

## 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 carcass 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 genotypic 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.