Discussion

In general AMR was observed more frequently in C. coli isolates than in C. jejuni isolates, which is consistent with results for Campylobacter isolates recovered from meat samples of chicken broilers in 2018 published in the annual report on AMR provided by the EU (EFSA and ECDC, 2020). Also similar to the result from this study, among the C. jejuni and C. coli isolates recovered from poultry meat in the EU, the highest prevalence of resistance was noted for CIP and TET (overall EU percentages: 54–83%) while resistance to GEN in C. jejuni and C. coli from poultry meat or caecal contents was not observed in most EU countries or were present at very low levels. Resistance to STR was either not detected or detected at low to very low levels in C. jejuni isolates but at higher levels in C. coli isolates. We also found that the percentages of C. jejuni and C. coli isolates with resistance to the macrolide ERY were lower than the resistance levels to quinolones or tetracyclines but resistance to ERY was higher among C. coli compared to C. jejuni isolates. In the EU report resistance to ERY was also generally higher among C. coli isolates compared to C. jejuni isolates.

Valid comparisons of the percentages of resistant campylobacters over time, and factors affecting these, may be limited by elements of variation in sampling and laboratory methodology in the different studies included in this analysis. Thus we acknowledge that the data used in this study were not designed to determine differences in abundance of AMR in C. jejuni or C. coli between different chicken production types, sample types, UK or non-UK origins or seasons over time. In addition, some factors possibly affecting levels of AMR were not investigated here due to insufficient data, including age of the chicken at sampling, the slaughterhouse/company behind production and the chicken breed. However, we have no evidence that would suggest that these factors have confounded the results found for the trends in resistances presented. For example there is no evidence to suggest that there was any bias in terms of the age of chicken between earlier and later years. We expected that the chicken breed was highly correlated with chicken production type as free range and organic chicken are usually slower growing breeds, free-range typically grown for approximately 56 days and organic for approximately 81 days prior to slaughter as opposed to 35-40 days for conventional production systems (BPC, 2017). This could mean the associations found here between the level of resistance for some antimicrobials and chicken production type may relate to breed; but it was not possible to separate an effect of chicken production type from chicken breed in the data examined here. It has been noted that C. coli is more common in chicken reared with access to a range and that this could relate to breed and/or rearing aspects (Babacan, 2020). It is also possible that there are (unknown) selection biases in the isolates examined and they may not be truly representative of Campylobacter isolates from chicken in the UK. Nevertheless, it was striking how similar the percentages of AMR resistant isolates were between those derived from UK human isolates at similar time points and comparison between retail and slaughterhouse samples also demonstrated similar AMR profiles. Another caveat related to the degree of correlation between the datasets and methods used to obtain isolates. More isolates in the earlier datasets were recovered by enrichment followed by detection from mCCDA rather than by direct detection from mCCDA. This did represent a challenge to separate the effects of time from method, particularly in the data prior to 2009 from chicken meat. However all data beyond this point are consistently based isolates that were obtained by direct detection from mCCDA.

For some of the combinations of the Campylobacter spp. and antimicrobial, there was a change in the threshold concentration used to distinguish resistant from susceptible isolates over time. We used MIC distributions collated and published by the EU Community Reference Laboratory

(CRL) for AMR to adjust the percentages where this was relevant to enable comparisons over time to be made. Whilst we believe this represented a reasonable approach, it is possible that the adjustments made were inadequate to wholly compensate for changes in threshold concentrations. Nevertheless, even for the most significant impact of a change made (the change in the threshold concentration used to ascertain resistance to ERY in C. coli) even if we had applied an adjustment of twice the magnitude, this would not have changed the conclusions made for the trend analysis. In the case of resistance to ERY in C. coli for example, we concluded the percentage of resistant isolates was low to moderate with low levels of resistance in last 10 years; if we had applied an even greater adjustment in the earlier years this would have resulted in low level of resistance to ERY in C. coli in all years including low resistance in 2007 and 2008.

6.1 Quinolone resistance

This study found that the percentage of isolates with resistance to CIP significantly increased from 2001 to 2014 but thereafter stabilised within the current survey methodology employed. It is possible increased sampling would reveal the trend is still upwards, in particular for C. jejuni. The most recent data on CIP resistance from UK broiler flocks from 2020 (not included in this study database) has found 59.2% of C. jejuni isolates displayed a CIP resistant phenotype; an increase from 48.2% in 2018 (VARSS, 2021). A similar finding was presented for C. jejuni and C. coli isolates from broilers in Ireland (Lynch et al., 2020). Increased resistance to CIP in C. jejuni and C. coli from broilers has been observed in EU countries (EFSA and ECDC, 2020). An increasing trend in resistance to CIP was also documented for campylobacters recovered from human clinical samples across the EU (EFSA and ECDC, 2020). In the EU report, the C. jejuni and C. coli from human and animal origins in 2017–2018, showed very high to extremely high levels of resistance to fluoroquinolones.

Resistance to quinolones and fluoroquinolones is usually due to mutations in the gyrase gene, the C257T mutation in the gyrA gene being the major mechanism for resistance to CIP. The increase in resistance to quinolones in C. jejuni and C. coli is likely to be associated with increased usage of fluoroquinolones in poultry farms (Endtz, 1991; Agunos et al., 2013; Marshall et al., 2011) and resistance can rapidly emerge in poultry flocks (Humphrey et al., 2005). High levels of fluoroquinolone resistance have persisted in isolates from poultry even after discontinued use of these antibiotics (Price et al., 2005) and this could reflect clonal expansion of resistant lineages (Lopes et al., 2019; Lynch et al., 2020) as well as fitness benefits linked to the gyrA mutation (Haldenby et al., 2020), as the mutation may not result in a biological cost, quinolone-resistant strains could outcompete susceptible ones in chickens in the absence of a selective pressure.

In 2020, less than 0.01% (by weight) of antimicrobials used in poultry meat production (British Poultry Council (BPC) members) belonged to a group including fluoroquinolones and aminoglycosides (VARSS, 2021) a reduction from 2016, when 1% of antimicrobials used by BPC members were fluoroquinolones (VARSS, 2017). The use of fluoroquinolones declined by 99% from 2014 to 2019 for broilers in the UK (VARSS, 2020) with a stop on the use of fluoroquinolones as a prophylactic for day old broilers in 2016 (VARSS, 2016). However, the levels of resistance to fluoroquinolones in Campylobacter from chicken in this study have yet to show any decrease over time (Figure 1). This might be seen as a disconcerting outcome for stakeholders despite the effort invested in driving antimicrobial usage down via the stewardship initiative, in particular for the HP-CIAs, including fluoroquinolones and macrolides. It is possible that more time is needed before an effect of reduced fluoroquinolones usage is seen in Campylobacter population resident in the broiler production system. There may be undefined mechanisms that are sustaining (in the absence of selective pressure) fluoroquinolone resistant Campylobacter within the broiler production system as discussed earlier. It is important to note that macrolide resistance remains at very low levels which alongside improved biosecurity and welfare on broiler farms is likely to relate to the antibiotic stewardship initiative.

6.1.1 C. jejuni

The percentage of C. jejuni isolates with resistance to CIP or NAL increased significantly over time with resistance to CIP increasing from 13% in 2001 to 52% in 2018 and with 58.5% of isolates resistant from carcases in 2020. A very similar level of CIP resistance (59.2%) was detected in C. jejuni isolates from random UK slaughterhouse caecal samples in 2020 representing an increase from the 48.2% resistance in 2018 (VARSS, 2021). Further monitoring would be needed to establish if this may indicate a renewed increase in the level of resistance to CIP in C. jejuni isolates, especially considering the relatively small sample sizes in the data from 2020. Persistence of CIP resistant isolates may relate to certain Campylobacter lineages being more likely to have CIP resistance, for example MLST clonal complexes (ST-354, ST-446, and ST-464) have been associated with resistance to CIP and quinolones in general (Cody et al., 2012; Oxford University, 2021) and such lineages may increase in frequency over time (Lopes et al., 2019) possibly due to fitness advantages.

An increasing trend was also reported for human cases in the UK with 5.5% of C. jejuni being resistant to CIP from 1997-1998 rising to 45.1% in isolates from 2015-2018 (Oxford University, 2021). Similarly, an increase from 1993–1996 to 2008–2009 was reported for C. jejuni isolates with the gyrA (T86I) among isolates from clinical cases in the UK (Haldenby et al., 2020).

Across the EU, resistance among C. jejuni isolates from broilers was extremely high (73.5%) in 2018 (EFSA and ECDC, 2021). Resistance to CIP among C. jejuni isolates from human cases in the EU was very high and detected in 59.3% of isolates in 2018 and in 61.5% of isolates in 2019.

Whilst investigating factors which possibly affecting resistance to CIP, we found that the percentages of C. jejuni with resistance to CIP or NAL was slightly higher in winter and spring compared to summer months. It is possible that resistance to quinolones is more common in some lineages of Campylobacter and that those were more frequent colonisers of chicken in winter and spring months. There is very limited understanding of how season relates to the types of Campylobacter colonising chicken flocks although C. coli was reported as more common during summer months compared to other months in UK chicken (PHE, 2020; Lawes et al., 2012; Arnold et al., 2014). It was unlikely to relate to different usages of antimicrobial across the seasons as arguably we should have seen a similar result for C. coli. The OR for resistance to CIP was slightly higher for frozen samples, but resistance to NAL was not affected by sample state indicating a weak if even real effect. While it is possible that there could be an increased probability of CIP resistant lineages colonising chicken destined for frozen sales there is to our knowledge no data on whether some flocks would be more likely to be sold as frozen meat or, even if there could be any association between the types of campylobacters that colonise such flocks.

6.1.2 C. coli

The percentage of C. coli isolates with resistance to CIP or NAL increased significantly over time with resistance to CIP increasing from 15% in 2001 to over 50% in 2017. The percentage of CIP resistant isolates remained stable between 2014 and 2018 and 52% of isolates were resistant in the sample from 2020.
An increasing trend was also reported for C. coli isolates from human cases in the UK with 6% of isolates with resistance to CIP from cases in 1997-1998 rising to 37% in human isolates from 2015-2018 (Oxford University, 2021). An increase in the prevalence of C. coli isolates with the gyrA (T86I) SNPs among isolates of clinical cases was also reported in the UK from 1993–1996 to 2008–2009 (Haldenby et al., 2020). The percentage of C. coli isolates from chicken with resistance to CIP appeared to be slightly higher compared to contemporaneous isolates from humans cases. It is likely that this relates to isolates from human cases having contributions from non-chicken sources e.g. cows and pig, where the percentage of isolates with resistance to CIP

may be lower than for chicken (Oxford University, 2021).

In comparison the very high percentage (51%) of C. coli isolates from chicken in the UK with resistance to CIP, across the EU, resistance to CIP among C. coli isolates from broilers was extremely high (86.7%) in 2018 (EFSA and ECDC, 2021). Resistance to CIP among C. coli isolates from human cases in the EU was very high and detected in 65.2% in 2018 (EFSA and ECDC, 2020).

The OR for resistance to CIP and NAL was higher for C. coli isolated from free-range than from standard chicken. This may relate to different C. coli lineages colonising outdoor as opposed to indoor reared chicken and that the percentage of CIP resistant isolates may differ between such lineages. It is also possible that this could relate to differences in usage of antimicrobials for the different production types and/or an impact of breeder flocks, but a reduction in usage has been reported across all production sectors (VARSS, 2020). Free-range and organic systems use slower growing breeds and live longer, which might influence the lineages of C. coli that are dominating the microbiota of the chickens in the later stages of production. Compared to spring months the percentages of C. coli with resistance to CIP or NAL was slightly higher in autumn months. It is possible that resistance to quinolones is associated with particular lineages that appear to become more dominate in UK flocks in the summer and autumn months (Lawes et al., 2012; Arnold et al., 2014).

6.2 Macrolide resistance

The percentages of isolates from chicken in the UK with resistance to ERY was low to very low in the years from 2001 to 2020. This was consistent with the low percentage of isolates from human cases in the UK with resistance to ERY (Oxford University, 2021). However, resistance to ERY was generally higher among C. coli compared to C. jejuni isolates. Resistance to ERY is mainly the result of mutations in the ribosomal proteins L4 and L22 in one or several copies of the ribosomal RNA genes, such as A2074G, A2075G and A2074C in the 23S rRNA target gene. Usually these mutations result in a biological cost (Wang et al., 2014), probably explaining the relatively low prevalence of macrolide-resistant C. jejuni. Methyltransferases, Erm(B) and Erm(N), have also been shown to confer resistance to ERY (Jehanne et al., 2021) and the Erm enzymes are able to methylate 23S rRNA to decrease the binding of macrolides. The ermB gene was not detected in the isolates (1636 C. jejuni and 464 C. coli isolates) from 2012 to 2020, subjected to WGS in this study.

6.2.1 C. jejuni

The percentages of C. jejuni with resistance to ERY from chicken in the UK remained low and has been below 5% since 2001. Data collected since 2012 from across the EU also found low to very low percentages of C. jejuni with resistance to ERY (0.4% in 2012, 5.9% in 2014, 1.3 % in 2016 and 1.3 % in 2018) from broilers (EFSA and ECDC, 2015; EFSA and ECDC, 2016; EFSA and ECDC, 2018; EFSA and ECDC 2020). In the UK, resistance to ERY in C. jejuni recovered from broiler flocks has been below ? 0.6% in the years monitored from 2014 to 2020 (VARSS, 2021). The percentage of C. jejuni isolates from human cases with determinants predicting resistance to ERY was also very low (0.4%) in 3,945 isolates tested from the UK in 2015-2018 (Oxford University, 2021).

Minor, but significant effects of sample state and season were noted. It is possible that the more frequent detection of C. jejuni with resistance to ERY in frozen samples may relate to colonisation patterns of chicken destined for frozen products and/or that freezing may favour survival of ERY resistant isolates, but we found no evidence for either. The lower percentages of C. jejuni with resistance to ERY found in autumn months may reflect that colonisation patterns is related to season i.e. that C. jejuni lineages with resistance to ERY were less likely to colonise chicken in

autumn but there is very little understanding of whether AMR profiles in C. jejuni colonising chicken flocks may differ between seasons. Alternatively, unrecognised sample bias (for example non-even sampling across seasons) may affect this such as frozen samples being correlated with season or with non-UK produce.

6.2.2 C. coli

The analysis of C. coli isolates from chicken in the UK demonstrated that resistance to the macrolide ERY has remained between low and moderate and stayed low beyond 2014. Across the EU resistance to ERY was also moderate to very low (11.2% in 2012, 14.5% in 2014, 1.2% in 2016 and 6.5% in 2018) in C. coli isolates from broilers (EFSA and ECDC, 2015; EFSA and ECDC, 2016; EFSA and ECDC, 2018; EFSA and ECDC 2020). Here, temporarily resistance appeared to be higher in 2007 and 2008 but this could reflect sample type and/or methodologies used (see below). The percentage of C. coli isolates from UK human cases with determinants predicting resistance to ERY was also low (4.1%; n = 535) in isolates from specimens tested between 2015-2018 (Oxford University, 2021).

Our results suggested that C. coli detected in free-range or organic broiler chicken samples were less likely to have resistance to ERY. This may reflect different frequencies of resistance to ERY in the dominant C. coli lineages colonising outdoor as opposed to indoor reared chicken; it is also possible that the colonising campylobacter types relate to breeds, which is different for standard and non-standard chicken, or the age of the birds at slaughter. Antimicrobial usage is currently strictly controlled in all UK production systems, however there may be scope variations in usage in different systems due to disease challenge.

Lower odds ratios were noted for C. coli with resistance to ERY in samples from caecal samples. This impact of sample type may relate to different methodologies used for isolates from caecal compared to other samples. The retail samples were much more diverse especially early survey samples that included samples from whole/portions, frozen/fresh as well as skin-on/off ones. It is possible that the isolates obtained from the retail samples arise from more diverse populations due to cross-contamination/selective pressures during slaughter affecting campylobacter populations to a larger extent than for caecal samples. The vast majority of isolates from caeca were obtained without prior enrichment unlike the majority of isolates from earlier retail samples, that were obtained by enrichment. It is possible that this could have affected the types of isolates obtained for the AMR testing. It is also important to note that the largest adjustment made for the percentages of resistant isolates was for resistance to ERY in C. coli, but no adjustment was factored into the multivariate analysis for this. In addition, AMR testing of isolates from caecal samples was carried out using MIC testing while isolates from other samples were mainly tested using break-point testing. Additional exclusive analysis of the caecal isolate data may help inform the impact of sample type on resistance observed.

6.3 Tetracycline resistance

This study found that the percentage of isolates with resistance to TET increased significantly from 27% in 2001 to over 60% in 2014 and the level was then relatively stable. A similar finding was presented for C. jejuni and C. coli isolates from broilers in the Republic of Ireland (Lynch et al., 2020). Increased levels of C. jejuni and C. coli with resistance to TET in broilers have been observed in EU countries (EFSA and ECDC, 2020). An increasing trend in resistance to TET was also documented for campylobacters recovered from human clinical samples across the EU (EFSA and ECDC, 2020). In the EU report the C. jejuni and C. coli from human and animal origins in 2017–2018, showed very high to extremely high levels of resistance to TET. The same report also recognised an increasing trend in resistance to TET in C. jejuni from broiler chicken flocks at slaughter in the UK, amongst ten other EU member states.

Resistance to tetracyclines is usually due to expression of tetO as it mediates resistance to TET by offering ribosomal protection by binding to an unoccupied site. The gene tet(O) responsible for expression of TetO is commonly carried on the pTet plasmid but has also been detected in the chromosome. Considering the transmissibility of such resistance plasmids within bacterial populations even in the absence of TET usage, Campylobacter may be an important reservoir for these resistance genes. This emphasises the importance of monitoring resistance to be able to assess the risk of genes conferring resistance to other bacteria. Similar to resistance against CIP, resistance to TET has been shown to be more common within certain multilocus sequence types (STs) (e.g. ST982) highlighting the importance of clonal expansion of resistant lineages and the role that mobile genetic elements play in disseminating and maintaining resistance to TET. For meat producing poultry in the UK (BPC stewardship members), usage of tetracyclines declined markedly from approximately 31 tonnes in 2014 to approximately 3.9 tonnes in 2019 (VARSS, 2020). However, the antimicrobial still accounts for 12% of all antimicrobials given to poultry meat producing birds in 2020, which is down from 48% in 2014 (VARSS, 2021).

6.3.1 C. jejuni

The trend in resistance to TET in C. jejuni isolates showed an increase from 2001 to 2014 but was then stable despite usage declining for broilers from 2014. However, considering broiler flock data alone (caecal samples), there was a modest increase recognised in the UK from 2014 (58% resistant) to 2018 (65%) (EFSA and ECDC, 2020) and the prevalence in 2020 has increased again to 67% (VARSS, 2020). An increasing trend was also reported for human cases in the UK with around 20% of C. jejuni being resistant to TET in 1997-1998 rising to over 40% of isolates in 2015-2018 (Oxford University, 2021). Similarly, an increase in the percentage of C. jejuni isolates with the tetO gene was reported from 1993–1996 to 2008–2009 among isolates from clinical cases in the UK (Haldenby et al., 2020).

Smaller but significant effects of sample category and season were also noted. C. jejuni with resistance to TET were more likely to be detected in caecal samples and it is possible that the sample category effect could relate to different methodologies used for isolates from caecal compared to other samples. The retail samples were much more diverse especially early survey samples that included samples from whole and chicken portions as well as frozen /non-frozen ones. It is possible that the isolates obtained from the retail samples arose from more diverse populations due to cross-contamination and/or selective pressures during slaughter and processing affecting campylobacter populations to a larger extent than for caecal samples. For example, C. jejuni with resistance to TET may not persist in the food production chain as well as C. jejuni that are sensitive to TET, although there is no published evidence/literature to support this. The vast majority of isolates from caeca were also obtained without prior enrichment unlike the majority of isolates from earlier retail samples and it is possible that this could have affected the types of isolates obtained for AMR testing. An exclusive analysis of caecal samples or samples that were only tested by direct culture would help determine if sample type or isolation methods have an impact on the recovery of TET resistant C. jejuni. In addition, AMR testing of isolates from caecal samples was carried out using MIC testing while isolates from other samples were mainly tested using break-point method. The percentage of C. jejuni with resistance to TET was slightly higher in winter months. It is possible that resistance to TET is more common in the Campylobacter types that colonise chicken in winter but there is very limited understanding of how season relates to the types of C. jejuni that colonise broiler flocks.

6.3.2 C. coli

The trend in resistance to TET in C. coli isolates showed an increase from 2001 to 2014 but was then stable. Smaller but significant effects of chicken production type and season were also noted. The risk for resistance to TET was higher for free-range compared to standard chicken. This could relate to different C. coli lineages colonising outdoor as opposed to indoor reared

chicken and that the level of TET resistance differs for such lineages. The different lineages may arise as a result of different exposure between outdoor and indoor chicken and/or breed/breeder flock colonisation factors. It is also possible that this could relate to differences in usage of antimicrobials for the different production types, but overall a significant reduction in TET usage has been reported from 2014 onwards. The percentage of C. coli with resistance to TET was slightly higher in autumn months. It is possible that resistance to TET is more common in C. coli types that colonise chicken in autumn but there is very limited understanding of how season relate to what types of Campylobacter colonise chicken flocks.

6.4 Aminoglycoside resistance in C. jejuni and C. coli

We found that the percentages of C. jejuni and C. coli isolates with resistance to the aminoglycosides GEN and STR were much lower than resistance levels to quinolones or tetracyclines. The percentages for resistance to GEN was extremely low for all years analysed. Importantly from a public health perspective, GEN can be used to treat Campylobacter spp. systemic infections in humans, justifying monitoring of resistance to this antimicrobial. Our results were consistent with the extremely low percentage of isolates from human cases in the UK with resistance to GEN found in only 0.1% of 3945 C. jejuni and in none of 435 C. coli isolates from 2015-2018 (Oxford University, 2021). Across the EU resistance to GEN was also very low in C. jejuni (0.1% in 2016 and 0.3% in 2018) and very low to low in C. coli (0.6% in 2016 and 2.1% in 2018) in isolates from broilers (EFSA and ECDC, 2018; EFSA and ECDC, 2020). In a survey of C. jejuni from caecal samples of UK broiler flocks in 2020, no isolates with the GEN resistant phenotype were observed (VARSS, 2020). In the UK in years from 2009 to 2020, according to VARSS report there was no change in sales of the group of antibiotics that included aminoglycosides.

6.4.1 Resistance to streptomycin in C. jejuni

The percentages of C. jejuni isolates from chicken in the UK with resistance to STR has remained low to very low since 2001 and no significant increasing or decreasing trend was detected. This is similar to the finding for C. jejuni isolates from human cases in the UK from 2015-2018 where only 0.5% had genetic determinants predicting resistance to STR (Oxford University, 2021). In a survey of C. jejuni from caecal samples of UK broiler flocks in 2020, only 0.6% of C. jejuni isolates displayed the STR resistant phenotype (VARSS, 2020). Across the EU resistance to STR was also low in C. jejuni isolates from broilers (0.1% in 2016 and 0.3% in 2018) although some modest increasing (nine MSs) and decreasing trends (seven MSs) from 2009 to 2019 were observed (EFSA and ECDC, 2018; EFSA and ECDC, 2020; EFSA and ECDC, 2021).

6.4.2 Resistance to streptomycin in C. coli

The percentages of C. coli isolates from chicken in the UK with resistance to STR remained low to moderate since 2007 and no significant increasing or decreasing trend was detected. This is similar to the finding for C. coli isolates from human cases in the UK from 2015-2018 where 10.8% of isolates had genetic determinants predicting resistance to STR (Oxford University, 2021). In the EU resistance to STR was moderate in C. coli isolates from broilers (15.4% in 2016 and 15.6% in 2018) with an increase in resistance detected in two MSs and a decrease in three MSs from 2009 to 2019 (EFSA and ECDC, 2018; EFSA and ECDC, 2020; EFSA and ECDC, 2021).

6.5 MDR in C. jejuni and C. coli

This study found that MDR in C. jejuni remained very low for all years from 2001 to 2020 with an overall average of 0.8% of isolates with a MDR phenotype. The percentage (1.6%) of MDR

isolates in the period 2016-2018 was similar to the percentage (1.3%) detected in broilers from European countries in 2018 (EFSA and ECDC, 2020). In 2017, MDR was detected in 0.9% of Campylobacter from human cases in the EU (EFSA and ECDC, 2019).

In agreement with other studies, we detected MDR more frequently in C. coli (6.8%) compared to C. jejuni (0.8%). In the period from 2016 to 2018, MDR phenotypes were observed in 8.6% of the C. coli isolates which is very similar to the proportion of C. coli with MDR profiles (8%) observed across six reporting member states in Europe (EFSA and ECDC, 2020). Higher risk of MDR strains may relate to acquisition of MDR genome island and plasmids (Tang et al., 2017).

6.6 Conclusions

- significant increases in prevalence of resistance were seen for the antimicrobials CIP/NAL and TET beyond the initial baseline period from 2001-2005 for following timepoints. Resistance to ERY was detected at low or very levels over the time period analysed with the exception of C. coli between 2006-2010 when moderate levels are reported. No significant trends were observed for STR and GEN (resistance was rare to low in all years). Similar temporal profiles were observed for C. jejuni and C. coli, however CIP resistance in C. jejuni may have increased more in recent years than for C. coli. As CIP is a HP-CIA antimicrobial this is concerning. The data analysed in this study may not have had sufficient power to demonstrate any very recent upward trend of a smaller magnitude in prevalence and new data would be needed to establish any future trend in CIP resistance in C. jejuni.
- there were weaker effects associated with a number of other factors. For example, as chicken from non-standard production appeared to be associated with a higher probability of C. coli with resistance to CIP and TET. However, resistance to ERY appeared lower in samples from chicken reared as free range or organic. Further weak effects associated with season as in the summer season was more likely to have higher levels of CIP/NAL and TET resistant C. coli. Yet, the summer season appeared protective for resistance towards CIP/NAL and TET in C. jejuni, but autumn was a risk for ERY resistance. Chicken from non-UK production had a slightly higher level of C. coli isolates with resistance to CIP/NAL. Frozen chicken was a risk factor for resistance to ERY in C. jejuni (but was protective for TET) but as data for frozen chicken did not extend beyond 2009 it is not possible to determine if this is a current risk factor. There were also weak effects of differing resistance profiles for Campylobacter isolates from caecal samples relative to whole carcase samples for some antimicrobials. It is possible that this could relate to a variable capacity for persistence within the meat production chain in different lineages that are also associated with AMR resistance profiles.
- this study has provided an overview of the resistance profile for Campylobacter within the poultry production system over the past two decades (from 2001 to 2020). Although general trends have been identified here, the findings are caveated by the influence of subtle differences in terms of sampling and testing methodology across the different studies. With further analysis it should be possible to assess the impact of these differences and reduce the uncertainties associated with comparing the datasets across all the studies.
- the study has demonstrated the potential for WGS to provide data that is just as comparable as the phenotypic data. There is real potential for WGS to replace phenotypic AMR testing, in terms of efficiency and the possibility to provide further data such as campylobacter lineage (using cgMLST analysis) to identify and monitor for emerging and problematic lineages of Campylobacter in the poultry production system.
- the prevalence of MDR Campylobacter was low and relatively stable in C. jejuni and C. coli in the years from 2001 to 2020 but considering the potential for resistance to CIP, ERY and TET and possible aminoglycosides, surveillance should be maintained.

6.7 Recommendations

- continued monitoring of AMR in C. jejuni and C. coli from chicken sampled at retail and from slaughterhouses is needed as the proportion of resistant strains is dynamic for a HP-CIA antibiotic (flouroquinolones)
- undertake further targeted studies to investigate the validity of key findings and trends from this study, in terms of increasing resistance, and the impact genome sequencing of isolates with MDR and/or high-level resistance to ERY or CIP should be implemented to evidence the genes involved, detect resistant clones and for comparison to human isolates
- undertaking phenotypic testing at intervals for a subset of isolates could help ensure new resistance is not missed; an annual literature review could support the addition of any new resistance genes discovered to routine pipelines used to predict AMR
- assess the scope for combining research activity with existing monitoring activies to maximise the output from the monitoring resource used. For example, this could investigate the potential for a national survey utilising Campylobacter isolates that are already recovered by the poultry industry as part of routine flock testing prior to slaughter and carcase testing for the process hygiene criteria. A systematic process for submitting a representative sub-sample of isolates from routine poultry industry testing (and associated meta-data) could allow AMR and type profiles to be established to monitor the emergence of resistant lineages within the industry. It could be linked to human systems to monitor the public health risks
- considering the significantly higher prevalence of certain AMR profiles in C. coli, monitoring must continue to be specific for C. coli and C. jejuni and test for AMR in a significant number of C. coli isolates to enable analysis of trends
- it may be useful to undertake a further detailed multivariable analysis of UK produced chicken only, and in particular analysis of the past decade to assess any impact of the concerted efforts of the poultry industry to achieve sustainable levels of antimicrobial use in broiler production
- undertake an analysis of genome sequence-based MLST types against the AMR profiles to establish any role of clonal expansion.
- the possible effects of season, chicken production system and and sample type, suggest carefully designed sampling plans are important for future robust monitoring of AMR in C. jejuni and C. coli
- further analysis of the datasets collated for this report, to determine any effect of isolation method and susceptibility testing method may provide a more comprehensive insight to any potential bias due to methodologies
- despite the use of antimicrobials in UK poultry production reducing dramatically in the past decade this has not been accompanied by reductions in resistance rates for all antimicrobials, and it would be insightful to review if the current data gathering efforts in terms of antimicrobial usage (AMU) and AMR are sufficient to detect subtle associations between AMU and AMR when they exist
- it would be useful to investigate if there are other underlying factors/mechanisms promoting resistance for example, enhanced fitness, co-selection by other antimicrobials/disinfectants or therapeutic practices (pencillins have been increasingly used in broiler production in the past 5 years and exposures to disinfectants may be more frequent now with enhanced biosecurity practices farms)
- further robust analyses of the meta-data could identify the key variables that may be maintaining the population of resistant Campylobacter in the poultry meat production system.