Scientific background

Campylobacter species, especially Campylobacter jejuni (C. jejuni), are the main cause of human bacterial gastroenteritis in the developed world and it is estimated that there are in excess of half a million cases and 80,000 general practitioner consultations annually in the UK (Strachan et al., 2010; Tam et al., 2012).

Source-attribution studies, outbreak investigations and case-control reports all indicate that chicken meat is a key foodborne vehicle for Campylobacter spp. infection (Tam et al., 2009; Danis et al., 2009; Friedman et al., 2004; Mullner et al., 2009; Sheppard et al., 2009; University of Oxford, 2021). Consumption of undercooked poultry or cross-contamination from raw poultry meat is believed to be an important vehicle of infection (EFSA, 2009). Raw chicken meat is frequently contaminated with Campylobacter spp. and a decrease in the exposure levels from this source is likely to reduce the number of human cases of campylobacteriosis.

The UK Food Standards Agency (FSA) agreed with industry to reduce Campylobacter spp. contamination in raw chicken, and as part of this activity, also to monitor antimicrobial resistance (AMR) in campylobacters recovered from chicken in the UK. Resistance to quinolone and tetracycline (TET) has increased over the years in Europe and Campylobacter resistance levels are evaluated by EU reference centres and reported annually in the EU Summary Report on Antimicrobial Resistance in Zoonotic and Indicator Bacteria from Humans, Animals and Food (for example in ECDC and EFSA, 2021).

The monitoring of AMR in Campylobacter spp. has focused on C. jejuni and C. coli and in countries belonging to the EU is carried out as part of the Commission Decision 2020/1729/EU or preceding mandates (2003/99, 2013/652). Monitoring and reporting of AMR in C. jejuni isolates recovered from caecal samples of broilers is mandatory (in even numbered years from 2014 to 2020) but the monitoring of AMR in C. coli isolates recovered from food-producing animals is performed on a voluntary basis. C. coli is more often resistant than C. jejuni to important antimicrobials and so there has been encouragement to monitor AMR levels in C. coli, in fact it is now mandatory from 2021 onwards (2020/1729/EU). As C. coli are more likely to exhibit resistance to antimicrobials than C. jejuni it is important to determine trends for C. coli and C. jejuni as separate species (EFSA and ECDC, 2016). AMR in Campylobacter spp. from poultry, especially to fluoroquinolones (FQ), has raised some health concerns relating to the occurrence of resistance in human isolates.

Antibiotic treatment of campylobacteriosis is only advised for patients with severe or persistent illness under guidance from the National Institute for Health and Care Excellence, as most patients recover without any treatment. Macrolides are long-established drugs of choice to treat campylobacteriosis when clinically appropriate, with fluoroquinolones as an alternative (Aarestrup et al., 2008; Silva et al., 2011). Antibiotics have been and continue to be used in agriculture and there is strong evidence to suggest that collectively these have led to the emergence of resistant Campylobacter spp. (Van Boeckel et al., 2015; Asuming-Bediako et al., 2019). In the USA, the prevalence of FQ resistant Campylobacter rose from 1.3% in 1992 to 40.5% in 2001 and an increase in prevalence of macrolide-resistant C. jejuni and C. coli has also been reported in the USA, with C. coli more likely to exhibit resistance to ERY. Lower levels of FQ resistance are present in samples from Australia, where agricultural usage of FQs is much lower.

Campylobacter spp. isolates from 38% of cases associated with one UK hospital in 2008 were resistant to CIP (Cody et al., 2010). This represented an increase from 2004 where 25% of

isolates were resistant to CIP, unlike resistance to ERY that had remained at an equivalent level (at approximately 2.5% of isolates). An increased prevalence of isolates with resistance to CIP has also been reported in the USA (Zhao et al., 2010). It is unclear whether infection with FQ-resistant Campylobacter spp. has adverse clinical consequences, such as prolonged post-infection complications, and studies published to date have produced conflicting results (Engberg, 2004; Evans et al., 2009). As stated above, where Campylobacter spp. infection warrants treatment with an antimicrobial, the drugs of choice are usually macrolides and FQs (Skirrow and Blaser, 2000). It is therefore, particularly important to ascertain any change in resistance to these groups of antimicrobials.

As risks associated with antimicrobial use in food producing animals have been recognised, mitigation steps have been implemented against the proliferation and dissemination of resistance genes and resistant bacteria in the food chain and environment and ultimately to people. In 2006, the EU withdrew approval for the use of antibiotics as growth promoters in poultry feed although therapeutic treatment with antibiotics is still allowed (Castanon, 2007). From 2012, the British Poultry Council (BPC), who's members account for almost 90% of all poultry meat producers in the UK, have developed an antibiotic stewardship program with an aim to ensure sustainable antibiotic use that can maintain animal health and welfare and antimicrobial efficacy (BPC Poultry report 2021). The poultry industry has cooperated with government on monitoring of antimicrobial usage from 2014 which is now published annually by the Veterinary Medicines Directorate in the VARSS report. These reports have evidenced the progress made in the industry towards sustainable and responsible use of antimicrobials, which is now within the targets set by the Responsible Use of Medicines in Agriculture Alliance (RUMA) (RUMA, 2021). In the poultry meat sector, there has been a 74% reduction in the use of antimicrobials since the antibiotic stewardship started in 2012 and a 95.5% reduction in the use of High Priority – Critically Important Antibiotics (HP-CIA). This has been a major effort by the industry, making improvements to husbandry and biosecurity plus using risk-based prescribing to reduce the demand for treatments overall. The VARSS reports document this progress but note that since 2017, the levels of antimicrobial consumption have generally flattened out to a sustainable level according to RUMA targets (VARSS, 2020). The analysis of AMR data collated from the past two decades, have provided an opportunity to assess the impact of the key changes in AMU over the same timeframe.

It is imperative for public health to obtain accurate data on the prevalence of AMR in campylobacters from chicken as these represent a major route of exposure to consumers. The AMR profiles in Campylobacter from chicken have been determined based on phenotypic methods via breakpoint (BP) testing and minimum inhibitory concentration (MIC) testing, but more recently also predicted from genome sequence data. Here these methods were used for Campylobacter spp. recovered from caecal contents of chicken and carcasses at slaughter and from chicken at retail sale. Integration of AMR data across the food chain will provide a better understanding of how AMR is emerging and help understand disseminating AMR from animal production to humans.

The European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) have jointly issued a Technical Document entitled 'EU protocol for harmonised monitoring of antimicrobial resistance in human Salmonella and Campylobacter isolates' (EFSA and ECDC, 2016) to provide standardisation of antimicrobial susceptibility testing methods. Within this document, the panel of antimicrobials for testing Campylobacter spp. isolates from animal and food sources includes two antimicrobials, nalidixic acid (NAL) and streptomycin (STR), which are not included in the protocol for human isolates. The Technical Document states that "The difference in the antimicrobials which are not on both panels is not considered a critical issue as the most important agents are included in both Panels" (EFSA and ECDC, 2016). The interpretation of results from animal and food isolates is based on the epidemiological cut-off value (ECOFF), which is different from the clinical breakpoint approach for human isolates. EFSA and ECDC recognise this within the Technical Document and state the following:

> Another difference between the protocols is that clinical breakpoints would primarily be used as the interpretive criteria for human isolates while ECOFFs are used for animal and food isolates. This reflects the difference in the reason for performing antimicrobial sensitivity testing (AST), with treatment of clinical illness being the primary focus for testing in human isolates and early detection of acquired resistance and increased resistance in zoonotic bacteria being the goal for AST in animal and food isolates. Quantitative data can however be reliably compared as the data can then be interpreted with either clinical breakpoints or ECOFFs, depending on the purpose of the analysis. An important consideration in relation to comparison of data is that only dilution susceptibility test data (Minimum Inhibitory Concentration (MIC) expressed in mg/L) are accepted in the monitoring in animals and food. Consideration has been given to adopting an MIC only policy also for human isolates, however the costs of testing all isolates by MIC methods are likely to be prohibitive for many."
> (EFSA and ECDC, 2016)

The work presented here aimed to ascertain what proportions of the C. jejuni and C. coli isolates from chicken examined between 2001 and 2020 were resistant to a range of antimicrobial agents relevant to public health. The level of AMR in Campylobacter found in chicken samples in the UK (with isolates obtained from chicken flocks at slaughter via caecal samples or from chicken carcases and chicken meat sampled either at the post-chill stage in the slaughterhouse or up to ten days later at the point of retail) was investigated alongside factors possibly affecting the levels and trends of AMR in Campylobacter spp.. The work has resulted in the creation of a detailed catalogue of AMR profiles of Campylobacter isolates with associated data including year of isolation, type of chicken production, sample type and other sample data to allow further analyses opportunities for interested stakeholders. The focus for this report has been to ascertain levels of AMR in the C. jejuni and C. coli isolates obtained from chicken in the UK from 2001 to 2020. Analysis of seasonality and differences between outdoor and indoor rearing and between organic and non-organic chicken were examined. The role of sample type was also investigated and the proportions of AMR was determined for isolates obtained from caecal samples collected from chicken at slaughter or samples from chicken carcasses sampled post-chill or chicken meat sampled at retail sale.

The project has utilised AMR data from both phenotypic testing or predicted from analysis of whole genome sequence (WGS) data. Validated bioinformatics pipelines were used to determine the presence of genes or specific mutations known to confer resistance to four classes of antibiotics: fluoroquinolones (gyrA mutation), macrolides (23s mutation; the presence of erm genes was also established but this gene was not part of the initial validation study; determination of very rare cmeABE mutations was not included)), tetracyclines (presence of tetO gene) and aminoglycosides (multiple different genes that predict resistance to GEN or STR). The detection of these AMR genes and mutations has been validated in-house by UKHSA to correspond to phenotypic resistance to CIP/NAL, ERY (a macrolide), TET and, GEN and STR (both aminoglycosides), as determined by the EUCAST interpretative thresholds (Painset et al., 2020).

In summary, the objectives were:

- to create a detailed database/catalogue of Campylobacter isolates, their AMR profiles and associated sample data from farm to fork
- to ascertain the percentages of resistant C. jejuni and C. coli isolates obtained from chicken sampled in the UK from 2001 to 2020 and analyse trends
- to determine if the percentages of resistant isolates were different between different types of chicken
- to determine if the proportion of isolates with different AMR profiles changed between caecal samples and carcase samples
- to determine other factors associated with AMR in campylobacters (using mathematical modelling as appropriate).