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Project status: Completed
Project code: FS307031
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Date published: 28 September 2022
DOI: https://doi.org/10.46756/sci.fsa.dud728

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Acknowledgements

The authors would like to thank the following people:

- All staff who was involved in the successful delivery of this project from the Animal and Plant Health Agency (APHA) and UKHSA through the data collection and analysis
- The Department of the Environment and Food and Rural Affairs (DEFRA) and the Veterinary Medicines Directorate (VMD) for support and funding for data collection
- Colleagues in UKHSA reference laboratory at Colindale for whole-genome-sequencing derived data.
- The FSA for funding this work

Abbreviations

Acronym	Definition of term
AMR	Antimicrobial resistance With terms used to describe the antimicrobial resistance levels according to EU zoonoses reports (EFSA, 2018), defined as: Rare: less than 0.1 % Very low: 0.1 % to 1.0 % Low: more than 1.0 % to 10.0 % Moderate: more than 10.0 % to 20.0 % High: more than 20.0 % to 50.0 % Very high: more than 50.0 % to 70.0 % Extremely high: more than 70.0 %
BPC	British Poultry Council
Broiler	Chicken reared for meat
ºC	Degrees Celsius
GBRU	Gastrointestinal Bacteria Reference unit
Cfu	Colony forming units
CI	Confidence Interval
CIP	Ciprofloxacin
ECOFF	Epidemiological Cut Off value (with respect to antimicrobial resistance)
ERY	Erythromycin
EU	European Union
FSA	Food Standards Agency
FSS	Food Standards Scotland
G	Gram
GEN	Gentamicin
HP-CIA	High Priority Critically Important Antibiotics
H	Hours
ISO	International Organisation for Standardisation
L	Litre
mCCDA	modified Charcoal Cefoperazone Deoxycholate Agar
MIC	Minimum Inhibitory Concentration
mg	Milligram

Acronym	Definition of term
NA	Not applicable
NAL	Nalidixic Acid
RUMA	Responsible use of medicines in Agriculture Alliance
spp.	Species
STR	Streptomycin
TET	Tetracycline
UKAS	United Kingdom Accreditation Service
UKHSA	UK Healthy Security Agency (formerly Public Health England)
WGS	Whole Genome Sequencing

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Project Summary

Campylobacter spp. are the most common bacterial cause of foodborne illness in the UK, with chicken considered to be the most important vehicle of transmission for this organism. It is estimated there are 500,000 cases of campylobacteriosis in the UK annually, with Campylobacter jejuni (C. jejuni) and Campylobacter coli (C. coli) accounting for approximately 91% and 8 % of infections, respectively.

Although severe infection in humans is uncommon, treatment is seldom needed for human infection but usually involves the administration of a macrolide (e.g., azithromycin) or a fluoroquinolone (e.g., ciprofloxacin). An increased rate of resistance in Campylobacter in chicken to such antimicrobials could limit effective treatment options for human infections and it is therefore important to monitor changes in rates of resistance over time.

In this report we analysed trends in antimicrobial resistance (AMR) in C. jejuni and C. coli isolated from chicken in the UK. The chicken samples were from chicken reared for meat (ie. broiler chicken as opposed to layer chicken (ie. egg-laying chicken)) and included chicken sampled at slaughterhouses as well as from retail stores in the UK. Datasets included AMR results from retail surveys of Campylobacter spp. on chicken sampled in the UK from various projects in the time period from 2001 to 2020.

In the retail surveys, samples were obtained from stores including major and minor retail stores throughout the UK (in proportion to the population size of each nation) and Campylobacter spp. testing was performed using standard methods with the majority of isolates obtained from direct culture on standard media (mCCDA).

Data from national scale surveys of broiler chicken, sampling caecal contents and carcase neckskins at slaughterhouses, undertaken by APHA in 2007/2008, and between 2012 and 2018 were also included in the study. In the APHA-led surveys, Campylobacter were isolated using standard culture methods (culture onto mCCDA) and antimicrobial susceptibility testing was performed by a standard microbroth dilution method to determine the minimum inhibitory concentration (MIC) of isolates.

Care was taken when comparing data from different studies as there had been changes to the threshold used to determine if an isolate was susceptible or resistant to an antimicrobial in a small number of scenarios. Harmonised thresholds (using epidemiological cut-off (ECOFF) values)

were employed to assess AMR with appropriate adjustments made where required to allow meaningful comparisons of resistance prevalence over time. Data from additional isolates where resistance to antimicrobials were predicted from genome sequence data were also considered.

Findings

This report uses data that were collected and reported from a number of surveys using varied methods, including phenotypic tests compared with epidemiological cut-off value (ECOFFs) and predicted resistance analysis from whole genome sequences (WGS). The costs of testing all isolates by MIC methods to allow for direct antimicrobial sensitivity testing (AST) required for clinical comparison are prohibitive and do not add significant value to the work (EFSA and ECDC, 2016). As such, to avoid confusion when reporting on the trends of resistance prevalence this report the terms ‘resistance’ and ‘reduced susceptibility’ can be considered interchangeable.

Overall, this study found that resistance to quinolones (ciprofloxacin (CIP) and nalidixic acid (NAL)) and tetracycline (TET) was common in C. jejuni and C. coli from UK chicken. In comparison, resistance to erythromycin (ERY) and streptomycin (STR) was much rarer in the isolates examined and resistance to gentamicin (GEN) was very rare.

Ciprofloxacin and Nalidixic acid

An increase in the prevalence of C. jejuni isolates with phenotypic resistance to CIP from a mean value of 13% in the 2001 to 2005 period to 47% in the 2011 to 2018 period was found. Although the most recent trend still appeared to be moderately increasing, there were no further significant increases in the percentages of C. jejuni with resistance to CIP in the years from 2014 to 2018. Results based on prediction of resistance to CIP from genome sequence data appeared to be consistent with the AMR results based on phenotypic resistance. The most recent genome sequence data predicted resistance to CIP in 52.9% of isolates from 2018 to 2020 and was consistent with the phenotypic AMR results from 2014 to 2018, showing no significant change in the percentage of C. jejuni isolates with resistance to CIP since 2014. Continued testing could reduce the uncertainty in the trend as related to the most recent percentages observed. Resistance to CIP also increased in C. coli isolates, from 18% in the years 2001 to 2005 to 48% in the years 2016 to 2018, although with no significant change in the years from 2014 to 2018, based on phenotypic AMR data. Consistent with these results, genome sequence data predicted resistance to CIP in 43.7% of isolates from 2018 to 2020 and in 46.3% of isolates from 2012-2020. These datasets suggested there has been no significant change in the percentage of C. coli isolates with resistance to CIP post 2014. Similar observations were made regarding the resistance to the quinolone nalidixic acid (NAL) in the C. jejuni and C. coli isolates. Over time there was a significant increase in the percentage of NAL resistant C. jejuni from 16% of isolates in 2001 to 52% in 2018. The same trend was observed for C. coli, where the percentage of resistant strains increased from 16% in 2001 to 50% in 2017. Similar to the data for resistance to CIP the rate of increasing NAL resistance in both C. jejuni and C. coli appeared to decline from 2014.

Erythromycin

The percentage of C. jejuni isolates with phenotypic resistance to erythromycin (ERY) was low or very low across the period with a peak of 4% resistant in 2008 and was <2% in 2018. Furthermore, analysis of genome sequence data did not detect any predicted ERY resistance in the C. jejuni 1,636 isolates from slaughterhouse and retail chicken samples tested between 2012 and 2020. No obvious increasing or decreasing trends were observed. Resistance to ERY in C. coli isolates appeared to reach a moderate peak in 2007/2008 with approximately 15% of isolates

showing a resistant phenotype but post 2014 resistance levels were either low or very low for each year with no significant increasing or decreasing trends in resistance detected. Analysis of genome sequence data predicted resistance to ERY in 1.7% of C. coli isolates from slaughterhouse and retail chicken samples tested between 2012 and 2020.

Tetracycline

The percentage of C. jejuni isolates with phenotypic resistance to tetracycline (TET) rose significantly from from 27% in 2001, to 66% in 2018 but with no increasing trend in recent years (2014-2018). Results based on prediction of resistance to TET from sequence data were consistent with the AMR results based on phenotypic resistance and predicted resistance to TET in 59.1% of C. jejuni isolates from slaughterhouse and retail chicken samples between 2012 and 2020 and in 61.4% of retail samples from 2018 to 2020 alone. These data suggested no significant change in the percentage of C. jejuni isolates with resistance to TET since 2014. Resistance to TET had also increased significantly in C. coli isolates with phenotypic resistance to TET increasing from 23% in 2001, to over 55% post 2013. Genome sequence data detected predicted resistance to TET in 68.3% of C. coli isolates from slaughterhouse and retail samples tested between 2012 and 2020 and in 66.3% of retail samples from 2018 to 2020 alone. Analysis of both genoptypic and phenotypic data did not demonstrate any significant increases post 2014 when analysed separately.

Gentamicin

A gentamicin (GEN) resistant phenotype was only found in two C. jejuni and two C. coli through the entire study that included the 5,267 C. jejuni and 1,997 C. coli isolates from between 2001 and 2018. It was clear that resistance to GEN was very rare in C. jejuni or C. coli obtained chicken in the UK. In addition, resistance to GEN was not predicted from genome sequence data of 2,100 C. jejuni and C. coli isolates from between 2012 and 2020.

Streptomycin

A streptomycin (STR) resistant phenotype was present in C. jejuni at low or very low levels, with only the years of 2017 and 2018 seeing levels greater than 1%. Analysis of genome sequence data followed a similar pattern with predicted resistance to STR detected in only 0.2% of C. jejuni isolates from slaughterhouse and retail chicken samples tested between 2012 and 2020. No obvious trends on increasing or decreasing resistance were observed. Phenotypic resistance to STR was more common in C. coli with moderate levels of 15% observed in 2013 and 2014, but with lower levels observed after this time. Analysis of genome sequence data predicted resistance to STR in 1.7% of C. coli isolates from slaughterhouse and retail chicken samples tested between 2012 and 2020. No clear increasing or decreasing trend in resistance was observed using either method.

Factors associated with resistance

This study provided an overview of antimicrobial resistance in C. jejuni and C. coli from chicken broilers over the past two decades (from 2001 to 2020). Although general trends and risk factors 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. In particular the type of production system and season appeared to be associated to the level of AMR for some antimicrobials. Isolates originating from non-standard chicken production were more likely to show resistance to CIP and TET for C. coli but were less likely to show resistance to ERY. Resistance to TET and

quinolones in C. coli was more common during summer months whereas AMR in C. jejuni appeared at a lower rate in summer months.

Multidrug resistance

Phenotypic multidrug resistant profiles (meaning a profile with resistance to at least three unrelated antimicrobial classes) were observed at very low levels in C. jejuni (0.8%) and at low levels in C. coli (6.8%). There was no significant evidence of an increasing trend in the occurrence of MDR phenotypes in C. jejuni or C. coli isolates over the course of this study.

Concluding remarks

In summary, the data have indicated that since 2014, there have been no significant increases in resistance to the antimicrobials examined. It is possible that this is related to the significant reductions in usage of antimicrobials (AMU) undertaken by the poultry industry in the past decade. However, more data is required to provide convincing evidence that historical increases in the prevalence of quinolone and tetracycline resistance have ceased. It is recommended that trends in AMR in Campylobacter spp. isolates from chicken sampled in the UK continue to be monitored to identify any decreases in resistance as well as any increasing resistance of concern, particularly to ERY and co-resistance to CIP/NAL and ERY.

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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).

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Methods

4.1 Data sources and recovery methods for isolates

The analysis presented was based on collated data from eight studies based on phenotypic AMR results and data from two studies based on predicted AMR profiles from genome sequence data. The databases with AMR data have been included in an excel file detailing all isolates from these studies (Appendix 1). An overview of the source datasets is presented in Table 1a and Table 1b.

The so-called PHE (now known as UKHSA) 1, 2 and 3 datasets represented datasets from the earliest FSA projects investigating AMR in Campylobacter spp. recovered from retail chicken that included portions and frozen chicken meat samples. The PHE 4 dataset represented Campylobacter spp. isolates from retail chicken during the FSA funded project FS102121 (PHE, 2015; PHE, 2017; PHE, 2018; PHE 2019; all under the project title of “A microbiological survey of Campylobacter contamination in fresh whole UK produced chilled chickens at retail sale”); all were speciated and AMR tested at the UKHSA Gastrointestinal Bacteria Reference Unit (GBRU).

Table 1a. Overview of datasets used to analyse trends in AMR in C. jejuni and C. coli detected in chicken in the UK.

Data set	Sampling	Type of samples	Isolate recovery(a)	Total number of samples, selection of isolates	AMR method
APHA1, 2007 to 2008	Broiler (chicken) flocks at slaughter, sampling weighted to sample proportionally based on slaughterhouse throughput. More than 85% of UK broiler production reflected in the sampling frame, stratified to ensure year round coverage.	Caecal contents, pooled from 10 broiler chickens collected at slaughter. Each batch of broilers originated from a single farm house.	From direct culture (mCCDA agar) or enrichment followed by culture (mCCDA)	In 2007, from 240 Campylobacter positive batches, 190 C. jejuni and 55 C. coli isolates were tested. In 2008 from 180 Campylobacter positive batches, 143 C. jejuni and 45 C. coli isolates were tested.	MIC
APHA2, 2012 to 2016	Broiler flocks at slaughter, weighted to sample proportionally based on abattoir throughput. More than 85% of UK broiler production reflected in the sampling frame, stratified to ensure year round coverage.	A) Caecal contents, pooled from 10 broilers. Each batch of broilers originated from a single farm house. B) Neck-skin sample from a single carcase chicken sampled post-chill.	A and B: From direct culture (on mCCDA)	A) C. jejuni isolates were selected at random for testing in 2013 (n=61), 2014 (n=166), 2016 (n=180). In 2013, 33 C. coli were selected for testing B) WGS was applied to 112 C. jejuni and 37 C. coli isolated in 2012 (randomised selection of isolates but stratified to ensure year round coverage). In 2013, 2014, 2015 all available isolates were selected with 252 C. jejuni/65 C. coli in 2013, 235 C. jejuni/67 C. coli in 2014, 264 C. jejuni /40 C. coli in 2015	A) MIC B) WGS predicted
APHA3, 2018	Broiler flocks at slaughter weighted to sample proportionally based on throughput. More than 60% of UK broiler production reflected in the sampling frame, stratified to ensure year round coverage.	Single broiler caecal content.Each broiler was from a single chicken house.	Direct from mCCDA	170 C. jejuni selected at random but stratified to ensure year round coverage.	MIC

Table 1b. Overview of datasets used to analyse trends in AMR in C. jejuni and C. coli detected in chicken in the UK.

Data set	Sampling	Type of samples	Isolate recovery(a)	Total number of samples, selection of isolates	AMR method
PHE1, FSA 2001 survey	Retail – designed to reflect market share	Fresh/frozen, whole/portions, skin/meat, UK retail (with a small % non-UK origin)	Direct from mCCDA or via enumeration on mCCDA	In total 4866 samples; of 2697 campylobacter-positive samples 1208 C. jejuni and 421 C. coli were tested (aiming for one isolate per sample; limited random drop-out due to isolate die-off). 982 isolates from enrichment; 647 isolates direct from mCCDA	Break-point
PHE2, FSA 2007-2008 survey	Retail – designed to reflect market share	Fresh/frozen, whole/portions, skin/meat, UK retail (with a small % non-UK origin)	Detection (enrichment then mCCDA)	In total 3274 samples were tested; 1358 were campylobacter-positive; from these 803 C. jejuni and 714 C. coli were tested for AMR (aiming for one isolate per sample; limited random drop-out)	Break- point

Data set	Sampling	Type of samples	Isolate recovery(a)	Total number of samples, selection of isolates	AMR method
PHE3, CLASSP 2004-2007 survey	Retail – random sampling from retail stores	Fresh/ frozen, whole only, UK retail (with a small % non-UK origin)	Enrichment (Bolton broth and mCCDA)	In total 2264 samples were tested; 1804 were campylobacter-positive; from these 800 C. jejuni and 389 C. coli were tested for AMR; (aiming for one isolate per sample – limited random drop-out due to isolate die-off)	Break- point
PHE4, FSA 2014-2018 survey	Retail – probably reflecting market share; neck-skin	Fresh, whole only, UK	Direct enumeration (mCCDA)	~13000. Every nth(b) isolate (organic and free-range bias)	Break- point
PHE5, FSA-MIC 2017 survey	Retail – reflecting market share; meat/skin	Fresh/frozen, whole/ portions (with a small % non-UK origin)	Direct enumeration (mCCDA)	Several picks per sample. All attempted (random drop-outs due to isolate die off); one isolate of each C. jejuni and C. coli used from each sample.	MIC
PHE6WGS, 2018-2020 survey	Retail – only from non-major retail stores; neck-skin	Fresh, whole chicken of UK origin	Direct enumeration (mCCDA)	One per sample; random drop-out due to isolate die-off	WGS predicted

(a) ISO 10272-1:2006 and ISO 10272-2:2006; (b) Random non-recoverable isolates replaced by next available isolate.

In the PHE 4 dataset isolates were obtained from fresh, raw, whole UK produced chicken collected from retail stores across the UK. Samples were collected from different types of stores including both major as well as minor retailers and details of samples can be found elsewhere (PHE, 2015; PHE, 2017a; PHE, 2018a; PHE, 2019). A proportion of isolates were tested for their antimicrobial susceptibility properties; every tenth isolate (or next viable isolate) were included, although selection was adjusted to ensure representation of producer premises and retailers as deduced from market share data. In an attempt to improve representation from free range and organic chicken, all recoverable isolates (i.e. able to grow after frozen storage) from organic and a high proportion of isolates from free range chicken were included. The PHE samples were collected from the point of retail and consisted of whole carcases or portions, and in some studies frozen samples were included. All PHE testing was carried out on skin or meat samples.

The source of the Campylobacter isolates in each dataset was from (broiler) chicken. The APHA datasets contained isolates from samples collected from structured slaughterhouse surveys representative of UK broiler production at the time, accounting for > 60% of UK broiler throughput (APHA dataset 1: Lawes et al., 2012; APHA dataset 2: Lawes, 2017; APHA dataset 3: VARSS, 2019). For these datasets caecal samples were collected from flocks at slaughter and carcases were collected after chilling for neck-skin sampling. Within each APHA dataset the isolates selected for MIC testing were representative of the wider selection of isolates collected from the parent survey.

The detailed description of laboratory methodology used in each dataset was published previously (APHA dataset 1, Lawes et al., 2012; APHA dataset 2, Lawes, 2017; APHA dataset 3, VARSS, 2019; PHE, 2016; PHE, 2017b; PHE, 2018b; PHE, 2020; PHE, 2021). In general, for all studies, Campylobacter spp. were isolated using methodology based on ISO-10272. This was mainly by direct culture on mCCDA agar (as described in ISO-10272 part 2) or for a subset of samples via enrichment broth and culture onto mCCDA or Preston agars (as described in ISO-10272 part 1). While we cannot rule out sampling bias in the source datasets there is no evidence to suggest they would present a biased sample of Campylobacter isolates from chicken over the time frame studied (from 2001 to 2020), although coverage was not continuous. All samples were collected by trained personnel and all testing laboratories participate in External Quality Assurance schemes and operate comprehensive internal quality assurance schemes as part of the requirements of their accreditation to ISO 17025/2005 and were assessed annually by the United Kingdom Accreditation Service (UKAS). All analyses were performed by trained and

competent staff in UKAS accredited laboratories operating an appropriate quality management system. The UKAS accreditation pertaining to the phenotypic antimicrobial susceptibility testing at PHE was according to the ISO 1518:2012 standard. Phenotypic AMR testing at APHA was compliant with the EU decisions and technical guidance at the time of testing, with laboratories participating in external quality assurance exercises to verify assay performance.

Two additional datasets with genome sequence-based AMR data contained isolates not already present in the phenotypic datasets. One dataset represented isolates (773 C. jejuni and 255 C. coli) from chicken sampled between August 2018 and October 2020 from retail stores not part of major chains (as part of project FS102121; PHE, 2021). The other dataset represented isolates (863 C. jejuni and 209 C. coli) obtained from chicken neck-skin samples at slaughter and were sampled between 2012 and 2015 (as part of FSA project FS241051 and subsequently genome sequenced as part of FSA project FS101013; University of Oxford, 2021). For both these datasets genome sequencing of one isolate from every sample testing positive for Campylobacter spp. was attempted (for a limited number of the samples testing positive for campylobacters initially, no isolate was tested for AMR due to loss of viability after frozen storage).

In total AMR profiles were predicted by genome sequencing for 1,636 C. jejuni and 464 C. coli isolates.

4.2 MIC and breakpoint harmonisation and adjustments

All minimum inhibitory concentration (MIC) testing was by the microbroth dilution method, using the sensititre system. MIC testing at APHA laboratories was in compliance with the relevant EU Commission Decision and EFSA technical specifications, in place at the time of sample collection. All breakpoint testing carried out at GBRU was done using Muller Hinton Agar containing specified breakpoint concentrations of antimicrobials to determine resistance. Briefly this was performed as follows: preparation of a suspension of each isolate in sterile saline to McFarland 0.5 turbidity and inoculation onto the surface of each of the antimicrobial containing agars. An isolate was considered resistant when growth was detected on the agar containing the antimicrobial, but scored sensitive if no growth was observed and the corresponding antimicrobial-free plate showed pure growth from the suspension applied.

The prediction of antimicrobial resistance by analysis of whole genome sequence data, generated by standard methods, was via the UKHSA pipeline (Painset et al., 2020). In general the antimicrobial resistance profiles created within the collated datasets were as recommended in the ECDC and EFSA protocol for harmonised monitoring of antimicrobial resistance in human Salmonella and Campylobacter isolates (EFSA and ECDC, 2016) and the EU Commission decision (Decision 2013/652/EU). However, across the datasets there was some variation in the criterium to define a resistant organism. To allow comparison of resistance rates between the different datasets, it was necessary to adopt a harmonised approach for the determination of a resistant C. jejuni or C. coli isolate. In this study the ECOFF thresholds defined by ECDC for CIP, ERY, GEN and TET were used to define a resistant isolate. These did match the thresholds defined in EU Commission decision with the exception of the C. jejuni threshold for TET; the difference was very minor (1 mg/l to 2 mg/l) and extremely unlikely to affect determinations of trends on TET resistance. For STR and NAL the thresholds were defined by the EU decision, as there are no thresholds specified by ECDC. This approach aligns with thresholds used to validate the calling of AMR genetic determinants via the UKHSA pipeline for these antimicrobials (Painset et al., 2020; although resistance to NAL was not included in validation for the pipeline calling of genetic determinants for AMR). The harmonised MIC thresholds and threshold used in this study are presented in Table 2.

Table 2. Harmonised MIC and break point (BP) thresholds used in this study in mg/l.

Antimicrobial	C. jejuni MIC	C. jejuni BP	C. coli MIC	C. coli BP
Ciprofloxacin	>0.5	0.5	>0.5	0.5
Nalidixic acid	>16	16	>16	16
Erythromycin	>4	4	>8	8
Tetracycline	>2*	2	>2	2
Gentamicin	>2	2	>2	2
Streptomycin	>4	4	>4	4

*EFSA interpretative threshold is >1 = resistant

In some of the earlier PHE datasets that were based on breakpoint testing, different thresholds applied compared to the current harmonised thresholds shown in Table 2. To allow comparison between earlier and more recent datasets, an adjustment factor for the earlier data was calculated. In summary, when an original threshold was higher than the harmonised threshold, there would be an underestimate of resistance occurrence in the original data set. As an example, the effect of changing the threshold for GEN and C. jejuni is outlined in Table 3. If the harmonised threshold was 2 but in an earlier dataset a threshold of 4 was used, any isolate with an MIC of 4 would change from being sensitive to resistant with the harmonised threshold. Therefore, the adjustment needs to account for the proportion of isolates determined sensitive by the original threshold (equivalent to an MIC of 4 or less) that could have an MIC value of 4.

Table 3. The effect of changing the threshold for gentamicin resistance (expressed as the break point (BP)) on a population of C. jejuni.

MIC	Number of isolates (a)	Susceptible (S) or Resistant (R) if MIC is >2)	Expected outcome with harmonised BP (2)	S/R outcome with original BP of 4	Outcome change?
?0.016	0	S	No growth (S_)	No growth (S_)	No
0.03	7	S	No growth (S_)	No growth (S_)	No
0.06	20	S	No growth (S_)	No growth (S_)	No
0.12	625	S	No growth (S_)	No growth (S_)	No
0.25	3593	S	No growth (S_)	No growth (S_)	No
0.5	6264	S	No growth (S_)	No growth (S_)	No
1	708	S	No growth (S_)	No growth (S_)	No
2	35	S	No growth (S_)	No growth (S_)	No
4	**1**	**R**	**Growth (R_)**	**No growth (S_)**	**Yes - S to R**
8	2	R	Growth (R_)	Growth (R_)	No
16	0	R	Growth (R_)	Growth (R_)	No
32	2	R	Growth (R_)	Growth (R_)	No
64	41	R	Growth (R_)	Growth (R_)	No

MIC	Number of isolates (a)	Susceptible (S) or Resistant (R) if MIC is>2)	Expected outcome with harmonised BP (2)	S/R outcome with original BP of 4	Outcome change?
128	0	R	Growth (R_)	Growth (R_)	No
256	0	R	Growth (R_)	Growth (R_)	No
>512	0	R	Growth (R_)	Growth (R_)	No

(a) MIC distribution source (EUCAST, 2012) (Eucast MIC data 2012)

EUCAST provide distributions of MIC values for C. jejuni and C. coli, and these were used to estimate the proportion of these isolates in a population (Table 3). A plausible assumption was made that the distribution generated by EUCAST was representative of the distributions of Campylobacter in the datasets in this study. In this example of 11,298 C. jejuni isolates and resistance to GEN, 11,253 isolates had an MIC value of 4 or lower, whilst only one isolate had an MIC value of four. Hence the percentage of sensitive isolates (original threshold) that should be re-classified to resistant is 0.01% and 99.99% of the original sensitive isolates would remain sensitive. In this example the change from original to adjusted will be minor (Table 3). A similar process is followed when the original threshold is lower than the harmonised threshold, which leads to an overestimation of resistance in the original dataset. This process was repeated for each antimicrobial for C. jejuni and for C. coli. A summary of the estimated adjustments required is presented in Table 4, and it is apparent that in most cases the adjustments were neglible. The one exception is for C. coli and ERY, whereby the adjustment results in the number of resistant isolates being reduced by approximately 25% for some of the earlier PHE datasets.

Table 4. Adjustment to the percentage of resistant Campylobacter jejuni and C. coli isolates taking into account changes in threshold concentrations to ensure harmonisation.

Antimicrobial	Species	Original threshold	Harmonised threshold	Adjustment direction	Estimated adjustment to compare with data with predating thresholds
Ciprofloxacin	C. jejuni	1	0.5	Sensitive decrease	Sensitive x 0.9963
Ciprofloxacin	C. coli	1	0.5	Sensitive decrease	Sensitive x 0.9917
Erythromycin	C. coli	4	8	Resistant decrease	Resistant x 0.7457
Tetracycline	C. jejuni	8	2	Sensitive decrease	Sensitive x 0.9841
Tetracycline	C. coli	8	2	Sensitive decrease	Sensitive x 0.9789
Gentamicin	C. jejuni	4	2	Sensitive decrease	Sensitive x 0.9999
Gentamicin	C. coli	4	2	Sensitive decrease	Sensitive x 0.9996

In this study the definition of multidrug resistance (MDR) was defined in accordance with that used in the 2014 antimicrobial resistance report for the EU (EFSA and ECDC, 2016), specifically this is organisms that display resistance to at least three different classes of antimicrobial.

In summary data analysis was performed to:

1. Explore the trends in resistance to six antimicrobials for C. coli and C. jejuni isolates from chicken sampled in the UK from 2001 to 2020.

2. Adjust the percentages of resistant isolates for the analysis to explore the impact of changes to the antimicrobial thresholds over time.
3. Investigate the relationship between antimicrobial resistance and other variables, for example chicken production type and season.
4. Also analyse WGS derived resistance data to determine any impact of including those on trends.

Data
A file from the database was up-loaded with the following fields:

- Unique sample ID
- Sample dataset name
- Sampling year
- Sampling month
- Sample type (caecal/slaughterhouse carcass/retail fresh carcass/retail fresh portion/retail frozen carcass/retail frozen portion)
- Sample production type (Standard/Free range/Organic)
- Sample origin (UK/Other)
- Campylobacter species (C. jejuni/C. coli)
- Ciprofloxacin (S/R)
- Nalidixic acid (S/R)
- Erythromycin (S/R)
- Tetracycline (S/R)
- Gentamicin (S/R)
- Streptomycin (S/R) (not available for all isolates)

Descriptive analysis
AMR trends for C. jejuni and C. coli (separately), were plotted for samples (regardless of sample type) from the UK. Confidence intervals in figures show the likely range of the results allowing for the number of samples taken. The 95% confidence intervals mean that we would expect the true prevalence to fall within the lower and upper confidence limits 95% of the time.

4.3 Statistical analysis

The percentage of resistant isolates (using both phenotypic and genome-sequenced based AMR data) over time were presented in figures created in MS Excel 2013. All other statistical analyses were performed in STATA 15.

Pearsons chi squared tests were used to investigate the relationship between eight categorical exposure variables and the antimicrobial resistance outcomes based on phenotypic data. Exposure variables included: sampling year, sampling year category (derived from sampling year, considered as an alternative to sampling year), sample category (caeca, whole bird, portions), production category (conventional, free range, organic), origin category (UK, non-UK), sampling month, season (derived from sampling month (December, January and February = Winter), considered as an alternative to sampling month). Processing plant origin was not included in the analysis as there was insufficient data available to analyse this factor.

Season was available for all except for seven isolates; a small proportion of samples were frozen -these were all from retail in the years from 2001- 2008.

Univariate analysis was performed to explore the unadjusted odds ratio for antimicrobial resistance in each risk factor category against a baseline category. The strength of association between each risk factor and the antimicrobial resistance in question was used to determine the stepwise order in which variables were included in the multivariable model. Only variables with a p value of < 0.25 were tested in the multi variable model. Mantel-Hanzel odds were used look for

evidence that the sampling year acted as an effect modifier on the other variables included in the model. The final multivariable logistic regression model for each antimicrobial was created in a stepwise fashion, testing the strength of the model with each new exposure variable against the preceding model using a likelihood-ratio test. Where the inclusion of a variable significantly improved the fit of the model to the data, it was included, and the next variable was tested. Where no significant improvement was made it was rejected from the final model. The final model was tested using a Goodness of fit test and by calculating the area under the ROC curve.

Cross tabulations were analysed by the calculation of Clopper-Pearson exact 95% confidence intervals for the proportion in each category. In addition, the Pearson chi square test of association has been used to test the null hypothesis of no association between the measured variable and AMR in Campylobacter. Fisher's exact test was used for individual comparisons when sample sizes were small.

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Results

The analysis of trends in AMR in the C. jejuni and C. coli isolates from chicken was performed for samples obtained from 2001 to 2020 in the UK, collected through various projects. In total, phentoypic antimicrobial resistance data for 5,267 C. jejuni and 1,997 C. coli isolates collected between 2001 and 2018 inclusive were analysed. Based on this data alone, on average for all years, 29% of the C. jejuni and 32% of the C. coli isolates examined were resistant to ciprofloxacin (CIP) but only 1.6% of the C. jejuni and 13.2% of the C. coli isolates were resistant to erythromycin (ERY). For the years from 2001 to 2018, 48.2% of both the C. jejuni and the C. coli isolates examined were resistant to tetracycline (TET). Resistance to streptomycin (STR) was detected in 1.0% of the C. jejuni and 8.9% of the C. coli isolates. Only four isolates tested were resistant to gentamicin (GEN), two each of C. jejuni and C. coli, with 95% CI of 0.00 - 0.07 and 0.00 - 0.18, respectively.

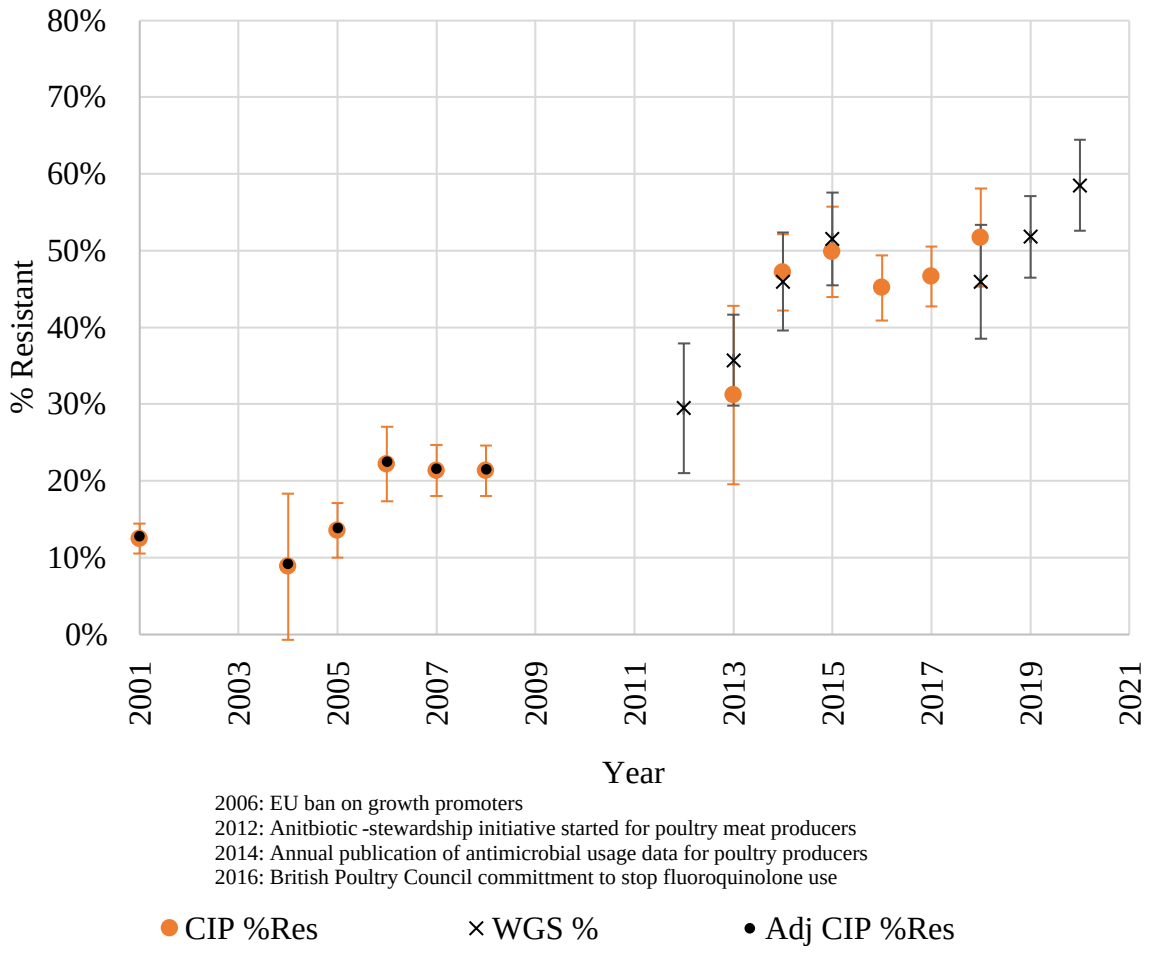
Results from two datasets, containing additional isolates collected from carcases between 2012-2015 and 2018 to 2020 with AMR predicted from genome sequence data were also examined and results presented.

5.1 Trends in resistance to ciprofloxacin and nalidixic acid

5.1.1 C. jejuni

The percentage of C. jejuni isolates from chicken in the UK (retail and slaughterhouse samples) from 2001 to 2020 with resistance to CIP is shown in Figure 1.

Figure 1. Trend in the percentages of ciprofloxacin (CIP) resistant C. jejuni isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2020. Orange circles are based on phenotypic data. The black dots represent adjusted percentages of resistant isolates to account for the higher threshold concentration used in the years before 2009. The crosses represent percentages of resistant isolates based on the presence of genetic determinants.



The percentage of C. jejuni isolates with resistance to CIP increased significantly over time, from 13% in 2001 to 52% in 2018, this upward trend is apparent in Figure 1. Modest observations post 2015 provided some percentage estimates with a relatively wide margin of uncertainty and so not able to statistically confirm any apparent increase in very recent years, however this may be possible if data from both phenotypic and genotypic testing in the later years were combined for analysis. There was a change in the threshold concentration used to distinguish phenotypically resistant isolates from 2009 and the percentages of resistant isolates prior to this change were adjusted (described in detail in section 2.1) to enable comparability of data before and after this change. The impact of the adjustments was negligible as can be seen from the adjusted vs. unadjusted values plotted. Results for the percentages of isolates with resistance to CIP based on genome sequence data from additional samples were consistent with the results based on the phenotypic data (Figure 1). The percentage of isolates with genetic determinants predicting resistance to CIP was detected in 52.9% of samples on average in the years from 2018 to 2020, similar to the percentages determined by phenotypic testing from 2014 to 2018. Taking the data from 2020 (n = 265) alone however, 58.5% of C. jejuni isolates had a genetic determinant predicting resistance to CIP and this percentage was significantly higher than the percentage of resistant isolates in 2018 (p = 0.03; Fishers exact test; based on analysis of the combined number of isolates including both genome sequence and phenotypic data from 2018, n = 408). Continued monitoring would be needed to establish if there may be evidence of an increased percentage of CIP resistant C. jejuni in the most recent years.

The univariate (as reflected by the unadjusted OR) and multivariate (as reflected by the adjusted OR) regression analysis for resistance to CIP in C. jejuni investigated the role of sample year (categorised as shown), season, sample type (caeca/whole carcass/ portions), sample state

(fresh/frozen) and chicken production type (standard or free-range or organic) (Table 5). Only data based on phenotypic testing were included in this analysis. It was not possible to incorporate the harmonised threshold adjustment to theses analyses, however the impact of the adjustment was neglible for all combinations of antimicrobial and Campylobacter spp., with the exception of ERY and C. coli.

In the multivariate analysis only the year category of isolation, season and sample state remained significant. The year category had the largest odds ratio and from the years 2001-2005 the percentage of C. jejuni with resistance to CIP increased from 13% to 47% in the years 2011 to 2018.

Table 5. Results of uni- and multivariable logistic regression analyses to identify significant risk factors for ciprofloxacin (CIP) resistance (based on phenotypic data) amongst C. jejuni isolates sampled at UK retail and slaughterhouses between 2001 and 2018.

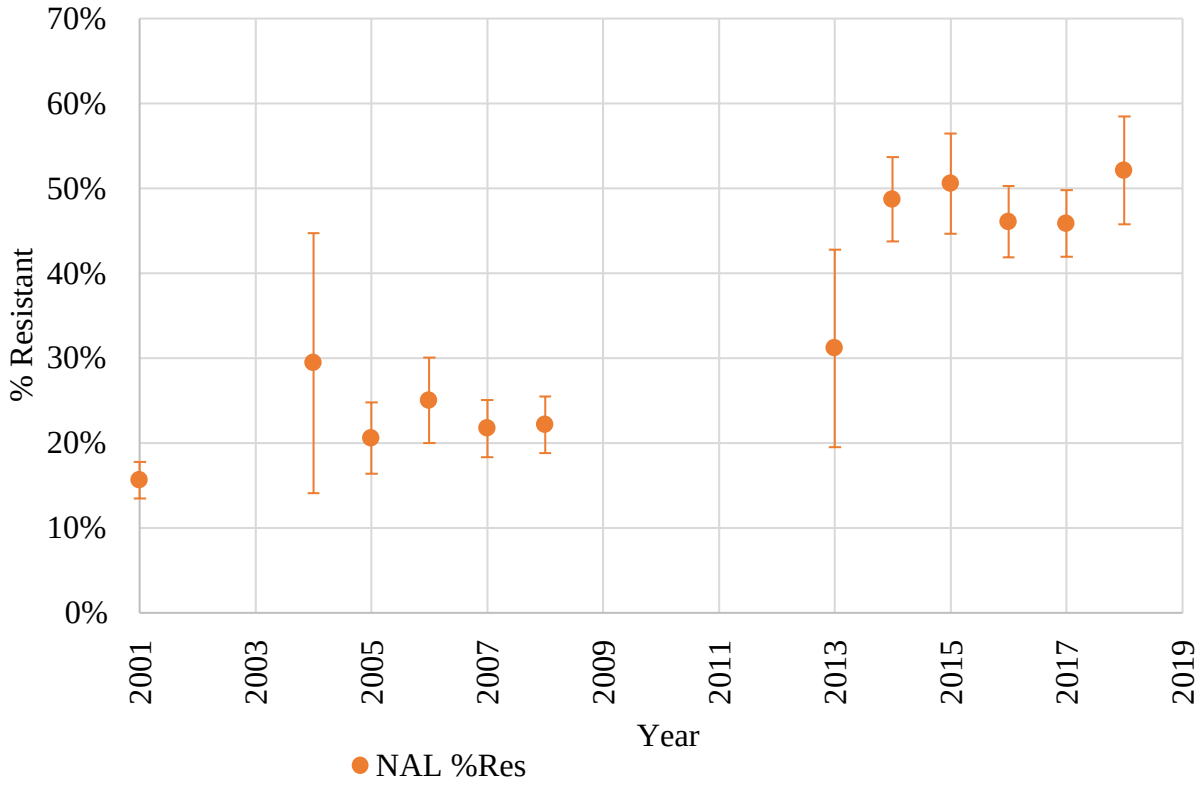
CIP category factors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
2001-2005	1,612	13%	1 (ref)	NA	1 (ref)	NA
2006-2010	1,530	22%	1.8 (1.5-2.2)	<0.001	1.9 (1.5-2.3)	<0.001
2011-2015	726	47%	5.8 (4.8-7.2)	<0.001	6.5 (5.2-8.2)	<0.001
2016-2018	1,401	47%	5.9 (4.9-7.0)	<0.001	6.3 (5.1-7.7)	<0.001
Spring	1,940	23%	1 (ref)	NA	1 (ref)	NA
Summer	1,284	28%	1.2 (1.1-1.5)	0.007	0.8 (0.7-1.0)	0.036
Autumn	1,180	35%	1.8 (1.5-2.1)	<0.001	0.9 (0.7-1.1)	0.198
Winter	865	37%	1.9 (1.6-2.3)	<0.001	1.2 (1.0-1.4)	0.098
Fresh sample	4,919	30%	1 (ref)	NA	1 (ref)	NA
Frozen sample	348	20%	0.6 (0.5-0.8)	<0.001	1.5 (1.1-1.9)	0.010
unknown	2	0%	NA	NA	NA	NA
Total	5,269	29%	NA	NA	NA	NA

NA: not applicable

Season was a significant factor with summer having a slightly reduced risk of detecting CIP resistant C. jejuni compared to the baseline of spring, and winter and frozen samples were also slightly more likely to yield resistant C. jejuni when the other factors in the model had been adjusted for. Including sample type category (whole/portions/caeca) did not improve the overall fit of the multivariable model to the data, however the results of the univariate analysis showed that portions were significantly less likely to have CIP resistance than whole birds (OR = 0.43, 95% CI 0.36-0.51; p<0.001). However, after adjusting for year category, sample category was not significantly associated with CIP resistance (OR = 0.99, 95% CI 0.82-1.20; p=0.929). The analysis demonstrated that there was no significant difference in the percentage of C. jejuni isolates with resistance to CIP between the different sample categories (i.e. caecal contents, whole carcasses or portions). Nor was there any significant difference between isolates obtained from organic, free-range or standard chicken or between samples of UK and non-UK origin.

Resistance to nalidixic acid (NAL) in C. jejuni showed a similar increasing trend for the isolates from UK retail and slaughterhouse samples (Figure 2).

Figure 2. Trend in the percentages of nalidixic acid (NAL) resistant C. jejuni isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2018.



his is expected as the key resistance mutation in gyrA confers resistance to both CIP and NAL. There was a significant increase over time from 16% of isolates with resistance to NAL in 2001 to 52% in 2018. Similar to the results for resistance to CIP there was no significant change in the percentage of isolates with resistance to NAL in the time period from 2014 to 2018. Validated data for prediction of resistance to NAL from genetic determinants were not available

The logistic regression analysis for resistance to NAL showed similar results to those obtained for CIP with no significant difference in the percentage of C. jejuni isolates with resistance to NAL between the different sample categories or chicken production type (Table 6). In the multivariate analysis only the year category of isolation and season remained significant and the year category had the largest odds ratio for NAL. From the years 2001-2005 the percentage of C. jejuni with resistance to NAL increased from 17% to 47% in the period 2016-2018. Season remained significant in the multivariate model with the summer period being protective (OR = 0.8, p-value 0.008) compared to a baseline of spring, and winter was a significant risk factor (OR = 1.2, p-value 0.028).

Table 6. Results of uni- and multivariable logistic regression analyses to identify significant risk factors for nalidixic acid (NAL) resistance amongst C. jejuni isolates sampled at UK retail outlets and slaughterhouses between 2001 and 2018.

NAL factors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	p value
2001-2005	1,612	17%	1 (ref)	NA	1 (ref)	NA
2006-2010	1.530	23%	1.4 (1.2-1.7)	<0.001	1.4 (1.2-1.7)	<0.001
2011-2015	726	48%	4.4 (3.6-5.3)	<0.001	4.6 (3.8-5.7)	<0.001
2016-2018	1,401	47%	4.2 (3.6-5.0)	<0.001	4.2 (3.5-5.1)	<0.001
Spring	1,940	26%	1 (ref)	NA	1 (ref)	NA
Summer	1,284	28%	1.1 (1.0-1.3)	0.166	0.8 (0.7-0.9)	0.008
Autumn	1,180	36%	1.6 (1.4-1.8)	<0.001	0.9 (0.7-1.0)	0.138

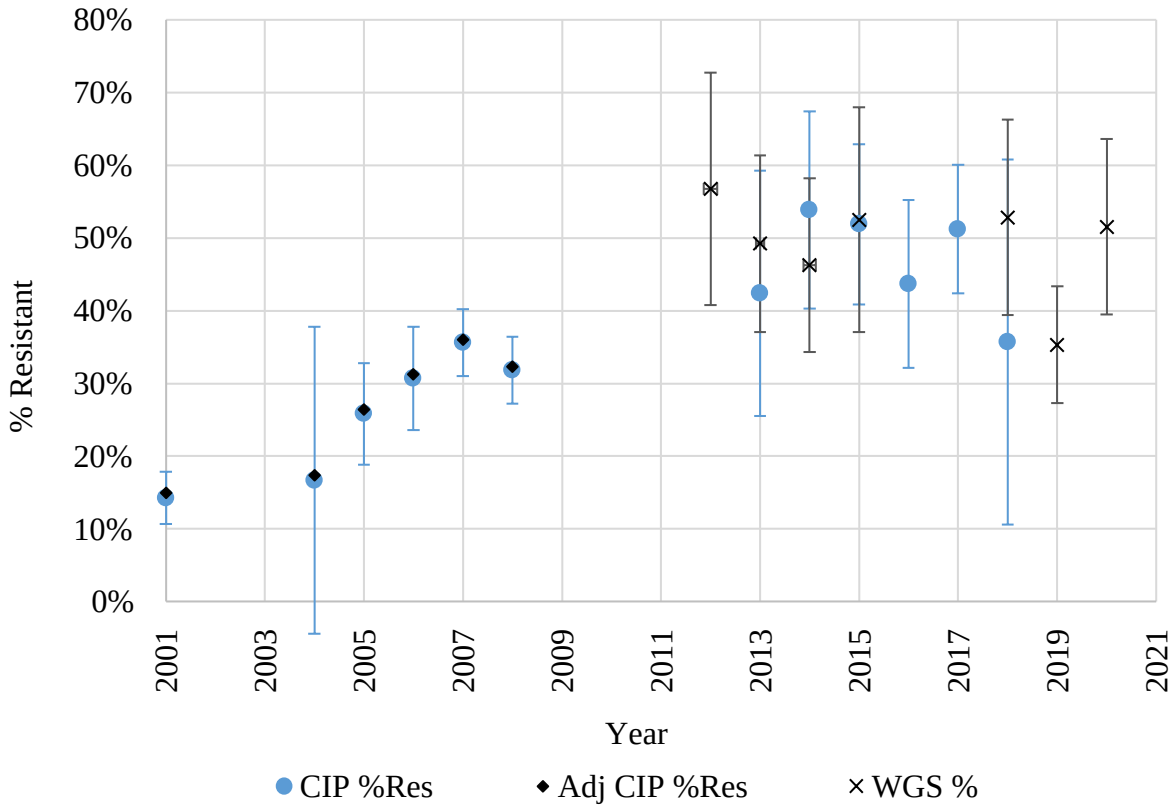
NAL factors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	p value
Winter	865	39%	1.8 (1.5-2.2)	<0.001	1.2 (1.0-1.5)	0.028
Total	5,269	31%	NA	NA	NA	NA

Including the sample type and state categories did not improve the overall fit of the multivariable model to the data, however the results of the univariate analysis for both variables were significant. Isolates from portions were less likely to have NAL resistance than isolates from whole chicken (OR = 0.43, 95% CI 0.36-0.51; p<0.001), while frozen samples were less likely to yield isolates with NAL resistance than isolates from fresh samples (OR = 0.64, 95% CI 0.49-0.82, p=0.001) but neither variable was significant after adjusting each for year category (portions OR = 0.88, 95% CI 0.73-1.05, p=0.168 and frozen OR = 1.29, 95% CI 0.99-1.69, p=0.064).

5.1.2 *C.coli*

The percentage of C. coli isolates from UK chicken from 2001 to 2018 with resistance to CIP is shown in Figure 3. Similar to the data obtained for C. jejuni, the percentage of isolates with resistance to CIP increased significantly over time, from 15% in 2001 to a peak of 51% in 2017. However, there was no significant difference in the percentages of C. coli with resistance to CIP over the years from 2014 to 2018.

Figure 3. Trend in the percentages of ciprofloxacin (CIP) resistant C. coli isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2020. Blue circles are based on phenotypic data. The black dots represent adjusted percentages of resistant isolates to account for the higher threshold concentration used in earlier compared to the later years. The crosses represent percentages of resistant isolates based on the presence of genetic determinants for CIP resistance.



As for C. jejuni, there was a change in the threshold concentration used to distinguish resistant and sensitive isolates from 2009 and the adjusted percentage for C. coli was also plotted in Figure 3. The impact of the adjustments was negligible as can be seen from the adjusted and the unadjusted values plotted. Results for the percentages of isolates with resistance to CIP based genome sequence data from additional samples were consistent with the results based on the phenotypic data (Figure 3).

Genetic determinants predicting resistance to CIP were detected in 43.7% of isolates collected between 2018 to 2020 (n = 252; 95% CI 37.4-50.0), similar to the percentage of resistant isolates determined by phenotypic testing from 2014 to 2018 and with no significant difference (Fishers exact test) in the percentage of resistant isolates in 2014 compared to 2020 suggesting no change in the percentage of C. coli isolates with resistance to CIP since 2014.
In the univariate and multivariate regression analysis there was no significant difference in the percentage of C. coli isolates with resistance to CIP between the different sample categories (Table 7).

Table 7. Results of uni- and multivariable logistic regression analyses to identify significant risk factors for resistance to ciprofloxacin (CIP) (based on phenotypic data) amongst C. coli isolates sampled at UK retail and slaughterhouses between 2001 and 2018.

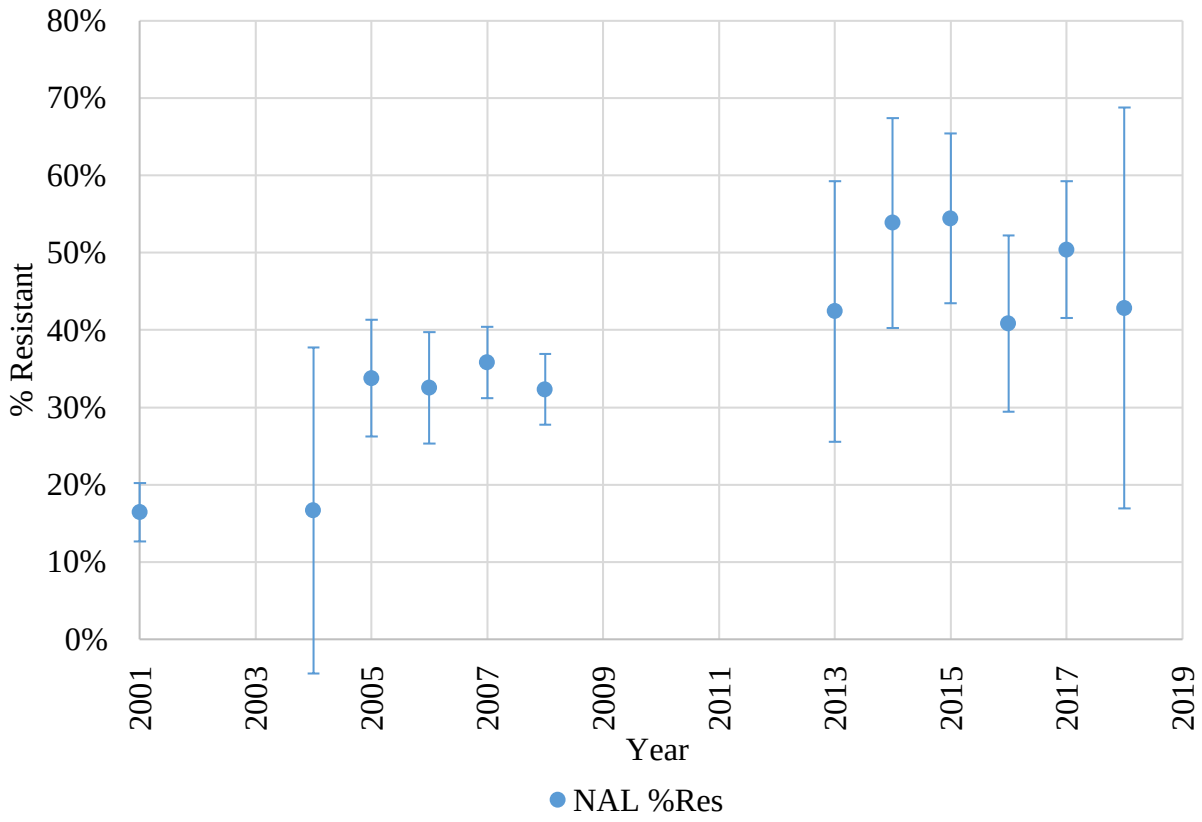
CIP category factors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
2001-2005	590	18%	1 (ref)	NA	1 (ref)	NA
2006-2010	1,034	34%	2.3 (1.8-3.0)	<0.001	2.1 (1.6-2.7)	<0.001
2011-2015	164	51%	4.6 (3.2-6.7)	<0.001	3.7 (2.5-5.4)	<0.001
2016-2018	209	48%	4.1 (2.9-5.8)	<0.001	3.2 (2.2-4.7)	<0.001
Standard	1,661	29%	1 (ref)	NA	1 (ref)	NA
Free range	245	48%	2.2 (1.7-2.9)	<0.001	1.9 (1.4-2.5)	<0.001
Organic	77	45%	2.0 (1.3-3.2)	0.003	2.2 (1.3-3.5)	0.001
Unknown	14	36%	1.3 (0.4-4.0)	0.602	1.5 (0.5-4.6)	0.483
Spring	565	22%	1 (ref)	NA	1 (ref)	NA
Summer	649	35%	1.9 (1.5-2.4)	<0.001	1.4 (1.1-1.9)	0.010
Autumn	513	41%	2.4 (1.9-3.2)	<0.001	1.8 (1.3-2.4)	<0.001
Winter	270	32%	1.7 (1.2-2.3)	0.001	1.2 (0.8-1.7)	0.315
UK	1,878	32%	1 (ref)	NA	1 (ref)	NA
Non-UK	92	40%	1.4 (0.9-2.2)	0.099	2.1 (1.3-3.3)	0.001
Unknown	27	26%	0.7 (0.3-1.8)	0.506	1.2 (0.5-3.0)	0.691
Total	1,997	32%	NA	NA	NA	NA

In the multivariate analysis the year of isolation, production type, season and country of origin remained significant. The year category had the largest odds ratio and from the years 2001-2005 the percentage of C. coli with resistance to CIP increased from 18% to 48% in the years from 2016 to 2018. Significant ORs were noted for chicken production type, season and chicken origin.The proportion of C. coli with resistance to CIP was higher in samples from free range and organic birds, from summer and autumn and from non-UK chicken. Analysis of the genome sequence data from 2018-2020 also found an increased percentage of C. coli with resistance to CIP in isolates from free range compared to standard chicken ($p < 0.0001$). Similarly, C. coli with resistance to CIP predicted by genome sequence data were more common in isolates from free range compared to standard chicken carcases from UK slaughterhouses in 2012-2015 ($p < 0.0001$). Including the sample category did not improve the overall fit of the multivariable model, however the results of the univariate analysis showed that isolates from portions were significantly less likely to have CIP resistance than isolates from whole birds (OR = 0.66, 95% CI

0.54-0.81; p<0.001). However, after adjusting for year category, sample category was not significantly associated with CIP resistance (OR = 0.88, 95% CI 0.71-1.11; p = 0.285).

Resistance to NAL in C. coli showed a similar increasing trend as for CIP (Figure 4) from 2001 to 2018 with an increase over time from 16% of isolates with resistance to NAL in 2001 to 50% in 2017. Similar to the data for resistance to CIP there was no significant change in the percentage of isolates with resistance to NAL in the time period from 2014 to 2018.

Figure 4. Trend in the percentages of nalidixic acid (NAL) resistant C. coli isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2018.



As expected the analysis for resistance to NAL in C. coli showed similar results as those observed for resistance to CIP (Table 8). In the multivariate analysis the year category of isolation, production type, season, sample category and chicken origin remained significant. The year category had the largest odds ratio and from the years 2001-2005 the percentage of C. coli with resistance to NAL increased from 22% to 47% in the years 2016 to 2018. Lower significant odds ratios were noted for production type, season, sample type and chicken origin, where the percentages of C. coli with resistance to NAL were lower for standard birds, in spring months, for caecal samples and for UK produced chicken.

Table 8. Results of uni- and multivariable logistic regression analyses to identify significant risk factors for nalidixic acid (NAL) resistance (based on phenotype data) amongst C. coli isolates sampled at UK retail and slaughterhouses between 2001 and 2018.

NAL category factors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
2001-2005	590	22%	1 (ref)	NA	1 (ref)	NA

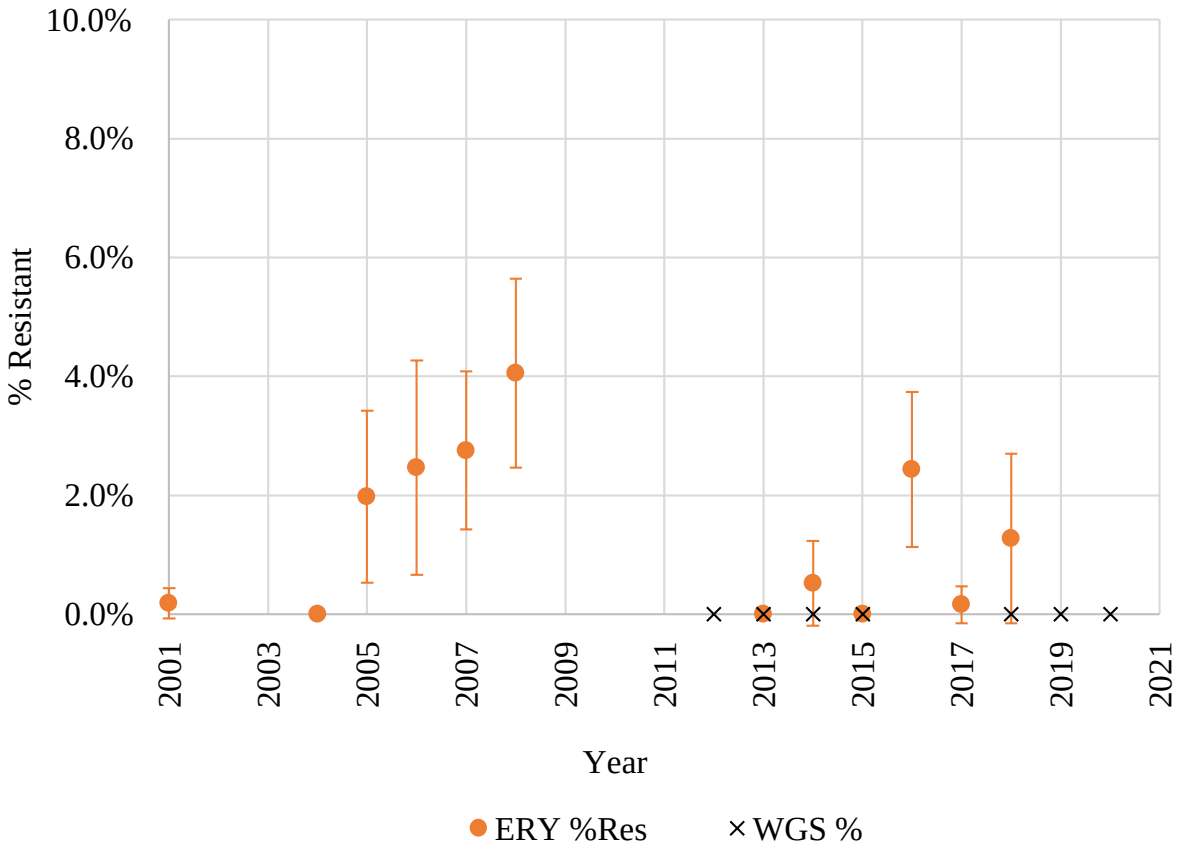
NAL categoryfactors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
2006-2010	1,034	35%	2.0 (1.6-2.5)	<0.001	1.8 (1.4-2.3)	<0.001
2011-2015	164	52%	3.9 (2.7-5.6)	<0.001	3.4 (2.3-5.1)	<0.001
2016-2018	209	47%	3.2 (2.3-4.5)	<0.001	2.5 (1.7-3.6)	<0.001
Standard	1,661	31%	1 (ref)	NA	1 (ref)	NA
Free range	245	47%	2.0 (1.5-2.6)	<0.001	1.7 (1.3-2.3)	<0.001
Organic	77	48%	2.1 (1.3-3.3)	0.002	2.2 (1.4-3.5)	0.001
Unknown	14	43%	1.7 (0.6-4.8)	0.343	1.8 (0.6-5.5)	0.267
Spring	565	24%	1 (ref)	NA	1 (ref)	NA
Summer	649	36%	1.7 (1.3-2.2)	<0.001	1.4 (1.1-1.8)	0.017
Autumn	513	41%	2.1 (1.6-2.8)	<0.001	1.7 (1.3-2.3)	<0.001
Winter	270	36%	1.7 (0.9-1.9)	0.001	1.3 (0.9-1.9)	0.112
Whole	1,199	37%	1 (ref)	NA	1 (ref)	NA
Portion	663	28%	0.7 (0.5-0.8)	<0.001	1.0 (0.8-1.2)	0.782
Caeca	133	31%	0.8 (0.5-1.1)	0.165	0.6 (0.4-0.9)	0.024
Unknown	2	0%	NA	NA	NA	NA
UK	1,878	33%	1 (ref)	NA	1 (ref)	NA
Non-UK	92	42%	1.5 (1.0-2.2)	0.074	2.0 (1.3-3.0)	0.003
Unknown	27	26%	0.7 (0.3-1.7)	0.420	1.0 (0.4-2.5)	1
Total	1,997	34%	NA	NA	NA	NA

5.2 Trends in resistance to erythromycin

5.2.1 *C Jejuni*

The percentages of C. jejuni isolates from UK chicken from 2001 to 2018 with resistance to ERY are shown in Figure 5. The percentage of C. jejuni isolates with resistance to ERY did not show a significant increasing trend of over time with resistance below 5% in all years. The highest percentage was detected in 2008 with 4% of isolates showing resistance to ERY.

Figure 5. Trend in the percentages of erythromycin (ERY) resistant C. jejuni isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2020. Orange circles are based on phenotypic data. The crosses represent percentages of resistant isolates based on the presence of genetic determinants predicting resistance to ERY. Note no error bars applicable for datapoints with no resistant isolates.



Results for the percentages of isolates with resistance to ERY based genome sequence data were consistent with the results based on the phenotypic data (Figure 5). In the most recent samples from 2018 to 2020 no resistance to ERY was detected in 773 C. jejuni isolates tested (95% CI 0.00-0.48). This supported the phenotypic results from previous years showing very low percentages of resistance to ERY in C. jejuni isolates since 2014. In the multivariate analysis the year category of isolation, sample state and season remained significant. The year category had the largest odds ratio and in the years 2006-2010 the percentage of C. jejuni with resistance to ERY was 3.6% compared to less than 2% in the years before or after. Lower odds ratios were noted for sample state and season, where the percentages of C. jejuni with resistance to ERY were lower for fresh samples and for samples tested in autumn months. Including sample country of origin did not include the overall fit of the multivariable model, however the results of the univariate analysis showed that non-UK chicken were significantly more likely to have ERY resistance than UK chicken (OR = 3.20, 95% CI 1.52-6.74; p=0.002). However, after adjusting for sample state (fresh/frozen), origin was not significantly associated with ERY resistance (OR = 1.81, 95% CI 0.76-4.29; p=0.179).

Table 9. Results of uni- and multivariable logistic regression analyses to identify risk factors for erythromycin (ERY) resistance amongst C. jejuni isolates sampled at UK retail and slaughterhouses between 2001 and 2018.

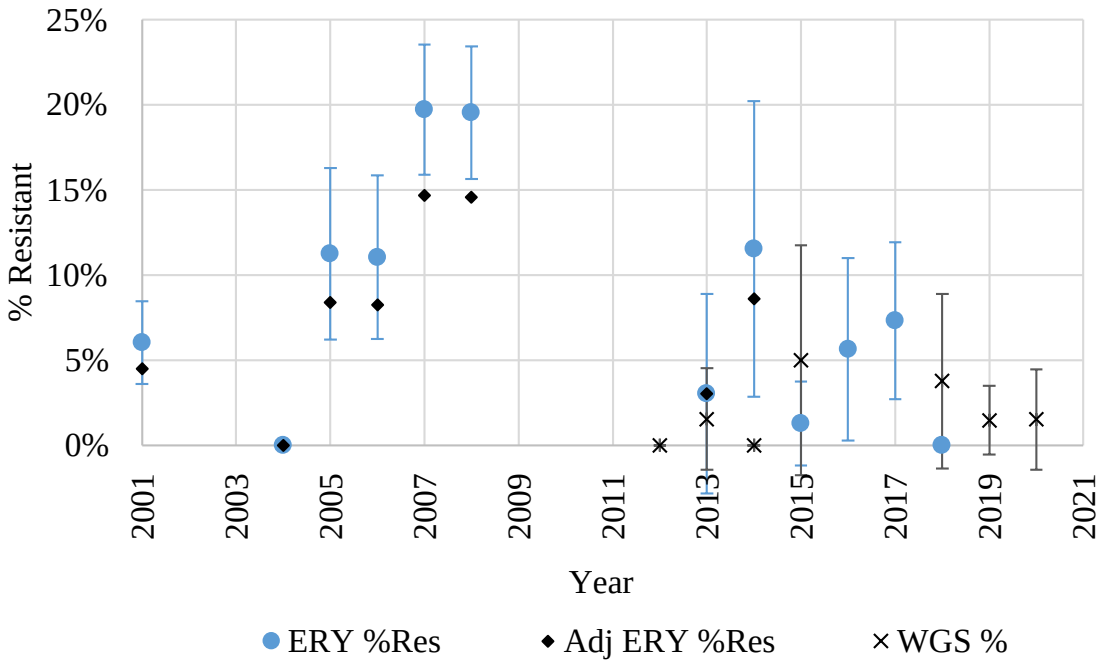
ERY category factors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
2001-2005	1,612	0.6%	1 (ref)	NA	1 (ref)	NA
206-2010	1,530	3.6%	6.0 (3.0-11.8)	<0.001	8.6 (4.2-17.5)	<0.001
2011-2015	726	0.3%	0.4 (0.1-2.0)	0.293	0.9 (4.2-17.5)	0.914
2016-2018	1,401	1.2%	2.0 (0.9-4.3)	0.091	3.6 (1.6-8.5)	0.003
Fresh	4,919	1.4%	1 (ref)	NA	1 (ref)	NA

ERY categoryfactors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Frozen	348	4.3%	3.2 (1.8-5.6)	<0.001	3.5 (1.9-6.4)	<0.001
Unknown	2	0%	NA	NA	NA	NA
Spring	1,940	1.9%	1 (ref)	NA	1 (ref)	NA
Summer	1,284	1.5%	0.8 (0.4-1.3)	0.364	0.6 (0.3-1.1)	0.085
Autumn	1,180	0.6%	0.3 (0.1-0.7)	0.004	0.3 (0.1-0.6)	0.001
Winter	865	2.4%	1.3 (0.7-2.2)	0.372	0.8 (0.5-1.4)	0.455
Total	5,269	1.6%	NA	NA	NA	NA

5.2.2 *C.coli*

There was no increasing trend in the percentages of C. coli isolates with resistance to ERY over time (Figure 6). The percentages with resistance to ERY were low except for data from 2007 and 2008 where level of resistance was moderate. There was a change in the threshold concentration used to distinguish resistant and sensitive isolates from 2009. The impact of the adjustments was a 25% reduction in the proportion of resistant isolates as can be seen from the adjusted vs. unadjusted values plotted (Figure 6).

Figure 6. Trend in the percentages of erythromycin (ERY) resistant C. coli isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2020. Blue circles are based on phenotypic data. The black dots represent adjusted percentages of resistant isolates to account for the change in threshold concentration used in earlier compared to the later years. The crosses represent percentages of resistant isolates based on the presence of genetic determinants predicting resistance to ERY. Note no error bars applicable for datapoints with no resistant isolates.



When considering the adjusted percentages there was no clear trend for resistance to ERY with resistance below 15% in all years and below 10% since 2014. More recent results based on prediction of resistance to ERY from genome sequence data in isolates from retail chicken in the UK showed that the percentage of isolates with determinants predicting resistance to ERY was detected in 1.96% of samples tested from 2018 to 2020 (n = 255; 95% CI 0.64-4.52). This supported the phenotypic results showing no significant increase in the percentage of C. coli

isolates with resistance to ERY since 2014.
The univariate and multivariate analysis (based on the unadjusted values) demonstrated that there was no significant difference in the percentage of C. coli isolates with resistance to ERY between seasons but the year of isolation, production method and sample type remained significant (Table 10). The year category had the largest odds ratio and in the years 2006-2010 the percentage of C. coli with resistance to ERY was 19% compared to 5-7% in the years before or after, however it should be noted adjusted data for the earlier time period was could not be included in the regression analysis. Applying the adjustment to the percentage found in the earlier years would reduce the percentage to 14%.

Table 10. Results of uni- and multivariable logistic regression analyses to identify risk factors for erythromycin (ERY) resistance (based on phenotype data) amongst C. coli isolates sampled at UK retail and slaughterhouses between 2001 and 2018.

ERY category factors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
2001-2005	590	7%	1 (ref)	NA	1 (ref)	NA
2006-2010	1,034	19%	3.1 (2.2-4.4)	<0.001	3.6 (2.5-5.1)	<0.001
2011-2015	164	5%	0.7 (0.3-1.5)	0.311	1.1 (0.5-2.3)	0.895
2016-2018	209	7%	1.0 (0.5-1.9)	0.978	1.3 (0.7-2.5)	0.413
Standard	1,661	15%	1 (ref)	NA	1 (ref)	NA
Free range	245	6%	0.4 (0.2-01.6)	<0.001	0.4 (0.2-0.7)	0.002
Organic	77	4%	0.2 (0.1-0.8)	0.015	0.3 (0.1-0.9)	0.036
Unknown	14	29%	2.3 (0.7-7.5)	0.152	2.7 (0.8-9.3)	0.118
Whole	1,199	13%	1 (ref)	NA	1 (ref)	NA
Portion	663	16%	1.3 (1.0-1.7)	0.077	1.2 (0.9-1.5)	0.298
Caeca	133	1%	0.1 (0.0-0.4)	0.003	0.0 (0.0-0.3)	0.001
Unknown	2	0%	NA	NA	NA	NA
Total	1,997	13%	NA	NA	NA	NA

A lower odds ratio was noted for C. coli with resistance to ERY in samples from free-range and organic chicken. However, in neither of the data sets where ERY resistance was predicted from genome sequence data (the 2012-2015 data from slaughterhouse samples and in the retail data from 2018-2020) was there a significant difference between samples from free range and standard retail chicken. Lower odds ratios were noted for C. coli with resistance to ERY in samples from caecal samples. Including sample origin (UK/non-UK) did not improve the overall fit of the multivariable model to the data, however the results of the univariate analysis showed that non-UK birds were significantly more likely to have ERY resistance than UK birds (OR = 1.78, 95% CI 1.05-3.00; p=0.031). However, after adjusting for sample category (which was included in the multivariable model), origin was not significantly associated with ERY resistance (OR = 1.65, 95% CI 0.98-2.78; p=0.062).

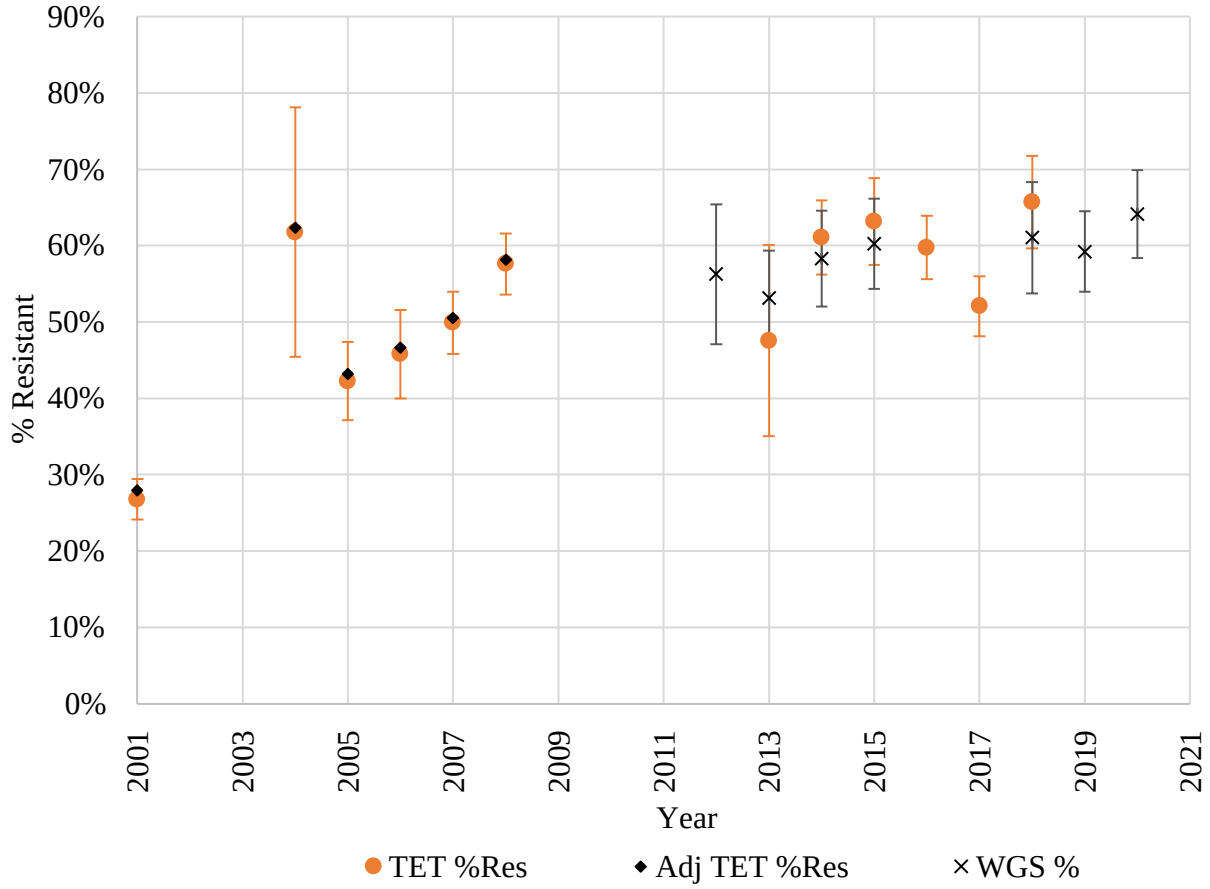
5.3 Trends in resistance to tetracycline

5.3.1 C. jejuni

The percentage of C. jejuni isolates from UK chicken from 2001 to 2020 with resistance to tetracycline (TET) is shown in Figure 7.

Figure 7. Trend in the percentages of tetracycline (TET) resistant C. jejuni isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2020. Orange circles are based on phenotypic data. The black dots represent adjusted percentages of resistant isolates to account for the higher threshold concentration used in earlier

compared to the later years. The crosses represent percentages of resistant isolates based on the presence of genetic determinants predicting resistance to TET.



The level of TET increased significantly over time, from 27% in 2001 to 66% in 2018. There was a stable level of resistance to TET in C. jejuni in the years from 2014 to 2020. The impact of the adjustments made for the percentages of resistant isolates in the years before 2009 was negligible as can be seen from the adjusted vs. the unadjusted values. Results for percentages of isolates with resistance to TET based on genome sequence data from additional samples were consistent with the results based on the phenotypic data.

The percentage of isolates with genetic determinants predicting resistance to TET was detected in 61.4% of samples tested from 2018 to 2020 (n = 773; 95% CI 57.8-64.8) similar to the percentages based on phenotypic testing from 2014 to 2018, showing no significant (Fisher exact test) change in the percentage of C. jejuni isolates with resistance to TET since 2014.

In the multivariate analysis the year category of isolation, sample state, season and sample category remained significant (Table 11). The year category had the largest odds ratio and from the years 2001-2005 the percentage of C. jejuni with resistance to TET increased from 32% to 57% in years from 2016 to 2018. The lower odds ratios for resistance were noted for frozen samples relative to fresh samples, samples collected in summer relative to spring and in chicken portions relative to whole birds. Increased resistance to tetracycline was observed in C. jejuni collected in winter months relative to spring and in caecal sample relative to whole bird samples.

Including sample country of origin did not improve the overall fit of the multivariable model to the data, but the results of the univariate analysis showed that non-UK birds were significantly less likely to have resistance to TET than UK birds (OR 0.57, 0.41-0.78; p<0.001). However, after adjusting for either sample state category, year category or sample category, origin was no longer

significantly associated with TET resistance (p>0.05).

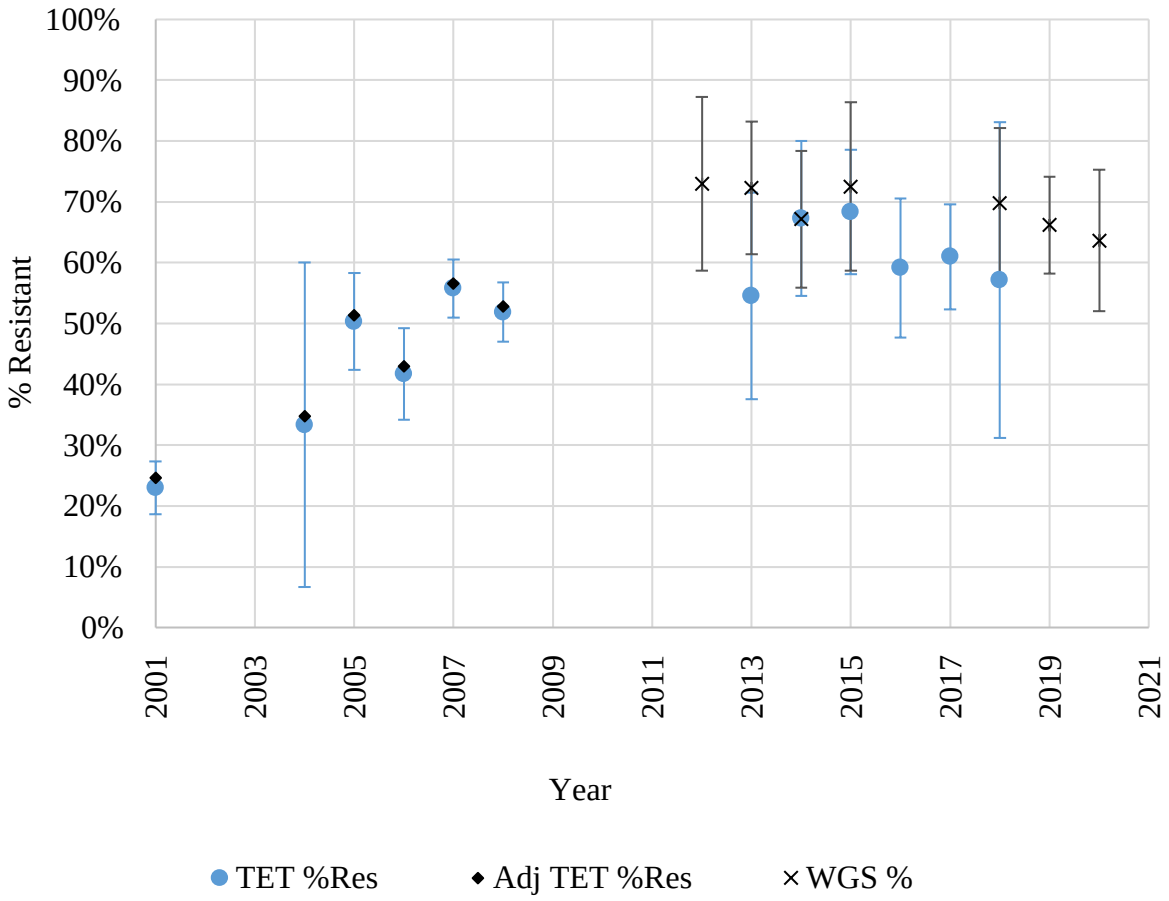
Table 11. Results of uni- and multivariable logistic regression analyses to identify significant risk factors for tetracycline resistance (based on phenotypic data) amongst C. jejuni isolates sampled at UK retail and slaughterhouses between 2001 and 2018.

TET category factors	Number of isolates	Percentage resistant	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
2001-2005	1,612	32%	1 (ref)	-	1 (ref)	-
2006-2010	1,530	52%	2.3 (2.0-2.7)	<0.001	2.0 (1.7-2.3)	<0.001
2011-2015	726	61%	3.4 (2.8-4.0)	<0.001	2.8 (2.2-3.4)	<0.001
2016-2020	1,401	57%	2.9 (2.5-3.4)	<0.001	2.3 (1.9-2.7)	<0.001
Fresh	4,919	50%	1 (ref)	-	1 (ref)	-
Frozen	348	30%	0.4 (0.3-0.6)	<0.001	0.7 (0.5-0.9)	0.002
Unknown	2	50%	1.0 (0.1-16.3)	0.989	1.0 (0.1-16.9)	0.976
Spring	1,940	44%	1 (ref)	-	1 (ref)	-
Summer	1,284	42%	1.0 (0.8-1.1)	0.504	0.7 (0.5-0.9)	<0.001
Autumn	1,180	53%	1.4 (1.2-1.7)	<0.001	0.9 (0.7-1.0)	0.111
Winter	865	61%	2.1 (1.7-2.4)	<0.001	1.3 (1.1-1.6)	0.002
Whole	3,118	49%	1 (ref)	-	1 (ref)	-
Portion	1,224	35%	0.6 (0.5-0.6)	<0.001	0.8 (0.7-0.9)	0.003
Caeca	909	62%	1.7 (1.2-2.0)	<0.001	1.3 (1.2-1.6)	<0.001
Unknown	18	17%	0.2 (0.1-0.7)	0.012	0.3 (0.1-1.2)	0.094
Total	5,269	48%	-	-	-	-

5.3.2 C. *coli*

The percentage of C. coli isolates from UK chicken from 2001 to 2018 with resistance to TET is shown in Figure 8. The percentage of C. coli isolates with resistance to TET increased significantly over time, from 23% in 2001, to over 55% in all years after 2013. However, there was no increasing trend in the percentage of C. coli with resistance to TET in the years from 2014 to 2018. The impact of the adjustments made for the percentages of resistant isolates in the years before 2009 was negligible as can be seen from the adjusted vs. the unadjusted values. Results for the percentages of isolates with resistance to TET based genome sequence data were consistent with the results based on the phenotypic data (Figure 8).

Figure 8. Trend in the percentages of tetracycline (TET) resistant C. coli isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2020. Blue circles are based on phenotypic data. The black dots represent adjusted percentages of resistant isolates to account for the higher threshold concentration used in earlier compared to the later years. The crosses represent percentages of resistant isolates based on the presence of genetic determinants for TET resistance.



The percentage of isolates with genetic determinants predicting resistance to TET was detected in 66.3% of samples tested from 2018 to 2020 (n = 255; 95% CI: 60.1-72.1) similar to the percentages based on phenotypic testing from 2014 to 2018, showing no significant change in the percentage of isolates with resistance to TET since 2014.

In the multivariate analysis the sample year category, production type and season remained significant factors associated with TET resistance (Table 12). The year category had the largest odds ratio and from the years 2001-2005 the percentage of C. coli with resistance to TET increased from 32% to 60-65% in years from 2011 to 2018.

Table 12. Results of uni- and multivariable logistic regression analyses to identify significant risk factors for tetracycline (TET) resistance (based on phenotype data) amongst C. coli isolates sampled at UK retail and slaughterhouses between 2001 and 2018.

TET category factors	Number of isolates	Percentage resistant	Unadjusted OR (95%, CI)	p value	Adjusted OR (95% CI)	p value
2001-2005	590	32%	1 (ref)	NA	1 (ref)	NA
2006-2010	1,034	52%	2.3 (1.9-2.8)	<0.001	2.0 (1.6-2.5)	<0.001
2011-2015	164	65%	4.0 (2.7-5.7)	<0.001	3.0 (2.1-4.4)	<0.001
2016-2020	209	60%	3.2 (2.3-4.4)	<0.001	2.4 (1.7-3.4)	<0.001
Standard	1,661	46v	1 (ref)	NA	1 (ref)	NA
Free range	245	62%	1.9 (1.5-2.5)	<0.001	1.7 (1.3-2.3)	<0.001
Organic	77	53%	1.3 (0.8-2.1)	0.214	1.4 (0.9-2.3)	0.142
Unknown	14	36%	0.7 (0.2-2.0)	0.445	0.7 (0.2-2.0)	0.483
Spring	565	36%	1 (ref)	NA	1 (ref)	NA

TET category factors	Number of isolates	Percentage resistant	Unadjusted OR(95%, CI)	p value	Adjusted OR (95% CI)	p value
Summer	649	51%	1.8 (1.4-2.3)	<0.001	1.4 (1.1-1.8)	0.009
Autumn	513	57%	2.3 (1.8-2.9)	<0.001	1.6 (1.3-2.2)	<0.001
Winter	270	50%	1.7 (1.3-2.3)	<0.001	1.2 (0.9-1.7)	0.233
Total	1,997	48%	NA	NA	NA	NA

Higher odds ratios were noted in free range relative to standard chicken and for summer and autumn relative to spring. Analysis of the genome sequence data (taking into account isolates from carcasses sampled at UK slaughterhouses in 2012-2015 and from retail chicken between 2018-2020) also found a significant difference (p = 0.02; Fishers exact test) in the percentage of C. coli with resistance to TET in free range compared to chicken from standard production. Including sample category did not improve the overall fit of the multivariable model to the data, but the results of the univariate analysis showed that portions were significantly less likely to have TET resistance than samples from whole birds (OR 0.64, 0.52-0.77; p < 0.001). However, after adjusting for year category, production type and season (which were included in the multivariable model), sample category was not significantly associated with TET resistance (OR 0.84, 0.68-1.03; p = 0.101).

5.4 Trends in resistance to aminoglycosides

5.4.1 Resistance to gentamicin

Resistance to gentamicin (GEN) was detected in two (0.04%; 95%CI: 0.00-0.14%) of the 5,269 C. jejuni isolates from 2001-2018 based on phenotypic data. One of these isolates originated from a whole frozen chicken collected at retail in 2005, the other from a caecal sample collected from a slaughterhouse in 2018. Additional results based on prediction of resistance to GEN from genome sequence data were also considered. No genetic determinants predicting resistance to GEN was detected in a total of 1,626 C. jejuni isolates from chicken samples between 2012 and 2020 (95% CI: 0.00-0.23%).

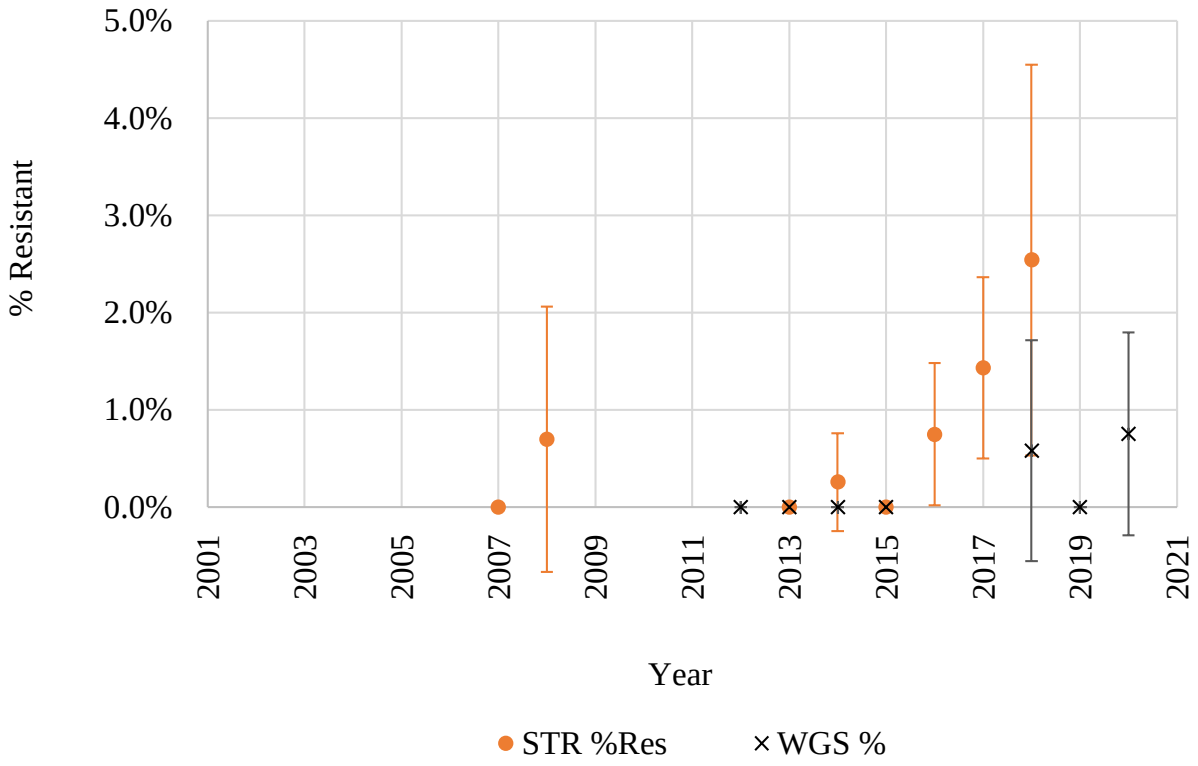
Resistance to GEN was detected two of the 2,034 C. coli isolates (0.10%; 95% CI: 0.01-0.35%) from 2001 and 2018, tested by phenotypic methods. Both isolates originated from frozen chicken breast portions labelled as of UK origin and collected at retail in 2001. Additional results based on prediction of resistance to GEN from genome sequence data in C. coli isolates from samples of chicken were also considered. No genetic determinants predicting resistance to GEN was detected in a total of 474 isolates from chicken samples obtained between 2012 and 2020 (95% CI: 0.00-0.78%). Due to this very rare occurrence of resistance to GEN no figures for trends nor multivariable analysis were included in this study.

5.4.2 Resistance to streptomycin *C jejuni*

There was no significant increase or decrease in the percentages of C. jejuni isolates with resistance to STR over the time period analysed (Figure 9). Resistance was zero or below 1% between 2007 and 2016 and 1.4% in 2017 (95% CI 0.5-2.4). Recent results based on prediction of resistance to STR from sequence data in C. jejuni isolates from samples of chicken at retail sale tested between 2018 and 2020 showed resistance to STR in three isolates (0.4%).

Figure 9. Trend in the percentages of streptomycin (STR) resistant C. jejuni isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2020. Orange circles are based on phenotypic data. The crosses represent percentages of STR resistant isolates based on the presence of genetic determinants. Note no error bars applicable

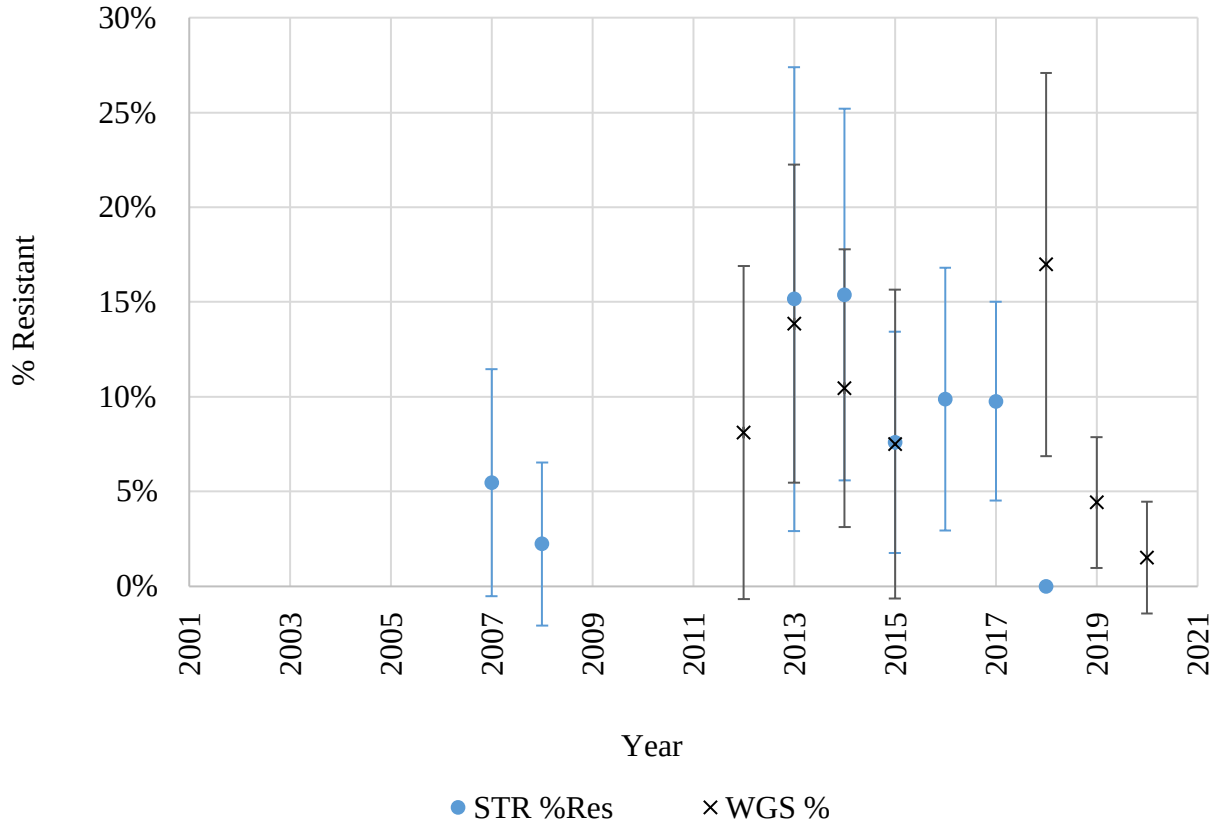
for datapoints with no resistant isolates.



5.4.3 Resistance to streptomycin in C *coli*

There was no significant increase or decrease in the percentage of C. coli isolates with resistance to STR in the data analysed (Figure 10). Resistance ranged from 2% to 15% between 2007 and 2017, with no apparent temporal trend. Recent results based on prediction of resistance to STR from genome sequence data in C. coli isolates from chicken at retail samples in the UK detected 16 isolates with resistance to STR (6.3%; 95% CI 3.3-9.5) in samples tested between 2018 and 2020.

Figure 10. Trend in the percentages of streptomycin (STR) resistant C. coli isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2007 and 2020. Blue circles are based on phenotypic data. The crosses represent percentages of resistant isolates based on the presence of genetic determinants predicting resistance to STR. Note that no error bars were applicable for data points with no resistant isolates.



5.5 Multidrug resistance

Multidrug resistance (MDR), defined as reduced susceptibility to at least three unrelated antimicrobial classes of drugs (guided by the definition from EFSA/ECDC), was found in 136 of the C. coli (6.8%) and 42 of the C. jejuni (0.8%) isolates based on phenotypic data. The proportion of MDR isolates detected in chicken samples originating in the UK, compared to those originating outside the UK was explored. Non-UK samples were predominately included in datasets prior to 2009. All UK (n = 4,435) and non-UK (n = 262) samples collected between 2001 and 2008, were examined. Significantly more non-UK samples had MDR profiles (4.96%) than samples originating in the UK (2.28%) (p = 0.012; Fisher's exact test).

5.5.1 C *jejuni*

There were very few observations of MDR in C. jejuni, with a study average based on phenotype data of just 0.8%. There was no evidence to suggest that MDR had increased in C. jejuni over the study period from 2001 to 2020 (Table 13).

Table 13. Number of C. jejuni isolates with multidrug resistant (MDR; defined as resistance to three or more unrelated antimicrobials) profiles (percentage resistant (R), 95% confidence intervals (CI)).

Time period	Total number of MDR isolates resistant to STR ERY and TET (%R, 95% CI)	Number of isolates resistant to STR ERY and TET (%R, 95% CI)	Number of isolates resistant to ERY TET and CIP (%R, 95% CI)	Number of isolates resistant to STR TET and CIP (%R, 95% CI)
2001-2005	2 (0.1, 0-0.3)	0 (0, 0-0)	2 (0.1, 0-0.3)	0 (0, 0-0)
2006-2010	16 (1, 0.5-1.6)	0 (0, 0-0)	15 (1, 0.5-1.5)	1 (0.1, 0.1-0.2)
2011-2015	2 (0.3, 0.1-0.7)	0 (0, 0-0)	2 (0.3, 0.1-0.7)	0 (0, 0-0)

Time period	Total number of MDR isolates resistant to STR ERY and TET (%R, 95% CI)	Number of isolates resistantto STR ERY and TET (%R,95% CI)	Number of isolates resistantto ERY TET and CIP (%R, 95%CI)	Number of isolates resistantto STR TET and CIP (%R, 95%CI)
2016-2018	22 (1.6, 0.9-2.2)	1 (0.1, 0.1-0.2)	5 (0.4, 0-0.7)	16 (1.1, 0.6-1.7)
All years	42 (0.8, 0.6-1)	1 (0,0-0.1)	24 (0.5, 0.3-0.6)	17 (0.3, 0.2-0.5)

None of the C. jejuni isolates tested had simultaneous resistance to CIP, ERY, STR and TET or to CIP, ERY and STR. The percentages of isolates with co-resistance to CIP and ERY varied between 0.2% (95% CI 0.0-0.4) in the time period from 2001-2005 to 1.8% (95% CI 0.0-0.4) in the time period from 2006 to 2010 and was 0.7% (95% CI 0.5-1.0) in the recent period from 2016-2018. Of the 1,636 C. jejuni isolates from 2012 to 2020 examined by genome sequencing none were co-resistant to CIP and ERY but three isolates were resistant to STR, TET and CIP (one from a retail sample in 2018 and two from retail samples in 2020).

5.5.2 C. *coli*

There was no evidence from this study to suggest that MDR had increased in C. coli during the study period from 2001 to 2020 (Table 14). However, the proportion of MDR isolates based on phenotypic data alone was significantly higher within C. coli (6.8%) compared to within C. jejuni (0.8%) ($p < 0.001$; Fishers exact test). The most common resistance profile detected was simultaneous resistance to CIP, ERY and TET.

Table 14. Number of C. coli isolates with multidrug resistant (MDR; defined as resistance to three or more unrelated antimicrobials) profiles (percentage resistant (R), 95% confidence intervals (CI)).

Time period	Total number of MDR isolates (%R, 95% CI)	Number of isolates resistant to STR ERY, TET, CIP (%R, 95% CI)	Number of isolates resistant to STR ERY and TET (%R, 95% CI)	Number of isolates resistant to ERY TET and CIP(%R, 95% CI)	Number of isolates resistant to STR, TET, CIP (%R, 95% CI)	Number of isolates resistant to STR ERY and CIP (%R, 95% CI)
2001-2005	11 (1.9, 0.8-3)	0 (0,0-0)	0 (0,0-0)	11 (1.9, 0.8-3.0)	0 (0,0-0)	0 (0,0-0)
2006-2010	86 (8.3, 6.6-10)	0 (0,0-0)	0 (0,0-0)	83 (8, 6.4-9.7)	3 (0.3, 0-0.6)	0 (0,0-0)
2011-2015	21 (12.8, 7.7-17.9)	3 (1.8, 0.2-3.9)	3 (1.8, 0.2-3.9)	7 (4.3, 1.2-7.4)	17 (10.4, 5.7-15)	3 (1.8, 0.2-3.9)
2016-2018	18 (8.6, 4.8-12.4)	1 (0.5, 0.5-1.4)	1 (0.5, 0.5-1.4)	4 (1.9, 0.1-3.8)	15 (7.2 3.7-10.7)	1 (0.5, 0.5-1.4)
All years	136 (6.8, 5.7-7.9)	4 (0.2, 0-0.4)	4 (0.2, 0-0.40	105 (5.3, 4.3-6.2)	35 (1.8, 1.2-2.3)	4 (0.2, 0-0.4)

The percentages of isolates that were (solely) co-resistant to CIP and ERY varied between a low of 1.9% (95% CI 0.8-3.0) in the time period from 2001-2005 to 9.5% (95% CI 7.7-11.3) in the years from 2006 to 2010 and was 6.1% (95% CI 5.0-7.1) in recent years from 2016-2018. Of the 464 C. coli isolates from 2012 to 2020 examined by genome sequencing none were exclusively co-resistant to CIP and ERY but 26 isolates were resistant to STR, TET and CIP and five were resistant to CIP, ERY and TET while two isolates were resistant to CIP, ERY, STR and TET.

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.

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Appendices

8.1 Appendix 1: List of isolates with AMR data based on phenotype

ODS
View Appendix I: List of isolates with AMR data based on phenotype as ODS(Open in a new window) (411.11 KB)

8.2 Appendix 2: List of isolates with AMR profiles based on WGS

ODS
View Appendix 2: List of isolates with AMR profiles based on WGS as ODS(Open in a new window) (65.1 KB)