1): 151–64. https://doi.org/10.1016/S0168-1605(02)00027-2.

Jorgensen, F., J. Ellis-Iversen, S. Rushton, S. A. Bull, S. A. Harris, S. J. Bryan, A. Gonzalez, and T. J. Humphrey. 2011. “Influence of Season and Geography on Campylobacter Jejuni and C. Coli Subtypes in Housed Broiler Flocks Reared in Great Britain.” Applied and Environmental Microbiology 77 (11): 3741–48. https://doi.org/10.1128/AEM.02444-10.

Juneja, Vijay K. 1997. “Thermal Destruction of Escherichia Coli 0157:H7 in Beef and Chicken: Determination of D- and z-Values.” International Journal of Food Microbiology 35: 231–37. https://doi.org/10.1016/S0168-1605(96)01237-8

Katsma, Wendelke E. A., Aline A. De Koeijer, Wilma F. Jacobs-Reitsma, Marie-Josée J. Mangen, and Jaap A. Wagenaar. 2007. “Assessing Interventions to Reduce the Risk of Campylobacter Prevalence in Broilers.” Risk Analysis 27 (4): 863–76. https://doi.org/10.1111/j.1539-6924.2007.00928.x.

Keener, K. M., M. P. Bashor, P. A. Curtis, B. W. Sheldon, and S. Kathariou. 2004. “Comprehensive Review of Campylobacter and Poultry Processing.” Comprehensive Reviews in Food Science and Food Safety 3 (2): 105–16. https://doi.org/10.1111/j.1541-4337.2004.tb00060.x.

Kemmett, K., N. J. Williams, G. Chaloner, S. Humphrey, P. Wigley, and T. Humphrey. 2014. “The Contribution of Systemic Escherichia Coli Infection to the Early Mortalities of Commercial Broiler Chickens.” Avian Pathology 43 (1): 37–42. https://doi.org/10.1080/03079457.2013.866213.

Kemp, G. Kere, M. L. Aldrich, M. L. Guerra, and K. R. Schneider. 2001. “Continuous Online Processing of Fecal- and Ingesta-Contaminated Poultry Carcasses Using an Acidified Sodium Chlorite Antimicrobial Intervention.” Journal of Food Protection 64 (6): 807–12. https://doi.org/10.4315/0362-028X-64.6.807.

Koga, Vanessa L., Renato P. Maluta, Wanderley D. da Silveira, Renan A. Ribeiro, Mariangela Hungria, Eliana C. Vespero, Gerson Nakazato, and Renata K. T. Kobayashi. 2019. “Characterization of CMY-2-Type Beta-Lactamase-Producing Escherichia Coli Isolated from Chicken Carcasses and Human Infection in a City of South Brazil.” BMC Microbiology 19 (1): 174. https://doi.org/10.1186/s12866-019-1550-3.

Kosmider, Rowena, Pádraig Nally, Robin Simons, Adam Brouwer, Susan Cheung, and Emma Snary. 2010. “EU0701: A UK VTEC O157 Risk Assessment Model for Meat Products,” 73.

Li, Xian-Zhi, Manisha Mehrotra, Shiva Ghimire, and Lateef Adewoye. 2007. “Beta-Lactam Resistance and Beta-Lactamases in Bacteria of Animal Origin.” Veterinary Microbiology 121 (3–4): 197–214. https://doi.org/10.1016/j.vetmic.2007.01.015.

Li, Y, H Yang, and BL Swem. 2002. “Effect of High-Temperature inside-Outside Spray on Survival of Campylobacter Jejuni Attached to Prechill Chicken Carcasses.” Poultry Science 81 (9): 1371–77. https://doi.org/10.1093/ps/81.9.1371.

Lindblad, M., H. Lindmark, S. Thisted Lambertz, and R. Lindqvist. 2006. “Microbiological Baseline Study of Broiler Chickens at Swedish Slaughterhouses.” Journal of Food Protection 69 (12): 2875–82. https://doi.org/10.4315/0362-028X-69.12.2875.

Löhren, Ulrich. 2012. “Overview on Current Practices of Poultry Slaughtering and Poultry Meat Inspection.” EFSA Supporting Publications 9 (6): 298E. https://doi.org/10.2903/sp.efsa.2012.EN-298.

Lu, Gang, Lingshuang Sun, Jiajun Ou, Haibin Xu, Liyan Wu, and Shoujun Li. 2018. “Identification and Genetic Characterization of a Novel Parvovirus Associated with Serum Hepatitis in Horses in China.” Emerging Microbes & Infections 7 (October). https://doi.org/10.1038/s41426-018-0174-2.

Luna-Guevara, J. J., M. M. P. Arenas-Hernandez, C. Martínez de la Peña, Juan L. Silva, and M. L. Luna-Guevara. 2019. “The Role of Pathogenic E. Coli in Fresh Vegetables: Behavior, Contamination Factors, and Preventive Measures.” International Journal of Microbiology 2019 (November). https://doi.org/10.1155/2019/2894328.

Luo, Yaguang, Qiang He, and James L. McEvoy. 2010. “Effect of Storage Temperature and Duration on the Behavior of Escherichia Coli O157:H7 on Packaged Fresh-Cut Salad Containing Romaine and Iceberg Lettuce.” Journal of Food Science 75 (7): M390–97. https://doi.org/10.1111/j.1750-3841.2010.01722.x.

Lynch, M. F., R. V. Tauxe, and C. W. Hedberg. 2009. “The Growing Burden of Foodborne Outbreaks Due to Contaminated Fresh Produce: Risks and Opportunities.” Epidemiology and Infection 137 (3): 307–15. https://doi.org/10.1017/S0950268808001969.

Manges, A. R., and J. R. Johnson. 2012. “Food-Borne Origins of Escherichia Coli Causing Extraintestinal Infections.” Clinical Infectious Diseases 55 (5): 712–19. https://doi.org/10.1093/cid/cis502.

McEvoy, James L., Yaguang Luo, William Conway, Bin Zhou, and Hao Feng. 2009. “Potential of Escherichia Coli O157:H7 to Grow on Field-Cored Lettuce as Impacted by Postharvest Storage Time and Temperature.” International Journal of Food Microbiology 128 (3): 506–9. https://doi.org/10.1016/j.ijfoodmicro.2008.08.008.

McMillin, Kenneth W. 2017. “Advancements in Meat Packaging.” Meat Science 132 (October): 153–62. https://doi.org/10.1016/j.meatsci.2017.04.015.

Mead, G. C., W. R. Hudson, and M. H. Hinton. 1995. “Effect of Changes in Processing to Improve Hygiene Control on Contamination of Poultry Carcasses with Campylobacter.” Epidemiology & Infection 115 (3): 495–500. https://doi.org/10.1017/S0950268800058659.

Meldrum, R. J., D. Tucker, and C. Edwards. 2004. “Baseline Rates of Campylobacter and Salmonella in Raw Chicken in Wales, United Kingdom, in 2002.” Journal of Food Protection 67 (6): 1226–28. https://doi.org/10.4315/0362-028X-67.6.1226.

Mellata, Melha. 2013. “Human and Avian Extraintestinal Pathogenic Escherichia Coli : Infections, Zoonotic Risks, and Antibiotic Resistance Trends.” Foodborne Pathogens and Disease 10 (11): 916–32. https://doi.org/10.1089/fpd.2013.1533.

“Microbiological Hazards in Fresh Leafy Vegetables and Herbs: Meeting Report.” 2008. Geneva : Rome: WHO/FAO. http://www.fao.org/3/a-i0452e.pdf;

Miranda, Andreza Costa, Marilena Lepretti, Luigi Rizzo, Ivana Caputo, Vincenzo Vaiano, Olga Sacco, Wilton Silva Lopes, and Diana Sannino. 2016. “Surface Water Disinfection by Chlorination and Advanced Oxidation Processes: Inactivation of an Antibiotic Resistant E. Coli Strain and Cytotoxicity Evaluation.” Science of The Total Environment 554–555 (June): 1–6. https://doi.org/10.1016/j.scitotenv.2016.02.189.

Miranda, J.M., B.I. Vázquez, C.A. Fente, J. Barros-Velázquez, A. Cepeda, and C.M. Franco. 2008. “Evolution of Resistance in Poultry Intestinal Escherichia Coli During Three Commonly Used Antimicrobial Therapeutic Treatments in Poultry.” Poultry Science 87 (8): 1643–48. https://doi.org/10.3382/ps.2007-00485.

Mo, Solveig Sølverød, Anja Bråthen Kristoffersen, Marianne Sunde, Ane Nødtvedt, and Madelaine Norström. 2016. “Risk Factors for Occurrence of Cephalosporin-Resistant Escherichia Coli in Norwegian Broiler Flocks.” Preventive Veterinary Medicine 130 (August): 112–18. https://doi.org/10.1016/j.prevetmed.2016.06.011.

Mollenkopf, Dixie F., Brittany De Wolf, Sydnee M. Feicht, Johana K. Cenera, Christy A. King, Joany C. van Balen, and Thomas E. Wittum. 2018. “Salmonella Spp. and Extended-Spectrum Cephalosporin-Resistant Escherichia Coli Frequently Contaminate Broiler Chicken Transport Cages of an Organic Production Company.” Foodborne Pathogens and Disease 15 (9): 583–88. https://doi.org/10.1089/fpd.2017.2390.

Monaghan, J. M., J. C. Augustin, J. Bassett, R. Betts, B. Pourkomailian, and M. H. Zwietering. 2017. “Risk Assessment or Assessment of Risk? Developing an Evidence-Based Approach for Primary Producers of Leafy Vegetables To Assess and Manage Microbial Risks.” Journal of Food Protection 80 (5): 725–33. https://doi.org/10.4315/0362-028X.JFP-16-237.

Monaghan, Jim, John I Thomas, and Kaarin Goodburn. 2008. “Food Standards Agency Project B17007. A Review of the Published Literature Describing Foodborne Illness Outbreaks Associated with Ready to Eat Fresh Produce and an Overview of Current UK Fresh Produce Farming Practices.” Food Standard Agency.

Monaghan, J.M., and M.L. Hutchison. 2012. “Distribution and Decline of Human Pathogenic Bacteria in Soil after Application in Irrigation Water and the Potential for Soil-Splash-Mediated Dispersal onto Fresh Produce: Soil Distribution and Splash of Bacteria.” Journal of Applied Microbiology 112 (5): 1007–19. https://doi.org/10.1111/j.1365-2672.2012.05269.x.

Mukherjee, Avik, Dorinda Speh, and Francisco Diez-Gonzalez. 2007. “Association of Farm Management Practices with Risk of Escherichia Coli Contamination in Pre-Harvest Produce Grown in Minnesota and Wisconsin.” International Journal of Food Microbiology 120 (3): 296–302. https://doi.org/10.1016/j.ijfoodmicro.2007.09.007.

Murphy, Colleen P., Carolee Carson, Ben A. Smith, Brennan Chapman, Jayme Marrotte, Maggie McCann, Courtney Primeau, Parth Sharma, and E. Jane Parmley. 2018. “Factors Potentially Linked with the Occurrence of Antimicrobial Resistance in Selected Bacteria from Cattle, Chickens and Pigs: A Scoping Review of Publications for Use in Modelling of Antimicrobial Resistance (IAM.AMR Project).” Zoonoses and Public Health 65 (8): 957–71. https://doi.org/10.1111/zph.12515.

Musa, Laura, Patrizia Casagrande Proietti, Raffaella Branciari, Laura Menchetti, Sara Bellucci, David Ranucci, Maria Luisa Marenzoni, and Maria Pia Franciosini. 2020. “Antimicrobial Susceptibility of Escherichia Coli and ESBL-Producing Escherichia Coli Diffusion in Conventional, Organic and Antibiotic-Free Meat Chickens at Slaughter.” Animals : An Open Access Journal from MDPI 10 (7). https://doi.org/10.3390/ani10071215.

Mylius, Sido D., Maarten J. Nauta, and Arie H. Havelaar. 2007. “Cross-Contamination During Food Preparation: A Mechanistic Model Applied to Chicken-Borne Campylobacter.” Risk Analysis 27 (4): 803–13. https://doi.org/10.1111/j.1539-6924.2006.00872.x.

Nastasijević, I, B Lakićević, and Z Petrović. 2017. “Cold Chain Management in Meat Storage, Distribution and Retail: A Review.” IOP Conference Series: Earth and Environmental Science 85 (September): 012022. https://doi.org/10.1088/1755-1315/85/1/012022.

Nauta, M. 2003. “A Retail and Consumer Phase Model for Exposure Assessment of Bacillus Cereus.” International Journal of Food Microbiology 83 (2): 205–18. https://doi.org/10.1016/S0168-1605(02)00374-4.

Nauta, Maarten J., Wilma F. Jacobs-Reitsma, and Arie H. Havelaar. 2007. “A Risk Assessment Model for Campylobacter in Broiler Meat.” Risk Analysis 27 (4): 845–61. https://doi.org/10.1111/j.1539-6924.2006.00834.x.

Newell, D. G., K. T. Elvers, D. Dopfer, I. Hansson, P. Jones, S. James, J. Gittins, et al. 2011. “Biosecurity-Based Interventions and Strategies To Reduce Campylobacter Spp. on Poultry Farms.” Applied and Environmental Microbiology 77 (24): 8605–14. https://doi.org/10.1128/AEM.01090-10.

Newell, D. G., and C. Fearnley. 2003. “Sources of Campylobacter Colonization in Broiler Chickens.” Applied and Environmental Microbiology 69 (8): 4343–51. https://doi.org/10.1128/AEM.69.8.4343-4351.2003.

Newell, D. G., J. E. Shreeve, M. Toszeghy, G. Domingue, S. Bull, T. Humphrey, and G. Mead. 2001. “Changes in the Carriage of Campylobacter Strains by Poultry Carcasses during Processing in Abattoirs.” Applied and Environmental Microbiology 67 (6): 2636–40. https://doi.org/10.1128/AEM.67.6.2636-2640.2001.

Njage, Patrick M K, and Elna M Buys. 2015. “Pathogenic and Commensal Escherichia Coli from Irrigation Water Show Potential in Transmission of Extended Spectrum and AmpC β-Lactamases Determinants to Isolates from Lettuce.” Microbial Biotechnology 8 (3): 462–73. https://doi.org/10.1111/1751-7915.12234.

Northcutt, Jk, Me Berrang, Ja Dickens, Dl Fletcher, and Na Cox. 2003. “Effect of Broiler Age, Feed Withdrawal, and Transportation on Levels of Coliforms, Campylobacter, Escherichia Coli and Salmonella on Carcasses before and after Immersion Chilling.” Poultry Science 82 (1): 169–73. https://doi.org/10.1093/ps/82.1.169.

Northcutt, J.K., M.E. Berrang, D.P. Smith, and D.R. Jones. 2003. “Effect of Commercial Bird Washers on Broiler Carcass Microbiological Characteristics.” Journal of Applied Poultry Research 12 (4): 435–38. https://doi.org/10.1093/japr/12.4.435.

Odonkor, Stephen T., and Joseph K. Ampofo. 2013. “Escherichia Coli as an Indicator of Bacteriological Quality of Water: An Overview.” Microbiology Research 4 (1): e2–e2. https://doi.org/10.4081/mr.2013.e2.

Oliveira, Fernanda A.R., and Jorge C. Oliveira. 2019. Processing Foods. Quality Optimization and Process Assessment. First. CRC Press. https://www.routledge.com/Processing-Foods-Quality-Optimization-and-Process-Assessment/Oliveira-Oliveira/p/book/9780367455699.

Oliveira, M., I. Viñas, J. Usall, M. Anguera, and M. Abadias. 2012. “Presence and Survival of Escherichia Coli O157:H7 on Lettuce Leaves and in Soil Treated with Contaminated Compost and Irrigation Water.” International Journal of Food Microbiology 156 (2): 133–40. https://doi.org/10.1016/j.ijfoodmicro.2012.03.014.

Oliveira, Maria Aparecida de, Vanessa Maciel de Souza, Alzira Maria Morato Bergamini, and Elaine Cristina Pereira De Martinis. 2011. “Microbiological Quality of Ready-to-Eat Minimally Processed Vegetables Consumed in Brazil.” Food Control 22 (8): 1400–1403. https://doi.org/10.1016/j.foodcont.2011.02.020.

Pacholewicz, Ewa, Len J.A. Lipman, Arno Swart, Arie H. Havelaar, and Willem J.C. Heemskerk. 2016. “Pre-Scald Brushing for Removal of Solids and Associated Broiler Carcass Bacterial Contamination.” Poultry Science 95 (12): 2979–85. https://doi.org/10.3382/ps/pew257.

Pacholewicz, Ewa, Arno Swart, Maarten Schipper, Betty G. M. Gortemaker, Jaap A. Wagenaar, Arie H. Havelaar, and Len J. A. Lipman. 2015a. “A Comparison of Fluctuations of Campylobacter and Escherichia Coli Concentrations on Broiler Chicken Carcasses during Processing in Two Slaughterhouses.” International Journal of Food Microbiology 205 (July): 119–27. https://doi.org/10.1016/j.ijfoodmicro.2015.04.006.

Pacholewicz, Ewa, Arno Swart, Maarten Schipper, Betty G.M. Gortemaker, Jaap A. Wagenaar, Arie H. Havelaar, and Len J.A. Lipman. 2015b. “A Comparison of Fluctuations of Campylobacter and Escherichia Coli Concentrations on Broiler

Chicken Carcasses during Processing in Two Slaughterhouses." International Journal of Food Microbiology 205 (July): 119–27. https://doi.org/10.1016/j.ijfoodmicro.2015.04.006.

Palumbo, Mary S, James R Gorny, David E Gombas, Larry R Beuchat, Christine M Bruhn, Barbara Cassens, Pascal Delaquis, et al. 2007. "Recommendations for Handling Fresh-Cut Leafy Green Salads by Consumers and Retail Foodservice Operators." Food Protection Trends 27 (11): 892–98.

Park, C.-M., Y.-C. Hung, M.P. Doyle, G.O.I. Ezeike, and C. Kim. 2001. "Pathogen Reduction and Quality of Lettuce Treated with Electrolyzed Oxidizing and Acidified Chlorinated Water." Journal of Food Science 66 (9): 1368–72. https://doi.org/10.1111/j.1365-2621.2001.tb15216.x.

Park, Yoon Soo, Jennifer M. Adams-Haduch, Jesabel I. Rivera, Scott R. Curry, Lee H. Harrison, and Yohei Doi. 2012. "Escherichia Coli Producing CMY-2 β-Lactamase in Retail Chicken, Pittsburgh, Pennsylvania, USA." Emerging Infectious Diseases 18 (3): 515–16. https://doi.org/10.3201/eid1803.111434.

Parker, Daniel. 2018. "Responsible Antibiotic Use." Veterinary Record 182 (6): 172.2-173. https://doi.org/10.1136/vr.k578.

Payot, Sophie, Jean-Michel Bolla, Deborah Corcoran, Séamus Fanning, Francis Mégraud, and Qijing Zhang. 2006. "Mechanisms of Fluoroquinolone and Macrolide Resistance in Campylobacter Spp." Microbes and Infection 8 (7): 1967–71. https://doi.org/10.1016/j.micinf.2005.12.032.

Peyrat, M. B., C. Soumet, P. Maris, and P. Sanders. 2008. "Recovery of Campylobacter Jejuni from Surfaces of Poultry Slaughterhouses after Cleaning and Disinfection Procedures: Analysis of a Potential Source of Carcass Contamination." International Journal of Food Microbiology 124 (2): 188–94. https://doi.org/10.1016/j.ijfoodmicro.2008.03.030.

Pintar, Katarina, Angela Cook, Frank Pollari, André Ravel, Susan Lee, and J. A. Odumeru. 2007. "Quantitative Effect of Refrigerated Storage Time on the Enumeration of Campylobacter, Listeria, and Salmonella on Artificially Inoculated Raw Chicken Meat." Journal of Food Protection 70 (3): 739–43. https://doi.org/10.4315/0362-028X-70.3.739.

Pointon, A., M. Sexton, P. Dowsett, T. Saputra, A. Kiermeier, M. Lorimer, G. Holds, et al. 2008. “A Baseline Survey of the Microbiological Quality of Chicken Portions and Carcasses at Retail in Two Australian States (2005 to 2006).” Journal of Food Protection 71 (6): 1123–34. https://doi.org/10.4315/0362-028X-71.6.1123.

Ponce-Rivas, Elizabeth, María-Enriqueta Muñoz-Márquez, and Ashraf A. Khan. 2012. “Identification and Molecular Characterization of Class 1 Integrons in Multiresistant Escherichia Coli Isolates from Poultry Litter.” Applied and Environmental Microbiology 78 (15): 5444–47. https://doi.org/10.1128/AEM.00660-12.

Poulsen, Louise Ladefoged, Ida Thøfner, Magne Bisgaard, Jens Peter Christensen, Rikke Heidemann Olsen, and Henrik Christensen. 2017. “Longitudinal Study of Transmission of Escherichia Coli from Broiler Breeders to Broilers.” Veterinary Microbiology 207 (August): 13–18. https://doi.org/10.1016/j.vetmic.2017.05.029.

Projahn, Michaela, Ewa Pacholewicz, Evelyne Becker, Guido Correia-Carreira, Niels Bandick, and Annemarie Kaesbohrer. 2018. “Reviewing Interventions against Enterobacteriaceae in Broiler Processing: Using Old Techniques for Meeting the New Challenges of ESBL E. Coli?” BioMed Research International 2018 (October). https://doi.org/10.1155/2018/7309346.

Rasschaert, Geertrui, Lieven De Zutter, Lieve Herman, and Marc Heyndrickx. 2020. “Campylobacter Contamination of Broilers: The Role of Transport and Slaughterhouse.” International Journal of Food Microbiology 322 (June): 108564. https://doi.org/10.1016/j.ijfoodmicro.2020.108564.

Ravishankar, Sadhana, Libin Zhu, Carl W. Olsen, Tara H. McHugh, and Mendel Friedman. 2009. “Edible Apple Film Wraps Containing Plant Antimicrobials Inactivate Foodborne Pathogens on Meat and Poultry Products.” Journal of Food Science 74 (8): M440–45. https://doi.org/10.1111/j.1750-3841.2009.01320.x.

Red Tractor Assurance for Farms. 2017. “Crop Module: Lettuce (Field).” version 3.2 (Crop Risk Category 1). Red Tractor Assurance. https://assurance.redtractor.org.uk/contentfiles/Farmers-6570.pdf?_=635971851272779192.

Red Tractor Certified Standards for farms. 2019. “Fresh Produce Standards.” Version 4.1. https://assurance.redtractor.org.uk/contentfiles/Farmers-7058.pdf?_=637311856137223163.

Reich, Felix, Viktoria Atanassova, and Günter Klein. 2013. “Extended-Spectrum β-Lactamase– and AmpC-Producing Enterobacteria in Healthy Broiler Chickens, Germany.” Emerging Infectious Diseases 19 (8): 1253–59. https://doi.org/10.3201/eid1908.120879.

Ridley, A., V. Morris, J. Gittins, S. Cawthraw, J. Harris, S. Edge, and V. Allen. 2011. “Potential Sources of Campylobacter Infection on Chicken Farms: Contamination and Control of Broiler-Harvesting Equipment, Vehicles and Personnel.” Journal of Applied Microbiology 111 (1): 233–44. https://doi.org/10.1111/j.1365-2672.2011.05038.x.

RIVM. 2017. “MARAN 2017 Monitoring of Antimicrobial Resistant and Antibiotic Usage in Animals in the Netherlands in 2016.” https://www.wur.nl/upload_mm/9/b/4/fe79278b-9361-4912-8cba-03ce17fc086b_Maran%20report%202017.pdf.

Rosenquist, Hanne, Louise Boysen, Anne Louise Krogh, Annette Nygaard Jensen, and Maarten Nauta. 2013. “Campylobacter Contamination and the Relative Risk of Illness from Organic Broiler Meat in Comparison with Conventional Broiler Meat.” International Journal of Food Microbiology 162 (3): 226–30. https://doi.org/10.1016/j.ijfoodmicro.2013.01.022.

Rosenquist, Hanne, Niels L Nielsen, Helle M Sommer, Birgit Nørrung, and Bjarke B Christensen. 2003. “Quantitative Risk Assessment of Human Campylobacteriosis Associated with Thermophilic Campylobacter Species in Chickens.” International Journal of Food Microbiology 83 (1): 87–103. https://doi.org/10.1016/S0168-1605(02)00317-3.

Rosenquist, Hanne, Helle M. Sommer, Niels L. Nielsen, and Bjarke B. Christensen. 2006. “The Effect of Slaughter Operations on the Contamination of Chicken Carcasses with Thermotolerant Campylobacter.” International Journal of Food Microbiology 108 (2): 226–32. https://doi.org/10.1016/j.ijfoodmicro.2005.12.007.

Roth, Nataliya, Annemarie Käsbohrer, Sigrid Mayrhofer, Ulrike Zitz, Charles Hofacre, and Konrad J Domig. 2019. “The Application of Antibiotics in Broiler Production and the Resulting Antibiotic Resistance in Escherichia Coli: A Global Overview.” Poultry Science 98 (4): 1791–1804. https://doi.org/10.3382/ps/pey539.

Russell, Sm. 2003. “The Effect of Airsacculitis on Bird Weights, Uniformity, Fecal Contamination, Processing Errors, and Populations of Campylobacter Spp. and Escherichia Coli.” Poultry Science 82 (8): 1326–31. https://doi.org/10.1093/ps/82.8.1326.

Sales, Ronaldo de Oliveira, and Ernani Porto. 2007. “Bacterial Dissemination. Main Pathogens and Hygiene in Chicken Slaughter: A Review.” http://www.repositorio.ufc.br/handle/riufc/4751.

Sampers, Imca, Ihab Habib, Dirk Berkvens, Ann Dumoulin, Lieven De Zutter, and Mieke Uyttendaele. 2008. “Processing Practices Contributing to Campylobacter Contamination in Belgian Chicken Meat Preparations.” International Journal of Food Microbiology 128 (2): 297–303. https://doi.org/10.1016/j.ijfoodmicro.2008.08.024.

Sapers, Gerald M. 2001. “Efficacy of Washing and Sanitizing Methods for Disinfection of Fresh Fruit and Vegetable Products.” Food Technology and Biotechnology, 7. https://doi.org/10.1201/9781420031850.ch11

Scherer, Kathrin, Edda Bartelt, Christine Sommerfeld, and Goetz Hildebrandt. 2006. “Quantification of Campylobacter on the Surface and in the Muscle of Chicken Legs at Retail.” Journal of Food Protection 69 (4): 757–61. https://doi.org/10.4315/0362-028X-69.4.757.

Seol, Kuk-Hwan, Dong-Gyun Lim, Aera Jang, Cheorun Jo, and Mooha Lee. 2009. “Antimicrobial Effect of κ-Carrageenan-Based Edible Film Containing Ovotransferrin in Fresh Chicken Breast Stored at 5°C.” Meat Science 83 (3): 479–83. https://doi.org/10.1016/j.meatsci.2009.06.029.

Sheane, Richard, Catherine McCosker, and Rob Lillywhite. 2017. “Food Waste in Primary Production. A Preliminary Study on Strawberries and Lettuces.” WRAP. https://wrap.org.uk/sites/files/wrap/Food_waste_in_primary_production_report.pdf.

Slader, J., G. Domingue, F. Jørgensen, K. McAlpine, R. J. Owen, F. J. Bolton, and T. J. Humphrey. 2002. “Impact of Transport Crate Reuse and of Catching and Processing on Campylobacter and Salmonella Contamination of Broiler Chickens.” Applied and Environmental Microbiology 68 (2): 713–19. https://doi.org/10.1128/aem.68.2.713-719.2002.

Smet, Annemieke, An Martel, Davy Persoons, Jeroen Dewulf, Marc Heyndrickx, Boudewijn Catry, Lieve Herman, Freddy Haesebrouck, and Patrick Butaye. 2008. “Diversity of Extended-Spectrum β-Lactamases and Class C β-Lactamases among Cloacal Escherichia Coli Isolates in Belgian Broiler Farms.” Antimicrobial Agents and Chemotherapy 52 (4): 1238–43. https://doi.org/10.1128/AAC.01285-07.

Smith, James L., and Pina M. Fratamico. 2010. “Fluoroquinolone Resistance in Campylobacter.” Journal of Food Protection 73 (6): 1141–52. https://doi.org/10.4315/0362-028X-73.6.1141.

Söderström, Ann, A Lindberg, and Y Anderson. 2005. “EHEC O157 Outbreak in Sweden from Locally Produced Lettuce, August-September 2005.” Euro Surveillance 10 (38). https://www.eurosurveillance.org/content/10.2807/esw.10.38.02794-en#html_fulltext. https://doi.org/10.2807/esw.10.38.02794-en

Solomon, Ethan B., Sima Yaron, and Karl R. Matthews. 2002. “Transmission of Escherichia Coli O157:H7 from Contaminated Manure and Irrigation Water to Lettuce Plant Tissue and Its Subsequent Internalization.” Applied and Environmental Microbiology 68 (1): 397–400. https://doi.org/10.1128/AEM.68.1.397-400.2002.

Sommer, H. M., O. E. Heuer, A. I. V. Sørensen, and M. Madsen. 2013. “Analysis of Factors Important for the Occurrence of Campylobacter in Danish Broiler Flocks.” Preventive Veterinary Medicine 111 (1): 100–111. https://doi.org/10.1016/j.prevetmed.2013.04.004.

Soonthornchaikul, Nantika, Hemda Garelick, Huw Jones, Jenny Jacobs, David Ball, and Manika Choudhury. 2006. “Resistance to Three Antimicrobial Agents of Campylobacter Isolated from Organically- and Intensively-Reared Chickens

Purchased from Retail Outlets.” International Journal of Antimicrobial Agents 27 (2): 125–30. https://doi.org/10.1016/j.ijantimicag.2005.09.020.

Sproston, Emma L., Helen M. L. Wimalarathna, and Samuel K. Sheppard. 2018. “Trends in Fluoroquinolone Resistance in Campylobacter.” Microbial Genomics 4 (8). https://doi.org/10.1099/mgen.0.000198.

Suslow, T. V., M. P. Oria, L. R. Beuchat, E. H. Garrett, M. E. Parish, L. J. Harris, J. N. Farber, and F. F. Busta. 2003. “Production Practices as Risk Factors in Microbial Food Safety of Fresh and Fresh-Cut Produce.” Comprehensive Reviews in Food Science and Food Safety 2 (s1): 38–77. https://doi.org/10.1111/j.1541-4337.2003.tb00030.x.

Takeuchi, Kazue, Ashraf N. Hassan, and Joseph F. Frank. 2001. “Penetration of Escherichia Coli O157:H7 into Lettuce as Influenced by Modified Atmosphere and Temperature.” Journal of Food Protection 64 (11): 1820–23. https://doi.org/10.4315/0362-028X-64.11.1820.

Tamtam, Fatima, Folkert van Oort, Barbara Le Bot, Tuc Dinh, Sophie Mompelat, Marc Chevreuil, Isabelle Lamy, and Médard Thiry. 2011. “Assessing the Fate of Antibiotic Contaminants in Metal Contaminated Soils Four Years after Cessation of Long-Term Waste Water Irrigation.” Science of The Total Environment 409 (3): 540–47. https://doi.org/10.1016/j.scitotenv.2010.10.033.

Terry, Dr Leon A, Carlos Mena, Dr Adrian Williams, and Mr Nigel Jenney. 2011. “Fruit and Vegetable Resource Maps. Mapping Fruit and Vegetable Waste through the Retail and Wholesale Supply Chain.” WRAP.

Tsironi, Theofania, Efimia Dermesonlouoglou, Marianna Giannoglou, Eleni Gogou, George Katsaros, and Petros Taoukis. 2017. “Shelf-Life Prediction Models for Ready-to-Eat Fresh Cut Salads: Testing in Real Cold Chain.” International Journal of Food Microbiology 240 (January): 131–40. https://doi.org/10.1016/j.ijfoodmicro.2016.09.032.

Turner, C. 2002. “The Thermal Inactivation of E. Coli in Straw and Pig Manure.” Bioresource Technology 83 (2): 0. https://doi.org/10.1016/S0960-8524(02)00008-1.

Tyrrel, S. F., J. W. Knox, and E. K. Weatherhead. 2006. “Microbiological Water Quality Requirements for Salad Irrigation in the United Kingdom.” Journal of Food Protection 69 (8): 2029–35. https://doi.org/10.4315/0362-028X-69.8.2029.

Uhlig, Elisabeth, Crister Olsson, Jiayi He, Therese Stark, Zuzanna Sadowska, Göran Molin, Siv Ahrné, Beatrix Alsanius, and Åsa Håkansson. 2017. “Effects of Household Washing on Bacterial Load and Removal of Escherichia Coli from Lettuce and ‘Ready-to-eat’ Salads.” Food Science & Nutrition 5 (6): 1215–20. https://doi.org/10.1002/fsn3.514.

USDA. 2014. “Chicken from Farm to Table.” USDA.

Uyttendaele, Mieke, Lee-Ann Jaykus, Philip Amoah, Alessandro Chiodini, David Cunliffe, Liesbeth Jacxsens, Kevin Holvoet, et al. 2015. “Microbial Hazards in Irrigation Water: Standards, Norms, and Testing to Manage Use of Water in Fresh Produce Primary Production.” Comprehensive Reviews in Food Science and Food Safety 14 (4): 336–56. https://doi.org/10.1111/1541-4337.12133.

Valcour, James E., Pascal Michel, Scott A. McEwen, and Jeffrey B. Wilson. 2002. “Associations between Indicators of Livestock Farming Intensity and Incidence of Human Shiga Toxin-Producing Escherichia Coli Infection.” Emerging Infectious Diseases 8 (3): 252–57. https://doi.org/10.3201/eid0803.010159.

Varzakas, Theodoros H., and Ioannis S. Arvanitoyannis. 2008. “Application of ISO22000 and Comparison to HACCP for Processing of Ready to Eat Vegetables: Part I.” International Journal of Food Science & Technology 43 (10): 1729–41. https://doi.org/10.1111/j.1365-2621.2007.01675.x.

VMD/FSA/APHA. 2016. “Handling of Manure and Slurry to Reduce Antibiotic Resistance.” September 8, 2016. https://www.gov.uk/guidance/handling-of-manure-and-slurry-to-reduce-antibiotic-resistance.

Voidarou, C., D. Vassos, G. Rozos, A. Alexopoulos, S. Plessas, A. Tsinas, M. Skoufou, E. Stavropoulou, and E. Bezirtzoglou. 2011. “Microbial Challenges of Poultry Meat Production.” Anaerobe 17 (6): 341–43. https://doi.org/10.1016/j.anaerobe.2011.05.018.

Wallace, J. S., T. Cheasty, and K. Jones. 1997. “Isolation of Vero Cytotoxin-Producing Escherichia Coli O157 from Wild Birds.” Journal of Applied Microbiology 82 (3): 399–404. https://doi.org/10.1046/j.1365-2672.1997.00378.x.

Warren, R. E., V. M. Ensor, P. O’Neill, V. Butler, J. Taylor, K. Nye, M. Harvey, D. M. Livermore, N. Woodford, and P. M. Hawkey. 2008. “Imported Chicken Meat as a Potential Source of Quinolone-Resistant Escherichia Coli Producing Extended-Spectrum -Lactamases in the UK.” Journal of Antimicrobial Chemotherapy 61 (3): 504–8. https://doi.org/10.1093/jac/dkm517.

Warriss, P.D., L.J. Wilkins, S.N. Brown, A.J. Phillips, and V. Allen. 2004. “Defaecation and Weight of the Gastrointestinal Tract Contents after Feed and Water Withdrawal in Broilers.” British Poultry Science 45 (1): 61–66. https://doi.org/10.1080/0007166041668879.

Wassenaar, Trudy M., Manfred Kist, and Anno de Jong. 2007. “Re-Analysis of the Risks Attributed to Ciprofloxacin-Resistant Campylobacter Jejuni Infections.” International Journal of Antimicrobial Agents 30 (3): 195–201. https://doi.org/10.1016/j.ijantimicag.2007.01.019.

Wedderkopp, A., K. O. Gradel, J. C. Jørgensen, and M. Madsen. 2001. “Pre-Harvest Surveillance of Campylobacter and Salmonella in Danish Broiler Flocks: A 2-Year Study.” International Journal of Food Microbiology 68 (1–2): 53–59. https://doi.org/10.1016/s0168-1605(01)00463-9.

WHO. 2006. “Five Key to Safer Food Manual.” Geneva: WHO.

———. 2017. “Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics,” 7.

———. 2018. “E.Coli.” February 2018. https://www.who.int/news-room/fact-sheets/detail/e-coli.

Whyte, R., J. A. Hudson, and C. Graham. 2006. “Campylobacter in Chicken Livers and Their Destruction by Pan Frying.” Letters in Applied Microbiology 43 (6): 591–95. https://doi.org/10.1111/j.1472-765X.2006.02020.x.

Wimalarathna, Helen ML, Judith F Richardson, Andy J Lawson, Richard Elson, Richard Meldrum, Christine L Little, Martin CJ Maiden, Noel D McCarthy, and Samuel K Sheppard. 2013. “Widespread Acquisition of Antimicrobial Resistance among

Campylobacter Isolates from UK Retail Poultry and Evidence for Clonal Expansion of Resistant Lineages." BMC Microbiology 13 (July): 160. https://doi.org/10.1186/1471-2180-13-160.

World Health Organization, and Food and Agriculture Organization of the United Nations, eds. 2009. Risk Assessment of Campylobacter Spp. in Broiler Chickens. Technical Report. Microbiological Risk Assessment Series 12. Geneva: World Health Organization : Food and Agriculture Organization of the United Nations.

Yakovleva, Natalia, and Andrew Flynn. 2004. "Innovation and Sustainability in the Food System: A Case of Chicken Production and Consumption in the UK." Journal of Environmental Policy & Planning 6 (3–4): 227–50. https://doi.org/10.1080/1523908042000344096.

Yang, S., M. G. Leff, D. McTague, K. A. Horvath, J. Jackson-Thompson, T. Murayi, G. K. Boeselager, et al. 1998. "Multistate Surveillance for Food-Handling, Preparation, and Consumption Behaviors Associated with Foodborne Diseases: 1995 and 1996 BRFSS Food-Safety Questions." MMWR. CDC Surveillance Summaries: Morbidity and Mortality Weekly Report. CDC Surveillance Summaries 47 (4): 33–57.

Zhang, Lei, Jong Y. Jeong, Kishorekumar K. Janardhanan, Elliot T. Ryser, and Iksoon Kang. 2011. "Microbiological Quality of Water Immersion–Chilled and Air-Chilled Broilers." Journal of Food Protection 74 (9): 1531–35. https://doi.org/10.4315/0362-028X.JFP-11-032.

Zhao, P., T. Zhao, M. P. Doyle, J. R. Rubino, and J. Meng. 1998. "Development of a Model for Evaluation of Microbial Cross-Contamination in the Kitchen." Journal of Food Protection 61 (8): 960–63. https://doi.org/10.4315/0362-028x-61.8.960.

11. Appendix 2: chicken model

The overall structure of the chicken model is based on an existing QMRA model for *Campylobacter spp.* in broiler chicken developed by (WHO and FAO 2009) and adapted by Collineau et al. (2020) for Salmonella Heidelberg to follow the population-level prevalence and individual bird level of contamination throughout the model. In some cases, other existing models were used to inform specific equations as described in the following sections.

11.1 Production Module

On-farm practices have been identified as the highest risk for occurrence of antimicrobial resistance. The role of antimicrobial usage is well established, but there are other factors, which have an impact as well. The amount of information related to these other factors is very scarce and further studies are needed to characterize the contribution of specific practices within the various management systems on the occurrence of antimicrobial resistance (Murphy et al. 2018). This paucity of data influenced the choices and the definition of probability distributions described in Section 3 of the report.

The model starts at the flock production stage just prior to bird transport to slaughter. In each iteration of the stochastic model, a flock (or batch of birds) is randomly selected from the production population. The status of the flock is either positive or negative, as defined by the between-flock prevalence of AMR-gene-carrying bacteria (*Prev_f*). The within-flock prevalence depends on values found in the literature for a given farm type (*Prev_farm_type*) but could be modified based on antimicrobial usage (*F_AMU*) assuming that antimicrobial usage might for example change the overall between-flock prevalence of AMR-gene-carrying bacteria. In this study, antimicrobial usage is defined as the effect of the usage of certain classes of antimicrobials during the chicken life on the occurrence of AMR in the broiler farm. The antimicrobial classes of interest are

expected to be defined based on the bacteria and resistance gene considered in the analysis.

Characteristics of the flock in terms of age of the birds before slaughter, biosecurity practices, use of thinning and season of slaughter, are randomly selected based on the proportion of farms slaughtering young or old birds (*Prop_age*), slaughtering birds during the high-risk season for the microorganism of interest (*Prop_season),* implementing thinning before slaughter (*Prop_thin),* and poor biosecurity practices (*Prop_bios*).

Birds originating from negative flocks (B_*Flock_status* = n) are considered as not contaminated with AMR-gene-carrying bacteria at pre-harvest. For positive flocks (B_*Flock_status* = p), the within flock prevalence (wfp) representing the number of birds internally colonized by AMR-gene-carrying bacteria before transport to slaughterhouse (*Prev_wfp_col*) was estimated based on the average data available in the literature for the microorganism of interest (*Prev_wfp_col_base*), and risk factors like season of slaughter (*F_season*), flock age (*F_age*), farm biosecurity (*F_bios*) and use of thinning (*F_thin*) depending on the characteristics of the flock randomly selected (respectively *B_Season_status, B_Age_status, B_Bios_status*, and *B_Thin_status*).

During the transport to slaughter cross-contamination can occur either directly via contact between birds (in positive flocks only), or indirectly via carry-over of bacteria within a truck that had transported a contaminated flock earlier that day (in both positive and negative flocks). In a positive flock, the probability a negative bird becomes contaminated by an AMR-gene-carrying bacteria (*P_pos)* was derived from the probability of cross contamination within flock (ccwf), *P_ccwf,* assuming every contact leads to effective transmission and from the probability of cross-contamination between flock (ccbf) from a positive flock transported earlier that day (*P_ccbf*). The default value used for these variables in the chicken model were the one estimated by Collineau et al. (2020) based on Canadian data based on Salmonella but could be updated later with UK specific data as soon as data would become available. These variables could be

also set up to zero if, for example, the risk of bird cross contamination during transport was assumed to be null.

P_pos was applied to the proportion of non-contaminated birds before transport to estimate the increase in prevalence during transport, and subsequently the prevalence of contaminated birds after transport at the end of the production module (*Prev_prod_p).* In a negative flock, we assumed that no birds were contaminated by AMR-gene-carrying bacteria before transport and the prevalence of contaminated birds after transport (*Prev_prod_n*) equalled the probability of cross contamination from a positive flock transported earlier that day (*P_ccbf)*. Since 100% of the bacteria contamination from a prior flock will not contaminate the subsequent flock being transported a term to dampen the probability of contamination (*F_cross_trans*) was incorporated together with the number of flocks transported before the current lock (*N_transp*) to estimate *P_ccbf*.

The number of AMR-gene-carrying bacteria on positive birds from positive flocks after transport (*C_prod_p)* was estimated using the approach of Collineau et al. (2020) by applying a load increasing factor *F_transp* to the number of bacteria on bird exteriors at pre-harvest depending on the number of bacteria on birds exterior (*C_btp*) estimated based on the variables “Concentration in the barn environment of positive flocks” (*C_barn)* and “Amount of faeces on bird exterior at pre-harvest in positive flocks” (*Amount_fec*). In negative flocks, the number of bacteria on birds after transport (*C_prod_n)* was defined as the number of bacteria gained through cross-contamination, as birds were assumed not contaminated before transport.

The model framework representing the model variables related to the production module and their dependencies is presented in Figure 2. A detailed description of the estimated and calculated variables is available in Tables 1 and 2 respectively.

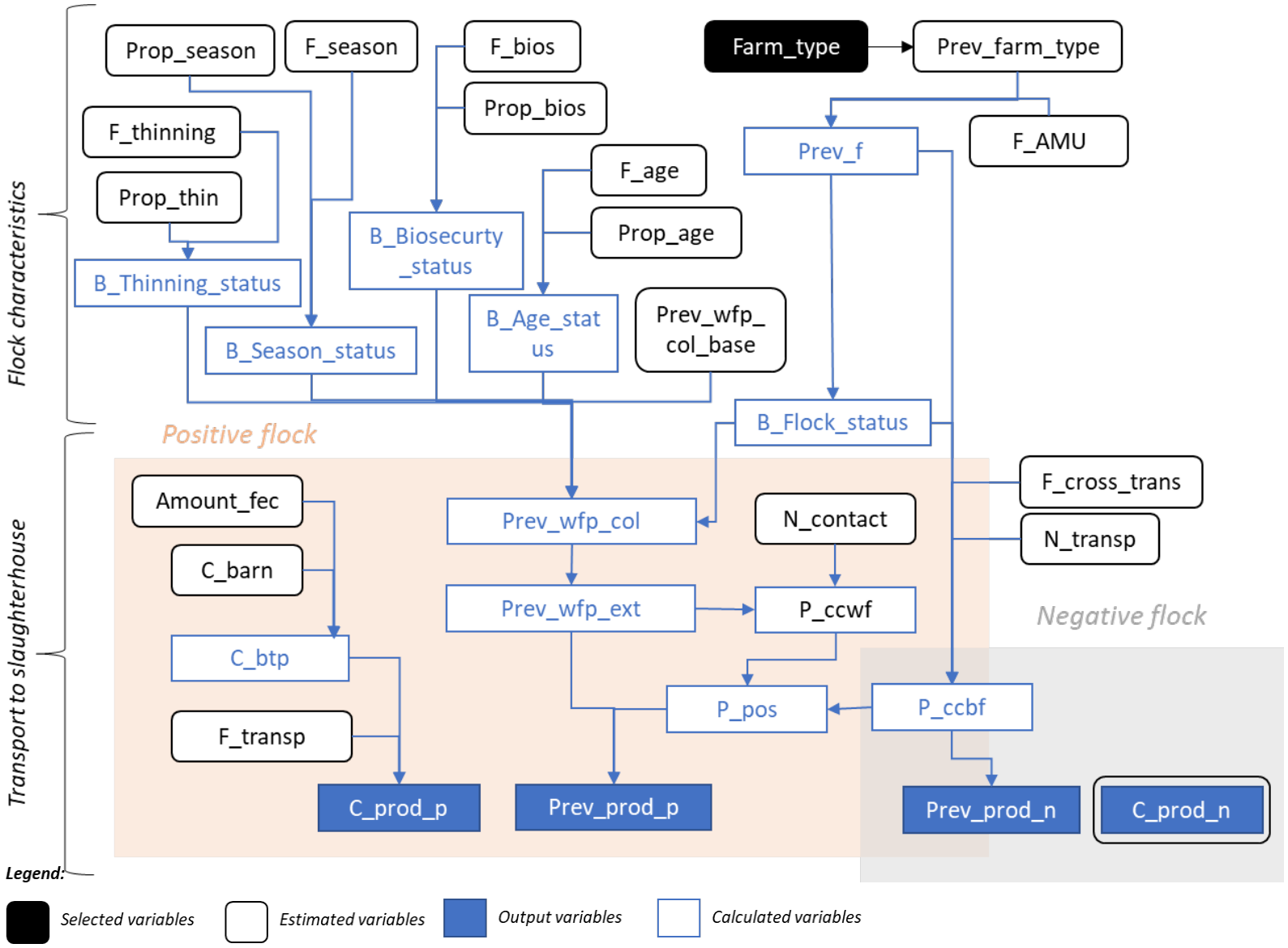


Figure 6: Model framework for the production module. Each node represents a model variable.

Table 18: List of estimated variables related to the production module

Domain	Variable Name	Description	Units
Other	*Prop_biosecurity*	Proportion of farms with poor biosecurity practices	Proportion
Other	*Prop_thinning*	Proportion of farms implementing thinning	Proportion
Other	*Amount_fec*	Amount of faeces on bird exterior at pre-harvest in positive flocks	g
Other	*N_transp*	Number of flocks transported prior to the current flock	Flocks
Other	*N_contact*	Number of contacts with contaminated birds during transport	-
Bacteria	*Prev_Farm_type*	Between Flock prevalence of AMR	Prevalence
Bacteria	*F_AMU*	Factor representing the impact of antimicrobial usage on between-flock prevalence of AMR	-
Bacteria	*F_thinning*	Factor representing the impact of thinning on contamination prevalence	Odds ratio
Bacteria	*F_biosecurity*	Factor representing the impact of poor biosecurity on contamination prevalence	Odds ratio
Bacteria	*Prop_age*	Proportion of birds slaughtered late. “Late” must be defined for each microorganism.	Proportion
Bacteria	*F_age*	Factor representing the impact of age on contamination prevalence	Odds ratio
Bacteria	*Prop_season*	Proportion of birds slaughtered during the high-risk season. “high risk season” must be defined for each microorganism.	Proportion
Bacteria	*F_season*	Factor representing the impact of high-risk season on contamination prevalence. “High risk season” must be defined for each microorganism.	Odds ratio

Domain	Variable Name	Description	Units
Bacteria	*Prev_wfp_col_base*	Average prevalence of birds from positive flock internally colonized at pre-harvest	Prevalence
Bacteria	*C_barn*	Concentration in the barn environment of positive flocks	CFU/g of faeces
Bacteria	*F_transp*	Factor representing the impact of transport of positive flocks on contamination load	-
Bacteria	*F_cross_trans*	Factor representing the probability of carryover contamination from a positive flock transported prior to the current flock.	Probability
Bacteria	*C_prod_n*	Number of bacteria on negative birds after transport due to cross contamination during transport	CFU/bird

Table 19: List of calculated variables related to the production module.

Variable Name	Description	Formula	Units	Source
Prev_f	Between flock prevalence of AMR1	Prev_farm_type *(1+F_AMU)	Prevalence	-
B_Flock_ status	Flock status	Binomial(1, Prev_f)	0 = negative, 1 = positive	-
B_Thin_stat us	Thinning status of the flock	Binomial(1, Prop_thinning)	0 = no thinning, 1 = thinning	-

Variable Name	Description	Formula	Units	Source
Prev_thinning	Increased within-flock prevalence associated with thinning	• ***If B_Thin_status = 1*** (F_thinning*Prev_wfp_col_base)/ (1 - Prev_wfp_col_base + (F_thinning*Prev_wfp_col_base)) • ***Else*** • Prev_wfp_col_base	Prevalence	-
B_Bios_status	Biosecurity status	Binomial(1, Prop_bios)	0 = good biosecurity 1 = poor biosecurity	-
Prev_biosecurity	Increased within-flock prevalence associated with poor biosecurity practices	• ***If B_Bios_status = 1*** (F_ biosecurity * Prev_thinning)/ (1 - Prev_thinning + (F_ biosecurity * Prev_thinning)) • ***Else*** Prev_thinning	Prevalence	-
B_Age_status	Flock age at slaughter	Binomial(1, Prop_age)	0 = young, 1 = old	
Prev_age	Increased within-flock prevalence associated with bird age	• ***If B_Age_status = 1*** (F_age * Prev_biosecurity)/ (1 - Prev_biosecurity + (F_ age * Prev_biosecurity))	Prevalence	-

Variable Name	Description	Formula	Units	Source
		• ***Else*** Prev_biosecurity		
B_Season_status	Season of slaughter	Binomial(1, Prop_season)	0 = low risk season, 1 = high risk season	
Prev_season	Increased within-flock prevalence associated with season of slaughter	• ***If B_Season_status = 1*** (F_season * Prev_age)/ (1 - Prev_age + (F_ season * Prev_age)) • ***Else*** Prev_age	Prevalence	-
C_btp	Number of bacteria on positive birds' exterior at pre harvest	C_barn * Amount_fec	CFU/bird	-
Prev_wfp_col	Prevalence of birds from positive flock internally colonized at pre-harvest	Prev_season	Prevalence	-
Prev_wfp_ext	Prevalence of birds externally contaminated at pre-harvest	Pert (0.03, *P_wfp_col*, 0.9)	Prevalence	(Colline au et al. 2020)

Variable Name	Description	Formula	Units	Source
P_ccbf	Probability of carry-over from a positive flock transported earlier on that day	$[1 - (1 - \text{Prev\_f})^{N\_transp}] \times$ F_cross_trans	Probability	(Bucher et al. 2012; Collineau et al. 2020)
P_ccwf	Probability of cross-contamination during transport	$1 - (1 - Prev\_wfp\_ext)^{N\_contact}$	Probability	(Bucher et al. 2012; Collineau et al. 2020)
P_pos	Probability a negative bird will become contaminated during transport	P_ccwf + P_ccbf - (P_ccwf * P_ccbf)	Probability	(Collineau et al. 2020)
Prev_prod_p	Prevalence of contaminated birds from positive flock after transport	Prev_wfp_ext + (1- Prev_wfp_ext)* P_pos	Prevalence	(Collineau et al. 2020)

Variable Name	Description	Formula	Units	Source
C_prod_p	Number of bacteria on positive birds after transport	C_btp * F_transp	CFU/bird	(Colline au et al. 2020)
Prev_prod_n	Prevalence of contaminated birds from negative flock after transport	P_ccbf	Prevalence	-

11.2 Processing Module

The poultry processing in large slaughterhouses is fast and greatly automated. UK is no exception. The technological advances have helped to reduce contamination during processing, however, there are still chances for bacteria contamination and spread at the slaughterhouse (Althaus, Zweifel, and Stephan 2017). In terms of antimicrobial-resistance, it is plausible that abattoir interventions may have varying effects but no data or very poor data (depending on the processing step) are currently available (Saatkamp, Gocsik, and Roskam 2018; Gonzalez-Zorn 2019) and further study are needed to understand the impact of interventions at abattoir on the occurrence of antimicrobial-resistant bacteria (Murphy et al. 2018). Because of the lack of relevant data, the processing was assumed to have the same effect on AMR and non-AMR bacteria. In addition, no change in bacteria contamination was assumed at the stunning and bleeding stage but changes occurred at scalding, defeathering, evisceration, washing, chilling, and portioning.

Interventions prior scalding have been shown to be effective in reducing bacteria contamination in chicken carcasses. Studies reported that brushing as well as plugging and suturing the vents of broiler prior scalding reduce the bacterial contamination in chicken carcass (Pacholewicz et al. 2016; Buhr, Berrang, and Cason 2003). However, the adoption of this practice is demanding and difficult to implement in current high throughput slaughterhouses (Parker, C Daniel 2020). This was confirmed during the stakeholder workshop and these practices were therefore not considered.

The scalding procedure is used to open the feather follicles to facilitate the removal of feathers. During the scalding stage, the within batch prevalence of contaminated carcasses is expected to increase while the bacteria load on carcasses reduce. The prevalence of contaminated birds after scalding (*Prev_scald_i*) is related to the incoming bacteria load on birds. Based on Collineau et al. (2020), the prevalence of contaminated carcasses was thus increased to 100% in the model when the incoming load on the carcass exceeded 5.5 to 6.5 log CFU; otherwise, prevalence was unchanged. The number of AMR-gene-carrying bacteria remaining on carcasses after

scalding (*C_scald_i*) depends on the scalding type used. Scalding schemes adopting different temperature ranges are possible, a hard scalding that includes water temperature from 60-66°C during an immersion time of 45-90s and a soft scalding with water temperature ranging between 51-54°C with immersion times for 120 to 210s (Projahn et al. 2018). In Europe generally, the scalding constant temperature varies from 50 to 65°C with immersion times between 60 and 210s. In addition, in EU, the use of chlorine is not allowed in higher concentrations than the ones used for potable water. Two different options for scalding either soft or hard could be selected by the model user by changing the values of the variable *Scalding_type*.

After scalding, carcasses are defeathered. During defeathering a proportion of organisms is washed off or removed with the feathers, but a number of organisms is also added via cross-contamination. This stage leads therefore to a reduction of bacterial contamination, and to carcasses cross-contamination between and within batch, as carcasses are exposed to residual from positive birds previously slaughtered. The reduction step was modelled by applying a reduction or increasing factor (*F_df)* to *C_scal_i*. The probability of occurrence of cross-contamination during defeathering (*P_cross_df_i)* was defined based on Collineau et al. (2020) and leads to an increase in both, the within batch prevalence of contaminated birds and the bacterial load after defeathering (respectively *Prev_df_i* and *C_df_i*) based on a factor named *R_df* and the average number of carcasses between a contaminated bird and a random bird at defeathering (*N_df_add_i*). *N_df_add_i* represents the effect of order at slaughter on the risk of cross contamination. Using the approach of Collineau (2020) it is estimated as 1 – *Prev_scal_i* but could be modified to represent the absence of risk of cross contamination by, for example, setting the numeric value of this variable to a very high number.

After defeathering, carcasses are eviscerated. The evisceration process involves removal of the feet, head and viscera of the birds, and the harvesting of edible offal. The process can be done either manually or mechanically. Manual evisceration can introduce human-borne contamination to the production line, while poorly calibrated machinery can also perforate the intestinal lining, leading to the spread of luminal

contents (Lu et al. 2018). This step is therefore associated with increased contamination and cross-contamination. In positive birds, contamination occurred if viscera are lacerated (*B_vis_cut =1*) and if the given bird was colonized (*B_bird_col* = 1). If contamination occurred, a load of bacteria (C_*spill_cfu*) was added to the bird (*C_visc_cut*). Birds from negative flocks were not contaminated via viscera rupture as they were, by definition, not internally colonized. The probability of cross-contamination during evisceration (*P_cross_ev_i*) was modelled as before using an increasing factor *F_ev* to obtain the within batch prevalence of contaminated carcasses (*Prev_ev_i*) and bacterial load (*C_ev_i*) after evisceration. As before, this variable represents hygienic practices of the slaughterhouse. It could be, for example, set up to zero if the risk of bird cross contamination during defeathering is assumed to be zero because of specific processing or hygienic practices. Findings from literature reviews show that the contamination along specific processing steps may vary between slaughterhouses due to risk management systems adopted (Pacholewicz et al. 2016).

After evisceration, carcasses are washed to decrease contamination load. The efficacy of carcass washing depends on a number of factors, including the number and type of washers, water pressure, nozzle arrangement, flow rate, line speed, water temperature, presence of sanitizing agents such as chlorine, and the use of surfactants (Lu et al. 2018). The within batch prevalence is however assumed to be unaffected at this step. In the UK, only one type of washing technique is used and is based on water bath (without chlorine). This technique is assumed to have only a physical effect on bacteria removal. Based on Collineau et al. (2020), the effect of using a second or third successive wash was assumed as less effective than the measured effect of a single wash. A reduction factor *F_wash_adj)* was thus applied to the effect of second and third washes, if present. The bacterial load after washing was represented by the variable *C_wash_i*.

After washing the carcasses are chilled and portioned. These steps were assumed to have no effect on the prevalence of contaminated carcasses but changed the bacterial contamination load. In the UK, only air pre-chilling is used. This type of pre-chilling was assumed to decrease bacterial load by a decreasing factor *Prop_ac* and to be not associated with cross-contamination (Collineau et al. 2020). The proportions of

remaining bacteria after air chilling (*C_chill_i*) was then calculated. The effect of portioning and skin removal on bacterial load was estimated as in Collineau et al. (2020) using the probability for a single cell to reside on the breast cap of a carcass (*P_skin*) and the proportion of cells transmitted from portion cap to meat (*Prop_cm*). The bacterial load and the prevalence of carcasses contaminated at the end of the processing modules were then calculated and named respectively *C_proc_i* and *Prev_proc_i.*

The model framework representing the variables in the processing module and their dependencies is presented in Figure 3. A detailed description of the estimated and calculated variables is available in Table 3 and Table 4 respectively.

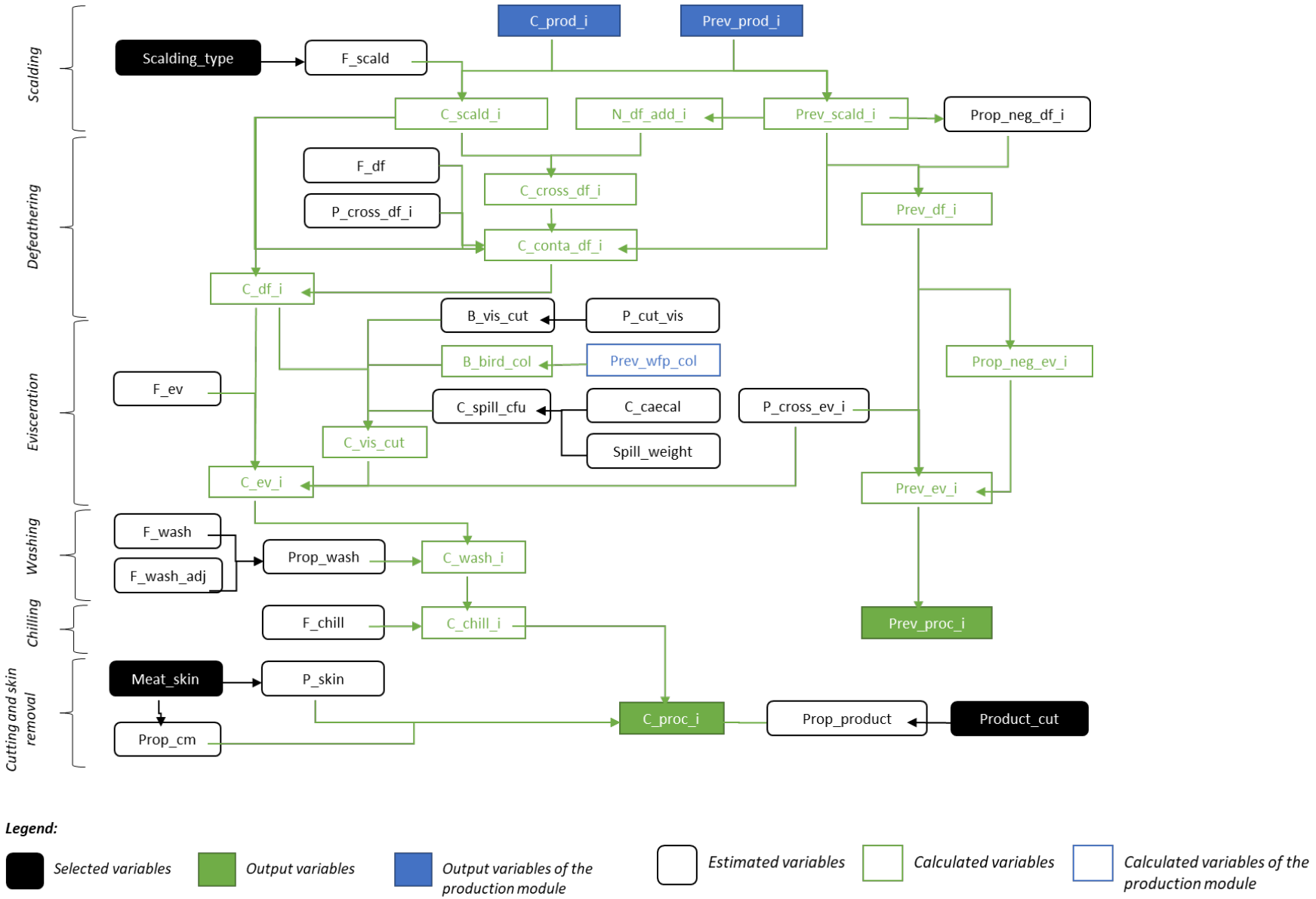

Scalding
Defeathering
Evisceration
Washing
Chilling
Cutting and skin removal
C_prod_i
Prev_prod_i
Scalding_type
F_scald
C_scald_i
N_df_add_i
Prev_scald_i
Prop_neg_df_i
F_df
P_cross_df_i
C_cross_df_i
C_conta_df_i
Prev_df_i
C_df_i
B_vis_cut
P_cut_vis
B_bird_col
Prev_wfp_col
Prop_neg_ev_i
F_ev
C_spill_cfu
C_caecal
P_cross_ev_i
Spill_weight
C_vis_cut
C_ev_i
Prev_ev_i
F_wash
F_wash_adj
Prop_wash
C_wash_i
F_chill
C_chill_i
Prev_proc_i
Meat_skin
P_skin
Prop_cm
C_proc_i
Prop_product
Product_cut
Legend:
Selected variables
Output variables
Output variables of the production module
Estimated variables
Calculated variables
Calculated variables of the production module

Figure 7: Model framework for the processing module. Each node represents a model variable. If i = n, the product comes from a negative flock. If i = p, the product comes from a positive flock

Table 20: List of estimated variables related to the processing module

Scalding

Variable type	Variable Name	Description	Units
Bacteria	*F_scald*	Reduction factor of bacterial load on carcass during after scalding	logCFU/ml

Defeathering

Variable type	Variable Name	Description	Units
Bacteria	*F_df*	Reduction or increasing factor of bacterial load on carcass during defeathering	logCFU
Bacteria	*P_cross_df_p*	Probability of cross-contamination to occur during defeathering for birds from positive flocks	Probability
Bacteria	*P_cross_df_n*	Probability of cross-contamination to occur during defeathering for birds from negative flocks	Probability

Evisceration

Bacteria	*F_df*	**Reduction or increasing factor of bacterial load on carcass during defeathering**	**logCFU**
Other	*P_cut_vis*	Probability that viscera are lacerated during evisceration	Probability
Other	*spill_weight*	Caecal content spilled in case of viscera rupture	g
Bacteria	*P_cross_ev_p*	Probability of cross-contamination to occur during evisceration for positive flocks	Probability
Bacteria	*F_ev*	Percentage of additive bacteria load increase due to cross-contamination between carcasses during evisceration	Proportion
Bacteria	*C_caecal*	Mean (90%CI) bacteria concentration in caecal content	CFU/g
Bacteria	*C_spill_cfu*	Number of bacteria added in case of viscera rupture	CFU

Washing

Bacteria	*F_df*	**Reduction or increasing factor of bacterial load on carcass during defeathering**	**logCFU**
Bacteria	*F_wash*	Load reduction or increasing factor after water washing (only water used in UK)	Log CFU
Bacteria	*F_Wash_adj*	Dampening or increasing factor for each successive wash	-

Chilling

Bacteria	*F_df*	**Reduction or increasing factor of bacterial load on carcass during defeathering**	**logCFU**
Bacteria	*F_chill*	Load reduction or increasing factor after air pre-chilling (only washing technique used in UK)	Log CFU

Cutting and skin removal

Bacteria	*F_df*	**Reduction or increasing factor of bacterial load on carcass during defeathering**	**logCFU**
Bacteria	*P_skin*	Probability that a single cell resides on portion cap	Probability
Bacteria	*Prop_cm*	Proportion of cells transmitted from portion cap to meat	-
Other	*Prop_product*	What fraction of the raw product represents the final processed product?	Proportion

Table 21: List of calculated variables related to the processing module

Scalding

Variable name	Description	Formula	Units	Source
Prev_scald_i	Prevalence of externally contaminated birds after scalding	• ***If C_prod_i > Uniform($10^{5.5}$, $10^{6.5}$)*** • 1 • ***Else*** • P_ prod _i	Prevalence	(McCarthy et al. 2019)
C_scald_i	Number of bacteria remaining after scalding	• ***If Scalding_type = "soft"*** • C_prod_i * 10^(-F_scald_soft) • ***If Scalding_type = "hard"*** C_prod_i * 10^(-F_scald_hard)	CFU/carcass	-

Defeathering

Variable name	Description	Formula	Units	Source
N_df_add_i	Average number of carcasses between seeder bird and random bird at defeathering	1/ Prev_scald_i	-	(Collineau et al. 2020)
C_cross_df_i	Number of bacteria added to birds following	C_scal_i *10^(a_df * log(N_df_add_i) + b_df)	CFU/carcass	(Hartnett et al. 2001)

Variable name	Description	Formula	Units	Source
	a contaminated bird during defeathering			
Prop_neg_df_i	Proportion of negative carcasses becoming positive via cross-contamination during defeathering	• ***If B_cross_df_01_i =1****:* 1- Prev_scald_i • ***Else:*** 0	Proportion	(Collineau et al. 2020)
Prev_df_i	Prevalence of contaminated carcasses after defeathering	• ***If P_cross_df_01_i =1:*** • Prev_scal_i + Prop_neg_df_i • ***Else:*** Prev_scal_i	Prevalence	(Collineau et al. 2020)
B_cross_df_01_i	Does cross-contamination occur during defeathering on a positive product?	Binomial(1, P_cross_df_i)	0 = no, 1=yes	(Collineau et al. 2020)
C_conta_df_i	Number of bacteria on carcasses given cross-contamination during defeathering	Discrete({C_scald_i x 10^{F_df} + C_cross_df_i, C_cross_df_i}; {Prev_scald_i * P_cross_df_i, [1-Prev_scald_i] * P_cross_df_i})	CFU	(Collineau et al. 2020)

Variable name	Description	Formula	Units	Source
C_df_i	Number of bacteria on carcasses after defeathering	• ***If P_cross_df_01_i =1:*** • C_conta_df_i • ***Else:*** C_scald_i* 10^{F_df}	CFU/carcass	(Collineau et al. 2020)

Eviscerations

Variable name	Description	Formula	Units	Source
B_vis_cut	Are the viscera lacerated?	Binomial(1, P_cut_vis)	1=cut, 0 =intact	(Collineau et al. 2020)
B_bird_col	Is the bird colonized?	Binomial(1, Prev_wfp_col)	0= no, 1=yes	(Collineau et al. 2020)
C_spill_cfu	Number of bacteria added in case of viscera rupture	C_caecal * spill_weight	CFU/carcass	(Collineau et al. 2020)
C_vis_cut	Number of bacteria on carcass after potential viscera laceration	• ***If B_vis_cut =1 & B_bird_col=1:*** • C_df_i + C_spill_cfu • ***Else:*** • C_df_i	CFU/carcass	-

Variable name	Description	Formula	Units	Source
P_cross_ev_n	Probability of cross-contamination to occur during evisceration for negative flocks	P_cross_df_n	Probability	(Collineau et al. 2020)
Prop_neg_ ev_i	Proportion of negative carcasses becoming positive via cross contamination during evisceration	(1-Prev_df_i) * P_cross_ev_i	Proportion	(Collineau et al. 2020)
B_cross_ev_ 01_i	Does cross-contamination occur at evisceration?	Binomial(1, P_cross_ev_i)	0=no, 1=yes	(Collineau et al. 2020)
C_ev_i	Number of bacteria on carcasses from positive flocks after evisceration	• ***If B_cross_ev_01_p =1:*** • C_visc_cut + C_visc_cut *F_ev/100 • ***Else:*** • C_visc_cut	CFU/carcass	-
Prev_ev_i	Prevalence of contaminated carcasses flocks after evisceration	• ***If B_cross_ev_01_i =1:*** • Prev_df_i + Prop_neg_ev_i • ***Else:*** • Prev_df_i	Prevalence	-

Washing

Variable name	Description	Formula	Units	Source
Prop_wash	Proportion of cells remaining after washing	(10^{F_wash}) x (1+F_Wash_adj)	Proportion	(Collineau et al. 2020)
C_wash_i	Number of bacteria remaining after washing	C_ev_i * Prop_wash	CFU/carcass	-

Chilling

Variable name	Description	Formula	Units	Source
Prev_ proc _i	Prevalence of contaminated carcasses after chilling	Prev_ev_i	Prevalence	(Collineau et al. 2020)
C_chill_i	Number of bacteria remaining after chilling	C_wash_i - C_wash_i *F_chill/100	CFU/carcass	(Collineau et al. 2020)

Cutting and skin removal

Variable name	Description	Formula	Units	Source

C_proc_i	Number of bacteria on a random product after portioning and skin removal	• ***If Product_cut = "no":***
		• C_chill_i
		• ***Else:***
		  • ***If Meat_skin ="skin off"***
		  • C_chill_i * P_skin* Prop_cm /Prop_fraction
		  • ***Else***
		  C_chill_i / Prop_fraction
			CFU/food item	(Collineau et al. 2020)

11.3 Post-processing Module

After processing, chicken meat is subjected to retail storage, consumer transport, and home storage. We assumed that no cross contamination occurs in this module and the prevalence of contaminated food item at the end of the post-processing module (*Prev_pproc_i*) equals *Prev_proc_i*. Bacterial growth may however occur depending on temperature variations occurring in the post-processing module. If the temperature at retail (*T_retail*), during transport home (*T_trans*) or home storage (*T_fridge*) exceed the minimal growth temperature of the selected bacteria (*T_growth_min*), growth factors (*F_retail, F_trans,* and *F_fridge*) depending on the optimal growth temperature (*T_growth_opt*) and the minimum generation time in food product (*Time_gen_min*) were applied to the incoming load of bacteria *C_proc_i*. The resulting load of bacteria (*C_ret_i*) was reduced or increased of a factor *F_pack* depending if the meat was sold in a modified atmosphere packaging (*Pack_type*). Based on Collineau et al. (2020), adjustments were also made to prevent products with very low contamination levels from being carried forward to the consumer stage of the model. The minimum load on contaminated product was set to 1 CFU per food item meaning that products contaminated with less than 1 CFU at the end of the module are considered to have 0 CFU. Similarly, a maximum possible number of CFU on a piece of meat (*C_max*) was defined based on estimated maximum population density (*C_MDP*) and size of product (*Size*) and used to adjust the bacteria load of contaminated product at the end of the post-processing module (*C_pproc_i*).

The model framework representing the model variables related the post-processing module and their dependencies is presented in Figure 4. A detailed description of the estimated and calculated variables is available in Table 5 and Table 6 respectively.

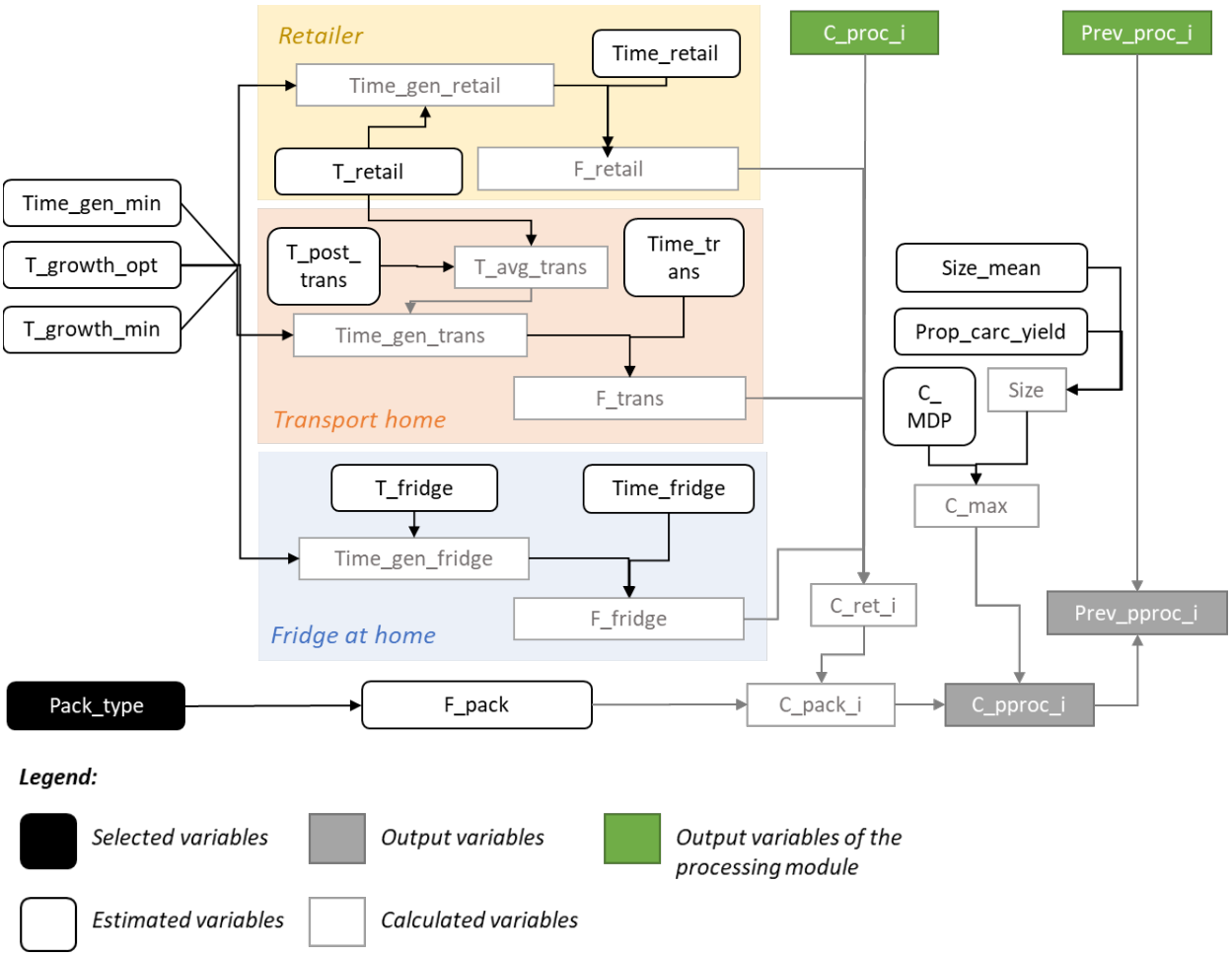


Figure 8: Model framework for the post-processing module. Each node represents a model variable. If i = p, the product comes from a positive flock

Table 22: List of estimated variables related to the post-processing module

Type of variable	Variable Name	Description	Units
Other	*Size_mean*	Breast or carcass size mean	g
Other	*Prop_carc_yield*	Carcass yield (proportion of lean meat)	Proportion
Other	*Time_retail*	Number of days stored at retail	days
Other	*T_retail*	Temperature at retail storage	Degree C
Other	*Time_trans*	Mean (90%CI) home transport duration	Minutes
Other	*T_post_trans*	Chicken temperature at the end of home transport	Degree C
Other	*Time_fridge*	Mean (90%CI) number of days refrigerated at home	days
Other	*T_fridge*	Home refrigeration temperature	Degree C
Bacteria	*T_growth_min*	Minimum growth temperature	Degree C
Bacteria	*T_growth_opt*	Optimal growth temperature	Degree C
Bacteria	*F_pack*	Reduction or increasing factor of bacterial load because of packaging	logCFU
Bacteria	*Time_gen_min*	Minimum generation time in food product	hours
Bacteria	*C_MPD*	Maximum population density	CFU / g

Table 23: List of calculated variables related to the post-processing module

Variable Name	Description	Calculus	Units	Source
F_retail	Growth factor of the microorganism on the selected food product at retail	Depends on each microorganism	CFU /g /hour	-
F_trans	Growth factor of the microorganism on the selected food product during transport	Depends on each microorganism	CFU /g /hour	-
F_fridge	Growth factor of the microorganism on the food product in the fridge of the consumer	Depends on each microorganism	CFU /g /hour	-
Size	Product size	• ***If Product_cut = "no"*** • Prop_carc_yield x Size_mean • ***Else*** Size_mean*Prop_product	g	-
C_max	Maximum possible number of CFU on a carcass or breast	C_MPD x Size	CFU/food item	(Collineau et al. 2020)
C_ret_i	bacteria load on a contaminated product	C_proc_i*G_retail * G_trans* G_fridge	CFU/food item	-

Variable Name	Description	Calculus	Units	Source
T_avg_trans	Average chicken temperature during home transport	(T_retail + T_post_trans)/2	Degree C	-
C_pack_i	Bacterial reduction load due to packaging	• **If *Pack_type = "MAP" & Time_retail + Time_trans+Time_fridge > 7 days*** C_ret_i x 10^{F_pack} • ***Else*** • C_ret_i	CFU/food item	-
C_pproc_i	Adjusted bacteria load on a contaminated product	• ***If C_pack_i < 1,*** 1 • ***If C_pack_i > C_max*** • C_max • ***Else*** C_pack_i	CFU/food item	(Collineau et al. 2020)
Prev_pproc_i	Prevalence of contaminated products after consumer storage	Prev_proc_i x (1 - Poisson (0, C_pproc_i))	Prevalence	(Collineau et al. 2020)

11.4 Home preparation Module

During home preparation, ingestion of antimicrobial resistant bacteria can occur via two parallel pathways: direct contamination and cross-contamination. Direct contamination represents the ingestion of a contaminated food item and is possible, in case of chicken meat, when undercooking occurs (*P_undercook*). If no undercooking occurs, we assumed that no bacteria cells survive. In case of undercooking, a proportion of cells can survive in the so-called 'protected area'. The number of cells remaining after cooking *C_cook_i* was derived as in Collineau et al. (2020) using the proportion of cells in the protected area (*Prop_protec)* and a logarithmic reduction of cells in the protected area. The latter is dependent on the exposure time (*Time_protec)* and the decimal reduction time (*R_ref)* at temperature in the protected area (*T_protec)*. The probability of cross-contamination occurrence (*P_h_wash*) is related to kitchen hygiene and was modelled using the 'drip-fluid' model proposed by (WHO and FAO 2009) and adapted by Collineau et al. (2020). A transfer factor (*tsf*) was defined as the product of the proportion of in the fluid (*Prop_fluid*) and the ratio of a volume of fluid (*V_ing*) ingested out of a total volume (*V_dil*) of fluid dripping off the chicken product. *V_dil* depends if the chicken carcass was portioned or not (*Product_cut*). The adjustments of contamination prevalence and load described after retail were applied again after cooking and home preparation to ensure that only doses greater than or equal to 1 CFU were considered in the risk assessment. The model framework representing the model variables related to the home preparation module and their dependencies is presented in Figure 5. A detailed description of the estimated and calculated variables is available in Tables 7 and 8 respectively.

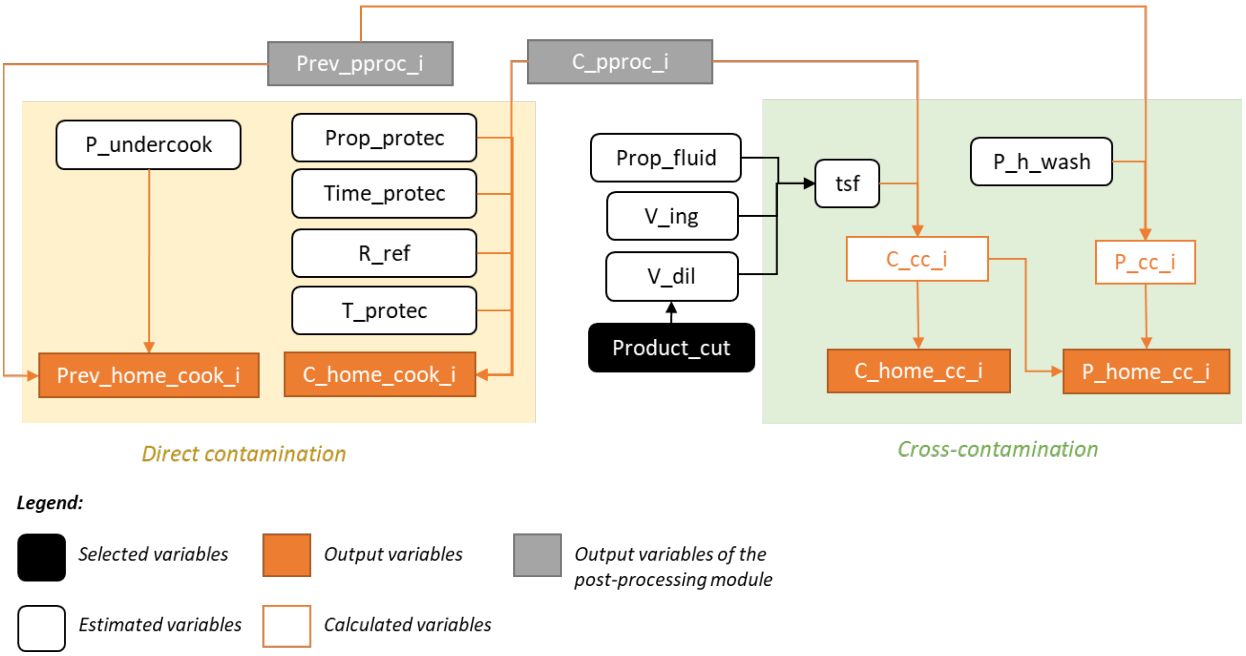


Figure 9: Model framework for the home preparation module. Each node represents a model variable. If i = p, the product comes from a positive flock

Table 24: List of estimated variables related to the home preparation module

Type of variable	Variable Name	Description	Units
Other	*P_undercook*	Mean (90%CI) probability of undercooking to occur	Probability
Other	*Time_protec*	Exposure time at exposure temperature in the protected area	Minutes
Other	*T_protec*	Exposure temperature during cooking in the "protected area"	Degree C
Other	*P_h_wash*	Probability of cross-contamination to occur (related to kitchen hygiene, represent the probability of not washing hands during chicken food preparation)	Probability
Other	*V_dil_carc*	Volume of fluid diluting for a whole carcass	ml

Type of variable	Variable Name	Description	Units
Other	*V_ing*	Volume of fluid ingested	ml
Bacteria	*Prop_protec*	Proportion of cells in the “protected area”	-
Bacteria	*Prop_loose*	Proportion of cells loosely attached	-

Table 25: List of calculated variables related to the home-preparation module

Variable Name	Description	Formula	Units	Source
V_dil	Volume of fluid diluting for a piece of chicken meat	• **If Product_cut = "no"** V_dil_carc • **Else** Size/Size_mean x V_dil_carc	ml	(Collineau et al. 2020; WHO and FAO 2009)
tsf	Transfer factor	Prop_loose x V_ing/V_dil	-	(Collineau et al. 2020)
R_ref	Decimal reduction time at the exposure temperature in the protected area	10^(-0.139 x T_protec + 8.58)	Minutes	(WHO and FAO 2009)
Prev_home_cook_i	Prevalence of contaminated servings after product preparation	P_undercook *Prev_pproc_i	Probability	(Collineau et al. 2020)
C_home_cook_i	Number of bacteria on one undercooked portion post product preparation	10^(Prop_protec *log(C_ pproc _i) – Time_protec/R_ref	CFU/food item	(Collineau et al. 2020)

Variable Name	Description	Formula	Units	Source
P_cc_i	Probability of exposure through cross contamination	P_h_wash *Prev_ pproc _i	Probability	(Collineau et al. 2020)
P_home_cc_i	Adjusted probability of exposure through cross contamination	P_cc_i * (1 - Poisson (0, C_cc_i))	Probability	(Collineau et al. 2020)
C_cc_i	Number of bacteria ingested by cross contamination	tsf * C_ pproc _i	CFU/food item	(Collineau et al. 2020)
C_home_cc_i	Adjusted number of bacteria ingested by cross contamination	• ***If C_cc_i < 1*** 1 • ***Else*** C_cc_i	CFU/food item	(Collineau et al. 2020)

11.5 References

Althaus, Denise, Claudio Zweifel, and Roger Stephan. 2017. “Analysis of a Poultry Slaughter Process: Influence of Process Stages on the Microbiological Contamination of Broiler Carcasses.” *Italian Journal of Food Safety* 6 (4). https://doi.org/10.4081/ijfs.2017.7097.

Bucher, O., A. Fazil, A. Rajić, A. Farrar, R. Wills, and S. A. McEWEN. 2012. “Evaluating Interventions against *Salmonella* in Broiler Chickens: Applying Synthesis Research in Support of Quantitative Exposure Assessment.” *Epidemiology and Infection* 140 (5): 925–45. https://doi.org/10.1017/S0950268811001373.

Buhr, Rj, Me Berrang, and Ja Cason. 2003. “Bacterial Recovery from Breast Skin of Genetically Feathered and Featherless Broiler Carcasses Immediately Following Scalding and Picking.” *Poultry Science* 82 (10): 1641–47. https://doi.org/10.1093/ps/82.10.1641.

Collineau, Lucie, Brennan Chapman, Xu Bao, Branavan Sivapathasundaram, Carolee A. Carson, Aamir Fazil, Richard J. Reid-Smith, and Ben A. Smith. 2020a. “A Farm-to-Fork Quantitative Risk Assessment Model for Salmonella Heidelberg Resistant to Third-Generation Cephalosporins in Broiler Chickens in Canada.” *International Journal of Food Microbiology*, February, 108559. https://doi.org/10.1016/j.ijfoodmicro.2020.108559.

———. 2020b. “A Farm-to-Fork Quantitative Risk Assessment Model for Salmonella Heidelberg Resistant to Third-Generation Cephalosporins in Broiler Chickens in Canada.” *International Journal of Food Microbiology* 330 (October): 108559. https://doi.org/10.1016/j.ijfoodmicro.2020.108559.

Gonzalez-Zorn, Bruno. 2019. “D3.4_Intervention Strategies That Influence Horizontal Gene Transfer and Stability of Antimicrobial Resistance Genes in the Food Chain.” Project report. EFFORT. http://www.effort-against-amr.eu/media/download_gallery/EFFORT_D3.4_Intervention_Strategies_that_influence_horizontal_gene_transfer_R1.pdf.

Hartnett, E., L. Kelly, D. Newell, M. Wooldridge, and G. Gettinby. 2001. “A Quantitative Risk Assessment for the Occurrence of Campylobacter in Chickens at the Point of Slaughter.” *Epidemiology & Infection* 127 (2): 195–206. https://doi.org/10.1017/S0950268801005866.

Lu, Gang, Lingshuang Sun, Jiajun Ou, Haibin Xu, Liyan Wu, and Shoujun Li. 2018. “Identification and Genetic Characterization of a Novel Parvovirus Associated with Serum Hepatitis in Horses in China.” *Emerging Microbes & Infections* 7 (October). https://doi.org/10.1038/s41426-018-0174-2.

McCarthy, Z, Fazil A, Ryan Sd, Wu Jh, and Munther D. 2019. “An individual-carcass model for quantifying bacterial cross-contamination in an industrial three-stage poultry scalding tank.” *Journal of Food Engineering* 262: 142–53.

Murphy, Colleen P., Carolee Carson, Ben A. Smith, Brennan Chapman, Jayme Marrotte, Maggie McCann, Courtney Primeau, Parth Sharma, and E. Jane Parmley. 2018. “Factors Potentially Linked with the Occurrence of Antimicrobial Resistance in Selected Bacteria from Cattle, Chickens and Pigs: A Scoping Review of Publications for Use in Modelling of Antimicrobial Resistance (IAM.AMR Project).” *Zoonoses and Public Health* 65 (8): 957–71. https://doi.org/10.1111/zph.12515.

Pacholewicz, Ewa, Len J.A. Lipman, Arno Swart, Arie H. Havelaar, and Willem J.C. Heemskerk. 2016. “Pre-Scald Brushing for Removal of Solids and Associated Broiler Carcass Bacterial Contamination.” *Poultry Science* 95 (12): 2979–85. https://doi.org/10.3382/ps/pew257.

Parker, C Daniel. 2020. “Falling Resistance in E Coli Isolated from Broilers in the UK - ProQuest.” 2020. https://search.proquest.com/openview/3b807d50ddbf02be7b099c67d5119098/1?pq-origsite=gscholar&cbl=2041027.

Projahn, Michaela, Ewa Pacholewicz, Evelyne Becker, Guido Correia-Carreira, Niels Bandick, and Annemarie Kaesbohrer. 2018. “Reviewing Interventions against Enterobacteriaceae in Broiler Processing: Using Old Techniques for Meeting the New Challenges of ESBL E. Coli?” *BioMed Research International* 2018 (October). https://doi.org/10.1155/2018/7309346.

Saatkamp, H.W., E. Gocsik, and J.L. Roskam. 2018. “Chain Model and Report on Analysis of Strategies.” Project report. EFFORT. http://www.effort-against-amr.eu/media/EFFORT_D8.4_Chain_model_and_report_on_analysis_of_strategies_R1.pdf.

WHO, and FAO, eds. 2009. *Risk Assessment of Campylobacter Spp. in Broiler Chickens. Technical Report*. Microbiological Risk Assessment Series 12. Geneva: World Health Organization : Food and Agriculture Organization of the United Nations.

12. Appendix 3: lettuce model

The overall model structure is based on an existing QMRA model for *E. coli* in lettuce developed by Njage and Buys (2017) and Pang et al. (2017). In some cases, other existing models were used to inform specific modules as described in the following sections.

12.1 Production Module

The model starts at the lettuce production stage. In each iteration of the stochastic model, a batch of lettuce heads is randomly selected from the production population. Each randomly selected lettuce batch was associated with a season of harvesting and specific on-farm practices. Season of harvesting was randomly selected based on the proportion of farms harvesting lettuces during the high-risk season for the microorganism of interest (*Prop_season).* Similarly, the on-farm practices were defined using the proportion of farms with poor biosecurity practices (*Prop_bios*), and the proportion of farms using untreated manure (*Prop_fert*). It should be noted that at the time of writing, untreated manure are not allowed in the UK for growing crops. This parameter was included in the model for potential future applications but was considered having no effect on the case study investigated in the last part of this report. The prevalence of contaminated lettuces within this batch was defined by the prevalence of AMR-gene-carrying bacteria in lettuce at pre-harvest (*Prev_harv, or Prev_prod*). This prevalence was estimated by combining the baseline prevalence of contaminated lettuces at pre-harvest found in the literature (*Prev_base*), with risk factors related to the usage of untreated manure (*F_fert*), high-risk season (*F_season*), and poor biosecurity (*F_biosecurity*) on the randomly selected farm (*B_fert_status, B_season_status, and B_biosecurity_status*). In the production module related to the food product “lettuce”, we assumed that no cross-contamination between lettuces occurred.

The level of bacterial contamination of the contaminated lettuce heads was estimated based on the level of contamination of irrigation water, the pre-harvest holding time, the harvesting tool used, and field cooling. Irrigation water is considered the major risk factor of microbial contamination of crops (Gil et al. 2015) and the impact on bacteria load (*C_pw*) was modelled based on Pang *et al.* (2017) combining concentration of bacteria in irrigation water (*C_water*) and the volume of water remaining on lettuce after irrigation (*W_water*). Pre-harvest holding time, *Time_hold* (i.e., time since the last irrigation to harvest) is used as a risk-reducing strategy. The rate of microorganism inactivation during that time interval (*F_hold*) allowed us to estimate a bacterial load pre-harvest (*C_hold*).

Harvesting tools may influence bacteria contamination post harvesting (*C_prod*). The effect of harvesting tools was modelled based on Pang *et al*. (2017) by multiplying the average soil bacteria concentration (*C_soil*) with the quantity of soil attached on harvesting blades (*W_soil*). Like Pang *et al.* (2017), our model assumed that harvesting blades would evenly contaminate three consecutive heads of lettuce after contact with soil. The increase of bacteria concentration in lettuce due to contact with contaminated harvesting blades (*C_harv*) was calculated by dividing the number of cells transferred to lettuce by 3 times the average weight of one head of lettuce (*Size_mean*).

In the UK, lettuce is cooled down after harvest and stored to prevent dehydration (Terry et al. 2011). The effect of the field cooling phase on bacterial growth was modelled using a growth factor during field cooling (*G_field*) based on the temperature and time of field cooling (*T_field_cool* and *Time_field_cool* respectively). Our model assumes that this step in the production chain does not influence the prevalence of contaminated products but only the bacterial load. Growth models used for *E. coli* to calculate G*_field* were the same than the one used in the chicken model (van Gerwen and Zwietering 1998; Collineau et al. 2020). We assumed that when the temperature was below the minimal growth temperature, the number of bacteria remains constant. The contamination load per gramme (*C_prod*) was obtained by multiplying *C_harv* and *G_field*.

The model framework representing the model variables related to the production module and their dependencies is presented in Figure 2. A detailed description of the estimated and calculated variables is available in Tables 2 and 3 respectively.

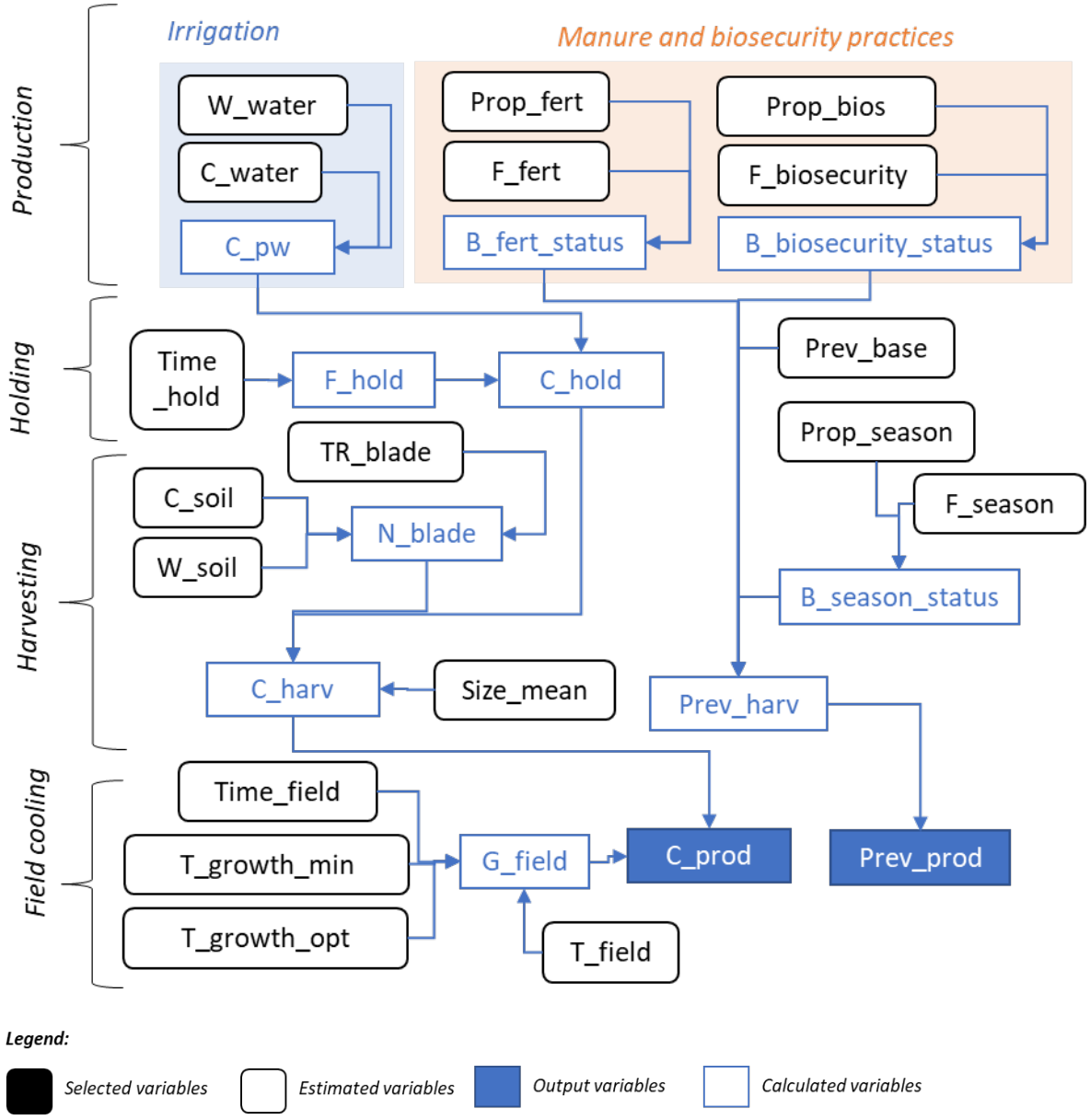


Figure 10: Model framework for the production module. Each node represents a model variable.

Table 26: **List of estimated variables related to the production module**

Domain	Variable Name	Description	Units
Other	*Prop_fert*	Proportion of lettuce fertilized with untreated manure.	Proportion
Other	*Prop_bios*	Proportion of farms with poor biosecurity practices	Proportion
Other	*W_soil*	Attached soil on harvesting blades	g
Other	*Size_mean*	Product size mean	g
Other	*W_water*	Volume of water remaining on lettuce after overhead irrigation	ml/g
Other	*TR_blade*	Transfer rate from harvesting blades to lettuce	-
Other	*Time_hold*	Time interval between last irrigation and harvest	days
Other	*Time_field*	Time of field cooling	hours
Other	*T_field*	Product temperature during field cooling	Degree C
Bacteria	*C_soil*	Average soil bacteria concentration	Log CFU/g
Bacteria	*C_water*	Concentration of bacteria in irrigation water	CFU/100mL
Bacteria	*F_fert*	Factor representing the impact of untreated manure on prevalence of contamination.	Odds ratio
Bacteria	*Prop_season*	Proportion of lettuce harvested during the high-risk season. “high risk season” must be defined for each microorganism.	Proportion
Bacteria	*F_biosecurity*	Factor representing the impact of poor biosecurity on contamination load.	Odds ratio
Bacteria	*F_season*	Factor representing the impact of high-risk season on prevalence of contamination. “High risk season” must be defined for each microorganism.	Odds ratio

Domain	Variable Name	Description	Units
Bacteria	*Prev_base*	Average prevalence of lettuce contaminated at pre-harvest	Prevalence
Bacteria	*Time_gen_min*	Minimum generation time in food product	hours
Bacteria	*T_growth_min*	Minimum growth temperature	°C
Bacteria	*T_growth_opt*	Optimal growth temperature	°C

Table 27: List of calculated variables related to the production module

Variable Name	Description	Formula	Units	Source
C_pw	Concentration after irrigation	C_water/100 *W_water	CFU/g	(Pang et al. 2017)
F_hold	Log reduction during holding time	-Time_hold/ (2.45/24))^0.3	Log CFU/g	(Pang et al. 2017)
C_hold	Log concentration after holding time	Log(C_pw) + F_hold	Log CFU/g	(Pang et al. 2017)
B_season_status	Season of harvesting	Binomial(1, Prop_season)	0 = low risk season 1 = high risk season	-
F_season01	Increased within batch prevalence associated with high risk season	• **If B_season_status = 1** (F_season * Prev_base)/ (1 - Prev_base + (F_ season * Prev_base)) • **Else** Prev_base	Prevalence	-
B_biosecurity_status	Biosecurity status	Binomial(1, Prop_biosecurity)	0 = good biosecurity 1 = poor biosecurity	-
F_biosecurity01	Increased within-batch prevalence associated with poor biosecurity practices	• **If B_biosecurity_status = 1** (F_ biosecurity * F_season01)/ (1 - F_season01+ (F_ biosecurity * F_season01)) • **Else**	Prevalence	-

Variable Name	Description	Formula	Units	Source
		• F_season01		
B_fert_status	Farm using untreated manure	Binomial(1, Prop_fert)	0 = treated manure 1 = untreated manure	-
F_fert01	Increased within-batch prevalence associated with usage of untreated manure	• **If B_fert_status = 1** (F_ fert * F_biosecurity01)/ (1 - F_biosecurity01+ (F_ fert * F_biosecurity01)) • **Else** F_biosecurity01	Prevalence	-
N_blade	Number of bacteria in soil attached on blade	C_soil *W_soil	CFU	(Pang et al. 2017)
Prev_harv	Prevalence of contaminated lettuce after harvesting	F_fert01	Prevalence	-
C_harv	Concentration of bacteria after harvest	10^{C_hold} + N_blade*TR_blade/(3*Size_ mean)	CFU/g	(Pang et al. 2017)
Prev_prod	Prevalence of contaminated lettuce	*Prev_harv*	Prevalence	-
G_field	Growth factor G during field cooling	• ***If T_field > T_growth_min*** exp(ln (2)* [(T_ field - T_growth_min)/ (T_growth_opt – T_growth_min)]^2	CFU /g /hour	(van Gerwen and Zwieterin g 1998; Collineau

Variable Name	Description	Formula	Units	Source
		* Time_field / Time_gen_min) • ***Else*** *1*		et al. 2020)
C_prod	Concentration of bacteria after field cooling	C_harv*G_ field	CFU/g	-

12.2 Processing Module

Once transferred to the processing module, lettuces can be washed and shredded. If washing with water occurred (*Product_wash* = 1), then it leads to a reduction (*D_wash_proc*) of bacterial contamination. Washing with water during the processing of bagged lettuce is a common practice to remove soil and gross debris. Washing practices will vary across the industry in the UK. Chlorine and other sanitisers can be added to the wash water but the main purpose of these is to control hygiene of the wash water rather reduce contamination on the produce. The CFA has published best practice protocols which aim to minimise the use of chlorine and ensure that soil is removed. These require RTE leafy salads to receive a primary and secondary wash in chlorinated water (<10 ppm and <25 ppm free chlorine respectively and <100 ppm total) followed by a potable water rinse ("Fresh Produce: Agency Advice on Re-Washing Ready to Eat Leafy Salads" 2008).

Microorganism present on contaminated lettuce may transfer to shredders, conveyor belts, flume tanks, shakers, and centrifuges during washing (if *Product_wash* = 1) and shredding (if *Product_cut* = 1).

If lettuces are not washed and not cut, we assumed that no cross contamination occurred.

Cross contamination occurring during washing and shredding was modeled based on (Pang et al. 2017) by calculating the number of bacteria transferred between lettuces and different processing surfaces (flume tank (*TR_flume*), shredder (*TR_shred*), shaker (*TR_shake*), centrifuge (*TR_centri*) and conveyor belts (*TR_convey*)). An additional factor representing the overall transfer coefficients from facilities to uncontaminated lettuces was also added (*TR_facility*). *TR_facility* represent the hygienic practices of the processing. Finally, the increase in prevalence due to cross-contamination (TR_overall) was estimated based on the literature to calculate the prevalence of contaminated lettuce at the end of the processing module (*Prev_proc*).

The average number of cells per contaminated product after processing depends on the type of product considered: either a lettuce head (*product_cut* = 0), or a bag of lettuce (*product_cut* =1). If the product considered is a lettuce head, the average number of cells per product at the end of the processing module (*C_proc*) was estimated based on the *Prev_proc,* the final number of cells in a lettuce head *(N_final)*. If the product considered is a bag of lettuce, *C_proc* was calculated based on the size of a bag of lettuce (*Size_bag*). For this calculation, we assumed that the leaves used to produce one bag of lettuce come all from the same batch of lettuce.

Our model assumes that cold storage occurring at the processing module is appropriate and has thus zero influence towards the prevalence of contaminated products and the bacterial load. The model framework representing the model variables related the processing module and their dependencies is presented in Figure 3. A detailed description of the estimated and calculated variables is available in Table 4 and 5 respectively.

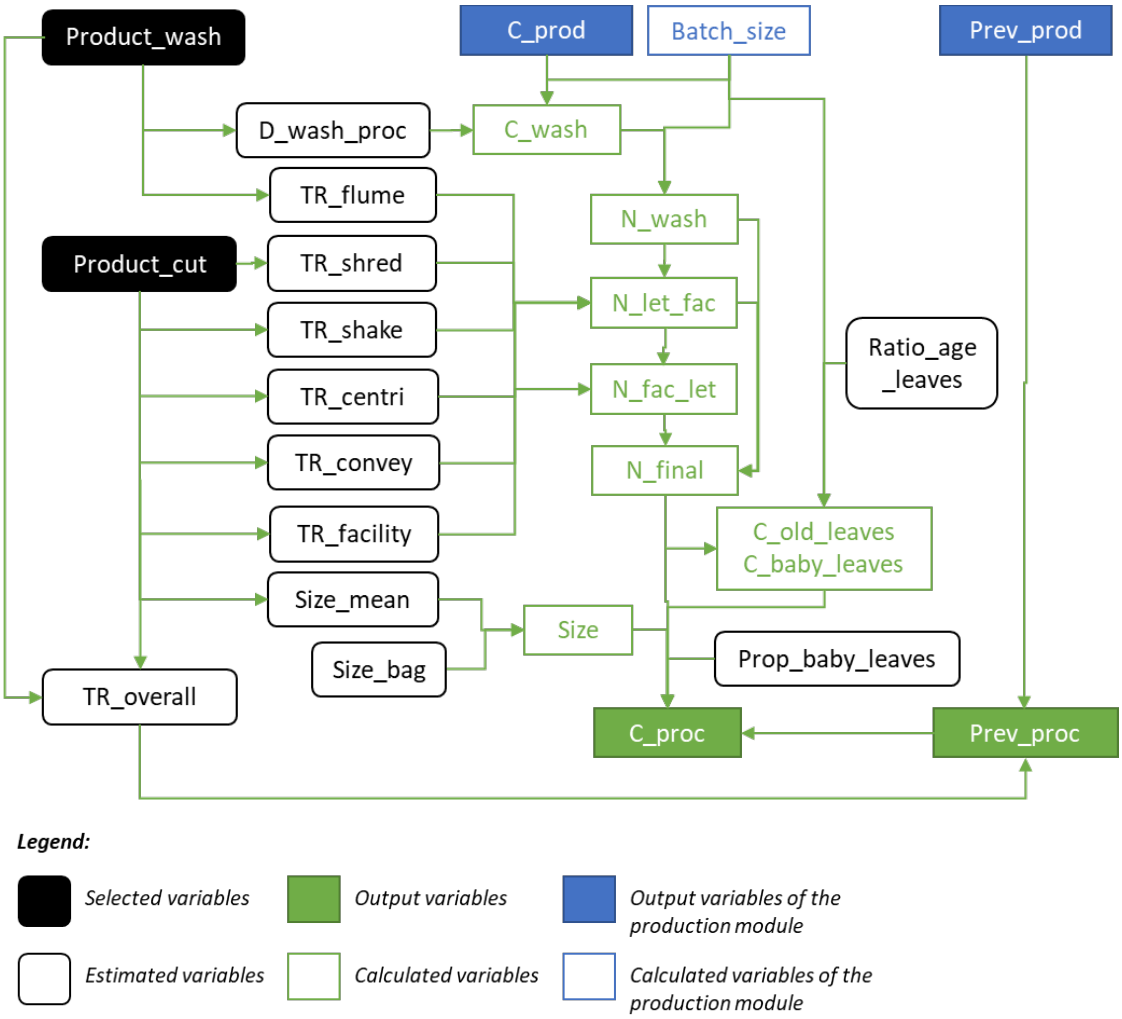


Figure 11: Model framework for the processing module. Each node represents a model variable.

Table 28: List of estimated variables related to the processing module

Domain	Variable Name	Description	Unit
Other	Prop_baby_leaves	Proportion of baby leaves in a bag of lettuce	Proportion
Other	Size_bag	Size of a bag of lettuce	g
Bacteria	*D_wash_proc*	Log reduction by washing with water	Log CFU /g

Domain	Variable Name	Description	Unit
Bacteria	*TR_flume*	Transfer from contaminated lettuce to flume	%
Bacteria	*TR_shred*	Transfer from contaminated lettuce to shredder	%
Bacteria	*TR_shake*	Transfer from contaminated lettuce to shaker	%
Bacteria	*TR_centri*	Transfer from contaminated lettuce to centrifuge	%
Bacteria	*TR_convey*	Transfer from contaminated lettuce to conveyor	%
Bacteria	*TR_facility*	Overall transfer coefficient from facilities to uncontaminated lettuce	%
Bacteria	*TR_overall*	Spread of contamination due to cross-contamination	-
Bacteria	*Ratio_age_leaves*	Ratio of the bacteria concentration in baby leaves to concentration of the same bacteria in old leaves.	-

Table 29: List of calculated variables related to the processing module

Variable Name	Description	Formulas	Unit	Source
C_wash	Concentration in a lettuce after washing	• **If *Product_wash* = 1** $C\_prod*10^{-D\_wash\_proc}$ • **Else** C_prod	CFU/g	(Pang et al. 2017)
N_wash	CFU in a unit batch after washing	C_wash*Prev_prod	CFU	(Pang et al. 2017)

N_let_fac	Number of cells transferred from lettuce to facility surfaces in a unit batch	• **If *Product_wash* = 1**
		  • **If *Product_cut* =1** N_wash*(TR_flume +TR_shred +TR_shake +TR_centri +TR_convey)
		  • **If *Product_cut* =0** N_wash*TR_flume
		• **Else** 0
			CFU	(Pang et al. 2017)

N_fac_let	Number of cells transferred from facility surfaces to lettuce in a unit batch	• **If *Product_wash* = 1** N_let_fac*TR_facility • **Else** 0	CFU	(Pang et al. 2017)
N_final	Number of cells in lettuce after processing in a unit batch	N_wash - N_let_afc + N_fac_let	CFU	(Pang et al. 2017)

Variable Name	Description	Formulas	Unit	Source
Prev_proc	Prevalence of contaminated lettuce after cross-contamination	• **If *Product_wash* = 1** • **If *Product_cut* =1** Prev_prod *TR_overall • **Else** Prev_prod	Prevalence	(Pang et al. 2017)
Size	Product size, either size of a lettuce head or size of a bag of lettuce	• ***If Product_cut = 1*** • Size_bag • ***Else*** • Size_mean	g	Authors' estimate
C_proc	Concentration of bacteria after processing in a product unit (either a lettuce head, or a bag of lettuce)	N_final/Prev_proc	CFU/g	Authors' estimate

12.3 Post-processing Module

After processing, lettuces are subjected to retail storage, consumer transport, and home storage. This part of the model is very similar to the one developed for chicken. The only difference is related to the product Size (*Size*). Growth models used for *E. coli* to calculate the growth factors of the microorganism on the selected food product during retail (*F_retail),* transport *(F_trans)* and fridge storage at home (*F_fridge)* were the same than the one used in the chicken model (van Gerwen and Zwietering 1998; Collineau et al. 2020). We assumed that when the temperature was below the minimal growth temperature, the number of bacteria remains constant. The model framework representing the model variables related the post-processing module and their

dependencies is presented in Figure 4. A detailed description of the estimated and calculated variables is available in Table 6 and 7 respectively.

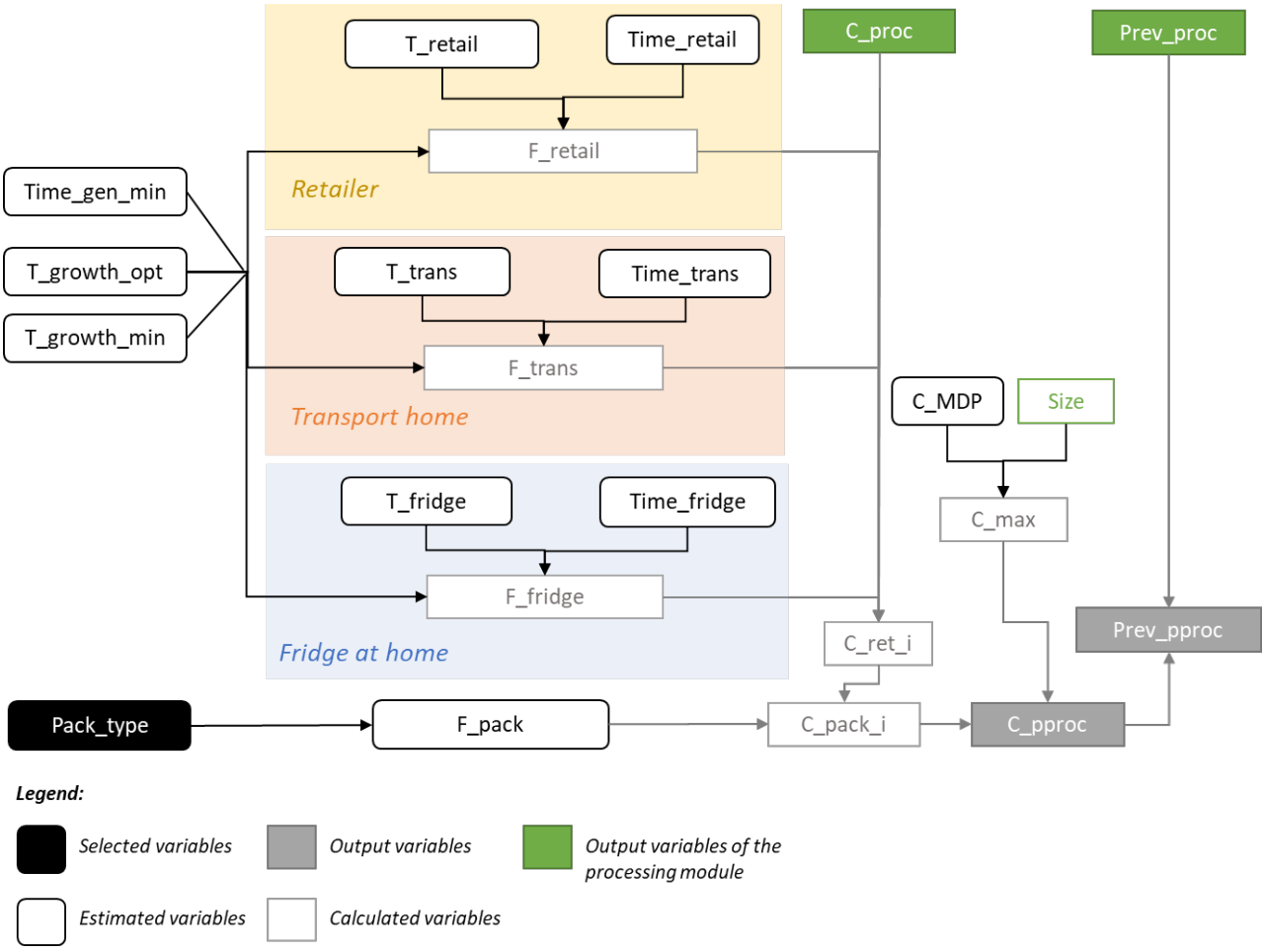


Figure 12: Model framework for the post-processing module. Each node represents a model variable.

Table 30: List of estimated variables related to the post-processing module

Domain	Variable Name	Description	Units
Other	*Time_retail*	Number of days stored at retail	days
Other	*T_retail*	Temperature at retail storage	Degree C
Other	*Time_trans*	Mean (90%CI) home transport duration	Minutes

Domain	Variable Name	Description	Units
Other	*T_post_trans*	Product temperature at the end of home transport	Degree C
Other	*T_avg_trans*	Average product temperature during home transport	Degree C
Other	*Time_fridge*	Mean (90%CI) number of days refrigerated at home	days
Other	*T_fridge*	Home refrigeration temperature	Degree C
Bacteria	*F_pack*	Reduction factor of bacterial load because of packaging	Log CFU
Bacteria	*C_MPD*	Maximum population density	CFU / g

Table 31: List of calculated variables related to the post-processing module

Variable Name	Description	Calculus	Units	Source
F_retail	Growth factor of the microorganism on the selected food product at retail	• ***If T_retail > T_growth_min*** exp(ln (2)* [(T_retail - T_growth_min)/ (T_growth_opt – T_growth_min)]^2 * Time_retail*24/ Time_gen_min) • ***Else*** *1*	CFU /g /hour	(van Gerwen and Zwietering 1998; Collineau et al. 2020)
F_trans	Growth factor of the microorganism on the	• ***If T_avg_trans > T_growth_min***	CFU /g /hour	(van Gerwen and Zwietering

Variable Name	Description	Calculus	Units	Source
	selected food product during transport	exp(ln (2)* [(T_trans - T_growth_min)/ (T_growth_opt – T_growth_min)]^2 * (Time_trans/60)/ Time_gen_min) • ***Else*** *1*		1998; Collineau et al. 2020)
F_fridge	Growth factor of the microorganism on the selected food product in the fridge of the consumer	• ***If T _fridge> T_growth_min*** exp(ln (2)* [(T_fridge - T_growth_min)/ (T_growth_opt – T_growth_min)]^2 * Time_fridge*24/ Time_gen_min) • ***Else*** *1*	CFU /g /hour	(van Gerwen and Zwietering 1998; Collineau et al. 2020)
C_max	Maximum possible number of CFU on a food product	C_MPD x Size	CFU	-
C_ret_i	bacteria load on a contaminated product	C_proc_i*G_retail*G_trans*G_fridge	CFU	-
C_pack_i	Bacterial reduction load due to packaging	• **If *Pack_type = "MAP" & Time_retail + Time_trans+Time_fridge > 7 days***	CFU	-

Variable Name	Description	Calculus	Units	Source
		$C\_ret\_i \times 10^{D\_pack}$ • ***Else*** C_ret_i		
C_pproc_i	Adjusted bacteria load on a contaminated product	• ***If C_pack_i < 1,*** 1 • ***If C_pack_i > C_max*** C_max/Size • ***Else*** C_pack_i/Size	CFU/g	-
Prev_pproc_i	Prevalence of contaminated products after consumer storage	Prev_proc_i x (1 - Poisson (0, C_pproc_i))	Prevalence	

12.4 Home preparation Module

During home preparation, ingestion of antimicrobial resistant bacteria can occur via two The main difference with the model developed for chicken meat is that lettuce was assumed to be always served raw, whereas chicken was always consumed cooked. It was thus assumed that only direct contamination via ingestion of contaminated product was relevant for the lettuce value chain. Washing the lettuce during home preparation may minimise final serving contamination but this effect is still poorly understood. The concentration of bacteria was assumed to depend on the bacteria load in the food product (*C_pproc_i*), the serving size *(Serv*), and a reduction factor (*D_wash_home*).

The adjustments of contamination prevalence and load described after retail were applied again after home preparation to ensure that only doses greater than or equal to 1 CFU were considered in the risk characterization. Finally, the probabilities of exposure to a positive serving

(i.e., meal with antimicrobial resistance bacteria) via direct contamination only was assessed *(P_exp_cool_i*).

The model framework representing the model variables related to the home preparation module and their dependencies is presented in Figure 5. A detailed description of the estimated and calculated variables is available in Table 8 and 9 respectively.

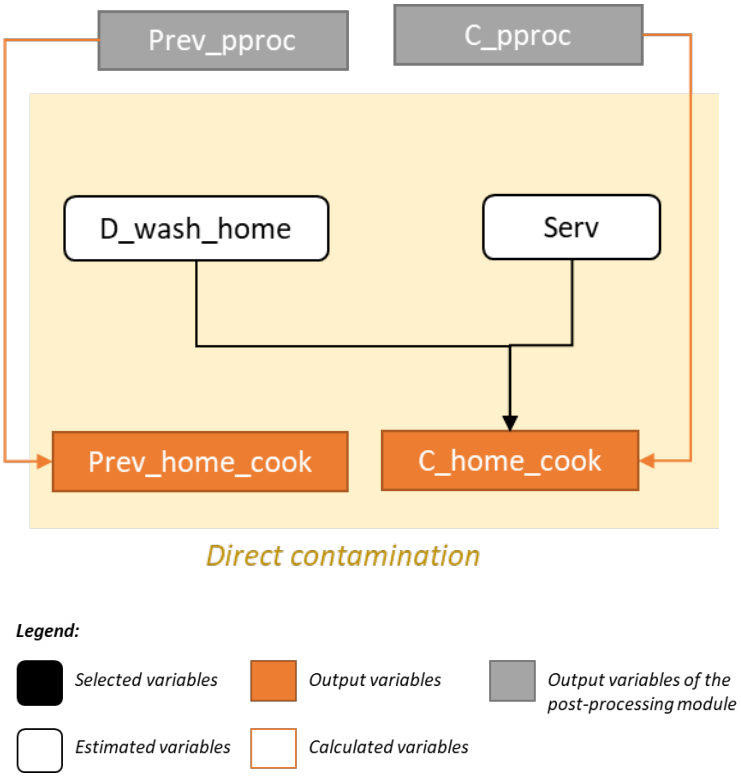


Figure 13: Model framework for the home preparation module. Each node represents a model variable.

Table 32: List of estimated variables related to the home preparation module

Domain	Variable Name	Description	Units
Other	*Serv*	Serving size	g
Bacteria	*D_wash_home*	Log reduction by washing at home with water	Log CFU /g

Table 33: List of calculated variables related to the home-preparation module

Variable Name	Description	Calculus	Units
Prev_home_cook	Prevalence of contaminated servings after product preparation	Prev_pproc	Probability
C_home_cook	Concentration of bacteria on one portion post product preparation	C_pproc*10– D_wash_home	CFU/g
N_home_cook	Number of of bacteria on one portion post product preparation	*C_home_cook* *Serv	CFU/serving

12.5 References

Collineau, Lucie, Brennan Chapman, Xu Bao, Branavan Sivapathasundaram, Carolee A. Carson, Aamir Fazil, Richard J. Reid-Smith, and Ben A. Smith. 2020. “A Farm-to-Fork Quantitative Risk Assessment Model for Salmonella Heidelberg Resistant to Third-Generation Cephalosporins in Broiler Chickens in Canada.” *International Journal of Food Microbiology* 330 (October): 108559. https://doi.org/10.1016/j.ijfoodmicro.2020.108559.

“Fresh Produce: Agency Advice on Re-Washing Ready to Eat Leafy Salads.” 2008. https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/committee/acm891revised.pdf.

Gerwen, S. J. van, and M. H. Zwietering. 1998. “Growth and Inactivation Models to Be Used in Quantitative Risk Assessments.” *Journal of Food Protection* 61 (11): 1541–49. https://doi.org/10.4315/0362-028x-61.11.1541.

Gil, Maria I., Maria V. Selma, Trevor Suslow, Liesbeth Jacxsens, Mieke Uyttendaele, and Ana Allende. 2015. “Pre- and Postharvest Preventive Measures and Intervention Strategies to Control Microbial Food Safety Hazards of Fresh Leafy Vegetables.” *Critical Reviews in Food Science and Nutrition* 55 (4): 453–68. https://doi.org/10.1080/10408398.2012.657808.

Njage, P.M.K., and E.M. Buys. 2017. “Quantitative Assessment of Human Exposure to Extended Spectrum and AmpC β-Lactamases Bearing E. Coli in Lettuce Attributable to Irrigation Water and Subsequent Horizontal Gene Transfer.” *International Journal of Food Microbiology* 240 (January): 141–51. https://doi.org/10.1016/j.ijfoodmicro.2016.10.011.

Pang, Hao, Elisabetta Lambertini, Robert L. Buchanan, Donald W. Schaffner, and Abani K. Pradhan. 2017. “Quantitative Microbial Risk Assessment for Escherichia Coli O157:H7 in Fresh-Cut Lettuce.” *Journal of Food Protection* 80 (2): 302–11. https://doi.org/10.4315/0362-028X.JFP-16-246.

Terry, Dr Leon A, Carlos Mena, Dr Adrian Williams, and Mr Nigel Jenney. 2011. “Fruit and Vegetable Resource Maps. Mapping Fruit and Vegetable Waste through the Retail and Wholesale Supply Chain.” WRAP.

13. Appendix 4: Case study 1 - *E. coli* carrying mutated CMY-2 gene in fresh portioned skin off chicken

13.1 Introduction

Inputs data for the model included quantitative information on the case study gathered through existing literature using PubMed and Google Scholar. The literature review focused on the most recent studies performed in Europe and, when available, in the UK. However, when no data were available other publications on research studies performed in other regions have been considered. The literature review strategy was based on inclusive criteria related to the case study and therefore the search terms consistently included "*E. coli*", and "*CMY-2* gene", among others.

Despite the initial bacteria contamination load in the production module was specific to *E. coli* carrying ampC beta-lactamase gene *CMY-2,* the lack of genotypic data was evident in other modules (i.e., Processing module). Data on AMR genes seem to be not routinely collected in studies and reports aiming at evaluating the bacterial load increase or reduction during processing steps in the slaughterhouses or in other sections of the production chain.

Because of the lack of data and evidence related to a different behaviour of *E. coli* carrying ampC beta-lactamase gene *CMY-2* compared to *E. coli* not carrying this resistance gene, our model assumes that AMR-gene-carrying bacteria have the same characteristics as bacteria not carrying AMR-gene. For example, the growth rate was estimated based on current knowledge of *E. coli* growth without considering possible changes due to the presence of the carrying ampC beta-lactamase gene *CMY-2.* It is plausible to assume that a bacterium with or without the AMR gene should be affected similarly by those processing practices which have a direct impact on the life of the pathogen. If this is not the case and new evidence is generated, the model can be updated by modifying the distributions of the parameter (something to be done in any

case depending on the hazard selected and the specific risk question). This assumption might however result in increased uncertainty in model outputs. Generally, a conservative approach was used to inform the model. Decisions regarding model inputs for which data were sparse or inconsistent between studies erred on the side of selecting either probability distributions built using most likely values (i.e., BetaPert, Triangular) or a range of possible values (i.e., Uniform) or selecting inputs that could provide a worst-case scenario (i.e., number of bacteria on negative birds' exteriors after transport - *C_prod_n*) (higher risk of exposure).

The values of the *selected variables* used in this case study are reported below:

- Product = Chicken
- Bacteria = *E. coli*
- Gene = ampC beta-lactamase gene *CMY-2*
- Pack_type = No packaging
- Farm_type = conventional
- Product_cut = portion
- Meat_skin = skin off
- Scalding type = soft

In the following sections, the inputs parameters for the different modules are shown in relation to the sources. Additional references can be found in the Appendix 1.

13.2 Estimated variables for Production module

Table 1 shows the list of the estimated input parameters for the Production module and the associated probability distributions, values and references. The parameters refer to flock characteristics and to transport to slaughterhouse. A number of the parameters describing the flock characteristics were not parameterized due to lack of specific data or because they were not relevant for *E. coli* but could be relevant for other hazards (i.e., *Campylobacter spp*) and therefore, for this specific case study, were set to 1 (no effect) or 0 depending on the parameter.

The factor representing the impact of antimicrobial usage on between-flock prevalence of AMR bacteria (*F_AMU*) in conventional farms was set to 1 (baseline) because no specific data were found to parametrize the level of correlation between AM usage and prevalence or resistant *E. coli*. This parameter can be adjusted if specific studies would generate the required data for UK. Simoneit et al, 2015 performed a literature review to assess the correlation between oral administration of antimicrobials and antimicrobial resistance in *E. coli* from chicken. Only seven papers were eventually selected, some of which rather old (1983, 1993) and with various level of quality. The authors concluded that the searched papers provided indications of positive association between AMU and AMR but could not be proved with advanced statistical methods. However, they acknowledged that the studies varied importantly concerning antimicrobial groups, dosage used, duration of treatment, resistance measurement and observation of effects and therefore they stressed the limitations of the study due to the inhomogeneity of the study designs.

In the UK, the latest results showed a decreasing trend (-70%) of AmpC-producing *E. coli* in broilers from 2016 to 2018 (EFSA/ECDC 2020). Data collected and published in early 2016 by the British Poultry Council also demonstrates that the industry has reduced overall antimicrobial use by 44% between 2012 and 2015, and that use of fluoroquinolones by the poultry industry was significantly reduced by 48% in 2015 compared with 2014. Similar decreasing trends situations were found in the Netherlands (RIVM 2017) and other EU countries. With regards to organic production, the literature review confirmed that organic farms do not use substantial amount of antimicrobials in the UK. There may be some situation/derogation where AM are being used to address specific health situation but in that case, antimicrobials are only given if absolutely necessary. The total amount given for a single farm should be minimal with an indiscernible effect on AMR1 prevalence.

The implementation of appropriate biosecurity measures is a key strategy to reduce the general use of antibiotics at farm level (FAO 2019) and also to reduce the burden of resistant *E. coli* in farms (Furtula et al. 2010). However, there are large gaps in the understanding of the most important risk factors and the most effective interventions.

Many different biosecurity measures can influence, with varying degree of effectiveness, the prevalence of *E. coli* and AMR level and therefore the integration of the different effects of different biosecurity measures in a unique parameter would not be adequate. For the same reasons and the lack of clearly defined categories (i.e., poor, medium, high) describing the implementation of specific biosecurity measures, both the proportion of farms with poor biosecurity (*Prop_biosecurity*) and the factor representing the impact of poor biosecurity on contamination load (*F_biosecurity)* were not parameterized in this model for both conventional and organic farms. These are important parameters and further efforts should be dedicated to define correct measures of the effect of relevant biosecurity measures.

In the UK, thinning is a common practice. Independent processors may organize multiple depopulation cycles before finally emptying a shed. These practices have shown to be potentially risky resulting in the spreading of bacteria in poultry population. No published papers were found on the microbial risk on *E. coli* and thinning in European settings that could help to parametrize the factor representing the impact of thinning on contamination load (*F_thinning*). According to the findings from the studies reviewed (Lindblad et al. 2006), there is no clear seasonality effect (*F_season*) on the risk of *E. coli* contamination on chicken carcasses. In addition, no effect of seasonality on resistant *E. coli* presence were found (Romero-Barrios et al. 2020).

The duration of the production value chain and the age of bird (*F_age*) at slaughter could be considered risk factors for the presence of AmpC-positive *E. coli*. However, age become less relevant when broilers reach the age of slaughter with the majority of birds positive. A study conducted by Dierikx et al (2013) showed that AmpC producing *E. coli* was found at all levels in the broiler production pyramid and that the prevalence of AmpC-positive broilers in the farm increased within the first week from 0-24% to 96-100% and remained 100% until slaughter (independent of the use of antibiotics) (Dierikx et al. 2013). Similar results were found in the Netherlands (Huijbers et al. 2014) and in Germany (Laube et al. 2013). We assumed this applied to both conventional and organic farming systems.

Since no data were found specifically relevant to the case study, the data to parametrize the concentration in the barn environment of positive flocks (*C_barn*) was modelled using data related to ESBL-producing *Escherichia coli* (Blaak et al. 2015).

During transport to the slaughterhouse, broilers can shed *E. coli* as well as other bacteria through excreta in the crates. Broiler litter is a source of multiple antibiotic-resistant *E. coli* and therefore, it should be considered as a significant reservoir (Furtula et al. 2010). Because no *E. coli* specific data were found to model the impact of transport of positive flocks on contamination load (*F_transport*), we estimated the point value from Collineau et al. (2020).

While we could not find data for modelling the number of *E. coli* on negative birds exterior after transport (*C_prod_n*), we used a triangular distribution representing a worst case scenario because it includes both positive and negative birds (Berrang and Northcutt 2005). It is a conservative approach since it is probably overestimating the risk of *E. coli* contamination.

Table 1: List of estimated variables related to the production module

Variable Name	Description	Value/Distribution	Units	Source
Prev_Farm_type	Between Flock prevalence of AMR1	• **If Farm_type = Conventional** Beta (26, 164) • **If Farm_type = Organic** Uniform (0.05-0.9)	Prevalence	(Parker, C Daniel 2020; Huijbers et al. 2014)
F_AMU	Factor representing the impact of antimicrobial usage on between-flock prevalence of AMR1	None		NA
F_biosecurity	Factor representing the impact of poor biosecurity on contamination prevalence	None		NA
Prop_biosecurity	Proportion of farms with poor biosecurity practices	0	Proportion	NA
F_thinning	Factor representing the impact of thinning on contamination prevalence	None		NA
Prop_thinning	Proportion of farms implementing thinning	0	Proportion	NA
F_season	Factor representing the impact of high-risk season on contamination prevalence. “High risk	None	-	(Lindblad et al. 2006)

Variable Name	Description	Value/Distribution	Units	Source
	season" must be defined for each microorganism.			
Prop_season	Proportion of birds slaughtered during the high-risk season. "high risk season" must be defined for each microorganism.	0	Proportion	NA
F_age	Factor representing the impact of age on contamination prevalence	None	-	No effect of age after 15 days (Dierikx et al. 2013; Huijbers et al. 2014; Laube et al. 2013)
Prop_age	Proportion of birds slaughtered late. "Late" must be defined for each microorganism.	0	Proportion	
Prev_wfp_col_base	Average prevalence of birds from positive flock internally colonized at pre-harvest	BetaPert (0.12, 0.42, 0.89)	Prevalence	(Ewers et al. 2012)
Amount_fec	Amount of faeces on bird exterior at pre-harvest in positive flocks	Triangular (1, 10, 50)	g	(Collineau et al. 2020)
C_barn	Concentration in the barn environment of positive flocks	Pert(1.8×10^{7}, $1.1*10^{9}$, 5.4×10^{9})	CFU/g of faeces	(Blaak et al. 2015)

Variable Name	Description	Value/Distribution	Units	Source
F_transp	Factor representing the impact of transport of positive flocks on contamination load	1.41		(Collineau et al. 2020)
N_transp	Number of flocks transported prior to the current flock	Uniform (0, 4)	Flocks	(O. Bucher et al. 2012a)
F_cross_trans	Factor representing the probability of carryover contamination from a positive flock transported prior to the current flock.	Uniform (0, 0.5)	-	(O. Bucher et al. 2012a)
N_contact	Number of contacts with contaminated birds during transport	Pert (1.5, 3, 4.5)	Count	(O. Bucher et al. 2012a)
C_prod_n	Number of bacteria on negative birds after transport due to cross contamination during transport	Triangular (4.4, 4.6, 4.8)	CFU/bird	(Berrang and Northcutt 2005)

13.3 Estimated variables for the Processing module

Table 2 shows the list of the estimated input parameters for the Processing module and the associated probability distributions, values and references. The parameters are grouped according to the different steps in the processing chain. The literature review highlighted data gaps or inconsistent results between studies on resistant *E. coli*. Some inconclusive or inconsistent results of studies regarding the main risk factors associated with the fluctuation of the *E. coli* concentration in the processing steps (Barco et al. 2014) could be due to the particular characteristics of these steps in the slaughterhouses and the implementation of the risk management practices (Pacholewicz et al. 2016). Major gaps in literature in this module hampered the selection of probability distributions of cross contamination of resistant *E. coli* in specific steps of processing. The probability of cross-contamination to occur during specific steps was based on authors estimate and (Collineau et al. 2020) if not specified differently in the table.

For the distributions of the probability that a single cell resides on portion cap (*P_skin*) and the proportion of cells transmitted from portion cap to meat (*Prop_cm*) we used data from (Nauta, Jacobs-Reitsma, and Havelaar 2007). This paper is focused on *Campylobacter spp.* and the data used refer to the probability for each *Campylobacter spp.* on the carcass to reside on the breast cap. Same unit (breast) applies here with the assumption that one chicken breast equal a quarter of whole carcass chicken (*Prop_product* = 0.25).

Table 2: List of estimated variables related to the processing module

Variable Name	Description	Value/Distribution	Units	Source
F_scald_soft	Proportion of cells remaining after soft scalding	Normal(4.16, 1.08)	log 10 CFU/ml	(Althaus, Zweifel, and Stephan 2017) (Cason, Hinton, and Ingram 2000)
F_scald_hard	Proportion of cells remaining after hard scalding	Normal(0.8, 0.4)	log 10 CFU/ml	(Althaus, Zweifel, and Stephan 2017) (Cason, Hinton, and Ingram 2000)
F_df	Reduction factor of bacterial load on carcass during defeathering	Triangular (- 1.60, -1.11, -0.62)	logCFU	(Belluco et al. 2016)
P_cross_df_p	Probability of cross-contamination to occur during defeathering for birds from positive flocks	0.5	Probability	Authors estimate
P_cross_df_n	Probability of cross-contamination to occur during defeathering for birds from negative flocks	Normal(0.02, 0.000557)	Probability	(Collineau et al. 2020)

Variable Name	Description	Value/Distribution	Units	Source
P_cut_vis	Probability that viscera are lacerated during evisceration	Triangular (0.14, 0.18, 0.23)	Probability	(Collineau et al. 2020)
spill_weight	Caecal content spilled in case of viscera rupture	Uniform (1,10)	g	(Collineau et al. 2020)
P_cross_ev_p	Probability of cross-contamination to occur during evisceration for positive flocks	Triangular (0.03, 0.07, 0.15)	Probability	(Collineau et al. 2020)
F_ev	Percentage of additive bacteria load increase due to cross-contamination between carcasses during evisceration	Triangular (0.06, 0.091, 0.12)	Percentage change in bacterial load	(Belluco et al. 2016)
C_caecal	Mean (90%CI) bacteria concentration in caecal content	Triangular (0.54, 8.69, 8.83)	CFU/g	(Robé et al. 2019)
F_wash	Load reduction factor after water washing (only water used in UK)	Pert (-0.96; - 0.62; - 0.28)	Log CFU	(Belluco et al. 2016)
F_Wash_adj	Dampening factor for each successive wash	Uniform (-0, -0.5)	-	(Collineau et al. 2020)

Variable Name	Description	Value/Distribution	Units	Source
F_chill	Load reduction factor after air pre-chilling (only washing technique used in UK)	-0.126	Percentage	(Buess et al. 2019)
P_skin	Probability that a single cell resides on portion cap	Beta (1, 3.15)	Probability	(Nauta, Jacobs-Reitsma, and Havelaar 2007)
Prop_cm	Proportion of cells transmitted from portion cap to meat	Pert (0.01, 0.02, 1)	Proportion	(Nauta, Jacobs-Reitsma, and Havelaar 2007)
Prop_product	What fraction of the raw product represents the final processed product?	0.25	Proportion	Authors estimate

13.4 Estimated variables for the post-Processing module

Growth models used for *E. coli* on chicken meat based on (van Gerwen and Zwietering 1998; Collineau et al. 2020) were used to calculate G*_retail, G_trans* and *G_fridge*. The equations used are presented in Table 3. We assumed that when the temperature was below the minimal growth temperature, the number of bacteria remains constant. In this case, F*_retail, F_trans* and *F_fridge* equalled 1.

Table 4 shows the list of the estimated input parameters for the post-Processing module and the associated probability distributions, values and references. The minimum generation time in food product (*Time_gen_min*) and the maximum population density (*C_MPD*) were based on the outputs from an exposure assessment of ESBL producing *Escherichia coli* through meat consumption of different type of meat (Evers et al. 2017). The maximum population density referred to ground beef.

Table 3: growth models used for E. coli on chicken meat

Variable Name	Description	Formula	Source
F_retail	Growth factor at retail	• ***If T_retail > T_growth_min*** exp(ln (2)* [(T_retail -T_growth_min)/ (T_growth_opt – T_growth_min)]^2 * Time_retail*24/ Time_gen_min) • ***Else*** • *1*	(van Gerwen and Zwietering 1998; Collineau et al. 2020)
F_trans	Growth factor during transport	• ***If T_avg_trans > T_growth_min*** exp(ln (2)* [(T_trans -T_growth_min)/ (T_growth_opt – T_growth_min)]^2 * (Time_trans/60)/ Time_gen_min) • ***Else*** *1*	(van Gerwen and Zwietering 1998; Collineau et al. 2020)

Variable Name	Description	Formula	Source
F_fridge	Growth factor at home	• ***If T _fridge> T_growth_min*** exp(ln (2)* [(T_fridge -T_growth_min)/ (T_growth_opt – T_growth_min)]^2 * Time_fridge*24/ Time_gen_min) • ***Else*** *1*	(van Gerwen and Zwietering 1998; Collineau et al. 2020)

Table 4: List of estimated variables related to the post-processing module

Variable Name	Description	Value/Distribution	Units	Source
Size_mean	Breast or carcass size mean	Normal (1495.6, 303.4)	g	(Chardon and Evers 2017)
Prop_carc_yield	Carcass yield (proportion of lean meat)	Uniform (0.6, 0.65)	Proportion	(Chardon and Evers 2017)
Time_retail	Number of days stored at retail	Triangular (1,3,7)	Days	(Collineau et al. 2020)
T_retail	Temperature at retail storage	Laplace (-6.67, 3.3333 19.44)	Degree C	(EcoSure 2007)
Time_trans	Mean (90%CI) home transport duration	Normal(69.6, 0.438)	Minutes	(Collineau et al. 2020)
T_post_trans	Chicken temperature at the end of home transport	Shifted Loglogistic Truncate (29.371, 16.763, -22.915, min = - 5.56, max = 20)	Degree C	(EcoSure 2007)
Time_fridge	Mean (90%CI) number of days refrigerated at home	Normal(2.2, 0.0203)	days	(Collineau et al. 2020)
T_fridge	Home refrigeration temperature	Laplace (-4.44, 5.3, 16.11)	Degree C	(Biglia et al. 2018; Evans and Redmond 2016; EcoSure 2007)

Variable Name	Description	Value/Distribution	Units	Source
T_growth_min	Minimum growth temperature	7	Degree C	(Food Standard Agency 2018)
T_growth_opt	Optimal growth temperature	Pert (35, 37, 40)	Degree C	(Food Standard Agency 2018)
F_pack	Reduction factor of bacterial load because of packaging	Triangular (-0.1, −0.2, −0.3)	logCFU	(Thomas et al. 2020)
Time_gen_min	Minimum generation time in food product	0.47	hours	(Evers et al. 2017)
C_MPD	Maximum population density	1.23E+05	CFU / g	(Evers et al. 2017)

13.5 Estimated variables for the Home-preparation module

Table 5 shows the list of the estimated input parameters for the Home-preparation module and the associated probability distributions, values and references. Considering the lack of specific data related to exposure temperature of E. coli, the probability distributions of the variables *Time_protec, T_protec, R_ref* were obtained from (FAO/WHO 2002), a risk assessment of Salmonella in eggs and broiler chicken. We believed that the similarity in the ideal temperature growth for *Salmonella spp.* and *E. coli* makes this assumption plausible.

Table 5: List of estimated variables related to the home preparation module

Variable Name	Description	Value/Distribution	Units	Source
P_undercook	Mean (90%CI) probability of undercooking to occur	Normal(0.40, 0.00212)	probability	(Collineau et al. 2020)
Time_protec	Exposure time at exposure temperature in the protected area	Pert(0.5, 1, 1.5)	Minutes	(FAO/WHO 2002)
T_protec	Exposure temperature during cooking in the protected area	Pert(60, 64, 65)	Degree C	(FAO/WHO 2002)
P_h_wash	Probability of cross-contamination to occur (related to kitchen hygiene,	Uniform(0.38, 1)	Probability	(Collineau et al. 2020; Bruhn 2014)

Variable Name	Description	Value/Distribution	Units	Source
	represent the probability of not washing hands during chicken food preparation)			
V_dil_carc	Volume of fluid diluting for a whole carcass	Uniform(150, 250)	ml	(Collineau et al. 2020; WHO and FAO 2009)
V_ing	Volume of fluid ingested	Uniform(0.5, 1.5)	ml	(Collineau et al. 2020; WHO and FAO 2009)
Prop_protec	Proportion of cells in the “protected area”	Pert (0.1, 0.16, 0.2)	Proportion	(FAO/WHO 2002)
Prop_loose	Proportion of cells loosely attached	Uniform(0.01, 0.1)	Proportion	(Collineau et al. 2020; WHO and FAO 2009)

13.6 Results

13.6.1 Risk of exposure

13.6.1.1 Overall risk

The overall risk of AMR exposure was estimated considering positive and negative flocks combined. The overall risk thus represents the average prevalence, and level of contamination, of contaminated serving given the estimated proportion of positive and negative flocks in the overall population. The Figure 1 presents the probability density functions associated with each outcome variables considered in the modelling framework (see list and definition of outcome variables in Table 2). Table 6 presents more specifically the numeric values of the mean and median of each outcome variables.

These results shown an overall decrease of the prevalence of contaminated products and level of contamination per contaminated product throughout the value chain. The median prevalence of contaminated serving after cooking equalled 5%. The probability of exposure through cross contamination was lower and equalled 0.00063%. The median number of AMR-gene-carrying bacteria per contaminated products was always low and below 3 CFU/item of interest. However, all the outcome variables presented highly skewed probability distributions. As an example, the CFU of AMR-gene-carrying bacteria /contaminated bird arriving at the slaughterhouse (C_prod) varied from 4.4 to 3.06 E+08 CFU/carcass with a median value of 4.62 CFU/carcass. Four of the outcome variables (i.e., Prev_prod, Prev_proc, Prev_pproc, Prev_home_cook) also shown two distinct peaks, which can be explained by the two different population considered together in these figures: the positive and negative flocks. The risk depending on the flock origin is discussed in the next chapter.

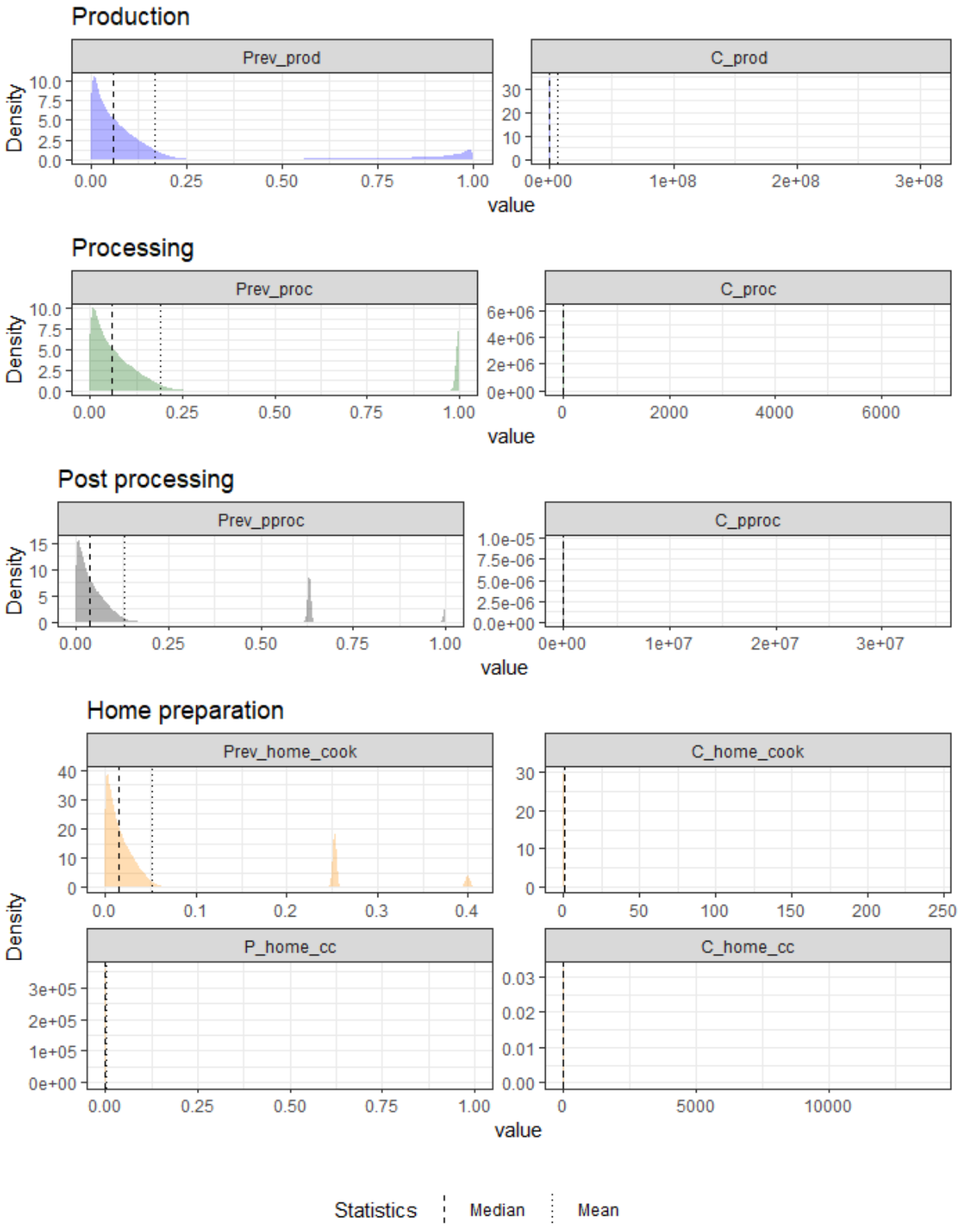


Figure 1: Probability distributions of the outcome variables associated with the production, processing, and post-processing modules after 100 000 simulations for both, the positive and negative flocks.

Table 6: Mean and median overall risk estimation per module

Module	Output variables	Unit	Mean	Median
Production	*Prev_prod*	Prevalence of birds contaminated with AMR-gene-carrying bacteria arriving at the slaughterhouse	0.17	0.06
Production	*C_prod*	CFU of AMR-gene-carrying bacteria / bird arriving at the slaughterhouse	6.4E+06	4.6
Processing	*Prev_proc*	Prevalence of carcasses contaminated with AMR-gene-carrying bacteria	0.19	0.06
Processing	*C_proc*	CFU of AMR-gene-carrying bacteria / carcasses	6.6	0.0
Post-processing	*Prev_pproc*	Prevalence of food item contaminated with AMR-gene-carrying bacteria	0.13	0.04
Post-processing	*C_pproc*	CFU of AMR-gene-carrying bacteria / food item	3.9E+03	1.0
Home preparation	*Prev_home_cook*	Prevalence of serving contaminated with AMR-gene-carrying bacteria after cooking	0.05	0.02
Home preparation	*C_home_cook*	CFU of AMR-gene-carrying bacteria ingested by food item	0.17	0.12
Home preparation	*P_home_cc*	Probability of exposure to AMR-gene-carrying bacteria through cross contamination	0.00	0.00

Module	Output variables	Unit	Mean	Median
Home preparation	*C_home_cc*	CFU of AMR-gene-carrying bacteria ingested by cross contaminated food item	2.27	1.0

13.6.1.2 Risk depending on the flock origin

The risk of AMR exposure was estimated separately for positive and negative flocks as these populations present very different baseline values in terms of within-flock prevalence of contamination, and contamination load per bird. The proportion of positive vs negative flocks was defined by the between flock prevalence estimated for conventional farms (*Prev_Farm_type)*: 13 703 (13.7%) and 86 297 (86.3%) positives and negative flocks were included in the analysis, respectively.

For the negative flocks, the median within flock prevalence of contaminated birds at the end of the production module was below 5% (Figure 2) while this prevalence was above 90% for the positive flocks (Figure 3). The prevalence of contaminated birds remained stable during the processing module for birds coming from negative flocks when this prevalence reached 100% (median) in positive flocks. This result is consistent with the fact that between flock cross contamination during processing was assumed to be low because in high throughput slaughterhouse the management practices in place reduce the risk of cross contamination.

In both cases, the prevalence of carcasses contaminated with AMR-gene-carrying bacteria drops after the post-processing module (i.e., median *Prev_pproc_n* = 3%, *Prev_pproc_p* = 63%). This result can be explained by the fact that adjustments made to prevent products with very low contamination levels from being carried forward to the consumer stage of the model were only implemented in the post-processing and home-preparation modules. Carcasses with very low contaminated levels were thus counted as contaminated carcasses in the production and processing modules, which may have overestimated the prevalence of contaminated carcasses in these modules.

The median prevalence of contaminated serving after cooking equalled 1.2% when the meat came from a negative flock, and 25.2% when the meat came from a positive flock. The median load of AMR-gene-carrying bacteria of this contaminated

serving was however low and equalled 0.0026 and 0.0029 CFU/piece of meat coming from negative or positive flocks respectively. The median probability of exposure through cross contamination was below 0.01% for both flocks. The median level of contamination with AMR-gene-carrying bacteria in case of exposure was also low and did not exceed 1 CFU/item of interest.

Figure 4 provides a more detailed overview of changes in mean and median within flock prevalence across the value chain in the two types of flocks. This figure shows that negative flocks are mostly contaminated by positive flocks during the transport to slaughterhouse: the within flock prevalence of contaminated birds remains stable during the processing module and drop only at the end of the post-processing module because of the adjustments made on low bacterial load as previously explained. Between flock cross contamination occurring during the processing module did not change the mean or median within flock prevalence. Cross contamination within flock occurring during scalding however increases the median within flock prevalence of positive flocks from 91% to 100%.

Similarly, Figure 5 and Figure 6 show the effect of scalding, washing, chilling and cutting and skin removal on the reduction of bacterial contamination. Defeathering and evisceration however tend to increase bacterial load due to cross contamination and/or contamination by faecal content leakage. The post-processing module also contribute to the increase in bacterial load because of temperature storage and transport above the minimum growth temperature of *E. coli* (i.e., 7°C).

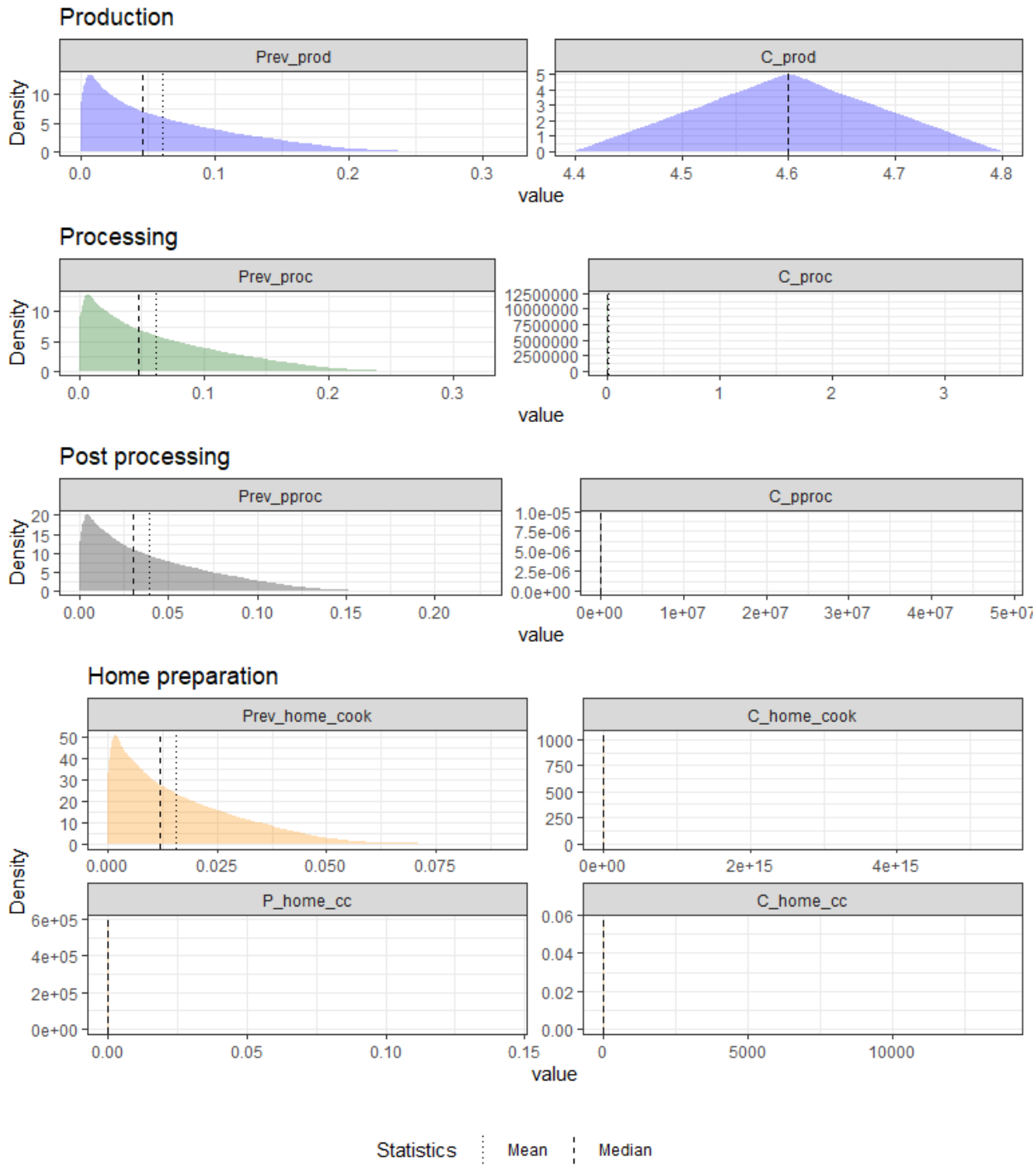


Figure 2: Probability distributions of the outcome variables associated with each module for birds coming from negative flocks after 100 000 simulations.

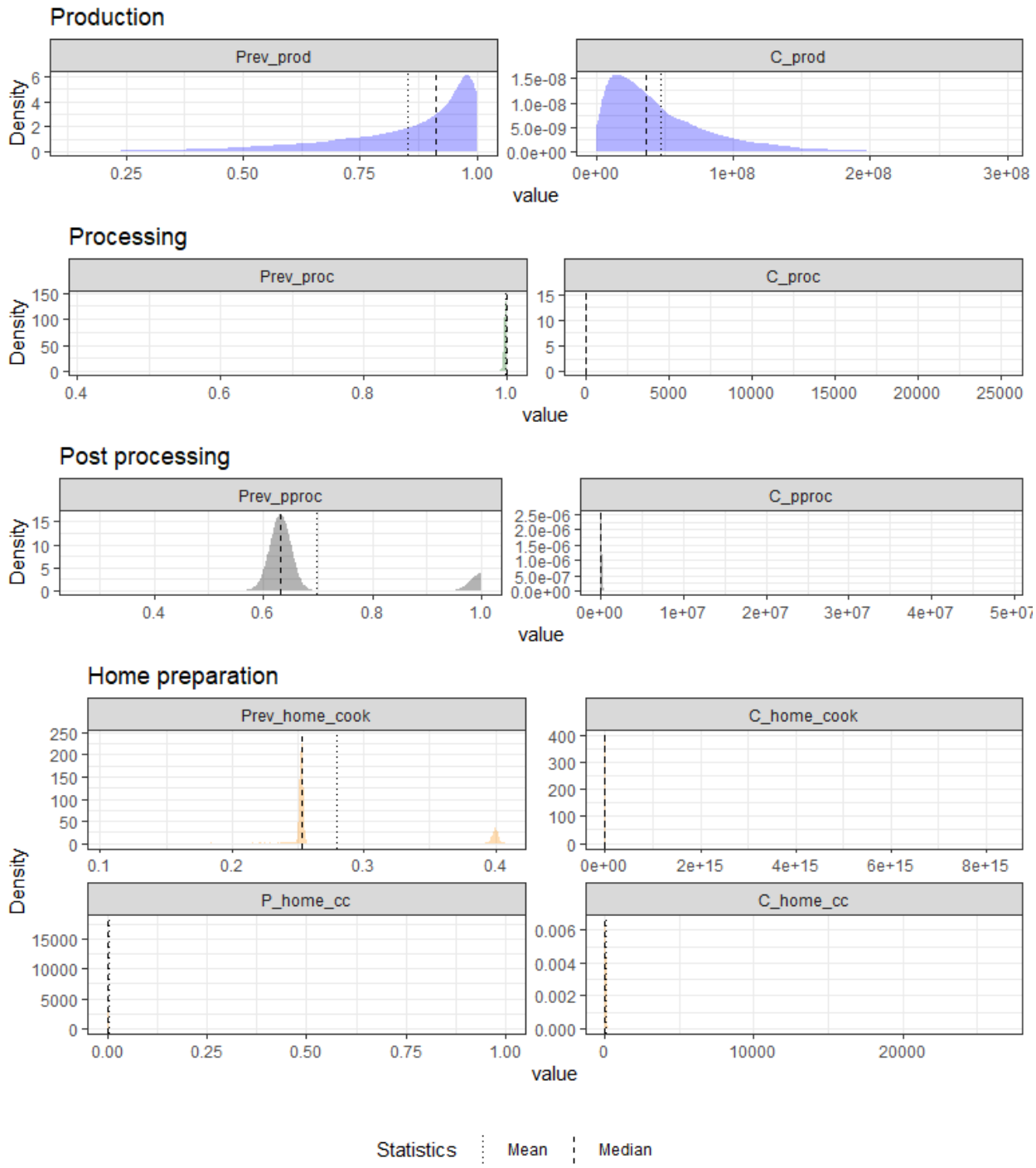


Figure 3: Probability distributions of the outcome variables associated with each module for birds coming from positive flocks after 100 000 simulations.

Figure 4: Mean and median within flock prevalence of contaminated item of interest from the production to the post-processing module

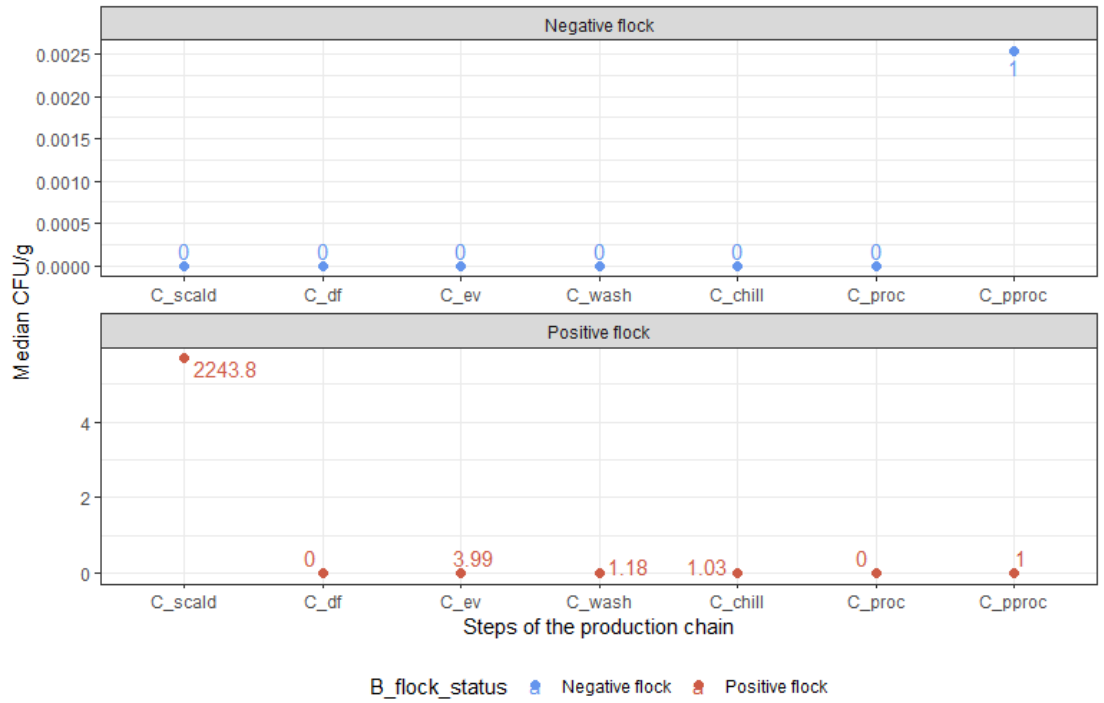


Figure 5: Median levels of bacterial contamination per gramme of contaminated item of interest from the production to the post-processing module

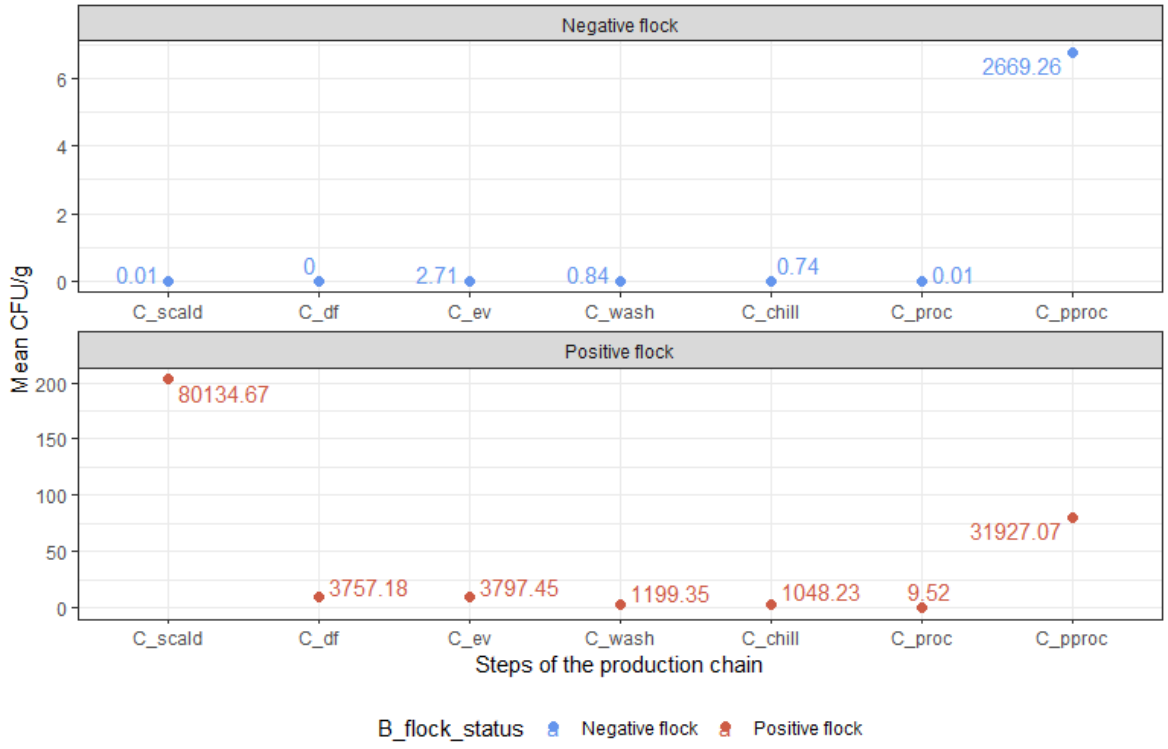


Figure 6: Mean levels of bacterial contamination per gramme of contaminated item of interest during the production module

13.6.2 Correlation analysis

The results of the correlation analysis performed for each outcome variables are presented in the Figure 7. Only estimated variables with spearman correlation coefficient ≥ |0.025| have been included in the figure. The estimated variables with a lower Spearman correlation coefficient were considered has having a negligible effect on the model outcomes.

Prevalence of contamination

All within flock prevalence estimations were highly influenced by three estimated variables (i.e., Spearman correlation coefficient > 0.6): the between farm prevalence (*Prev_farm_type),* the number of flocks transported before the current flock (*N_transp),* and the term for dampening the probability of carryover contamination from a positive flock transported prior to the current flock (*R_damp)*. The prevalence of contaminated product after cooking was also highly influence by the probability of undercooking (*P_undercook*) while the probability of exposure via cross-contamination was influenced by the kitchen hygiene (*P_h_wash*), the size of the meat (*Size_mean*), the proportion of bacteria in fluid extracted from this piece of meat (*Prop_fluid*), and the volume of contaminated fluid ingested by the consumer (*V_ing*).

The estimated variables with a large influence on the model outcome related to the prevalence of contaminated product are briefly discussed here.

The between farm prevalence (*Prev_farm_type*) was parameterized differently according to the production type (conventional vs organic). As a first step in the risk pathway and the conditional relationship between subsequent steps in the pathway its influence over the output model was expected. With regards the conventional production, the literature review provided indications on between and, above all, within farm prevalence from various EU countries showing a remarkable variability between studies and reports. Studies implemented in different European countries have shown an average proportion of about 42% (Minimum 12.2%, Maximum 89%) of *CMY-2* in ESBL/AmpC *E. coli* positive samples (Ewers et al. 2012). In the UK, data on AmpC-producing *E. coli* is being regularly collected in broilers at the slaughterhouse and retail level as part of the European monitoring program. The latest results showed a prevalence of 6.1 % of AmpC-producing *E. coli* in broilers, with a decreasing trend (-70%) from 2016 to 2018 (EFSA/ECDC 2020). Parker (2020) in 2019, reported that 25% (47/188) of sheds and 21.6% (79/365) of individual samples were confirmed as positive for ESBL/AmpC by PCR in the UK. The latter was used to build the probability distribution. Dierikx et al. (2013) in the Netherlands reported that the prevalence of ESBL/AmpC positive birds at broiler farms increased within the first week from 0–24% to 96–100% independent of the use of antibiotics and stayed 100% until slaughter. With regards the organic

production, the amount of information related to between farm prevalence were very limited. ESBL/AmpC-producing *E. coli* were detected in broilers on all 8 organic farms in the Netherlands, with a prevalence of ≥80% at the sample-level (Huijbers et al. 2014).

Due to the lack of specific data on ampC beta-lactamase gene *CMY-2* for these factors, the number of flocks transported before the current flock (*N_transp*), and the related term for dampening the probability of carry-over contamination from a positive flock transported prior to the current flock (*R_damp*) were estimated from Bucher et al 2012 (in Collineau et al. 2020) that evaluated the effectiveness of various interventions for Salmonella in broiler chicken, from grow-out farm to secondary processing in Canada. The level of uncertainty is therefore not negligible due to the different hazard and differences in production environment. It is difficult to anticipate whether these two parameters will hold the same strong effect on the outcome when based on more specific EU or UK centred data when available.

During home preparation, the probability of cross-contamination occurrence *(P_h_wash*) is related to kitchen hygiene and was modelled using the 'drip-fluid' model proposed by (WHO and FAO 2009) and adapted by Collineau et al. (Collineau et al. 2020). These same references were used in the current work to build probability distributions of the other variables *(Prop_fluid; P_h_wash; V_ing*) in the home-preparation module showing a high influence towards the outputs of the model.

Level of bacterial contamination

In terms of bacterial load contamination, many estimated variables have a large influence on the model outcome (i.e., Spearman correlation coefficient > |0.5|) and are briefly discussed here.

As mentioned earlier the number of Ampc-positive bacteria on negative birds after transport (*C_prod_n*) was modelled from Berrang et al. (2005) and represent the worst-case scenario since it includes both positive and negative birds. It is a conservative approach, and it is probably overestimating the risk of *E. coli* contamination, and therefore it was expected to have a high influential effect on the outcome.

In the reduction of meat bacterial contamination, the scalding phase is the most important processing step. This is in line with the results of Belluco et al. (2016) that reviewed the effect of the slaughtering process towards *E . coli* and *Enterobacteriaceae*. This is confirmed by the correlation analysis where the proportion of cells remaining after scalding (*Prop_scald_soft*) is strongly influencing the outputs in the processing and post-processing modules. In the post -processing module also the temperature at retail level *(T_retail*) and at home (*T_fridge*) are particularly important. These parameters were modelled, similarly to Collineau 2020, using the EcoSure (EcoSure 2007) and latest data from UK cold temperature database that gather data on cold temperature storage practices of food both in retail establishments and in UK consumer homes.

As expected, the cooking temperature and time (*Time_protect* and *T_protect* respectively) are influencing the safety of the food product and the parameters related to the “protected area” (i.e., the small internal portion of the chicken where the temperature is lowest), are all strongly influencing the output of the model. Due to lack of specific information on *E. coli*, these parameters were modelled using data from a risk assessment of *Salmonella* in Eggs and Broiler Chickens (FAO/WHO 2002).

The very large majority of the parameters were modelled as probability distributions to account for their uncertainty and variability. However few parameters used in the model were considered fixed when evidence found provided little doubt about their degree of variability (i.e., Minimum growth temperature- *T_growth_min* in the post-processing module**)** or when no information about variability and uncertainty could be found in the literature (for example, probability of cross-contamination to occur during defeathering for birds from positive flocks). Depending on the parameter and in relation to the hazard under study, it might be necessary to build probability distributions of these parameters to account for uncertainty.

Figure 7: Correlation of the model estimated variables with the outcome variables. Only estimated variables with spearman correlation coefficient ≥ |0.0.25| have been included in the tornado charts. The darker the color (either green or red), the stronger the correlation.

13.7 Discussion

At the time of writing, there is no published model investigating, at every step of the food production chain, the prevalence or level of bacterial contamination of chicken

carcasses contaminated by *E. coli* carrying the ampC beta-lactamase gene *CMY-2.* Comparing our results with existing the scientific literature is thus only indirect.

Change of prevalence of contaminated carcasses along the food chain

Our results shown that prevalence of carcass contamination remains stable or slightly increases during the processing module (see Figure 10). This results is consistent with the results obtained by Herman et al. (2003) who have shown that it is in general not possible for a slaughterhouse to avoid contamination of carcasses when status-positive animals were delivered. Supplementary contaminations can however occur during the processing module. In our study, the impact of cross contamination was mainly observed within positive flock at the scalding phase. This result is consistent with the fact that the probability of cross contamination was estimated as low in our model.

The drop in prevalence of carcasses contaminated with AMR-gene-carrying bacteria observed after the post-processing module is due to the adjustments made to prevent products with very low contamination levels from being carried forward to the consumer stage of the model. These adjustments were only implemented in the post-processing and home-preparation modules. Carcasses with very low contaminated levels were thus counted as contaminated carcasses in the production and processing modules, which may have overestimated the prevalence of contaminated carcasses in these modules.

Change of contamination load of contaminated carcasses along the food chain

Our results shown that the level of contamination decreases during the processing module 55) which is consistent with the existing literature showing that slaughterhouses play a key role in the reduction of meat bacterial contamination (Belluco et al. 2016). The scalding phase appeared as the most important processing step to decrease bacteria contamination (7*C_proc*), which is consistent with the results of Belluco et al. (2016).

Our results also indicated a slight increase in bacteria contamination during the post processing module. This is consistent with the fact that this where bacteria growth may occur if storage conditions are not appropriate. This result is supported by the

importance of storage temperature at retail (*T_retail*) and fridge temperature (*T_fridge)* on the number of bacteria at the end of the post-processing module, *C_pproc* .

As expected, the main factors affecting the bacteria load after cooking are the time and temperature of cooking of the meat protected are). This result is consistent with recommendations for consumers in terms of cooking of chicken meat.

13.8 References

Althaus, Denise, Claudio Zweifel, and Roger Stephan. 2017. “Analysis of a Poultry Slaughter Process: Influence of Process Stages on the Microbiological Contamination of Broiler Carcasses.” *Italian Journal of Food Safety* 6 (4). https://doi.org/10.4081/ijfs.2017.7097.

Ballou, Anne L., Rizwana A. Ali, Mary A. Mendoza, J. C. Ellis, Hosni M. Hassan, W. J. Croom, and Matthew D. Koci. 2016. “Development of the Chick Microbiome: How Early Exposure Influences Future Microbial Diversity.” *Frontiers in Veterinary Science* 3. https://doi.org/10.3389/fvets.2016.00002.

Barco, Lisa, Simone Belluco, Anna Roccato, and Antonia Ricci. 2014. “Escherichia Coli and Enterobacteriaceae Counts on Poultry Carcasses along the Slaughter Processing Line, Factors Influencing the Counts and Relationship between Visual Faecal Contamination of Carcasses and Counts: A Review.” *EFSA Supporting Publications* 11 (8): 636E. https://doi.org/10.2903/sp.efsa.2014.EN-636.

Belluco, S., L. Barco, A. Roccato, and A. Ricci. 2016. “Escherichia Coli and Enterobacteriaceae Counts on Poultry Carcasses along the Slaughterline: A Systematic Review and Meta-Analysis.” *Food Control* 60 (February): 269–80. https://doi.org/10.1016/j.foodcont.2015.07.033.

Berrang, M.E., and J.K. Northcutt. 2005. “Use of Water Spray and Extended Drying Time to Lower Bacterial Numbers on Soiled Flooring from Broiler Transport Coops.” *Poultry Science* 84 (11): 1797–1801. https://doi.org/10.1093/ps/84.11.1797.

Biglia, Alessandro, Andrew J. Gemmell, Helen J. Foster, and Judith A. Evans. 2018. “Temperature and Energy Performance of Domestic Cold Appliances in Households in England.” *International Journal of Refrigeration* 87 (March): 172–84. https://doi.org/10.1016/j.ijrefrig.2017.10.022.

Blaak, Hetty, Angela H. A. M. van Hoek, Raditijo A. Hamidjaja, Rozemarijn Q. J. van der Plaats, Lianne Kerkhof-de Heer, Ana Maria de Roda Husman, and Franciska M. Schets. 2015. “Distribution, Numbers, and Diversity of ESBL-Producing E. Coli in the Poultry Farm Environment.” *PLoS ONE* 10 (8). https://doi.org/10.1371/journal.pone.0135402.

Bogaard, A. E. van den. 2001. “Antibiotic Resistance of Faecal Escherichia Coli in Poultry, Poultry Farmers and Poultry Slaughterers.” *Journal of Antimicrobial Chemotherapy* 47 (6): 763–71. https://doi.org/10.1093/jac/47.6.763.

Bruhn, Christine. 2014. “Chicken Preparation in the Home: An Observational Study.” *Food Protection Trends* 34 (September): 318–30.

Bucher, O., A. Fazil, A. Rajić, A. Farrar, R. Wills, and S. A. McEWEN. 2012. “Evaluating Interventions against *Salmonella* in Broiler Chickens: Applying Synthesis Research in Support of Quantitative Exposure Assessment.” *Epidemiology and Infection* 140 (5): 925–45. https://doi.org/10.1017/S0950268811001373.

Buess, Simone, Katrin Zurfluh, Roger Stephan, and Claudia Guldimann. 2019. “Quantitative Microbiological Slaughter Process Analysis in a Large-Scale Swiss Poultry Abattoir.” *Food Control* 105 (November): 86–93. https://doi.org/10.1016/j.foodcont.2019.05.012.

Buhr, Rj, Me Berrang, and Ja Cason. 2003. “Bacterial Recovery from Breast Skin of Genetically Feathered and Featherless Broiler Carcasses Immediately Following Scalding and Picking.” *Poultry Science* 82 (10): 1641–47. https://doi.org/10.1093/ps/82.10.1641.

Cason, J. A., A. Hinton, and K. D. Ingram. 2000. “Coliform, Escherichia Coli, and Salmonellae Concentrations in a Multiple-Tank, Counterflow Poultry Scalder.”

Journal of Food Protection 63 (9): 1184–88. https://doi.org/10.4315/0362-028X-63.9.1184.

CDC. 2020. “E.Coli (Escherichia Coli).” February 2020. https://www.cdc.gov/ecoli/index.html.

Chardon, Jurgen E., and Eric G. Evers. 2017. “Improved Swift Quantitative Microbiological Risk Assessment (SQMRA) Methodology.” *Food Control* 73 (March): 1285–97. https://doi.org/10.1016/j.foodcont.2016.10.049.

Collineau, Lucie, Brennan Chapman, Xu Bao, Branavan Sivapathasundaram, Carolee A. Carson, Aamir Fazil, Richard J. Reid-Smith, and Ben A. Smith. 2020a. “A Farm-to-Fork Quantitative Risk Assessment Model for Salmonella Heidelberg Resistant to Third-Generation Cephalosporins in Broiler Chickens in Canada.” *International Journal of Food Microbiology*, February, 108559. https://doi.org/10.1016/j.ijfoodmicro.2020.108559.

———. 2020b. “A Farm-to-Fork Quantitative Risk Assessment Model for Salmonella Heidelberg Resistant to Third-Generation Cephalosporins in Broiler Chickens in Canada.” *International Journal of Food Microbiology* 330 (October): 108559. https://doi.org/10.1016/j.ijfoodmicro.2020.108559.

Deng, Hui, Hong-Bin Si, Shu-Yi Zeng, Jian Sun, Liang-Xing Fang, Run-Shi Yang, Ya-Hong Liu, and Xiao-Ping Liao. 2015. “Prevalence of Extended-Spectrum Cephalosporin-Resistant Escherichia Coli in a Farrowing Farm: ST1121 Clone Harboring IncHI2 Plasmid Contributes to the Dissemination of BlaCMY-2.” *Frontiers in Microbiology* 6 (November). https://doi.org/10.3389/fmicb.2015.01210.

Dierikx, Cindy M., Jeanet A. van der Goot, Hilde E. Smith, Arie Kant, and Dik J. Mevius. 2013. “Presence of ESBL/AmpC -Producing Escherichia Coli in the Broiler Production Pyramid: A Descriptive Study.” *PLoS ONE* 8 (11). https://doi.org/10.1371/journal.pone.0079005.

EC. 2017. “Final Report of an Audit Carried out in the United Kingdom from 21 March 2017 to 31 March 2017 in Order to Evaluate the Monitoring and Reporting of

Antimicrobial Resistance in Zoonotic and Commensal Bacteria in Certain Food-Producing Animal Population and Food." EC.

EcoSure. 2007. "EcoSure 2007 Cold Temperature Database." 2007. https://www.foodrisk.org/resources/display/21.

EFSA/ECDC. 2020. "The European Union Summary Report on Antimicrobial Resistance in Zoonotic and Indicator Bacteria from Humans, Animals and Food in 2017/2018." *EFSA Journal*, January. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.6007.

EVANS, ELLEN W., and ELIZABETH C. REDMOND. 2016. "Time-Temperature Profiling of United Kingdom Consumers' Domestic Refrigerators." *Journal of Food Protection* 79 (12): 2119–27. https://doi.org/10.4315/0362-028X.JFP-16-270.

Evers, Eric G., Annemarie Pielaat, Joost H. Smid, Engeline van Duijkeren, Francy B. C. Vennemann, Lucas M. Wijnands, and Jurgen E. Chardon. 2017. "Comparative Exposure Assessment of ESBL-Producing Escherichia Coli through Meat Consumption." *PLoS ONE* 12 (1). https://doi.org/10.1371/journal.pone.0169589.

Ewers, C., A. Bethe, T. Semmler, S. Guenther, and L.H. Wieler. 2012. "Extended-Spectrum β-Lactamase-Producing and AmpC-Producing Escherichia Coli from Livestock and Companion Animals, and Their Putative Impact on Public Health: A Global Perspective." *Clinical Microbiology and Infection* 18 (7): 646–55. https://doi.org/10.1111/j.1469-0691.2012.03850.x.

FAO. 2019. "Prudent and Efficient Use of Antimicrobials in Pigs and Poultry." Rome: FAO. https://doi.org/10.4060/CA6729EN.

FAO/WHO, Niels. 2002. "Risk Assessments of Salmonella in Eggs and Broiler Chickens." *International Journal of Food Microbiology* 91 (2): 223. https://doi.org/10.1016/S0168-1605(03)00369-6.

Food Standard Agency. 2018. "Chapter 2.8 Animal By-Products." Manual for Official Controls. Food Standard agency. https://www.food.gov.uk/business-guidance/manual-for-official-controls.

Furtula, V., FOR EXAMPLE, Farrell, F. Diarrassouba, H. Rempel, J. Pritchard, and M.S. Diarra. 2010. “Veterinary Pharmaceuticals and Antibiotic Resistance of Escherichia Coli Isolates in Poultry Litter from Commercial Farms and Controlled Feeding Trials.” *Poultry Science* 89 (1): 180–88. https://doi.org/10.3382/ps.2009-00198.

Gerwen, S. J. van, and M. H. Zwietering. 1998. “Growth and Inactivation Models to Be Used in Quantitative Risk Assessments.” *Journal of Food Protection* 61 (11): 1541–49. https://doi.org/10.4315/0362-028x-61.11.1541.

Gonzalez-Zorn, Bruno. 2019. “D3.4_Intervention Strategies That Influence Horizontal Gene Transfer and Stability of Antimicrobial Resistance Genes in the Food Chain.” Project report. EFFORT. http://www.effort-against-amr.eu/media/download_gallery/EFFORT_D3.4_Intervention_Strategies_that_influence_horizontal_gene_transfer_R1.pdf.

Hartnett, E., L. Kelly, D. Newell, M. Wooldridge, and G. Gettinby. 2001. “A Quantitative Risk Assessment for the Occurrence of Campylobacter in Chickens at the Point of Slaughter.” *Epidemiology & Infection* 127 (2): 195–206. https://doi.org/10.1017/S0950268801005866.

Herman, L., M. Heyndrickx, K. Grijspeerdt, D. Vandekerchove, I. Rollier, and L. De Zutter. 2003. “Routes for Campylobacter Contamination of Poultry Meat: Epidemiological Study from Hatchery to Slaughterhouse.” *Epidemiology & Infection* 131 (3): 1169–80. https://doi.org/10.1017/S0950268803001183.

Huijbers, P. M. C., E. A. M. Graat, A. P. J. Haenen, M. G. van Santen, A. van Essen-Zandbergen, D. J. Mevius, E. van Duijkeren, and A. H. A. M. van Hoek. 2014. “Extended-Spectrum and AmpC β-Lactamase-Producing Escherichia Coli in Broilers and People Living and/or Working on Broiler Farms: Prevalence, Risk Factors and Molecular Characteristics.” *Journal of Antimicrobial Chemotherapy* 69 (10): 2669–75. https://doi.org/10.1093/jac/dku178.

Koga, Vanessa L., Renato P. Maluta, Wanderley D. da Silveira, Renan A. Ribeiro, Mariangela Hungria, Eliana C. Vespero, Gerson Nakazato, and Renata K. T. Kobayashi. 2019. “Characterization of CMY-2-Type Beta-Lactamase-Producing

Escherichia Coli Isolated from Chicken Carcasses and Human Infection in a City of South Brazil." *BMC Microbiology* 19 (1): 174. https://doi.org/10.1186/s12866-019-1550-3.

Laube, H., A. Friese, C. von Salviati, B. Guerra, A. Käsbohrer, L. Kreienbrock, and U. Roesler. 2013. "Longitudinal Monitoring of Extended-Spectrum-Beta-Lactamase/AmpC-Producing Escherichia Coli at German Broiler Chicken Fattening Farms." *Applied and Environmental Microbiology* 79 (16): 4815–20. https://doi.org/10.1128/AEM.00856-13.

Li, Xian-Zhi, Manisha Mehrotra, Shiva Ghimire, and Lateef Adewoye. 2007. "Beta-Lactam Resistance and Beta-Lactamases in Bacteria of Animal Origin." *Veterinary Microbiology* 121 (3–4): 197–214. https://doi.org/10.1016/j.vetmic.2007.01.015.

Lindblad, M., H. Lindmark, S. Thisted Lambertz, and R. Lindqvist. 2006. "Microbiological Baseline Study of Broiler Chickens at Swedish Slaughterhouses." *Journal of Food Protection* 69 (12): 2875–82. https://doi.org/10.4315/0362-028X-69.12.2875.

Lu, Gang, Lingshuang Sun, Jiajun Ou, Haibin Xu, Liyan Wu, and Shoujun Li. 2018. "Identification and Genetic Characterization of a Novel Parvovirus Associated with Serum Hepatitis in Horses in China." *Emerging Microbes & Infections* 7 (October). https://doi.org/10.1038/s41426-018-0174-2.

Manges, A. R., and J. R. Johnson. 2012. "Food-Borne Origins of Escherichia Coli Causing Extraintestinal Infections." *Clinical Infectious Diseases* 55 (5): 712–19. https://doi.org/10.1093/cid/cis502.

McCarthy, Z, Fazil A, Ryan Sd, Wu Jh, and Munther D. 2019. "An individual-carcass model for quantifying bacterial cross-contamination in an industrial three-stage poultry scalding tank." *Journal of Food Engineering* 262: 142–53.

Mellata, Melha. 2013. "Human and Avian Extraintestinal Pathogenic *Escherichia Coli* : Infections, Zoonotic Risks, and Antibiotic Resistance Trends." *Foodborne Pathogens and Disease* 10 (11): 916–32. https://doi.org/10.1089/fpd.2013.1533.

Miranda, J.M., B.I. Vázquez, C.A. Fente, J. Barros-Velázquez, A. Cepeda, and C.M. Franco. 2008. “Evolution of Resistance in Poultry Intestinal Escherichia Coli During Three Commonly Used Antimicrobial Therapeutic Treatments in Poultry.” *Poultry Science* 87 (8): 1643–48. https://doi.org/10.3382/ps.2007-00485.

Murphy, Colleen P., Carolee Carson, Ben A. Smith, Brennan Chapman, Jayme Marrotte, Maggie McCann, Courtney Primeau, Parth Sharma, and E. Jane Parmley. 2018. “Factors Potentially Linked with the Occurrence of Antimicrobial Resistance in Selected Bacteria from Cattle, Chickens and Pigs: A Scoping Review of Publications for Use in Modelling of Antimicrobial Resistance (IAM.AMR Project).” *Zoonoses and Public Health* 65 (8): 957–71. https://doi.org/10.1111/zph.12515.

Nauta, Maarten J., Wilma F. Jacobs-Reitsma, and Arie H. Havelaar. 2007. “A Risk Assessment Model for Campylobacter in Broiler Meat.” *Risk Analysis* 27 (4): 845–61. https://doi.org/10.1111/j.1539-6924.2006.00834.x.

Pacholewicz, Ewa, Len J.A. Lipman, Arno Swart, Arie H. Havelaar, and Willem J.C. Heemskerk. 2016. “Pre-Scald Brushing for Removal of Solids and Associated Broiler Carcass Bacterial Contamination.” *Poultry Science* 95 (12): 2979–85. https://doi.org/10.3382/ps/pew257.

Parker, C Daniel. 2020. “Falling Resistance in E Coli Isolated from Broilers in the UK - ProQuest.” 2020. https://search.proquest.com/openview/3b807d50ddbf02be7b099c67d5119098/1?pq-origsite=gscholar&cbl=2041027.

Projahn, Michaela, Ewa Pacholewicz, Evelyne Becker, Guido Correia-Carreira, Niels Bandick, and Annemarie Kaesbohrer. 2018. “Reviewing Interventions against Enterobacteriaceae in Broiler Processing: Using Old Techniques for Meeting the New Challenges of ESBL E. Coli?” *BioMed Research International* 2018 (October). https://doi.org/10.1155/2018/7309346.

R Development Core Team. 2019. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org.

RIVM. 2017. “MARAN 2017 Monitoring of Antimicrobial Resistant and Antibiotic Usage in Animals in the Netherlands in 2016.” https://www.wur.nl/upload_mm/9/b/4/fe79278b-9361-4912-8cba-03ce17fc086b_Maran%20report%202017.pdf.

Robé, Caroline, Anja Blasse, Roswitha Merle, Anika Friese, Uwe Roesler, and Sebastian Guenther. 2019. “Low Dose Colonization of Broiler Chickens With ESBL-/AmpC- Producing Escherichia Coli in a Seeder-Bird Model Independent of Antimicrobial Selection Pressure.” *Frontiers in Microbiology* 10 (September). https://doi.org/10.3389/fmicb.2019.02124.

Romero-Barrios, Pablo, Anne Deckert, E. Jane Parmley, and Daniel Leclair. 2020. “Antimicrobial Resistance Profiles of Escherichia Coli and Salmonella Isolates in Canadian Broiler Chickens and Their Products.” *Foodborne Pathogens and Disease* 17 (11): 672–78. https://doi.org/10.1089/fpd.2019.2776.

Saatkamp, H.W., E. Gocsik, and J.L. Roskam. 2018. “Chain Model and Report on Analysis of Strategies.” Project report. EFFORT. http://www.effort-against-amr.eu/media/EFFORT_D8.4_Chain_model_and_report_on_analysis_of_strategies_R1.pdf.

Thomas, Christian, Annett Martin, Sachsenröder Jana, and Niels Bandick. 2020. “Effects of Modified Atmosphere Packaging on an Extended-Spectrum Beta-Lactamase–Producing Escherichia Coli, the Microflora, and Shelf Life of Chicken Meat.” *Poultry Science* 99 (12): 7004–14. https://doi.org/10.1016/j.psj.2020.09.021.

WHO. 2017. “Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics,” 7.

WHO, and FAO, eds. 2009. *Risk Assessment of Campylobacter Spp. in Broiler Chickens. Technical Report*. Microbiological Risk Assessment Series 12. Geneva: World Health Organization : Food and Agriculture Organization of the United Nations.

14. Appendix 5: Case study 2 – *Campylobacter spp*. in fresh portioned skin off chicken

14.1 Introduction

It was initially planned to investigate as case study Campylobacter spp. carrying the mutated GyrA gene. However, based on the results of the first deliverable of this project and as highlighted in the first case study, the amount of data currently available on *Campylobacter spp*. carrying GyrA gene is not sufficient to properly validate the results of our study with other studies. As a reminder this second case study focused on the risk of bacteria exposure and not the risk of AMR genes exposure.

The case study presented in this report only differs from the one presented in the previous report in terms of microorganism and resistance gene. Therefore, only the *estimated variables* associated with the domain “Bacteria” differed between the two cases studies. The *estimated variables* related to the domain “Bacteria” are specific to the **microorganism or resistance gene** of interest (for example, prevalence of the pathogen, minimal growth temperature, or bacterial concentration in caeca content), when the variables related to the domain “Other” are related to the **production chain or food product** of interest (for example, average size of carcasses, type of scalding technique, or average cooling temperature).

Inputs data for the model included quantitative information on the case study gathered through existing literature using PubMed and Google Scholar. The literature review focused on the most recent studies performed in Europe and, when available, in the UK. However, when no data were available other publications on research studies performed in other regions have been considered. As agreed with FSA, the risk assessment was performed at the bacteria level, and the literature review strategy was based on inclusive criteria related to the case study and

therefore the search terms consistently included "*Campylobacter spp*.", among others. The values of the *selected variables* used in this case study are reported bellow:

- Product = Chicken
- Bacteria = *Campylobacter spp.*
- Gene = None
- Pack_type = No packaging
- Farm_type = conventional
- Product_cut = portion
- Meat_skin = skin off
- Scalding type = soft

14.2 Estimated variables for Production module

The risk of carcass contamination is more strongly influenced by on-farm production practices compared with slaughterhouse activities with on-farm factors being 3.5 times more important than processing plant factors in the model developed by Hutchison et al. (2017; 2016). The production module is thus a critical module for assessing *Campylobacter spp.* contamination.

Table 1 shows the list of the estimated input parameters for the production module and the associated probability distributions, values and references. The parameters refer to flock characteristics and to transport to slaughterhouse. Only parameters in the "Bacteria" domain were reviewed, as parameters in the "Other" domain would be the same as AMR1. A few parameters describing the flock characteristics were not parameterized due to lack of specific data or because they were not relevant for *Campylobacter jejuni* but could be relevant for other hazards and therefore, for this specific case study, were set to no effect or 0 depending on the parameter.

Studies conducted in Denmark show an infection of 100% of organic broiler flocks, from 36.7% of conventional broiler flocks and from 49.2% of extensive indoor broiler flocks suggesting that organic broiler flocks constitute a strong potential for introduction of *Campylobacter spp.* (Heuer et al. 2001). The highest risk associated with organic farms has been also highlighted by Rosenquist et al. (2013) who show

that, in Denmark, the yearly mean prevalence being 54.2% (CI: 40.9–67.5) for organic and 19.7% (CI: 14.8–24.7) for conventional carcasses with obvious differences in all quarters of the year. No similar study was found for the UK context. Data from Rosenquiest et al. (2013) were used to build the probability distribution of the between flock prevalence (*Prev_Farm_type*). None of these studies specified resistance to the mutated GyrA gene.

With regards antimicrobial usage (*F_AMU*), this parameter was set to null because this case study was conducted at the bacteria level.

There is an increased risk of Campylobacter contamination with low biosecurity practices (*F_biosecurity*). In the studies assessed, none were specific to the GyrA mutation or resistance of Campylobacter species to fluoroquinolones. Only one study, Herman et al. (2003), specified the use of fluoroquinolones in the sample farms. This study characterized the different biosecurity measures which were grouped in seven categories, hatchery, animal material in the broiler house, broiler house hygiene, water in the broiler house, movable material, i.e., 'vectors', animal material in the environment, and non-animal material in the environment. The authors concluded that, due to poor biosecurity practices, there was a 133% increase in the proportion of positive flocks along the rearing process. Georgiev, Beauvais, and Guitian (2017) tested the hypothesis that enhanced biosecurity and other factors may prevent *Campylobacter spp.* caecal colonization of poultry batches at high levels (>123 000 c.f.u./g in pooled caecal samples). The farms selected were considered to apply standard production practices relative to other broiler farms and were therefore considered representative of UK poultry farms. Enhanced biosecurity reduced the odds of colonization at partial depopulation (thinning) [odds ratio (OR) 0·25, 95% confidence interval (CI) 0·14–0·47] and, to a lesser extent, at final depopulation (OR 0·47, 95% CI 0·25–0·89). We used the latter OR estimates to build the probability distribution of *F_biosecurity.* We did not differentiate between conventional and organic farming systems here with the assumption that the production type (conventional vs organic) in itself should not be considered as a proxy for the effectiveness of biosecurity measures (i.e., assuming conventional = high biosecurity and organic=poor biosecurity) when producers, as we assumed is

the case in UK, tend to be compliant with requirements related to biosecurity regardless of the production type.

In the United Kingdom, broiler flocks are usually thinned at about 4 or 5 weeks of age by removing 10 to 50% of the birds (Allen et al. 2008). This practice is used by the majority of large-scale producers (Allen et al. 2008). For logistical reasons (the number of houses on a farm or the processing plant operational schedules), the thinning process can take 1 to 2 days to complete (Allen et al. 2008). Although considered an essential part of many broiler operations, thinning may disrupt normal biosecurity measures and is stressful for the remaining birds. Thinning can be measured as the proportion of birds caught at that time for slaughter. It has been shown that thinning of the flocks increases the risk of *Campylobacter spp.* contamination of the flock (OR = 3.302; 95% CI 1.523-7.157; p = 0.002) (Hue et al. 2010; Hald, Rattenborg, and Madsen 2001). The introduction most likely occurs during catching of the first batch, and the microbiological quality of the end product depends not only on the risk of introduction of campylobacter into the chicken flock, but also on the speed with which catching and slaughtering of the flock can be completed (Hald, Rattenborg, and Madsen 2001). In their study in UK, Georgiev, Beauvais, and Guitian (2017) shown that where only 42% of batches raised under enhanced biosecurity that were colonized at thinning, this proportion increased to 65% (percentage increase of 54.7%) at final depopulation showing that thinning increased the risk of Campylobacter contamination. In farms with enhanced biosecurity, flocks that were thinned had more than twice the odds of colonization at depopulation than flocks that were not thinned. The OR for the 810 batches that had been thinned was 2.43 with 95% CI 1.34-4.42. This data was used to parameterize the factor representing the impact of thinning on contamination load (*F_thinning*).

With regards the influence of the season on the Campylobacter contamination levels (*F_season*), the seasonal variation in the level of Campylobacter contamination of fresh chicken are reported by many authors (Meldrum, Tucker, and Edwards 2004; Newell et al. 2011; F. Jorgensen et al. 2011; Wedderkopp, Rattenborg, and Madsen 2000). However, none of the references are specific to the GyrA mutation or fluoroquinolone resistance. The reason of the seasonality of Campylobacter infections in poultry is still unknown but it indicates that the relative importance of

potential reservoirs and transmission routes can change over the course of the year. One hypothesis is that the increased risk is related to the breeding period of flies (Newell et al. 2011). In Denmark, it appears that over 2/3 of the Campylobacter positive flocks during the seasonal peak may be due to flies, and there was a statistically significant reduction in flock positivity of over 60% in houses protected by fly nets (Newell et al. 2011). Campylobacter isolation showed variation throughout the year in Wales, with a peak in June and the lowest rates in January, March, and December (Meldrum, Tucker, and Edwards 2004). The seasonal risk peak generally occurs in late summer/ early autumn, but the timing, extent, and sharpness of this peak can vary between countries and may be related to the latitude of the country (Newell et al. 2011). A study in the UK, shown that the *Campylobacter spp*. prevalence of positive batches was significantly higher in July (54%; P = 0.01), August (55%; P = 0.005), and September (60%; P= 0.001) than during the rest of the year (range, 14 to 48%). The summer peak was slightly more pronounced in Southern Great Britain than in the other regions (F. Jorgensen et al. 2011). Wedderkopp et al. (2000), in Denmark, reported that the Odds Ratio for Campylobacter colonization at slaughter was highest in August (OR 5.6; 95% CI 4.0-7.8) and lowest in February (OR 0.8; 95% CI 0.6-1.2) and that 86% of the isolates were *Campylobacter jejuni*. The authors also reported the number of birds slaughtered in high and low risk seasons. This reference was used to build the probability distribution of *F_season.* The proportion of birds slaughtered during the high risk season (*Prop_season*) was modelled as a Uniform distribution taking into account the min and maximum proportion of birds slaughtered in different risk periods (Wedderkopp, Rattenborg, and Madsen 2000).

The effect of age (*F_age*) at slaughter as a risk factor for bacterial contamination is controversial and not modelled in this case study. In general, the infection of broiler flocks increases continuously during the rearing time (Herman et al. 2003) and (Hutchison et al. 2017) estimated that for each day a bird was farmed there was a mean increase in log10 *Campylobacter spp.* numbers of 0-331 CFU/g in the litter. Russa et al. (2005) considered age a confounding factor as the longer the birds are kept on farm the higher the chance of colonization of the broilers.

The probability distribution for the average prevalence of birds from positive flock internally colonized at pre-harvest *(Prev_wfp_col_base)* was based on frequency data of *Campylobacter spp*. from cecum and carcass (neck skin) samples from different production system in Portugal (Fraqueza et al. 2014).

While we could not find data for modelling the number of Campylobacter on negative birds exterior after transport (*C_prod_n*), we used a triangular distribution representing a worst case scenario because it includes both positive and negative birds (Berrang and Bailey 2009). It is a conservative approach since it is probably overestimating the risk of Campylobacter contamination.

Table 34: List of estimated variables related to the production module. Black = Variables associated with the domain "Bacteria", Grey = Variables associated with the domain "Other" (their value is the same than for AMR1).

Variable Name	Description	Value/Distribution	Units	Source
Prev_Farm_type	Between Flock prevalence of AMR1	• **If Farm_type = Conventional** • Pert (0.148, 0.197, 0.247) • **If Farm_type = Organic** • Pert (0.409, 0.542, 0.675)	Prevalence	(Rosenquist et al. 2013)
F_AMU	Factor representing the impact of antimicrobial usage on between-flock prevalence of AMR1	0	Prevalence	
F_biosecurity	Factor representing the impact of poor biosecurity on contamination prevalence	Pert (0.25, 0.47, 0.89)	Probability (OR) (reduced contamination prevalence due to enhanced biosecurity)	(Georgiev, Beauvais, and Guitian 2017)
Prop_biosecurity	Proportion of farms with poor biosecurity practices	0	Proportion	NA

Variable Name	Description	Value/Distribution	Units	Source
F_thinning	Factor representing the impact of thinning on contamination prevalence	Pert (1.34, 2.43, 4.42)	Probability (OR) (increased contamination prevalence due to thinning)	(Georgiev, Beauvais, and Guitian 2017)
Prop_thinning	Proportion of farms implementing thinning	Uniform(0.05, 0.09)	Proportion	(Georgiev, Beauvais, and Guitian 2017)
F_season	Factor representing the impact of high-risk season on contamination prevalence. “High risk season” must be defined for each microorganism.	Pert (4.0, 5.6, 7.8)	Probability (OR) (increased contamination prevalencedue to season)	(Wedderkopp, Rattenborg, and Madsen 2000)
Prop_season	Proportion of birds slaughtered during the high-risk season. “high risk season” must be defined for each microorganism.	Uniform (0.061, 0.093)	Proportion	(Wedderkopp, Rattenborg, and Madsen 2000)
F_age	Factor representing the impact of age on contamination prevalence	None	Probability (OR) (increased contamination prevalence due to bird age)	(Hutchison et al. 2017; Herman et al. 2003; Evans and Sayers 2000; Allen et al. 2008)

Variable Name	Description	Value/Distribution	Units	Source
Prop_age	Proportion of birds slaughtered late. “Late” must be defined for each microorganism.	0	Proportion	(Hutchison et al. 2017; Herman et al. 2003; Evans and Sayers 2000; Allen et al. 2008)
Prev_wfp_col_base	Average prevalence of birds from positive flock internally colonized at pre-harvest	• **If Farm_type = Conventional** • Uniform (0.79, 1) • **If Farm_type = Organic** • Uniform (0.83, 1)	Prevalence	(Fraqueza et al. 2014)
Amount_fec	Amount of faeces on bird exterior at pre-harvest in positive flocks	Triangular (1, 10, 50)	g	(Collineau et al. 2020)
C_barn	Concentration in the barn environment of positive flocks	Pert (3910, 4290, 4670)	CFU/kg of faeces	(Santini n.d.)
F_transp	Factor representing the impact of transport of positive flocks on contamination load	1.41		(Collineau et al. 2020)
N_transp	Number of flocks transported prior to the current flock	Uniform (0, 4)	Flocks	(O. Bucher et al. 2012a)

Variable Name	Description	Value/Distribution	Units	Source
F_cross_trans	Factor representing the probability of carryover contamination from a positive flock transported prior to the current flock.	Uniform(0.16, 0.46)	Probability (expressed as RR)	(Hansson et al. 2005)
N_contact	Number of contacts with contaminated birds during transport	Pert (1.5, 3, 4.5)	Count	(O. Bucher et al. 2012a)
C_prod_n	Number of bacteria on negative birds after transport due to cross contamination during transport	Triangular (1.6, 2.5, 3.4)	CFU/bird	(Berrang and Bailey 2009)

14.3 Estimated variables for the Processing module

Table 2 shows the list of the estimated input parameters for the Processing module and the associated probability distributions, values and references. The parameters are grouped according to the different steps in the processing chain.

During processing of broiler chickens, the level of Campylobacter contamination present on the broiler carcasses will fluctuate. According to the report of Campylobacter risk assessment (WHO and FAO 2009) in broilers, the relative changes during the processes are similar in the various studies analyzed. This despite the use of different methods for sampling and quantification and indicate therefore that the changes in concentrations of Campylobacter between processing steps in commercial broiler slaughter plants may be relatively uniform and consistent between studies.

With regards the scalding parameters (*Prop_scald* soft and hard) we used data from Hinton et al. (2004) that looked at concentration changes of campylobacter in a multi-tank scalding systems with three different temperatures (45, 50 and 57.2 °C). Temperature of tanks 2 and 3 were used as a proxy for soft and hard scalding respectively.

The input data to model the probability of cross-contamination to occur during defeathering for birds from positive and negative flocks (*P_cross_df*) were obtained from Berrang et al. (2001). The authors in this work aimed to determine, with three experiments, if the escape of contaminated feces from the cloaca during defeathering leads to an increase in Campylobacter numbers recovered from broiler carcasses. In the first and second experiments live positive broilers obtained from a commercial processor and free campylobacter flocks were used respectively.

The literature on effect of evisceration suggests that this step is associated with increased *Campylobacter spp.* load and risk of contamination. However, some studies also reported a reduction in Campylobacter load (see the literature review). The probability of cross-contamination to occur during evisceration for positive flock (*P_cross_ev_p*) was modelled as Beta distribution using data from Berrang and Dickens (2000). No data were found on the probability of cross-contamination to

occur during evisceration for negative flock (*P_cross_ev_n*) and the same probability distribution of *P_cross_ev_p* was used as a surrogate.

The load reduction factor after air pre-chilling (*F_Chill*) was parameterized using data from Rosenquist et al. (2006). The resulting distribution shows the percentage change reduction in bacterial load chilling.

For the distributions of the probability that a single cell resides on portion cap (*P_skin*) and the proportion of cells transmitted from portion cap to meat (*Prop_cm*) we used data from (Nauta, Jacobs-Reitsma, and Havelaar 2007) and the data used refer to the probability for each Campylobacter on the carcass to reside on the breast cap. The same unit (breast) applies here with the assumption that one chicken breast equals a quarter of whole carcass chicken (*Prop_product* = 0.25).

Table 35: List of estimated variables related to the processing module. Black = Variables associated with the domain "Bacteria", Grey = Variables associated with the domain "Other" (their value is the same than for AMR1).

Variable Name	Description	Value/Distribution	Units	Source
Prop_scald_soft	Proportion of cells remaining after soft scalding	Normal(4.31, 0.35)	log 10 CFU/ml	(Hinton, Cason, and Ingram 2004)
Prop_scald_hard	Proportion of cells remaining after hard scalding	Normal(2.54, 0.24)	log 10 CFU/ml	(Hinton, Cason, and Ingram 2004)
F_df	Reduction or increasing factor of bacterial load on carcass during defeathering	Pert (2.9, 3.0, 3.01)	logCFU	(Berrang et al. 2001)
P_cross_df_p	Probability of cross-contamination to occur during defeathering for birds from positive flocks	Beta (95+1, 120-95+1)	Probability	(Berrang et al. 2001)
P_cross_df_n	Probability of cross-contamination to occur during defeathering for birds from negative flocks	Beta (69+1, 120-69+1)	Probability	(Berrang et al. 2001)
P_cut_vis	Probability that viscera are lacerated during evisceration	Triangular (0.14, 0.18, 0.23)	Probability	(Collineau et al. 2020)
spill_weight	Caecal content spilled in case of viscera rupture	Uniform (1,10)	g	(Collineau et al. 2020)

Variable Name	Description	Value/Distribution	Units	Source
P_cross_ev_p	Probability of cross-contamination to occur during evisceration for positive flocks	Beta (26+1, 30-26+1)	Probability	(Berrang and Dickens 2000)
F_ev	Percentage of additive bacteria load increase due to cross-contamination between carcasses during evisceration	Triangular (0.116, 0.174, 0.232)	Proportion change in bacterial load	(Rosenquist et al. 2006)
C_caecal	Mean (90%CI) bacteria concentration in caecal content	Pert ($10^{6.9}$, $10^{7.3}$, $10^{7.7}$)	CFU/g	(Berrang, Buhr, and Cason 2000)
F_wash	Load reduction factor after water washing (only water used in UK)	Pert(−0.70, −0.54, −0.34)	Log10 CFU /carcass	(Dogan et al. 2019)
F_Wash_adj	Dampening factor for each successive wash	Uniform (0, 0.5)	-	(Collineau et al. 2020)
F_chill	Load reduction factor after air pre-chilling (only washing technique used in UK)	Triangular (-0.285, -0.2.4, -0.143)	Proportion	(Rosenquist et al. 2006)
P_skin	Probability that a single cell resides on portion cap	Beta (1, 3.15)	Probability	(Nauta, Jacobs-Reitsma, and Havelaar 2007)

Variable Name	Description	Value/Distribution	Units	Source
Prop_cm	Proportion of cells transmitted from portion cap to meat	Pert (0.01, 0.02, 1)	Proportion	(Nauta, Jacobs-Reitsma, and Havelaar 2007)
Prop_product	What fraction of the raw product represents the final processed product?	0.25	Proportion	Authors estimate

14.4 Estimated variables for the post-Processing module

Growth models used for *Campylobacter spp*. on chicken meat based on (van Gerwen and Zwietering 1998; Collineau et al. 2020) were used to calculate F*_retail, F_trans* and *F_fridge*. The equations used are presented in Table 3 and 4 show the list of the estimated input parameters for the Post-Processing module from the domain “Bacteria” and the associated probability distributions, values and references. The reduction of contamination load due to packaging was based on data obtained by Boysen et al. (2007) considering an oxygen-containing gas mixture, 70/30% O2/CO2 and an average time period under packaging conditions of 8 days. It should be noted that anaerobic packaging is usually not associated with significant reduction of *Campylobacter spp.* load (Boysen, Knøchel, and Rosenquist 2007).

Table 36: growth models used for Campylobacter spp. on chicken meat

Variable Name	Description	Formula	Source
F_retail	Growth factor at retail	• ***If T_retail > T_growth_min*** exp(ln (2)* [(T_retail - T_growth_min)/ (T_growth_opt – T_growth_min)]^2 * Time_retail*24/ Time_gen_min) • ***Else*** *1*	(van Gerwen and Zwietering 1998; Collineau et al. 2020)
F_trans	Growth factor during transport	• ***If T_avg_trans > T_growth_min*** exp(ln (2)* [(T_trans - T_growth_min)/ (T_growth_opt – T_growth_min)]^2 * (Time_trans/60)/ Time_gen_min) • ***Else*** *1*	(van Gerwen and Zwietering 1998; Collineau et al. 2020)

Variable Name	Description	Formula	Source
F_fridge	Growth factor at home	• ***If T _fridge> T_growth_min*** exp(ln (2)* [(T_fridge - T_growth_min)/ (T_growth_opt – T_growth_min)]^2 * Time_fridge*24/ Time_gen_min) • ***Else*** *1*	(van Gerwen and Zwietering 1998; Collineau et al. 2020)

Table 37: List of estimated variables related to the post-processing module. Black = Variables associated with the domain "Bacteria", Grey = Variables associated with the domain "Other" (their value is the same than for AMR1).

Variable Name	Description	Value/Distribution	Units	Source
Size_mean	Breast or carcass size mean	Normal (1495.6, 303.4)	g	(Chardon and Evers 2017)
Prop_carc_yield	Carcass yield (proportion of lean meat)	Uniform (0.6, 0.65)	Proportion	(Chardon and Evers 2017)
Time_retail	Number of days stored at retail	Triangular (1,3,7)	Days	(Collineau et al. 2020)
T_retail	Temperature at retail storage	Laplace (-6.67, 3.3333 19.44)	Degree C	(EcoSure 2007)
Time_trans	Mean (90%CI) home transport duration	Normal(69.6, 0.438)	Minutes	(Collineau et al. 2020)
T_post_trans	Chicken temperature at the end of home transport	Shifted Loglogistic Truncate (29.371, 16.763, -22.915,	Degree C	(EcoSure 2007)

Variable Name	Description	Value/Distribution	Units	Source
		min = - 5.56, max = 20)		
Time_fridge	Mean (90%CI) number of days refrigerated at home	Normal(2.2, 0.0203)	days	(Collineau et al. 2020)
T_fridge	Home refrigeration temperature	Laplace (-4.44, 5.3, 16.11)	Degree C	(Biglia et al. 2018; Evans and Redmond 2016; EcoSure 2007)
T_growth_min	Minimum growth temperature	30	Degree C	(FAO 2007)
T_growth_opt	Optimal growth temperature	Pert (30, 42, 45)	Degree C	(FAO 2007)
F_pack	Reduction factor of bacterial load because of packaging	Uniform (−2.6, -2.0)	logCFU	(Boysen, Knøchel, and Rosenquist 2007)
Time_gen_min	Minimum generation time in food product	2.1	hours	(Battersby et al. 2016)
C_MPD	Maximum population density	1096.6	CFU / g	(WHO and FAO 2009)

14.5 Estimated variables for the Home preparation module

Table 5 shows the list of the estimated input parameters for the Home-preparation module and the associated probability distributions, values and references. The proportion of cells in the protected area and the proportion of cells loosely attached was based on the model developed by (WHO and FAO 2009) for *Campylobacter spp.*

Table 38: List of estimated variables related to the home preparation module. Black = Variables associated with the domain "Bacteria", Grey = Variables associated

with the domain "Other" (their value is the same than for AMR1).

Variable Name	Description	Value/Distribution	Units	Source
P_undercook	Mean (90%CI) probability of undercooking to occur	Normal(0.40, 0.00212)	probability	(Collineau et al. 2020)
Time_protec	Exposure time at exposure temperature in the protected area	Pert(0.5, 1, 1.5)	Minutes	(FAO/WHO 2002)
T_protec	Exposure temperature during cooking in the protected area	Pert(60, 64, 65)	Degree C	(FAO/WHO 2002)
P_h_wash	Probability of cross-contamination to occur (related to kitchen hygiene, represent the probability of not washing hands during chicken food preparation)	Uniform(0.38, 1)	Probability	(Collineau et al. 2020; Bruhn 2014)
V_dil_carc	Volume of fluid diluting for a whole carcass	Uniform(150, 250)	ml	(Collineau et al. 2020; WHO and FAO 2009)
V_ing	Volume of fluid ingested	Uniform(0.5, 1.5)	ml	(Collineau et al. 2020; WHO and FAO 2009)

Variable Name	Description	Value/Distribution	Units	Source
Prop_protec	Proportion of cells in the “protected area”	Triangular(0.10,0.15,0.20)	Proportion	(WHO and FAO 2009)
Prop_loose	Proportion of cells loosely attached	Uniform(0.01, 0.1)	Proportion	(WHO and FAO 2009)

14.6 Results

14.6.1 Risk of exposure

14.6.1.1 Overall risk

The overall risk of bacteria exposure was estimated considering positive and negative flocks combined. The overall risk thus represents the average prevalence, and level of contamination, of contaminated serving given the estimated proportion of positive and negative flocks in the overall population. Figure 1 presents the probability density functions associated with each *outcome variables* considered in the modelling framework. Table 6 presents more specifically the numeric values of the mean and median of each outcome variables.

These results shown an overall increase of the prevalence of contaminated products and level of contamination per contaminated product during the processing module. The median prevalence of contaminated serving after cooking equalled 20%. The median probability of exposure through cross contamination was lower and equalled 1.0E-04%. The median number of bacteria per contaminated products was always low and below 3 CFU/item of interest. Similar to the first case study, all the outcome variables presented highly skewed probability distributions. Four of the outcome variables (i.e., *Prev_prod, Prev_proc, Prev_pproc, Prev_home_cook*) also shown distinct peaks, which can be explained by the two different population considered together in these figures (i.e., the positive and negative flocks) and the importance of cross contamination for Campylobacter spp. The risk depending on the flock origin is discussed in the next section.

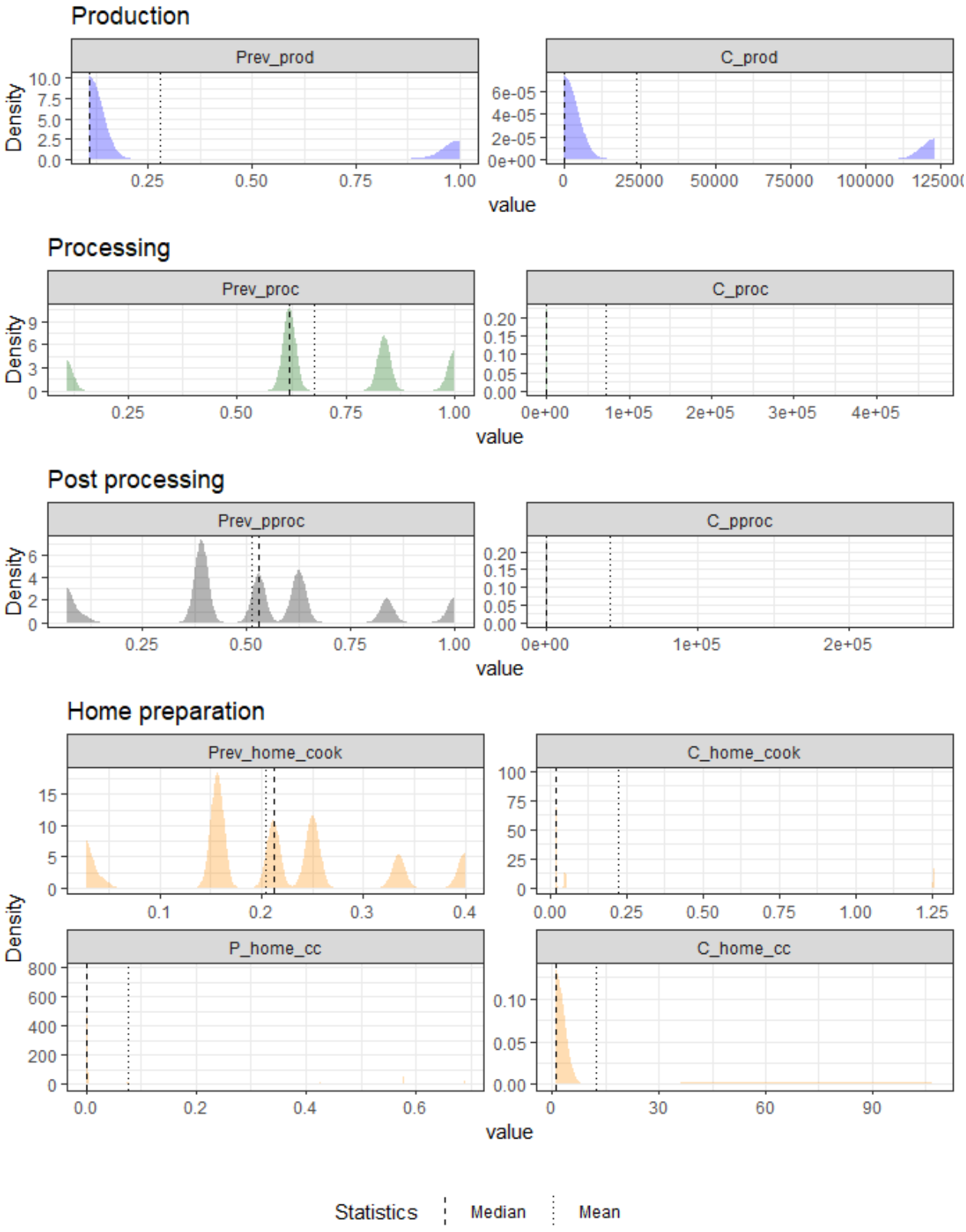


Figure 14: Probability distributions of the outcome variables associated with the production, processing, and post-processing modules after 100 000 simulations.

Table 39: Mean and median overall risk estimation per module

Module	Output variables	Unit	Mean	Median
Production	*Prev_prod*	Prevalence of birds contaminated with bacteria arriving at the slaughterhouse (%)	0.28	0.12
Production	*C_prod*	CFU of bacteria /bird arriving at the slaughterhouse	2.4E+04	2.6
Processing	*Prev_proc*	Prevalence of carcasses contaminated with bacteria (%)	0.68	0.68
Processing	*C_proc*	CFU of A bacteria / carcasses	7.5E+04	0
Post-processing	*Prev_pproc*	Prevalence of food item contaminated with bacteria (%)	0.50	0.52
Post-processing	*C_pproc*	CFU of bacteria /food item	2.5E+04	1
Home preparation	*Prev_home_cook*	Prevalence of serving contaminated with bacteria after cooking (%)	0.20	0.21
Home preparation	*C_home_cook*	CFU of bacteria ingested by contaminated food item	0.34	0.03
Home preparation	*P_home_cc*	Probability of exposure to bacteria through cross contamination (%)	0.07	0.00
Home preparation	*C_home_cc*	CFU of bacteria ingested by cross contaminated food item	9.25	1.0

14.6.1.2 Risk depending on the flock origin

The risk of bacteria exposure was estimated separately for positive and negative flocks as these populations present very different baseline values in terms of within-flock prevalence of contamination, and contamination load per bird. The proportion of positive vs negative flocks was defined by the between flock prevalence estimated for

conventional farms (*Prev_Farm_type)*: 19 823 (19.8%) and 80 177 (80.2%) positives and negative flocks were included in the analysis, respectively.

For the negative flocks, the median within flock prevalence of contaminated birds at the end of the production module was below 30% (Figure 2) while this prevalence was above 95% for the positive flocks (Figure 3). This is in line with results from several studies that assumed that either none or all birds in a flock are infected with Campylobacter at arrival to the slaughterhouse (see for example (Rosenquist et al. 2003)). This assumption can be made since it has been shown that the time from initial infection to a full-blown infection of all broilers in a flock occurs within a few days (Newell and Fearnley 2003; Hartnett et al. 2001; Katsma et al. 2007).

Several peaks can be observed in Figure 2 and Figure 3. These peaks are due to sub-group of birds being cross-contaminated at different points of the production chain. For example, the peak observed on the left in *Prev_proc* in Figure Figure 2 represents birds contaminated during defeathering. The peak in the middle represents birds contaminated not during defeathering but only during evisceration.

The prevalence of contaminated birds increased up to 60% during defeathering and evisceration for birds coming from negative flocks when this prevalence reached 100% (median) in positive flocks. This result is consistent with the fact that between flock cross contamination during these two processing steps was assumed to be high as confirmed by the literature accessed.

With regards to evisceration, in their study Berrang and Dickens (2000) confirmed that 86.7 % of birds sampled were Campylobacter positive when sampled post evisceration with an increase in number of contaminated birds compared to the previous step. While it is not possible to specifically impute the increase entirely to cross contamination this is a plausible co-cause as reported in other papers (Hue et al. 2010).

In both cases, the prevalence of carcasses contaminated with bacteria drops after the post-processing module (i.e., median *Prev_pproc_n* = 39%, *Prev_pproc_p* = 63%). As with the first case study, this result can be explained by the fact that model adjustments made to prevent products with very low contamination levels from being carried forward to the consumer stage of the model were only implemented in the post-processing and home-preparation modules. Carcasses with very low (<1CFU/carcass) contaminated levels were thus counted as contaminated carcasses in the production and processing

modules, which overestimates the prevalence of truly contaminated carcasses in these modules.

The median prevalence of contaminated serving after cooking equalled 17% when the meat came from a negative flock, and 25% when the meat came from a positive flock. The median load of bacteria of this contaminated serving was however low and was less than 1 CFU/piece of meat. The median probability of exposure through cross contamination was below 0.01% for both flocks. The median level of contamination with bacteria in case of exposure was also low and did not exceed 1 CFU/item of interest.

Figure 4 and Figure 5 provide a more detailed overview of changes in mean and median within flock prevalence across the value chain in the two types of flocks. These figures show that, in line with the impact of cross contamination during defeathering and evisceration, negative flocks are contaminated by positive flocks during these two steps.

Similarly, Figure 5 shows the effect of scalding, washing, chilling and cutting and skin removal on the reduction of bacterial contamination. Defeathering and evisceration however tend to increase bacterial load due to cross contamination and/or contamination by faecal content leakage. The post-processing module does not contribute to the increase in bacterial load because of temperature storage and transport below the minimum growth temperature of *Campylobacter spp.* (i.e., 30°C).

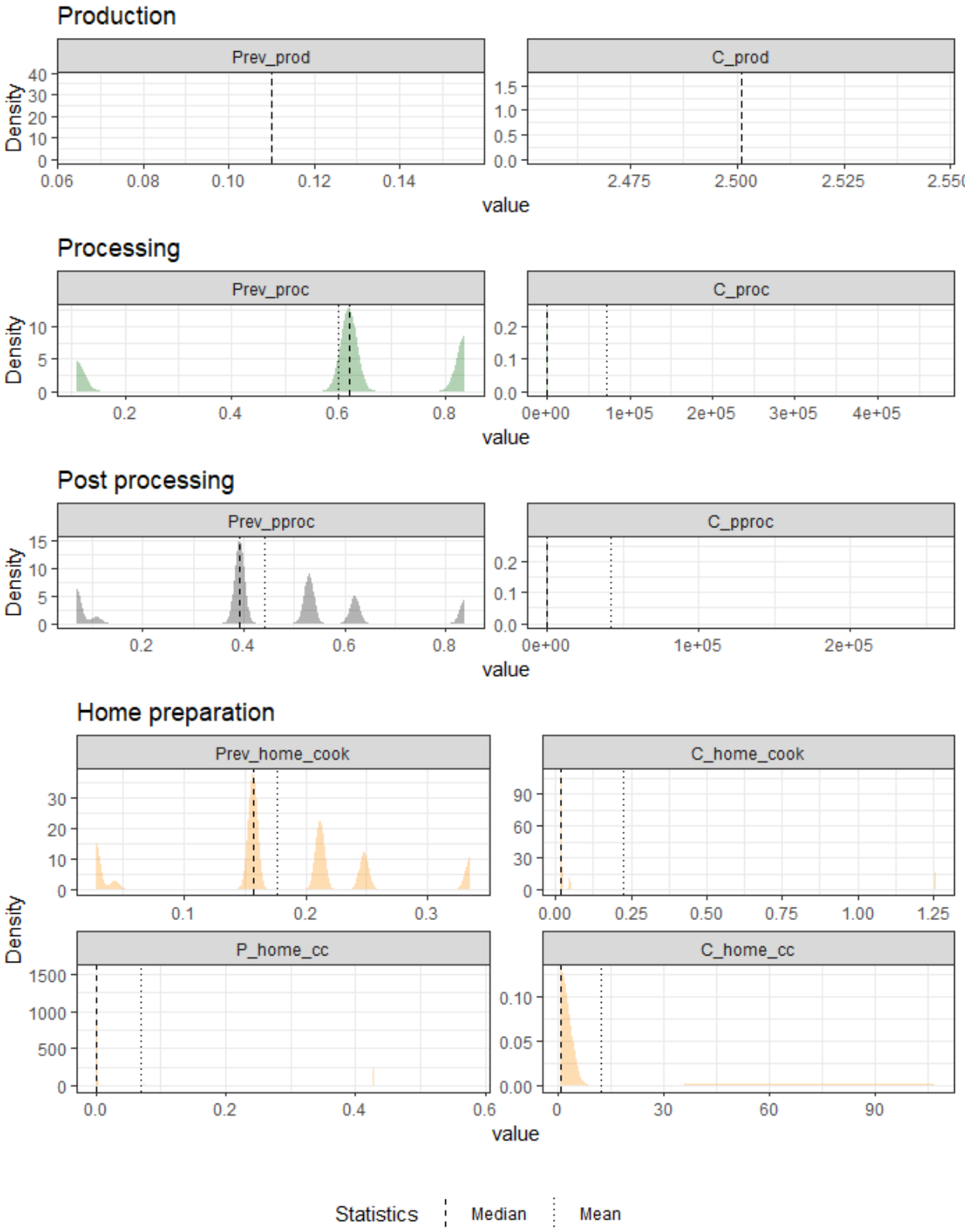


Figure 15: Probability distributions of the outcome variables associated with each module for birds coming from negative flocks after 100 000 simulations.

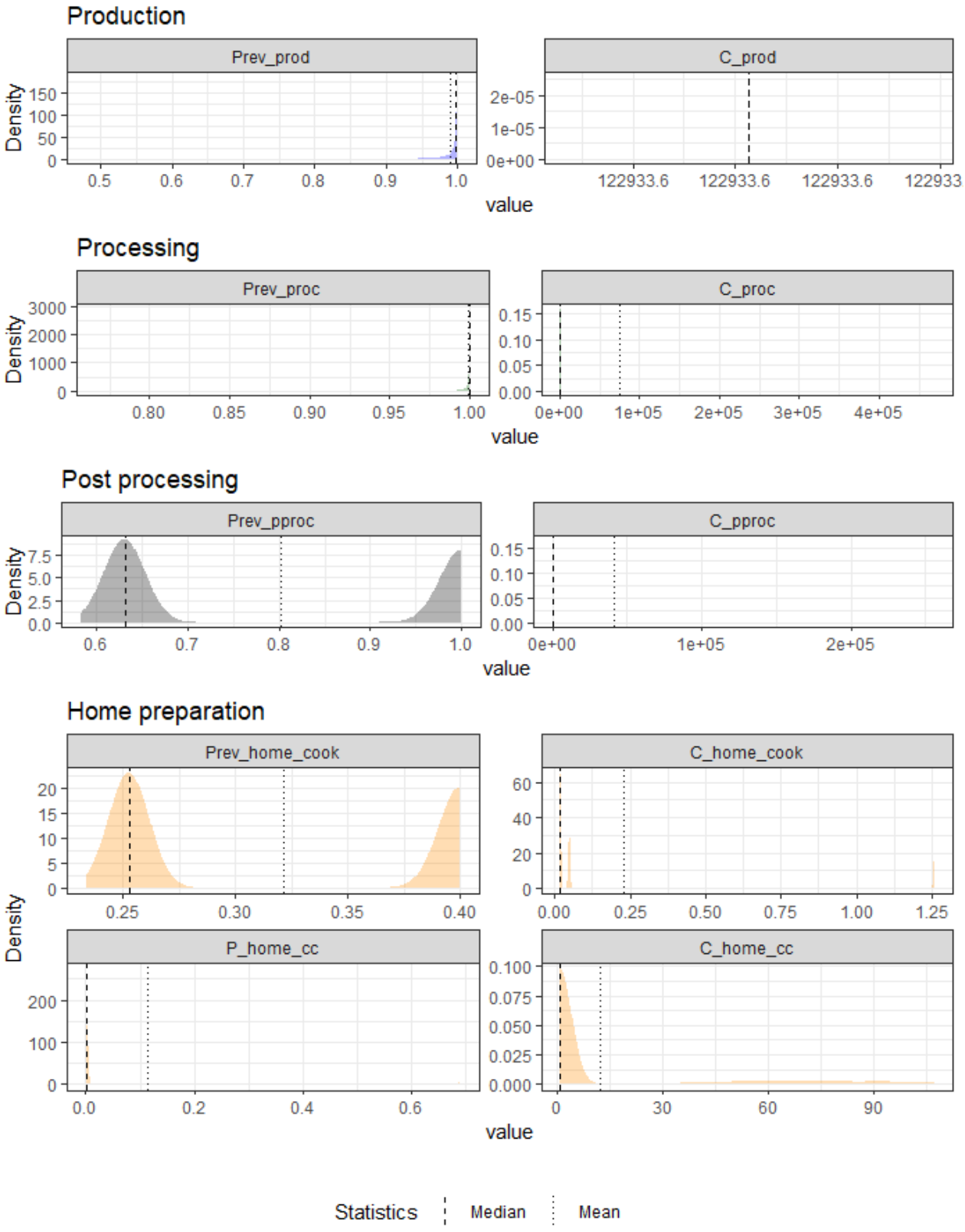


Figure 16: Probability distributions of the outcome variables associated with each module for birds coming from positive flocks after 100 000 simulations.

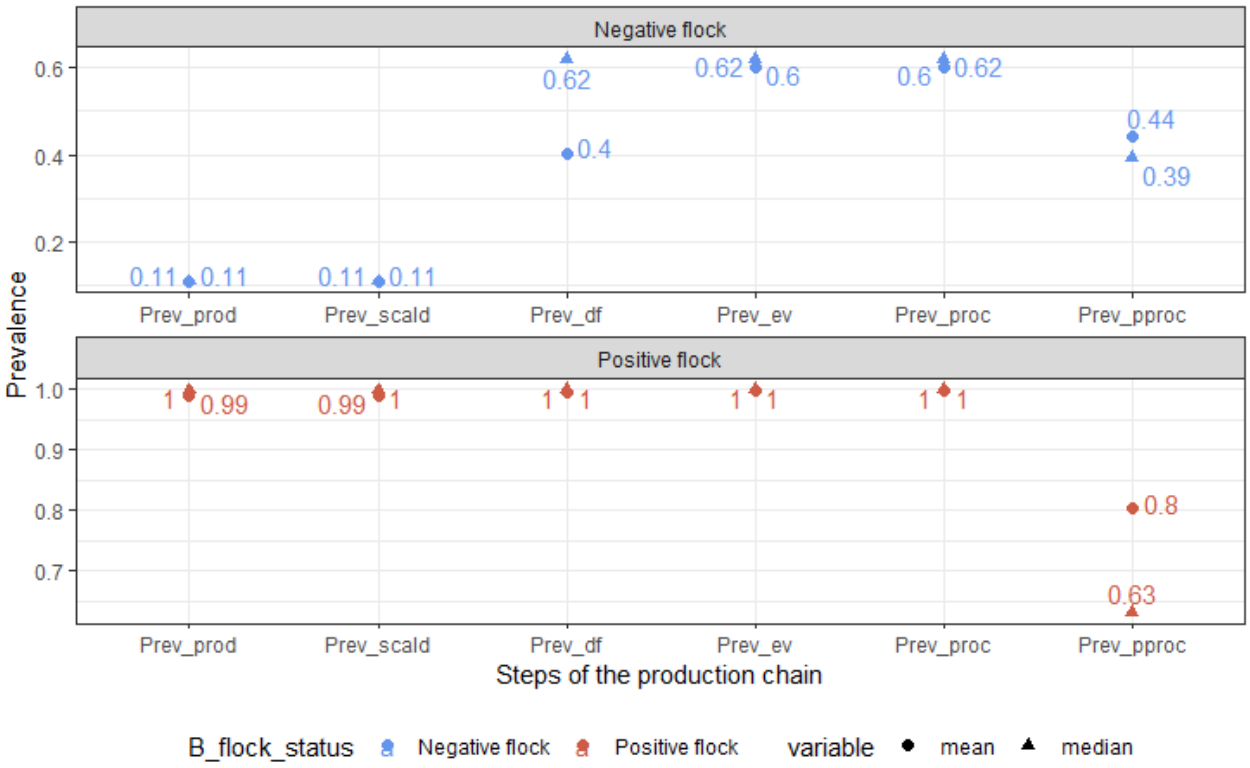


Figure 17: Mean and median within flock prevalence of contaminated item of interest from the production to the prost-processing module

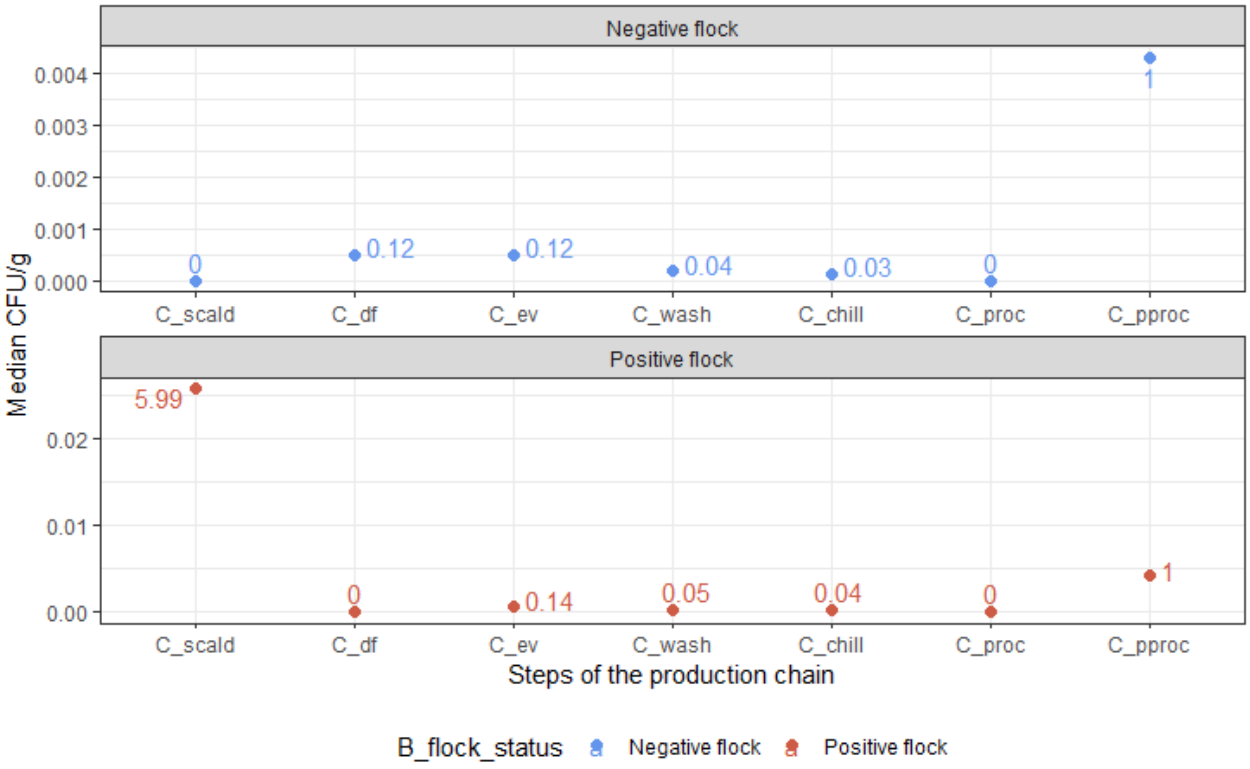


Figure 18: Median levels of bacterial contamination per gramme of contaminated item of interest from the production to the prost-processing module

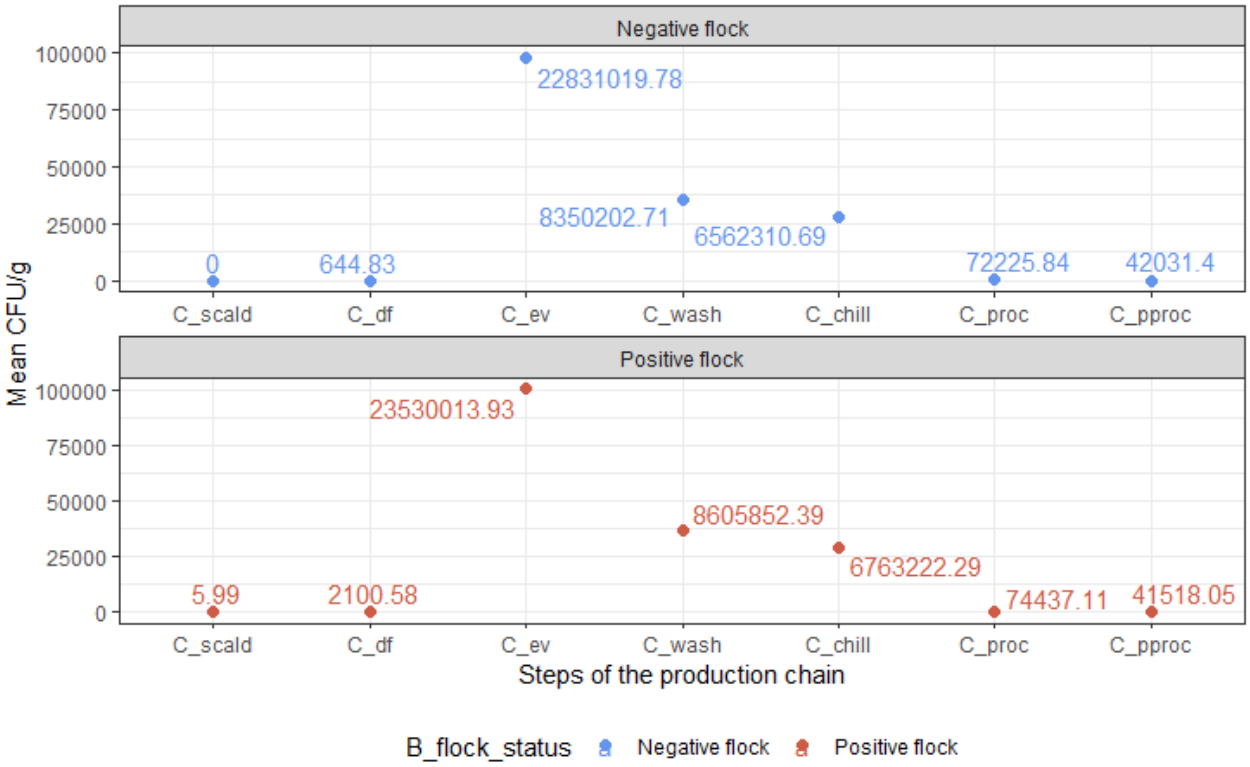


Figure 19: Mean and median levels of bacterial contamination per gramme of contaminated item of interest during the production module

14.6.2 Correlation analysis

The results of the correlation analysis performed for each outcome variables are presented in the Figure 7. Only estimated variables with spearman correlation coefficient ≥ |0.025| have been included in the figure. The estimated variables with a lower Spearman correlation coefficient were considered has having a negligible effect on the model outcomes.

Prevalence of contamination

Consistently with the results of AMR1, all within flock prevalence estimations were highly influenced by three estimated variables: the between farm prevalence (*Prev_farm_type),* the number of flocks transported before the current flock (*N_transp),* and the term for dampening the probability of carryover contamination from a positive flock transported prior to the current flock (*F_cross_trans)*. The probability of cross contamination

occurring in negative flocks during defeathering (*P_cross_df_n*) also had a major influence on the model outcome.

Due to the lack of specific data, the number of flocks transported before the current flock (*N_transp*), were estimated from Bucher et al. (2012) in Collineau et al. (2020) and the input data is identical. Therefore, the same limitation described in the first case studies applies. To model the probability of carryover contamination from a positive flock transported prior to the current flock (*F_cross_trans*) we used data from Hansson et al. (2005) to build a Uniform distribution with minimum and maximum ranges estimated by the results of two studies in Sweden in 2002 from slaughter groups aiming to determine the prevalence of Campylobacter-contaminated transport crates and to determine whether contaminated crates represent a risk for contamination of chickens during transport to slaughter. The authors concluded that crates represent a clear risk factor but alerted that the transportation time might be a confounding factor. In a study by Slader et al. (2002) broilers were in crates for about 2h and no Campylobacter was isolated from cloacal samples, indicating that 2h is probably not a sufficient time for intestinal colonization.

Berrang et al. (2001) implemented three sets of experiments to explore the increase in recovery of Campylobacter from broiler carcasses after defeathering *(P_cross_df_n*). Experiment one and two selected live broilers from a processing plant and campylobacter-negative flocks respectively. Their findings showing a high prevalence of contaminated carcasses after defeathering (79% and 57% respectively) in the two experiments were in line, according to the authors, with previously published reports that show that the incidence and numbers of Campylobacter recovered increase after the carcass is defeathered.

The prevalence of contaminated product after cooking was also highly influenced by the probability of undercooking (*P_undercook*) while the probability of exposure via cross-contamination was influenced by the kitchen hygiene (*P_h_wash*), the size of the meat (*Size_mean*), the proportion of bacteria in fluid extracted from this piece of meat (*Prop_fluid*), and the volume of contaminated fluid ingested by the consumer (*V_ing*).

Level of bacterial contamination

In terms of bacterial load contamination, many estimated variables have a large influence on the model outcome (i.e., Spearman correlation coefficient > |0.5|) and some are briefly discussed here.

The probability that a single cell resides on portion cap (*P_skin*) and the proportion of cells transmitted from portion cap to meat (*Prop_cm*) are influential in the processing and post-processing modules. To model this parameter, data from (Nauta, Jacobs-Reitsma, and Havelaar 2007) were used. The authors developed a quantitative microbiological risk assessment model describing the transmission of Campylobacter through the broiler meat production chain until consumption of a chicken breast fillet meal. The same unit (breast) applies here with the assumption that one chicken breast equals a quarter of whole carcass chicken (*Prop_product* = 0.25). In addition, our model assumes that the level of bacterial contamination is homogeneous in different parts of the carcass. The authors acknowledge that count data on the effects of cutting on the numbers of Campylobacter on chicken products were scarce and they interviewed only one expert to get the required information. Therefore, by using these parameters, we assumed the level of uncertainty related to these variables being relevant and further data are needed to properly challenge the effect of these variables. In addition, our model assumes that the level of bacterial contamination is homogeneous in different parts of the carcass. The model results represent therefore an average level of meat contamination. However, this may not be the case (i.e., neck flap skin tends to have higher campylobacter numbers per cm than thigh skin as a result of the way birds are suspended during the slaughter process, D. Parker personal communication). Our choice to not include in the model a specific parameter related to “part of carcass” aimed to simplify the data collection process for future users. Retrieving specific input data for different part of carcasses can be very challenging.

The load reduction factor after water washing (*F_wash*) is also influential in the processing modules and data used were obtained from a meta-analysis performed by Dogan et al. (2019). Because parameters were derived from a systematic review and metanalysis these data provide an unbiased estimate with the current pool of knowledge. The authors of this work aimed to evaluate the effectiveness of intervention strategies in processing plants in USA to protect the safety of chicken consumption and associated consumer health. In the UK, only water (without additives) is allowed and in this study the authors also modelled the impact of water without additives.

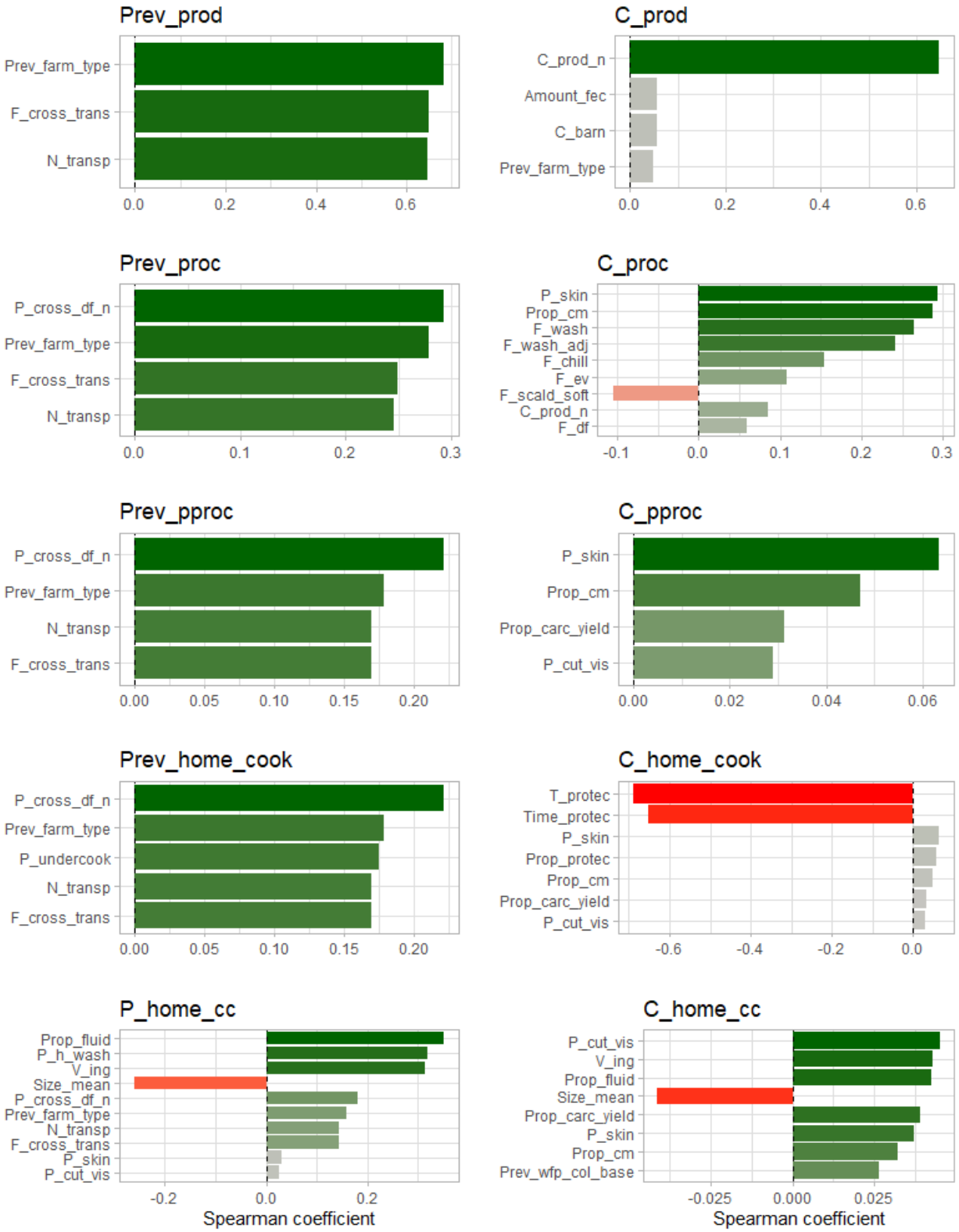


Figure 20: Correlation of the model estimated variables with the outcome variables. Only estimated variables with spearman correlation coefficient ≥ |0.025| have been included in the tornado charts. The darker the color (either green or red), the stronger the correlation.

14.7 Discussion

The major increase in prevalence of contaminated carcasses coming from negative flocks observed in our study during the processing module is consistent with results found in the literature (Allen et al. 2007; Dogan et al. 2019). Our estimated prevalence at the end of the processing module is however much higher than the one reported by these two studies (i.e., 60% vs 30%). This difference can be explained by the fact that, in our model, 75% of carcasses were contaminated at very low level (cf Figure 17 and Figure 18). If we adjust the prevalence at the end of the processing module assuming that all carcasses with a contamination level below 1 CFU/carcass are not contaminated, the mean prevalence obtained with our model drops to 21.3%, which is then fully consistent with Allen et al. (2007) and Dogan et al. (2019).

The mean bacteria contamination at the end of the processing module (considering negative and positive flocks together) estimated by our model (i.e., 3.0E+04 CFU/carcass, or 3.4 log10 CFU/carcass), was also consistent with what was estimated by Allen et al. (2007) (2.5 log10 CFU/carcass), Dogan et al. (2019) (2.5 log10 CFU/carcass), and Slader et al. (2002) (1.1 log10 CFU/carcass).

At the retailer level, the average prevalence of *Campylobacter spp.* contaminated chicken was estimated at 50%, which is consistent with the latest data available regarding the prevalence of contaminated fresh chicken in the UK (56%) (Jorgensen et al. 2019). The percentage of highly contaminated products, product with a bacteria load above 1000 CFU/g, at the retailer level estimated in our study equalled 6.4% and was consistent with the percentage of highly contaminated products estimated by (Jorgensen et al. 2019), 7%.

14.8 References

Allen, V. M., S. A. Bull, J. E. L. Corry, G. Domingue, F. Jørgensen, J. A. Frost, R. Whyte, A. Gonzalez, N. Elviss, and T. J. Humphrey. 2007. “Campylobacter Spp. Contamination of Chicken Carcasses during Processing in Relation to Flock Colonisation.” *International Journal of Food Microbiology* 113 (1): 54–61. https://doi.org/10.1016/j.ijfoodmicro.2006.07.011.

Allen, V. M., H. Weaver, A. M. Ridley, J. A. Harris, M. Sharma, J. Emery, N. Sparks, M. Lewis, and S. Edge. 2008. “Sources and Spread of Thermophilic Campylobacter

Spp. during Partial Depopulation of Broiler Chicken Flocks." *Journal of Food Protection* 71 (2): 264–70. https://doi.org/10.4315/0362-028X-71.2.264.

Battersby, Tara, Desmond Walsh, Paul Whyte, and Declan J. Bolton. 2016. "Campylobacter Growth Rates in Four Different Matrices: Broiler Caecal Material, Live Birds, Bolton Broth, and Brain Heart Infusion Broth." *Infection Ecology & Epidemiology* 6 (April). https://doi.org/10.3402/iee.v6.31217.

Berrang, M. E., R. J. Buhr, and J. A. Cason. 2000. "Campylobacter Recovery from External and Internal Organs of Commercial Broiler Carcass Prior to Scalding." *Poultry Science* 79 (2): 286–90. https://doi.org/10.1093/ps/79.2.286.

Berrang, M. E., R. J. Buhr, J. A. Cason, and J. A. Dickens. 2001. "Broiler Carcass Contamination with Campylobacter from Feces during Defeathering." *Journal of Food Protection* 64 (12): 2063–66. https://doi.org/10.4315/0362-028X-64.12.2063.

Berrang, M. E., and J. A. Dickens. 2000. "Presence and Level of Campylobacter Spp. on Broiler Carcasses Throughout the Processing Plant." *Journal of Applied Poultry Research* 9 (1): 43–47. https://doi.org/10.1093/japr/9.1.43.

Berrang, M.E., and J.S. Bailey. 2009. "On-Line Brush and Spray Washers to Lower Numbers of Campylobacter and Escherichia Coli and Presence of Salmonella on Broiler Carcasses during Processing." *Journal of Applied Poultry Research* 18 (1): 74–78. https://doi.org/10.3382/japr.2008-00067.

Biglia, Alessandro, Andrew J. Gemmell, Helen J. Foster, and Judith A. Evans. 2018. "Temperature and Energy Performance of Domestic Cold Appliances in Households in England." *International Journal of Refrigeration* 87 (March): 172–84. https://doi.org/10.1016/j.ijrefrig.2017.10.022.

Boysen, Louise, Susanne Knøchel, and Hanne Rosenquist. 2007. "Survival of Campylobacter Jejuni in Different Gas Mixtures." *FEMS Microbiology Letters* 266 (2): 152–57. https://doi.org/10.1111/j.1574-6968.2006.00525.x.

Bruhn, Christine. 2014. "Chicken Preparation in the Home: An Observational Study." *Food Protection Trends* 34 (September): 318–30.

Bucher, O., A. Fazil, A. Rajić, A. Farrar, R. Wills, and S. A. McEWEN. 2012a. "Evaluating Interventions against *Salmonella* in Broiler Chickens: Applying Synthesis Research in Support of Quantitative Exposure Assessment." *Epidemiology and Infection* 140 (5): 925–45. https://doi.org/10.1017/S0950268811001373.

———. 2012b. "Evaluating Interventions against Salmonella in Broiler Chickens: Applying Synthesis Research in Support of Quantitative Exposure Assessment."

Epidemiology & Infection 140 (5): 925–45. https://doi.org/10.1017/S0950268811001373.

Bucher, Oliver, Lisa Waddell, Judy Greig, and Ben A. Smith. 2015. “Systematic Review-Meta-Analysis of the Effect of Chilling on Campylobacter Spp. during Primary Processing of Broilers.” *Food Control* 56 (October): 211–17. https://doi.org/10.1016/j.foodcont.2015.03.032.

Chardon, Jurgen E., and Eric G. Evers. 2017. “Improved Swift Quantitative Microbiological Risk Assessment (SQMRA) Methodology.” *Food Control* 73 (March): 1285–97. https://doi.org/10.1016/j.foodcont.2016.10.049.

Collineau, Lucie, Brennan Chapman, Xu Bao, Branavan Sivapathasundaram, Carolee A. Carson, Aamir Fazil, Richard J. Reid-Smith, and Ben A. Smith. 2020. “A Farm-to-Fork Quantitative Risk Assessment Model for Salmonella Heidelberg Resistant to Third-Generation Cephalosporins in Broiler Chickens in Canada.” *International Journal of Food Microbiology* 330 (October): 108559. https://doi.org/10.1016/j.ijfoodmicro.2020.108559.

Dogan, Onay Burak, Jennifer Clarke, Fabio Mattos, and Bing Wang. 2019. “A Quantitative Microbial Risk Assessment Model of Campylobacter in Broiler Chickens: Evaluating Processing Interventions.” *Food Control* 100 (June): 97–110. https://doi.org/10.1016/j.foodcont.2019.01.003.

EcoSure. 2007. “EcoSure 2007 Cold Temperature Database.” 2007. https://www.foodrisk.org/resources/display/21.

EVANS, ELLEN W., and ELIZABETH C. REDMOND. 2016. “Time-Temperature Profiling of United Kingdom Consumers’ Domestic Refrigerators.” *Journal of Food Protection* 79 (12): 2119–27. https://doi.org/10.4315/0362-028X.JFP-16-270.

Evans, S. J, and A. R Sayers. 2000. “A Longitudinal Study of Campylobacter Infection of Broiler Flocks in Great Britain.” *Preventive Veterinary Medicine* 46 (3): 209–23. https://doi.org/10.1016/S0167-5877(00)00143-4.

FAO. 2007. “Food Safety Risk Profile for Campylobacter Species in Broiler (Young) Chickens.” FAO. http://www.fao.org/tempref/codex/Meetings/CCFH/CCFH40/fh40rpcb.pdf.

FAO/WHO, Niels. 2002. “Risk Assessments of Salmonella in Eggs and Broiler Chickens.” *International Journal of Food Microbiology* 91 (2): 223. https://doi.org/10.1016/S0168-1605(03)00369-6.

Fraqueza, M.J., A. Martins, A.C. Borges, M.H. Fernandes, M.J. Fernandes, Y. Vaz, R.J.B. Bessa, and A.S. Barreto. 2014. “Antimicrobial Resistance among

Campylobacter Spp. Strains Isolated from Different Poultry Production Systems at Slaughterhouse Level." *Poultry Science* 93 (6): 1578–86. https://doi.org/10.3382/ps.2013-03729.

Georgiev, M., W. Beauvais, and J. Guitian. 2017. "Effect of Enhanced Biosecurity and Selected On-Farm Factors on Campylobacter Colonization of Chicken Broilers." *Epidemiology & Infection* 145 (3): 553–67. https://doi.org/10.1017/S095026881600251X.

Gerwen, S. J. van, and M. H. Zwietering. 1998. "Growth and Inactivation Models to Be Used in Quantitative Risk Assessments." *Journal of Food Protection* 61 (11): 1541–49. https://doi.org/10.4315/0362-028x-61.11.1541.

Hald, B., E. Rattenborg, and M. Madsen. 2001. "Role of Batch Depletion of Broiler Houses on the Occurrence of Campylobacter Spp. in Chicken Flocks." *Letters in Applied Microbiology* 32 (4): 253–56. https://doi.org/10.1046/j.1472-765x.2001.00896.x.

Hansson, I., M. Ederoth, L. Andersson, I. Vågsholm, and E. Olsson Engvall. 2005. "Transmission of Campylobacter Spp. to Chickens during Transport to Slaughter." *Journal of Applied Microbiology* 99 (5): 1149–57. https://doi.org/10.1111/j.1365-2672.2005.02689.x.

Herman, L., M. Heyndrickx, K. Grijspeerdt, D. Vandekerchove, I. Rollier, and L. De Zutter. 2003. "Routes for Campylobacter Contamination of Poultry Meat: Epidemiological Study from Hatchery to Slaughterhouse." *Epidemiology & Infection* 131 (3): 1169–80. https://doi.org/10.1017/S0950268803001183.

Heuer, O. E., K. Pedersen, J. S. Andersen, and M. Madsen. 2001. "Prevalence and Antimicrobial Susceptibility of Thermophilic Campylobacter in Organic and Conventional Broiler Flocks." *Letters in Applied Microbiology* 33 (4): 269–74. https://doi.org/10.1046/j.1472-765X.2001.00994.x.

Hinton, Arthur, J.A. Cason, and Kimberly D. Ingram. 2004. "Tracking Spoilage Bacteria in Commercial Poultry Processing and Refrigerated Storage of Poultry Carcasses." *International Journal of Food Microbiology* 91 (2): 155–65. https://doi.org/10.1016/S0168-1605(03)00377-5.

Hue, Olivier, Sophie Le Bouquin, Marie-José Laisney, Virginie Allain, Françoise Lalande, Isabelle Petetin, Sandra Rouxel, et al. 2010. "Prevalence of and Risk Factors for Campylobacter Spp. Contamination of Broiler Chicken Carcasses at the Slaughterhouse." *Food Microbiology* 27 (8): 992–99. https://doi.org/10.1016/j.fm.2010.06.004.

Hutchison, M. L., M. J. Taylor, M. A. Tchòrzewska, G. Ford, R. H. Madden, and T. G. Knowles. 2017. “Modelling-Based Identification of Factors Influencing Campylobacters in Chicken Broiler Houses and on Carcasses Sampled after Processing and Chilling.” *Journal of Applied Microbiology* 122 (5): 1389–1401. https://doi.org/10.1111/jam.13434.

Jorgensen, F., J. Ellis-Iversen, S. Rushton, S. A. Bull, S. A. Harris, S. J. Bryan, A. Gonzalez, and T. J. Humphrey. 2011. “Influence of Season and Geography on Campylobacter Jejuni and C. Coli Subtypes in Housed Broiler Flocks Reared in Great Britain.” *Applied and Environmental Microbiology* 77 (11): 3741–48. https://doi.org/10.1128/AEM.02444-10.

Jorgensen, Frieda, Andre Charlett, Craig Swift, and Nicolae Corcionivoschi. 2019. “A Microbiological Survey of Campylobacter Contamination in Fresh Whole UK-Produced Chilled Chickens at Retail Sale.” Project FS102121. FSA.

Meldrum, R. J., D. Tucker, and C. Edwards. 2004. “Baseline Rates of Campylobacter and Salmonella in Raw Chicken in Wales, United Kingdom, in 2002.” *Journal of Food Protection* 67 (6): 1226–28. https://doi.org/10.4315/0362-028X-67.6.1226.

Nauta, Maarten J., Wilma F. Jacobs-Reitsma, and Arie H. Havelaar. 2007. “A Risk Assessment Model for Campylobacter in Broiler Meat.” *Risk Analysis* 27 (4): 845–61. https://doi.org/10.1111/j.1539-6924.2006.00834.x.

Newell, D. G., K. T. Elvers, D. Dopfer, I. Hansson, P. Jones, S. James, J. Gittins, et al. 2011. “Biosecurity-Based Interventions and Strategies To Reduce Campylobacter Spp. on Poultry Farms.” *Applied and Environmental Microbiology* 77 (24): 8605–14. https://doi.org/10.1128/AEM.01090-10.

Rosenquist, Hanne, Louise Boysen, Anne Louise Krogh, Annette Nygaard Jensen, and Maarten Nauta. 2013. “Campylobacter Contamination and the Relative Risk of Illness from Organic Broiler Meat in Comparison with Conventional Broiler Meat.” *International Journal of Food Microbiology* 162 (3): 226–30. https://doi.org/10.1016/j.ijfoodmicro.2013.01.022.

Rosenquist, Hanne, Helle M. Sommer, Niels L. Nielsen, and Bjarke B. Christensen. 2006. “The Effect of Slaughter Operations on the Contamination of Chicken Carcasses with Thermotolerant Campylobacter.” *International Journal of Food Microbiology* 108 (2): 226–32. https://doi.org/10.1016/j.ijfoodmicro.2005.12.007.

Russa, A. D., A. Bouma, J. C. M. Vernooij, W. Jacobs-Reitsma, and J. A. Stegeman. 2005. “No Association between Partial Depopulation and Campylobacter Spp.

Colonization of Dutch Broiler Flocks." *Letters in Applied Microbiology* 41 (3): 280–85. https://doi.org/10.1111/j.1472-765X.2005.01751.x.

Santini, Cecilia. n.d. "Characterization of Probiotic Strains: An Application as Feed Additives in Poultry against Campylobacter Jejuni | Elsevier Enhanced Reader." Accessed February 26, 2021. https://doi.org/10.1016/j.ijfoodmicro.2010.03.039.

Slader, J., G. Domingue, F. Jørgensen, K. McAlpine, R. J. Owen, F. J. Bolton, and T. J. Humphrey. 2002. "Impact of Transport Crate Reuse and of Catching and Processing on Campylobacter and Salmonella Contamination of Broiler Chickens." *Applied and Environmental Microbiology* 68 (2): 713–19. https://doi.org/10.1128/aem.68.2.713-719.2002.

Wedderkopp, A., E. Rattenborg, and M. Madsen. 2000. "National Surveillance of Campylobacter in Broilers at Slaughter in Denmark in 1998." *Avian Diseases* 44 (4): 993–99. https://doi.org/10.2307/1593078.

WHO, and FAO, eds. 2009. *Risk Assessment of Campylobacter Spp. in Broiler Chickens. Technical Report*. Microbiological Risk Assessment Series 12. Geneva: World Health Organization : Food and Agriculture Organization of the United Nations.

15. Appendix 6: Case study 3 - *E. coli* in outdoor grown pre-cut and pre-washed lettuce

15.1 Introduction

Reasons for this food product choice included the higher susceptibility of microbial contamination of outdoor lettuce compared to indoor grown lettuce. Indoor lettuce is more protected from the outside environment (Holvoet et al. 2015) and is associated with the increased sale of pre-cut and pre-washed bags of salad in the UK (Sheane, McCosker, and Lillywhite 2017). Most of the lettuce grown in the UK is grown outdoors, however, about 20% is grown in glasshouses (British Leafy Salad Association 2021).

Like before, a conservative approach was used to inform the model, that is, decisions regarding model inputs for which data were sparse erred on the side of selecting inputs that provide a worst-case scenario.

The challenge for ensuring safe produce is greatest for those vegetable products that are eaten uncooked, such as leafy salad vegetables. Even low levels of pathogens on these products could result in a considerable disease burden (Monaghan et al. 2017). Importantly, the microbial contamination that occur at field production might not be eliminated during further processing steps (Tyrrel, Knox, and Weatherhead 2006; Sapers 2001). Most of the factors affecting the risk of *E. coli* contamination in outdoor grown lettuce might also apply to other bacteria

The values of the *selected variables* used in this case study are reported bellow:

- Product = Lettuce
- Bacteria = *E. coli*
- Gene = None
- Pack_type = packaging
- Product_cut = yes
- Product_washed =Pre-washed

15.2 Estimated variables for Production module

Table 1 shows the list of the estimated input parameters for the production module and the associated probability distributions, values and references. The parameters refer to the stages of farm production, harvesting and packing. A few parameters were not parameterized due to lack of specific data or because they were not relevant for E. coli but could be relevant for other hazards and therefore, for this specific case study, were set to no effect (1) or 0 depending on the parameter.

Pang et al. (2017) developed a QMRA model for *E. coli* O157:H7 in fresh-cut lettuce in the United States and evaluated the effects of different potential intervention strategies on the reduction of public health risk. Several variables used in this model (see Table 1) were parametrized using the same input probability distributions in Pang *et al.* (2017).

The mean prevalence of contaminated lettuce at pre-harvest (*Prev_base*) was defined at 5% based on (Holvoet et al. 2013). However, no data could be found regarding variability and/or uncertainty associated with this parameter. We thus modelled uncertainty by assuming that this variable follows a normal distribution of average 0.05 and standard deviation equals to 25% of 0.05, 0.015.

With regards the factors related to the harvest season, according to the British Leafy Salad Association, the UK season for whole-head lettuce begins around the middle of May and finishes at the end of October (British Leafy Salad Association 2021). No data were found on the actual proportion of lettuce harvested during the high-risk season (*Prop_season*); this parameter was therefore set to 0 in the current model.

Some studies conducted under outdoor conditions, have shown than seasonality affects the survival of *E. coli* in vegetables. A study conducted by Oliveira *et al.* (2012) in Spain, showed that the *E. coli* O157:H7 counts in lettuce leaves was higher in autumn than in spring. The differences in temperature and humidity between seasons were mentioned as possible factors influencing *E. coli* presence, as well as other factors such as solar radiation and soil composition. In a study conducted in USA, various green produce (including spinach) sampled in the fall or in spring was respectively over six times (OR=6.4) and about 1.4 times (OR= 1.36) as likely to be contaminated with *E. coli* as produce sampled in the winter; in the final model used

by the authors the analysis was limited to the 755 (82%) samples from the six produce items that were found in earlier analyses to have detectable concentrations of *E. coli* so spinach and other species were excluded (Ailes et al, 2008). We could not find specific data in the UK or in Europe on the factor representing the impact of high-risk season on prevalence of contamination (*F_season*) suitable to parameterize the model parameter, so the data (OR=6.4) from Ailes *et al.* (2008) was used to build a Pert probability distribution for this parameter.

Animal manure can be used as a fertilizer in crops (Bicudo and Goyal 2003). *E. coli* is part of the gut flora of many animal species and therefore, animal manure can be a source of contamination of *E. coli* for soil and crops (Bicudo and Goyal 2003; Smet et al. 2008). Pathogens such as *E. coli* O157 can survive for several months following the spreading of farm manures. In the UK, the use of untreated manure and slurry is discouraged in the production of ready to eat crops within 12 months of harvest and less than 6 months before planting (VMD/FSA/APHA 2016). We thus assume that no producer use untreated manure and the proportion of lettuce fertilized with untreated manure (*Prop_fert*) was estimated as zero.

Manures and other animal wastes are widely used in organic farming. Therefore, potentially, microbial contamination of organically grown plants may be higher than in conventional cultivation, where chemical treatments may reduce the microbial loading of the raw products (Szczech et al. 2018). Szczech *et al.* (20018) and other studies (Mukherjee et al, 2007, Luna-Guevara et al., 2019) further elaborate on this hypothesis. A study implemented in 14 organic (certified by accredited organic agencies), 30 semi-organic (used organic practices but not certified) and 19 conventional farms in Minnesota and Wisconsin (Mukherjee et al, 2007) were conducted to determine the prevalence of *E. coli* in pre-harvest fruits and vegetables. The use of animal wastes for fertilization of produce plants increased the risk of *E. coli* contamination in organic (OR=13.2, 95% CI=2.2–61.2) and semi-organic (OR=12.9, 95% CI=2.9–56.3) produce significantly. The low number of positive *E. coli* samples in conventional farming prevented any risk-factor analyses involving conventional farms. No data specific for the UK were found from the literature review suitable to parametrize this parameter. Adopting a conservative approach, we used data (OR=13.2) from the organic farming in Mukherjee *et al.* (2007) to parametrize the factor representing the possible impact of untreated manure on prevalence of contamination (*F_fert*). However, because *Prop_fert* was set to zero, the impact of

untreated manure on prevalence of contamination was actually considered as null in our model.

With regards to biosecurity measures adopted by producers, the UK fresh produce industry is characterized by very high production standards in response to the UK Food Safety Act (1990) (Monaghan et al, 2008). Many growers of fresh produce in the UK are required by their customers to apply strict standards of production to reduce the risks of microbial contamination (Finch, Samuel, and Lane 2014). The most applied scheme in the UK is the Red Tractor Fresh Produce (RTFP) Scheme, which include general standards for fresh produce (Red Tractor Certified Standards for farms 2019) and specifically for outdoor lettuce (Red Tractor Assurance for Farms 2017). Various factors are imputable for an increased risk of *E. coli* contamination including use of untreated manures and other animal wastes (see above), presence of wildlife, farmed animals and pests, worker health and hygiene practices.

Workers on the field can transfer microorganisms to fresh leafy vegetables by direct contact (EFSA 2014). Adequate hygienic practices of workers are essential to minimize the risk of contamination of leafy greens. This includes adequate hygiene, hand washing and drying, and if necessary, the use of gloves (Suslow et al. 2003). Furthermore, knives and cutting edges used to trim lettuce as well as containers used for transportation should be cleaned and disinfected to avoid cross-contamination (Codex Alimentarius Commission 2019). The presence of wildlife and pests represent a potential source of *E. coli* in field crops. The faeces of wild animals may be a source as well as flies and other insects of fresh products (Luna-Guevara et al. 2019). However, the risks posed by livestock, wildlife and pests for microbial contamination of lettuce crops depend on the prevalence and burden of pathogens carried by the hosts and their interaction with the production field (Holvoet et al. 2015). The situation may plausibly vary extensively at a national level depending on these factors. Specific recent data for the UK were not found in our literature search. Monaghan *et al*. (2008) reported that growers recognize the potential risk posed by livestock faeces. A common action taken to minimize access from domesticated animals was to maintain or improve fencing which was reported by 38% of businesses. To minimize access to the crops from wildlife, netting and fencing were employed by 62% of businesses. We could not find any data from the UK suitable to this case study related to the impact of poor biosecurity on contamination load (***F_biosecurity)***. However, Liu *et al.* (2016) studied the impact of climate and management variables on the contamination of preharvest

leafy greens with *E. coli* through the use of different statistical models in Belgium, Brazil, Egypt, Norway, and Spain. Management variables included also the presence of farm animals which, in the univariable analysis, was statistically associated with the presence of *E. coli* in the lettuce (OR=3.19). This estimate, despite referring to one management practice only, was used to parametrize (*F_biosecurity*) in the current case study.

The very large majority of producers have fully adopted standards to guarantee hygiene, based on the results from Monaghan *et al.* (2008). In reference to hygiene standards: 90% of producers received formal training on hygiene; hand washing was required by harvesting crew in 81% of producers; nearly all businesses (95%) provided toilet and hand wash facilities for all staff; nearly all businesses (95%) had a formal sickness policy and return to work procedure; almost all businesses (95%) regularly cleaned harvest trays and crates, and all businesses (100%) used dedicated harvest containers. The exceptions were related mostly to small producers. In general, specific data on proportion of farms with good or poor biosecurity measures in the UK is lacking. However, we assume that the proportion of farms with poor biosecurity practices (*Prop_biosecurity*) is low (<5%), given that leafy production in the UK is strongly regulated by safety standards (Monaghan et al. 2008).

Whole-head lettuce is cut and wrapped by hand, while baby leaf salads are generally harvested by a machine (British Leafy Salad Association 2021). To guarantee the best shelf-life lettuce need to be cooled as quickly as possible after harvesting. Growers aim to cool the salad leaves to 3°C within 3 hours of leaving the field. Terry *et al.* (2011) reported that in the UK, lettuce is cooled down to 4°C after harvest and stored with ca. 100% relative humidity to prevent dehydration and respiration. According to Gil *et al.* (2015) leafy greens must be cooled rapidly as soon as possible (less than 90 minutes) after harvest. The temperature of lettuce when it is received in the processing plant should be lower than 5°C (Varzakas and Arvanitoyannis, 2008). The temperature of field cooling (*T_field_cool)* was parameterized as a truncated Normal distribution (mean = 4°C, sd = 0.5, min = 3°C, max = 8°C).

We could not find specific data in the UK related to the duration of the process of field cooling (*Time_field_cool*). Since the gap between harvest and transportation to processing unit should be as short as possible to minimize the risk of quality loss of

vegetables (Codex Alimentarius: Code of Hygienic Practice for Fresh Fruits and Vegetable, 2003), it is likely that the field cooling is a reasonably short period after the harvesting. Variation related to production practices may exist between producers though. We therefore used a uniform distribution to parametrize this input data expressed in hours (minimum:3; maximum:8).

Table 40: List of estimated variables related to the production module. Grey = the value of these variables are the same than for the chicken value chain and the case study AMR1

Variable Name	Description	Value/Distribution	Unit	Source
C_water	Concentration of bacteria in irrigation water	Uniform(1, 235)	CFU/ 100m L	(Pang et al. 2017)
W_water	Volume of water remaining on lettuce after overhead irrigation	Truncated Normal(0.108, 0.019, min=0)	ml/g	(Pang et al. 2017)
Time_hold	Time interval between last irrigation and harvest	Triangular(2,4,8)	days	(Pang et al. 2017)
C_soil	Soil bacteria concentration	Truncated Normal(0.928, 1.11, max(3.67), min(0))	CFU/ g	(Pang et al. 2017)
W_soil	Attached soil on harvesting blades	10.22	g	(Pang et al. 2017)
Prop_season	Proportion of lettuce harvested during the high-risk season. 'high risk season' must be defined for each microorganism.	0	Prop ortion	NA

Variable Name	Description	Value/Distribution	Unit	Source
TR_blade	Transfer rate from harvesting blades to lettuce	0.013	-	(Pang et al. 2017)
F_season	Factor representing the impact of high-risk season on prevalence of contamination. 'High risk season' must be defined for each microorganism.	Pert (2.9, 6.4, 13.8)	Odds ratio	(Ailes et al, 2008)
Prop_biosecurity	Proportion of farms with poor biosecurity practices	Triangular (0.01, 0.03, 0.05)	Proportion	(Monaghan, 2008)
F_biosecurity	Factor representing the impact of poor biosecurity on contamination load.	Pert (1.7, 3.2, 5.9)	Odds ratio	(Liu et al, 2016)
Prop_fert	Proportion of lettuce fertilized with untreated manure.	0	Proportion	
F_fert	Factor representing the impact of untreated manure on prevalence of contamination.	Pert (2.2, 13.2, 61.2)	Odds ratio	(Mukherjee et al, 2007)
Prev_base	Average prevalence of lettuces contaminated at pre-harvest	Normal(0.05, 0.015)	Prevalence	(Holvoet et al. 2013)
Size_mean	Lettuce head size mean	Normal(662.6, 112.7)	g	(Njage and Buys 2017)

Variable Name	Description	Value/Distribution	Unit	Source
T_field	Temperature during field cooling	Truncated Normal(4, 0.5, min(3), max(8))	Celsius degrees	(British Leafy Salad Association, 2021); (Terry et al. 2011); (Varzakas and Arvanitoyannis, 2008)
Time_field	Duration of field cooling	Uniform (3,8)	Hours	Authors estimates
Time_gen_min	Minimum generation time in food product	0.47	hours	(Evers et al. 2017)
T_growth_min	Minimum growth temperature	7	Degree C	(Food Standard Agency 2018)
T_growth_opt	Optimal growth temperature	Pert (35, 37, 40)	Degree C	(Food Standard Agency 2018)

15.3 Estimated variables for the Processing module

Table 2 shows the list of the estimated input parameters for the Processing module and the associated probability distributions, values and references. Pathogens remained in the lettuce after the washing step (*D_wash_proc*) may transfer to flume tanks, shredders, shakers, centrifuges and conveyor belts. We used same distribution variables as in Pang

et al. (2017) and references therein since they were appropriate for this case study too. With regards the transfer rates, the authors used values described in Perez-Rodriquez *et al.* (2011). The authors performed a stochastic model to evaluate *E. coli* O157:H7 cross contamination in a processing line for fresh-cut lettuce. Transfer coefficients were estimated exclusively though experimental data obtained at low contamination levels (2 log cfu/g) and probability distributions were fitted to these experimental data. The spread of contamination due to cross contamination (*TR_overall*) was parametrized using data from an expert elicitation exercise in the US (U.S. Food and Drug Administration 2012).

Table 41: list of estimated variables related to the processing module

Variable Name	Description	Value/Distribution	Unit	Source
Size_bag	Size of a bag of lettuce	Uniform(100, 200)	g	Authors' estimate
D_wash_proc	Log reduction by washing with water	Pert(-1.4,-1,-0.6)	Log CFU /g	(Perez-Rodriguez et al. 2011)
TR_flume	Transfer from contaminated lettuce to flume	Triang(0, 0.0001, 0.0002)	Proportion	(Perez-Rodriguez et al. 2011)
TR_shred	Transfer from contaminated lettuce to shredder	Triang(0, 0.0002, 0.0002)	Proportion	(Perez-Rodriguez et al. 2011)
TR_shake	Transfer from contaminated lettuce to shaker	Triang(0, 0.0001, 0.0002)	Proportion	(Perez-Rodriguez et al. 2011)
TR_centri	Transfer from contaminated lettuce to centrifuge	Triang(0.0001, 0.0004, 0.0008)	Proportion	(Perez-Rodriguez et al. 2011)

Variable Name	Description	Value/Distribution	Unit	Source
TR_convey	Transfer from contaminated lettuce to conveyor	Triang(0, 0.001, 0.0024)	Proportion	(Perez-Rodriguez et al. 2011)
TR_facility	Overall transfer coefficient from facilities to uncontaminated lettuce	Triang(0.99, 0.1533, 0.1883)	Proportion	(Perez-Rodriguez et al. 2011)
TR_overall	Spread of contamination due to cross-contamination	Pert(1,1.2,2)	-	(U.S. Food and Drug Administration 2012)

15.4 Estimated variables for the post-Processing module

The table 3 shows the list of estimated variables related to the post-processing module. A large proportion of the variables used in this module were the same than in the chicken case studies as these variables were not specific to any food product or microorganism.

Table 42: List of estimated variables related to the post-processing module. Grey = the value of these variables are the same than for the chicken value chain and the case study AMR1

Variable Name	Description	Value/Distribution	Units	Source
Time_retail	Number of days stored at retail	Triangular (1,3,7)	Days	(Collineau et al. 2020)
T_retail	Temperature at retail storage	Laplace (-6.67, 3.3333 19.44)	Degree C	(EcoSure 2007)

Variable Name	Description	Value/Distribution	Units	Source
Time_trans	Mean (90%CI) home transport duration	Normal(69.6, 0.438)	Minutes	(Collineau et al. 2020)
T_post_trans	Chicken temperature at the end of home transport	Shifted Loglogistic Truncate (29.371, 16.763, -22.915, min = - 5.56, max = 20)	Degree C	(EcoSure 2007)
Time_fridge	Mean (90%CI) number of days refrigerated at home	Normal(2.2, 0.0203)	days	(Collineau et al. 2020)
T_fridge	Home refrigeration temperature	Laplace (-4.44, 5.3, 16.11)	Degree C	(Biglia et al. 2018; Evans and Redmond 2016; EcoSure 2007)
F_pack	Reduction factor of bacterial load because of packaging	Triangular (-0.1, −0.2, −0.3)	logCFU	(Thomas et al. 2020)
C_MPD	Maximum population density	10^7		(Pang et al. 2017)

15.5 Estimated variables for the Home preparation module

Because lettuce were assumed to be always consumed raw, only two estimated variables were estimated in the home-preparation module (see table 4). The average size of a serving (Serv) was based on Pang et al. (2017) and equalled 85 g.

No data could be found on the decimal reduction due to lettuce washing at home. We assumed that reduction of bacteria load would be similar to the one observed in the processing module.

Table 43: List of estimated variables related to the home-preparation module.

Variable Name	Description	Value/Distribution	Units	Source
D_wash_home	Log decimal reduction due to washing	Pert(-1.4,-1,-0.6)	Log CFU /g	Authors' estimate
Serv	Serving size	85	g	(Pang et al. 2017)

15.6 Results

15.6.1 Overall risk of exposure

The overall risk of *E. coli* bacteria exposure represents the average prevalence, and level of contamination, of contaminated servings, given the estimated proportion of positive and negative lettuces in the overall production population. Figure 6 presents the probability density functions associated with each *outcome variables* considered in the modelling framework. Table 5 presents more specifically the numeric values of the mean and median of each outcome variables.

These results show an overall slight increase of the prevalence of contaminated products and level of contamination per contaminated product during the processing module, in response to exposure to the AMR gene. The median prevalence of contaminated servings equalled 4%. The median number of bacteria per serving was always low (< 1 CFU/g) but some lettuces end up being highly contaminated at the end of the post-processing module due to bacteria growth either at retail or at home as illustrated by the value of the mean (> 3.7E+05 CFU/g) and the results of the correlation analysis.

Table 44: Mean and median overall risk estimation per module

Module	Output variables	Unit	Mean	Median
Production	*Prev_prod*	Prevalence of birds contaminated with bacteria arriving at the slaughterhouse	0.05	0.05
Production	*C_prod*	CFU of bacteria /bird arriving at the slaughterhouse	0	0
Processing	*Prev_proc*	Prevalence of carcasses contaminated with bacteria (%)	0.07	0.06
Processing	*C_proc*	CFU of A bacteria / carcasses	0	0
Post-processing	*Prev_pproc*	Prevalence of food item contaminated with bacteria (%)	0.04	0.04
Post-processing	*C_pproc*	CFU of bacteria /food item	4.0E+04	1
Home preparation	*P_home_cook*	Probability of exposure to bacteria through direct contamination (%)	0.04	0.04
Home preparation	*C_home_cook*	CFU of bacteria ingested by contaminated food item	3.7E+03	0.1

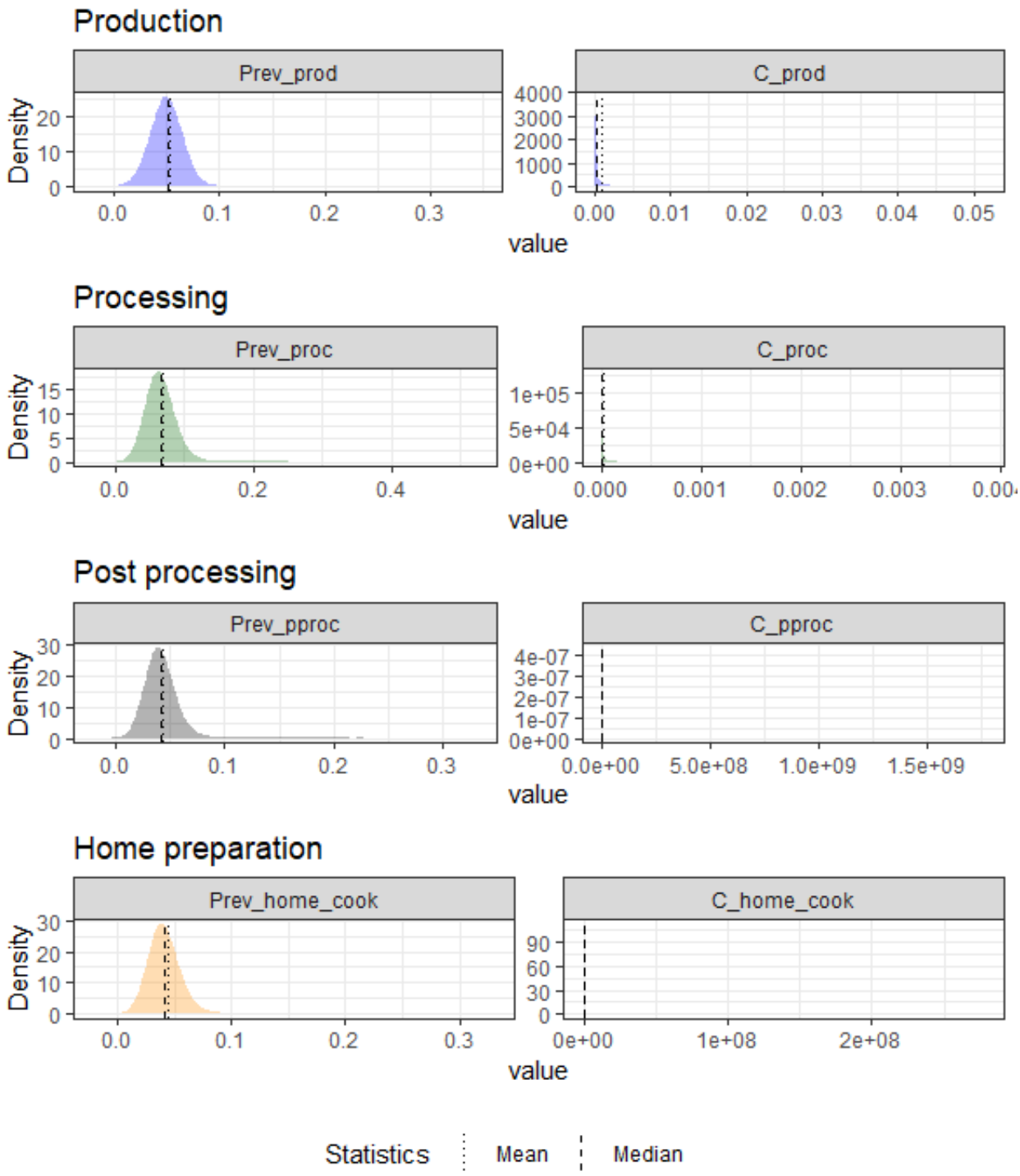


Figure 1: Probability distributions of the outcome variables associated with the production, processing, post-processing and home-preparation modules after 100 000 simulations.

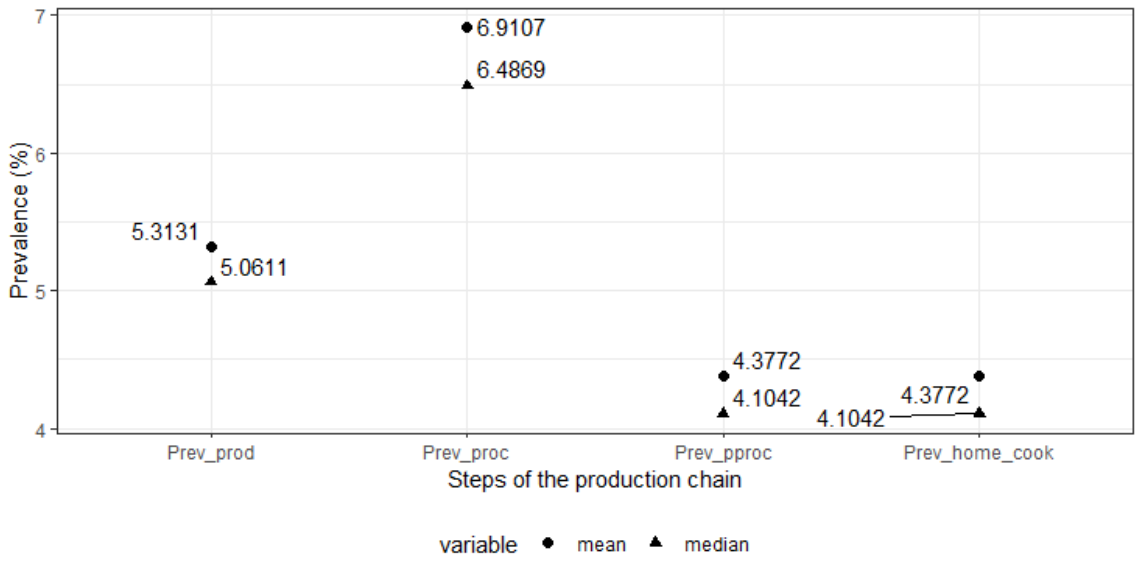


Figure 2: Mean and median prevalence of contaminated item of interest from the production to the home-preparation module

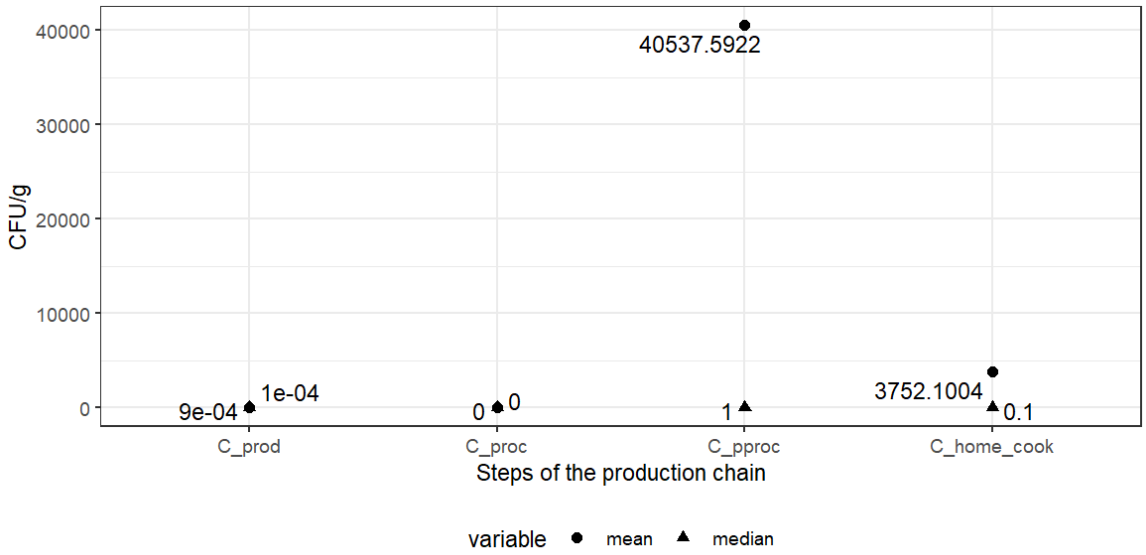


Figure 3: Median levels of bacterial contamination per gramme of contaminated item of interest from the production to the home-preparation module

15.6.2 Correlation analysis

The results of the correlation analysis performed for each outcome variables are presented in Figure 4. Only estimated variables with a Spearman correlation coefficient ≥ |0.025| have been included in the figure. The estimated variables with a lower Spearman correlation coefficient were considered as having a negligible effect on the model outcomes.

Prevalence of contamination

As expected, the prevalence of contaminated product throughout the production chain was mainly influenced by the baseline prevalence of contaminated lettuce (*Prev_base*). Steps of the processing module aiming at reducing the level of bacterial contamination do influence the number of bacteria per lettuce head but do not really influence the prevalence of contaminated products. The second variable with the highest impact on the prevalence of contaminated lettuce was the probability of cross contamination occurring during the processing phase (TR_overall).

Farm practices (*Prop_biosecurity*) also influence the results. With regards, the *Prop_biosecurity* parameter, the input data used to fit the distribution were based on one management practice only from studies in in Belgium, Brazil, Egypt, Norway, and Spain (Liu, Hofstra, and Franz 2016). We failed to retrieve input data describing the cumulative effect of multiple management practices. Therefore, the interpretation of the correlation analysis outputs related to this parameter should be considered with caution. In order to address the lack of data and specific variability in this parameter, further development of the lettuce model could consider the integration of specific individual biosecurity parameters particularly relevant for the lettuce production sector.

Level of bacterial contamination

In terms of bacterial load contamination, many estimated variables have a large influence on the model outcome (i.e., Spearman correlation coefficient > |0.5|) and some are briefly discussed here.

As expected, the concentration in bacteria of irrigation water and soil was strongly associated with the bacteria concentration at the production and processing level. The holding time decreased the level of bacteria concentration in the production module. However, the step of field cooling seems to have either no effect, or a negligible effect on the model outcome (Spearman correlation coefficient < |0.025|).

As also expected, all the transfer factors used in the processing module seem to have a significant impact on the outcome of this module. The figure 9 should be however interpreted carefully. Indeed, the apparently strong correlation coefficients of 1 or -1 are largely due to the nature of the equations used in this module: all these equations imply a monotonic relationship between the contamination load during the processing steps

(*C_proc*) and the various transfer factors considered. In this case, due to the monotonic relationship, it was plausible to observe a correlation coefficient of 1 or -1 between these variables. However, none of these parameters influence the outcomes of the two following modules suggesting that, if the correlation between the variables is strong, the actual influence of these transfer factors on the final level of lettuce contamination is small relative to other parameters.

The factors having the biggest impact on the level of lettuce contamination for the consumer are the temperature at retail. The quality of the washing of the lettuce before consumption appears also as a major step to reduce bacteria load on contaminated product. It should be noted, however, that no data could be found to precisely estimate the efficiency of home washing in reducing bacteria contamination. In our study we used the same value as reported at the processing level, but more information would be needed.

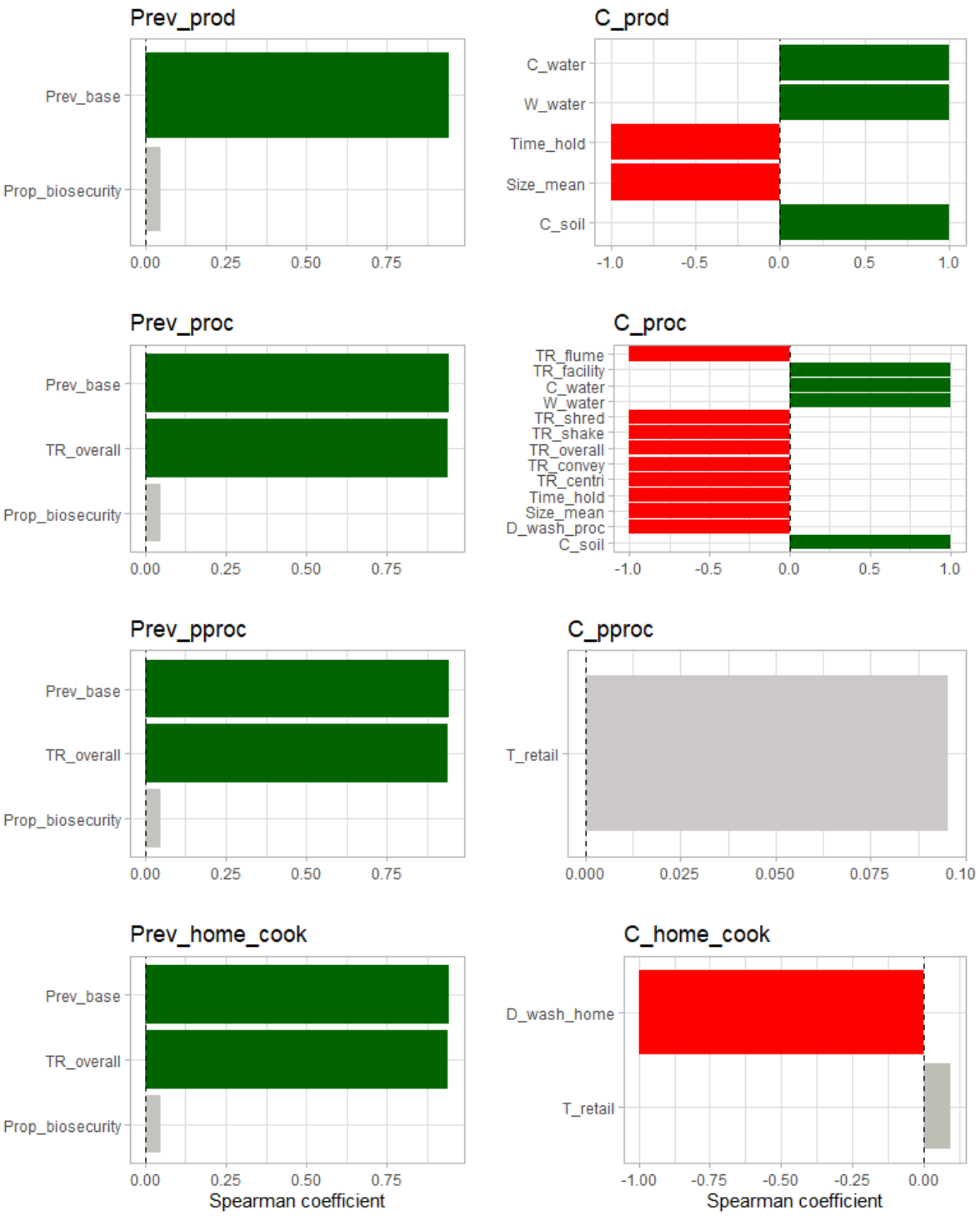


Figure 4: Correlation of the model estimated variables with the outcome variables. Only estimated variables with spearman correlation coefficient ≥ |0.025| have been included in the tornado charts. The darker the color (either green or red), the stronger the correlation.

15.7 Model validation and discussion

Validating the results of our model with the existing literature is challenging because the quantitative risk assessments published on the lettuce sector do not model the dynamic of interim prevalence or contamination load along the different steps of the production chain. Instead, these assessments present only final estimates related to the expected number of illness (see for example Pang et al. (Pa2017) or O'Flaherty et al. (2019)) making comparison with our own results impossible. The results retrieved from our literature search are related to specific type of *E. coli* growth in different production systems (for example, *E. coli* O157:H7 in Australia (Bozkurt et al. 2021)) or *E. coli* carrying antimicrobial resistance gene (for example, ESBL/AmpC positive *E. coli* in South Africa (Njage and Buys 2017)) making the comparison with our own study challenging.

Sagoo et al. (2001) reported a prevalence of 0.5% of unsatisfactory uncooked ready-to-eat organic vegetables sampled at retail in the UK. In this study, 'unsatisfactory' means that *E. coli* count was above 10^2 CFU/g. In our study the prevalence of contaminated servings equaled 4% but included all products with a bacterial contamination load above 1 CFU/g. Focusing only on the servings contaminated at more than 10^2 CFU/g like Sagoo et al., we would obtain a median prevalence of 'unsatisfactory' servings of 0.3% in line with their study results.

15.8 References

Biglia, Alessandro, Andrew J. Gemmell, Helen J. Foster, and Judith A. Evans. 2018. "Temperature and Energy Performance of Domestic Cold Appliances in Households in England." *International Journal of Refrigeration* 87 (March): 172–84. https://doi.org/10.1016/j.ijrefrig.2017.10.022.

Bozkurt, Hayriye, Tina Bell, Floris van Ogtrop, Kim-Yen Phan-Thien, and Robyn McConchie. 2021. "Assessment of Microbial Risk during Australian Industrial Practices for Escherichia Coli O157:H7 in Fresh Cut-Cos Lettuce: A Stochastic Quantitative Approach." *Food Microbiology* 95 (May): 103691. https://doi.org/10.1016/j.fm.2020.103691.

"British Leafy Salad Association." 2021. 2021. https://www.britishleafysalads.co.uk/know/faq.shtml#:~:text=The%20UK%20season%20for%20wholehead,will%20take%20place%20around%20April.

"Codex Alimentarius: Code of Hygienic Practice for Fresh Fruits and Vegetable." 2003. FAO, WTO. http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B53-2003%252FCXC_053e.pdf.

EcoSure. 2007. "EcoSure 2007 Cold Temperature Database." 2007. https://www.foodrisk.org/resources/display/21.

EVANS, ELLEN W., and ELIZABETH C. REDMOND. 2016. "Time-Temperature Profiling of United Kingdom Consumers' Domestic Refrigerators." *Journal of Food Protection* 79 (12): 2119–27. https://doi.org/10.4315/0362-028X.JFP-16-270.

Food Standard Agency. 2018. "Chapter 2.8 Animal By-Products." Manual for Official Controls. Food Standard agency. https://www.food.gov.uk/business-guidance/manual-for-official-controls.

Holvoet, Kevin, Imca Sampers, Benedicte Callens, Jeroen Dewulf, and Mieke Uyttendaele. 2013. "Moderate Prevalence of Antimicrobial Resistance in Escherichia Coli Isolates from Lettuce, Irrigation Water, and Soil." *Applied and Environmental Microbiology* 79 (21): 6677–83. https://doi.org/10.1128/AEM.01995-13.

Liu, Cheng, Nynke Hofstra, and Eelco Franz. 2016. "Impacts of Climate and Management Variables on the Contamination of Preharvest Leafy Greens with Escherichia Coli." *Journal of Food Protection* 79 (1): 17–29. https://doi.org/10.4315/0362-028X.JFP-15-255.

Monaghan, J. M., J. C. Augustin, J. Bassett, R. Betts, B. Pourkomailian, and M. H. Zwietering. 2017. "Risk Assessment or Assessment of Risk? Developing an Evidence-Based Approach for Primary Producers of Leafy Vegetables To Assess and Manage Microbial Risks." *Journal of Food Protection* 80 (5): 725–33. https://doi.org/10.4315/0362-028X.JFP-16-237.

Njage, P.M.K., and E.M. Buys. 2017. "Quantitative Assessment of Human Exposure to Extended Spectrum and AmpC β-Lactamases Bearing E. Coli in Lettuce Attributable to Irrigation Water and Subsequent Horizontal Gene Transfer." *International Journal of Food Microbiology* 240 (January): 141–51. https://doi.org/10.1016/j.ijfoodmicro.2016.10.011.

Pang, Hao, Elisabetta Lambertini, Robert L. Buchanan, Donald W. Schaffner, and Abani K. Pradhan. 2017. "Quantitative Microbial Risk Assessment for Escherichia Coli O157:H7 in Fresh-Cut Lettuce." *Journal of Food Protection* 80 (2): 302–11. https://doi.org/10.4315/0362-028X.JFP-16-246.

Perez-Rodrıguez, JD, Campos, E. T., Posada-Izquierdo, B. P., Ryser, A. L., and Buchholz, G. D. 2011. “A Mathematical Risk Model for Escherichia Coli O157:H7 Cross-Contamination of Lettuce during Processing | Elsevier Enhanced Reader.” https://doi.org/10.1016/j.fm.2010.06.008.

Sagoo, S. K., C. L. Little, and R. T. Mitchell. 2001. “The Microbiological Examination of Ready-to-Eat Organic Vegetables from Retail Establishments in the United Kingdom.” *Letters in Applied Microbiology* 33 (6): 434–39. https://doi.org/10.1046/j.1472-765X.2001.01026.x.

Szczech, Magdalena, Beata Kowalska, Urszula Smolińska, Robert Maciorowski, Michał Oskiera, and Anna Michalska. 2018. “Microbial Quality of Organic and Conventional Vegetables from Polish Farms.” *International Journal of Food Microbiology* 286 (December): 155–61. https://doi.org/10.1016/j.ijfoodmicro.2018.08.018.

Thomas, Christian, Annett Martin, Sachsenröder Jana, and Niels Bandick. 2020. “Effects of Modified Atmosphere Packaging on an Extended-Spectrum Beta-Lactamase–Producing Escherichia Coli, the Microflora, and Shelf Life of Chicken Meat.” *Poultry Science* 99 (12): 7004–14. https://doi.org/10.1016/j.psj.2020.09.021.

U.S. Food and Drug Administration. 2012. “Quantitative Risk Assessment (QRA) to Support the Proposed Produce Rule,” 18.

16. Appendix 7: Shiny App (see folder attached)

17. Appendix 8: User manual graphical user interface

17.1 Introduction

The objective of this user manual is to present how to use the Shiny App, or graphical user interface (GUI), developed in the project.

As a reminder, the modelling framework is organized in 4 distinct modules that represent steps in the risk pathway:

1. **Production module**: include all the relevant on-farm practices having an influence on the probability of presence of bacteria carrying AMR genes in food.
2. **Processing module:** include all the food transformation processes from raw product to manufactured product including packaging and their associated probabilities of reducing or increasing bacteria load and AMR genes contamination in food.
3. **Post-processing module**: focus on transport and storage practices at retail having an influence on bacteria load and AMR genes contamination level
4. **Home preparation module:** include the key consumers behavior (for example, washing lettuce or cooking meat) having an influence on the final AMR exposure which is a function of the prevalence and level of contamination of food units at the time of consumption.

Each module is made of four different types of variables:

- **Selected variables** = variables defined by the model user before running the analysis. They are used to define a particular model scenario, including the value chain and the hazard risk pathway considered in the risk analysis.
- **Estimated variables** = variables estimated by the model user based on the literature. They are often expressed as probability distributions.
- **Calculated variables** = variables calculated based on the value of the selected and estimated variables previously defined. For example, the number of bacteria on a portion of chicken meat after X days spent in a fridge at Y °C was calculated based on the estimated variable 'minimum growth temperature'.
- **Output variables** = special kind of calculated variables used to estimate the risk of AMR bacteria/gene exposure at the end of each module. They are the key variables used as results of the risk analysis. Their value is presented in terms of probability distribution, median and 95% prediction intervals. As key variables of interest, the output variables are also the target for the correlation analysis.

The first section of the document shows how to load and use the standalone Shiny App to run quantitative risk assessment models for different food product and

microorganisms. The second section presents the technical details of the structure of the Shiny App. Finally, the last section of the document shows how the Shiny App should be modified to either modify an existing production chain or add a new production chain.

17.2 Opening the GUI and run a model

17.2.1 Opening the Shiny App

Before you can load the Shiny App, you need to install on your computer:

- R https://cran.r-project.org/
- R Studio https://www.rstudio.com/products/rstudio/

Once this is done, get access to the folder named "*QRA_shinyApp*". This folder contains the Shiny App. Make sure the folder is unzipped before going to the next step.

All the files from the folder named "*QRA_shinyApp*" should be always kept together. This folder contains:

- A folder named "www", which contains all the images and scripts used by the App
- 3 R scripts named "ui.R", "server.R" and "global.R". These scripts must be open in R studio to be able to load the Shiny App.

To load the App, the 3 steps indicated in the Figure 1 must be followed.

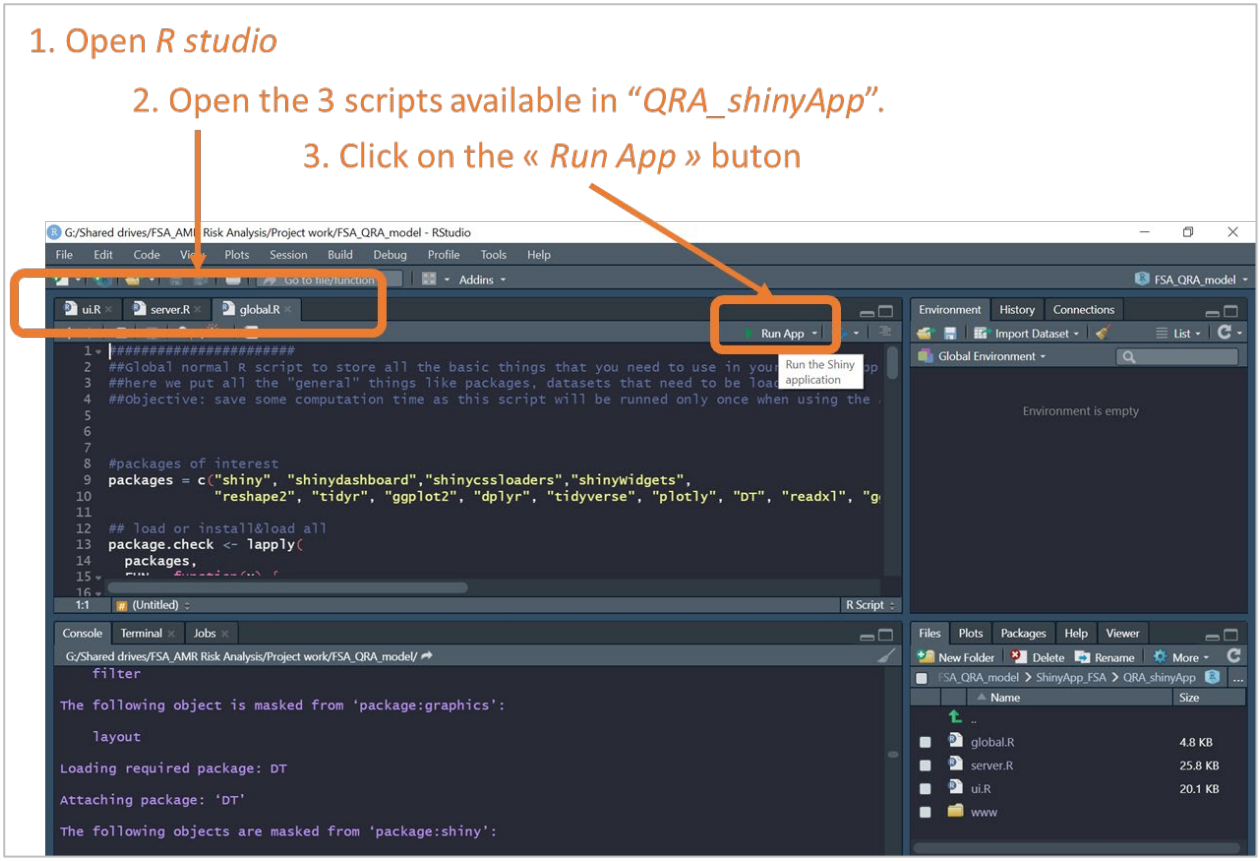


Figure 21: Steps to open the Shiny App

It should be noted that the step n°3 "Click on the Run App button" might take some time the first time the App is opened on a new computer as the program may have to install multiple packages. If the App does not open after the installations are finished, you may have to click on "*Reload App*" (same place than the "*Run App*" button) depending on the packages the computer had to install. This step should be much faster the next time a user will use the App on the same computer.

Once all the packages have been installed, the Shiny App should be opened as see on Figure 2.

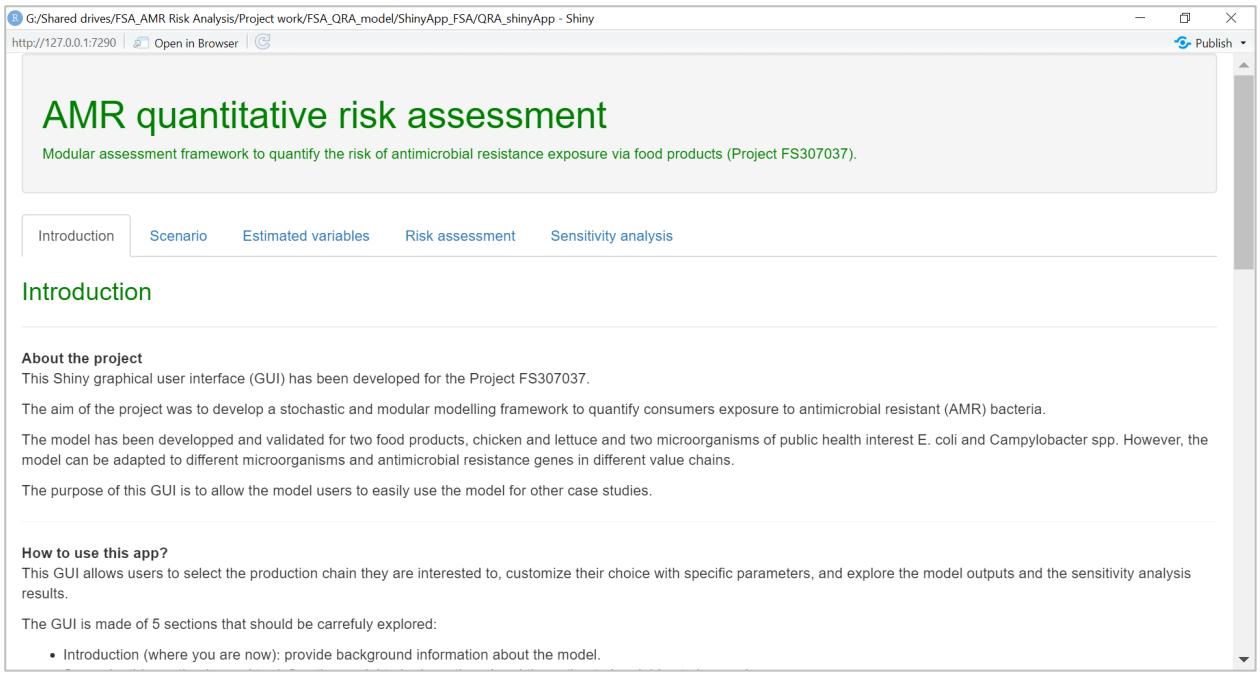


Figure 22: Front page of the Shiny App

17.2.2 Using the Shiny App

The GUI allows users to select the production chain they are interested to customize their choice with specific parameters and explore the model outputs and the correlation analysis results.

The GUI is made of 5 sections that should be carefully explored:

- Introduction: provide background information about the model.
- Scenario: this section is used to define the model to be investigated and the estimated variables to be used.
- Estimated variables: this section is used to perform simulation based on the data uploaded in the 'Scenario' tab.
- Risk assessment: this section runs the model and provides the results of the analysis.
- Correlation analysis: this section performs a correlation analysis to identify the most influential variables in the model.

The user of the GUI can just follow the instruction provided within the App to be able to run a model. The four main steps to be follow are presented in Figure 3.

Figure 23: The four steps to follow to run a quantitative risk assessment using the Shiny App

17.2.3 Defining the estimated used for the analysis

To be able to run a model, the user needs to provide the App with probability distribution for each estimated variables for the selected value chain. The probability distribution (and associated parameters) are provided to the App using a Excel Spreadsheet a (see Figure 4).

The spreadsheet provides information about every estimated variable included in the model for a given value chain. A template of spreadsheet has been developed per value chain included in the project (i.e., lettuce, and chicken). Examples on how to fill these templates have been provide for the 3 case studies investigated in the project (i.e., *E. coli* in chicken and lettuce, and *Campylobacter spp.* in chicken).

The template of spreadsheet is made of sixteen columns. The first columns are used to indicate for each estimated variable the module (column B) and variable type (column C) they belong to. The columns D to F provide the variables name, description, and unit. They are not meant to be modified by the user. The user is only supposed to fill the columns G to Q when defining the probability distribution associated with a certain variable (columns G and H), the parameters associated with the selected probability distribution (columns I to O), and when providing additional information when needed (columns P and Q).

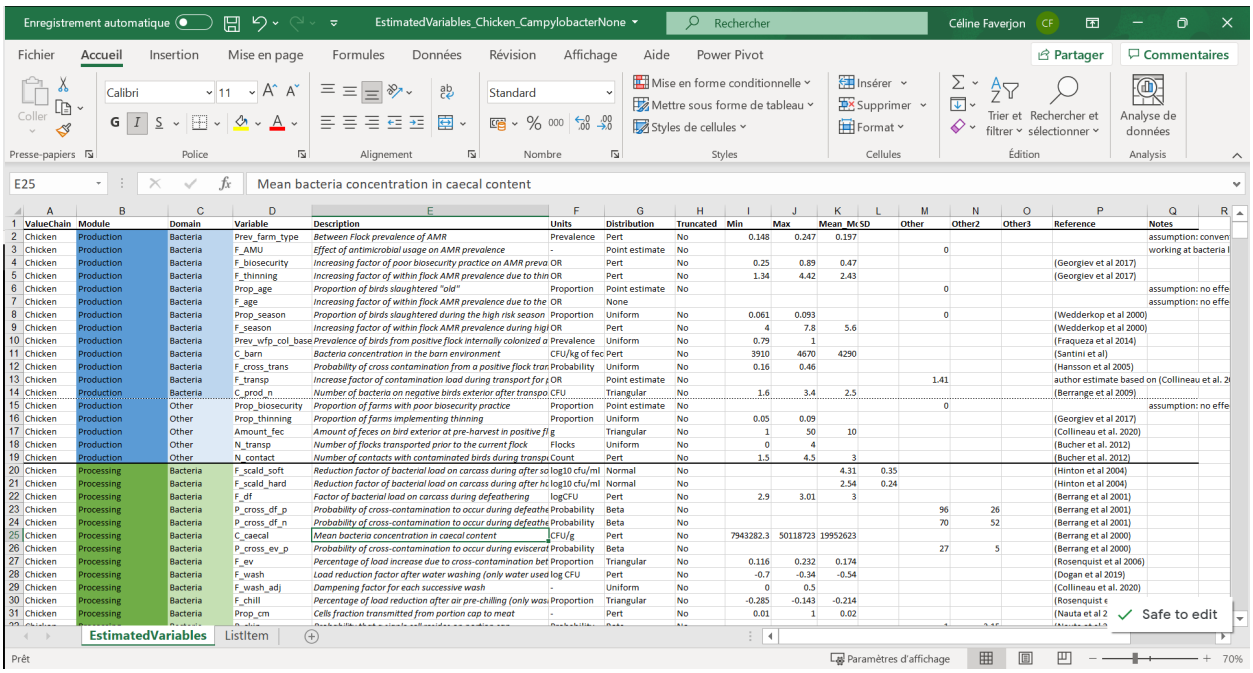


Figure 24: Excel spreadsheet to be filled and uploaded into the Shiny App. Example of the spreadsheet associated with the Chicken value chain.

The system allows for the selection of 14 different type of probability distributions. The distributions and their associated parameters are reported in Table 1.

Table 45: Probability distributions and associated parameters available in the GUI

Distribution	Parameters to be filled
Point estimate	Other
Uniform	Min, Max
Normal	Mean_Mode, SD
Pert	Min, Max, Mean_Mode
Triangular	Min, Max, Mean_Mode
Beta	Other (stand for the shape1 parameter), Other2 (stands for the shape2 parameter)
Laplace	Mean_Mode, Other (stands for the sigma parameter)
LogLogistic	Other (stands for the shape parameter), Other2 (stands for the scale parameter)
Shifted LogLogistic	Other (stands for the shape parameter), Other2 (stands for the scale parameter), Other3 (stands for the threshold parameter)

LogNormal	Mean_Mode, SD
Poisson	Other (stands for the lambda parameter)
Negative Binomial	Mean_Mode, Other (stands for the theta parameter)
Binomial	Other (stands for the probability parameter)
Gamma	Other (stands of the shape parameter), Other2 (stands for the scale parameter)

All the distributions but Point estimate can be defined as truncated or not. It should be noted that when a truncated probability distribution is selected, a minimum and a maximum value must always be selected. If the user wishes to only apply the truncation to one side of the distribution, we recommend that a very large (or small) number is entered on the other side of the distribution to make sure the model will run while not truncating the distribution. For example, if the user wants to select a zero truncated normal distribution of mean 4.3 and standard deviation 0.3, the user should enter the values 0 (standing for the zero truncation) and 100 (or any other large number) in the min and max columns respectively.

17.3 Technical presentation of the GUI

17.3.1 Packages

The following packages are required to be able to run the Shiny App: "shiny", “shinydashboard", "shinycssloaders", "shinyWidgets", "reshape2", "tidyr", "ggplot2", "dplyr", "tidyverse", "plotly", "DT", "readxl", "ggrepel", "gridExtra", "ggfortify", "mc2d", "MCSim", "stats", "EnvStats", "extraDistr", "remotes", and "FAdist".

All these packages will be automatically installed and loaded when opening the Shiny App in R.

17.3.2 R-scripts

The folder named “*QRA_shinyApp*” contains several scripts described in the Table 2. The Shiny App can navigate between the different scripts using a specific naming convention based on the product name. The product name is defined on line 96 in the *ui.R* script and is then used as a reference everywhere in the App to select the appropriate scripts or piece of code to be used for a selected value chain.

Example: the product names currently defined in the GUI are "Chicken" and "Lettuce". In the *global* script, a specific output table is defined for each product: *outputTab_Chicken, outputTab_Lettuce*. In the *server* script, new selected variables are added depending on the *ProductName* name. The name of the script defining the calculated variables for a given module and product refers to the *ProductName* name (for example, *Module_processingShiny_Chicken.R* for the product *Chicken*)

Table 46: R scripts associated with the Shiny App

Script name	Description
ui	Defines the structure of the User Interface (text, colors, layout, interactive features, etc.) and the selected variables shared among production chain (for example, packaging, cutting). This script is made of 5 main sections (one per tab of the GUI)
server	Defines server logic to read selected files in an appropriate order. This script is made of 4 sections, one for each tab of the App but the tab "Introduction".
global	Allows the App to load the required R packages and functions used by the model. This script also defines the outputs specific to each value chain.
Function_ProbaDistribution	Defines additional probability distributions than those already provided by existing R packages
Function_dataSimulation	Simulates data based on the probability distributions defined in the Excel spreadsheet
Module_productionShiny_*ProductName*	Defines relationships between estimated and selected variables and calculated variables in the production module of the product named *ProductName.*
Module_processingShiny_*ProductName*	Defines relationships between estimated and selected variables and calculated variables in the processing module of the product named *ProductName*

Script name	Description
Module_postprocessingShiny_ *ProductName*	Defines relationships between estimated and selected variables and calculated variables in the post-processing module of the product named *ProductName*
Module_homepreparationShiny_ *ProductName*	Defines relationships between estimated and selected variables and calculated variables in the home preparation module of the product named *ProductName*
Correlation_analysisShiny	Runs the correlation analysis

17.4 Modifications of the GUI

17.4.1 To update an existing production chain

Updating or modifying an existing production chain (i.e., chicken or lettuce) in the GUI is relatively easy and the user should follow the following steps:

- Add new *estimated variables* in the corresponding Excel spreadsheet (if there is a need for adding new estimated variables)
- Modify the *calculated variables* in the scripts *Module_productionShiny_productName.R, Module_processingShiny_productName.R, Module_postprocessingShiny_productName.R, or Module_homepreparationShiny_productName.R*
    - If a new *estimated variable* has been added, make sure to also include its relationship with the existing *calculated variables*
    - If no *estimated variable* has been added but the way *calculated variables* are calculated should be modified, just modify these scripts

When modifying an existing production chain, you should always make sure to use the same variable names everywhere: in the column D of the Excel spreadsheet, and in all the scripts *Module_modulenameShiny_productName.R* where changes should be made.

As long as the module outputs are not modified, nothing else needs to be modified in the GUI.

It should be noted that *estimated variables* are assumed to be independent variables while the *calculated variables* are assumed to be dependant from the values of the estimated and/or *calculated variables*. It is possible to turn variables currently considered as *estimated variables* into *calculated variables* if these variables are not considered independent anymore. The steps proposed above just have to be followed. An example is provided below.

Example: turn the *estimated variable* A into a calculated variable.

- Remove A from the Excel spreadsheet
- Add to the Excel spreadsheet new estimated variables, for example, X and Y, needed to calculate the value of A
- Add A to the corresponding *Module_modulenameShiny_productName.R* script and define its relationship with X and Y.

17.4.2 To add a new production chain

Adding a new production chain to the GUI is a project is a project in itself as new variables and new relationship between these variables must be defined for each module. There is no user-friendly way to include a new production chain in the GUI but it is feasible as long as the person making the change has R and R Shiny knowledge.

To add a new production chain the following scripts must be modified:

- **Ui.R.** Add a new product name (line 96): *Chicken, Lettuce, Newproduct*…
- **Global.R.** Add new output tab depending on the requirements of the new production chain (for example, *outputTab_Chicken, outputTab_Lettuce, outputTab_Newproduct*)
- **Server.R.** A large proportion of the script is generic, but some parts are specific to each *Newproduct*
    - You may need to add specific selected variables depending on the *Newproduct*
    - Parts of the script should be also updated depending on the new outputs associated with this *Newproduct*

- **Correlation_analysisShiny.R** . Because the outputs of the model can be different between product (for example, no cooking for lettuce), this script must be also modified when adding a new product.

The following scripts must be also created and added into the "*www*" folder:

- ***Module_productionShiny_Newproduct.R***
- ***Module_processingShiny_Newproduct.R***
- ***Module_postprocessingShiny_Newproduct.R***
- ***Module_homepreparationShiny_Newproduct.R***

Last but not least, when adding a new production chain, you should also create a new Excel spreadsheet to be able to enter the value of the estimated variables associated with the *Newproduct*.

Once the scripts have been properly modified, and new scripts and Excel spreadsheet specific to the *Newproduct* have been created, the Shiny App described can be used as GUI to run the new model.

18. Appendix 9: Comparison sensitivity analysis

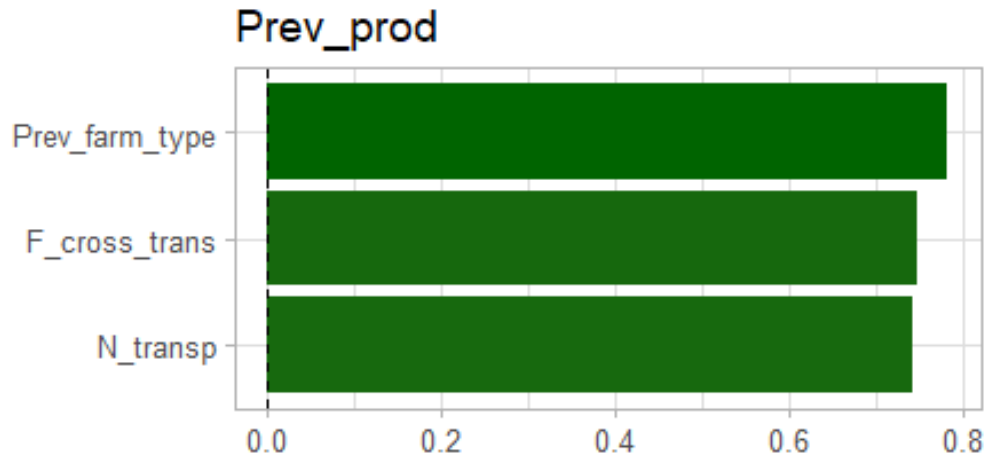
Two approaches of sensitivity analysis were compared:

- Approach 1: Global sensitivity analysis, or correlation analysis, looking at spearman rank correlation coefficient between estimated and outcome variables
- Approach 2: Sensitivity analysis estimating percentage of change in outcome variables depending on changes in the value of the estimated variables

The outcome variable *Prev_prod*, the prevalence of contaminated products at the end of the production module, in the chicken value chain considering the microorganism *E. coli* was used as a case study.

The results of the 1st approach have been extracted from the Appendix 4 and are presented in Figure 1. For the second approach, changes in four estimated variables were investigated (cf Table 1). For sake of simplicity, the magnitude of change in each variable was defined as follow: maximum value equaled two times the baseline value, and minimam value equaled the baseline value divided by two.The results of this second approach are presented in Figure 2.

In both cases, *Prev_farm_type, N_transp,* and *F_cross_trans* appeared highly correlated with *Prev_prod*. However, differences were observed when looking at the variable *C_prod_n*. Using the approach 1, variations in the variable *C_prod_n* were not highlighted as having a major impact (i.e., Spearman corelation coefficient < |0.025|) on the uncertainty associated with *Prev_prod*. On the other hand, the results of the approach 2 show that changes in this variable could have a major impact on the model outcome. It is likely that the magnitude of changes selected in the approach 2 has a major impact on these results such as the choice of probability distribution used in approach 1. This highlight the importance, in both cases, to carefully select the probability distribution associated with the baseline scenario and/or with with the scenario tested before interpreting the results of a sensitivity analysis. Going further in the comparison of the two approaches is challenging because they do not represent the same type of information.



Spearman correlation coefficiant

Figure 1. Correlation of the model estimated variables with the outcome variables. Only estimated variables with spearman correlation coefficient ≥ |0.0.25| have been included in the tornado charts. The darker the color (either green or red), the stronger the correlation.

Table 1. Baseline and new values of estimated variables tested for estimating the percentage of change in the outcome variable

Estimated variables	Baseline value	Minimum	Maximum
Prev_farm_type	Beta(26, 164)	Beta(13. 164)	Beta(52. 164)
N_transp	Uniform(0,4)	Uniform(0,2)	Uniform(2,8)
F_cross_trans	Uniform(0, 0.5)	Uniform(0, 0.25)	Uniform(0, 0.75)
C_prod_n	Triangular (4.4, 4.6, 4.8)	Triangular (8.8, 9.2, 9.6)	Triangular (2.2, 2.3, 2.4)

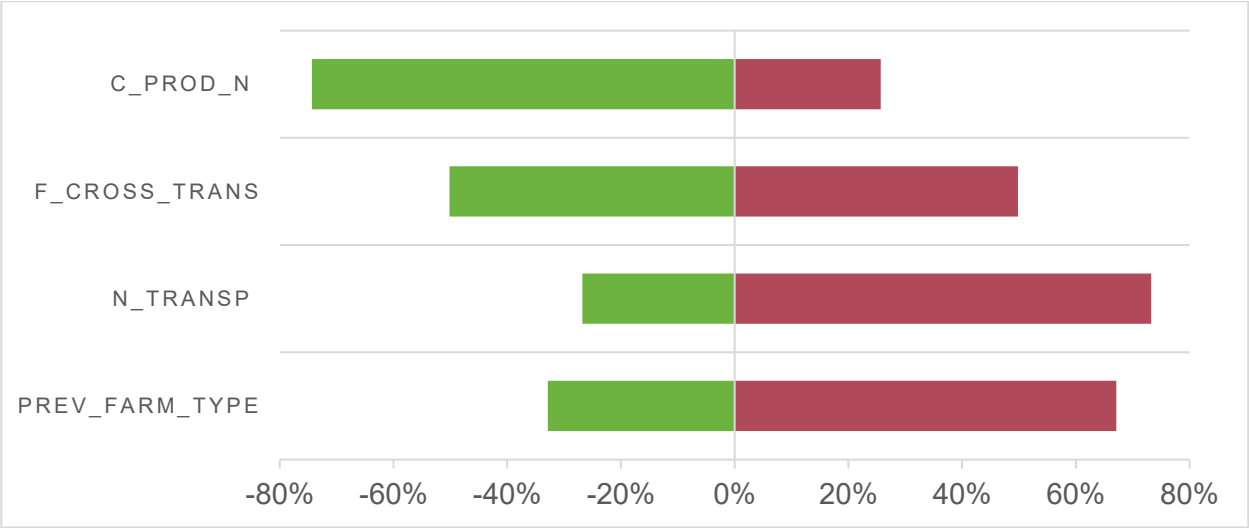


Figure 2. Percentage of change in *Prev_prod* compared to baseline scenario.

For enquiries about this publication, contact the Food Standards Agency.

Project reference: FS307037

Follow us on Twitter: @foodgov

Find us on Facebook: facebook.com/FoodStandardsAgency