



**FSA Project FS102121**

***Antimicrobial resistance in *Campylobacter jejuni* and  
*Campylobacter coli* from retail chilled chicken in the UK (Year  
3: 2016-17)***

Forming part of the project: A microbiological survey of  
*Campylobacter* contamination in fresh whole UK produced chilled  
chickens at retail sale (2015-18)

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## 1. Background

*Campylobacter* species, especially *Campylobacter 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.* 2011). Source-attribution studies, outbreak investigations and case-control reports all incriminate chicken meat as the key foodborne vehicle for *Campylobacter* spp. infection, with cross-contamination from poultry being identified as an important transmission route (Tam *et al.*, 2009; Danis *et al.*, 2009; Friedman *et al.* 2004; Mullner *et al.*, 2009; Sheppard *et al.*, 2009). 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 *Campylobacter* cases.

It has been reported that *C. coli* are more likely to exhibit resistance to antimicrobials than *C. jejuni* isolates and it is therefore important to determine trends for *C. coli* and *C. jejuni* as separate species (EFSA and ECDC, 2016). Antimicrobial resistance (AMR) in *Campylobacter* spp., especially to fluoroquinolones, has raised concern relating to transfer of resistance in cases impacting on the global increase of resistance seen in infectious organisms. *Campylobacter* spp. isolates from 38% of cases associated with one UK hospital in 2008 were resistant to ciprofloxacin (Cody *et al.*, 2010). This represented an increase from 2004 where 25% of isolates were resistant to ciprofloxacin, unlike resistance to erythromycin that had remained at an equivalent level (approximately 2.5% of isolates). Increased levels of ciprofloxacin-resistance have also been reported in the USA (Zhao *et al.*, 2010). It is unclear whether infection with quinolone-resistant *Campylobacter* spp. has adverse clinical consequences, such as prolonged post-infectious complications, and studies published to date have produced conflicting results (Engberg, 2004; Evans *et al.*, 2009). In cases where a *Campylobacter* spp. infection warrants treatment with an antimicrobial, the drugs of choice are usually macrolides and fluoroquinolones (Skirrow and Blaser, 2000). It is therefore, particularly important to ascertain any increase in resistance to these groups of antimicrobials in particular.

It is imperative for public health to obtain accurate data on the prevalence of antimicrobial-resistant campylobacters in retail chicken as these represent a close point of exposure to consumers. Breakpoint susceptibility testing has been used in a number of previous studies of *Campylobacter* spp. contamination of poultry flocks, carcasses at slaughter and meat samples at retail sale. Integration of antimicrobial resistance data across the food chain will provide a better understanding of how such antimicrobial resistance is emerging and disseminating 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 and streptomycin, 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 proportion of the *C. jejuni* and *C. coli* isolates from fresh whole retail chicken examined between August 2016 and July 2017 exhibited resistance to a range of antimicrobial agents relevant to public health. Resistance to streptomycin was included to allow a comparison to be made with the percentage of streptomycin resistant isolates found in previous survey years.

## 2. Methods

The survey protocol agreed with the FSA was used for sampling and *Campylobacter* spp. enumeration testing procedures (FSA, 2016).

### 2.1 Microbiological methods

*Campylobacter* spp. isolates recovered and confirmed during project FS102121 (A microbiological survey of campylobacter contamination in fresh whole UK produced chilled chickens at retail sale) were sent to the PHE Gastrointestinal Bacteria Reference Unit (GBRU) for speciation and archiving. A proportion of isolates (funding was available for recovering 600 cultures) were tested for their antimicrobial susceptibility properties by GBRU. Isolates were selected for testing as every tenth isolate (or next viable isolate) but selection was adjusted to ensure representation of producer premises and retailers as deduced from market share data (source: FSA provided Kantar Market share data 2015). If the tenth isolate did not meet the criteria, the 11<sup>th</sup>, then 12<sup>th</sup> etc. isolate was reviewed and used to ensure fair representation. A total of 585 isolates were tested. All recoverable (able to grow after frozen storage) organic and a high proportion of free range chicken isolates were included.

Muller Hinton Agar with the addition of 5% horse blood containing specified breakpoint concentrations of antimicrobials were used to determine resistance. Agar quality was monitored using control strains with known minimum inhibitory concentration results. The standard agar break-point testing method was used briefly described 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 antimicrobial, but scored sensitive if no growth was observed and the corresponding antimicrobial free plate showed pure growth from the suspension applied. Antimicrobial resistance profiles were determined using the ECOFF values (Table 2) as recommended in the ECDC and EFSA protocol for harmonised monitoring of antimicrobial resistance in human *Salmonella* and *Campylobacter* isolates (EFSA and ECDC, 2016). Multi-drug resistance was defined in accordance with that used in the 2014 antimicrobial resistance report for the EU (EFSA and ECDC, 2016). The main issues when comparing antimicrobial resistance data originating from different datasets are the use of different laboratory methods and different interpretive criteria of resistance. These issues have been addressed by the development of EFSA's guidelines for harmonised reporting of resistance in food-producing animals and food thereof.

The resistance monitoring performed under these guidelines utilises ECOFF values which separate the naive, susceptible bacterial populations from isolates that have developed reduced susceptibility to a given antimicrobial agent (Table 1).

For some antimicrobials the ECOFFs may differ from breakpoints used for clinical purposes, which are defined against a background of clinically relevant data.

The breakpoints used in this report were the ECOFF interpretative thresholds for AMR in *C. jejuni* and *C. coli* and the same as those used for the isolates tested from the second survey year (Table 2). Multidrug resistance was defined as reduced susceptibility to at least three antimicrobial classes as specified by the ECDC definition.



**Table 1. Antimicrobial groups and the compounds within them**

Antimicrobial Group	Antimicrobial(s) included
Aminoglycosides	Gentamicin, Streptomycin
Macrolides	Erythromycin
Quinolones	Ciprofloxacin, Nalidixic acid
Tetracyclines	Tetracycline

**Table 2. EUCAST interpretative thresholds for antimicrobial resistance in *C. jejuni* and *C. coli***

Antimicrobial	Species	ECOFF threshold (mg/l)
Erythromycin (Ery)	<i>C. jejuni</i>	> 4
	<i>C. coli</i>	> 8
Ciprofloxacin (Cp)	<i>C. jejuni</i>	> 0.5
	<i>C. coli</i>	> 0.5
Tetracycline (Tet)	<i>C. jejuni</i>	> 2
	<i>C. coli</i>	> 2
Gentamicin (G)	<i>C. jejuni</i>	> 2
	<i>C. coli</i>	> 2
Nalidixic acid (Nal)	<i>C. jejuni</i>	> 16
	<i>C. coli</i>	> 16
Streptomycin (S)	<i>C. jejuni</i>	> 4
	<i>C. coli</i>	> 4

The range of antimicrobials and breakpoints that were used to examine the isolates obtained from that used in the first survey year (PHE, 2016) was slightly different to that used in this study. In the first survey year cultures were also tested for resistance to chloramphenicol, kanamycin and neomycin. Furthermore, resistance plates at 1 and 5 mg ciprofloxacin/l, 16 mg erythromycin/l, 1 and 4 mg gentamicin/l, 32 mg nalidixic acid/l, 8 and 128 mg tetracycline and 2 mg streptomycin/l were omitted in this study. The rationale for this amended testing panel was to align with the standard ECOFF thresholds as recommended by EFSA and ECDC.

### 2.3 Quality Assurance

All laboratories participate in recognised External Quality Assurance schemes, including the FSA funded scheme for enumeration of *Campylobacter* species, as well as operating comprehensive internal quality assurance schemes as part of the requirements of their accreditation to ISO 17025/2005 as assessed annually by the United Kingdom Accreditation Service (UKAS). All analyses were performed by trained and competent staff in a UKAS accredited laboratory operating an appropriate quality management system.

### 3. Results

All results other than those pertaining to AMR have been submitted in the third survey year report to the FSA for peer-review. The AST results are presented in detail in Appendix I. The isolates collected from the third year of the survey (survey year 3) were collected from August 2016 to July 2017.

#### 3.1 Antimicrobial resistance in *C. jejuni* and *C. coli* from survey year three

A total of 489 *C. jejuni* and 96 *C. coli* isolates from 585 samples were tested for antimicrobial resistance. Testing identified that 41% of the *C. jejuni* and 52% of the *C. coli* isolates examined were resistant to ciprofloxacin but only two *C. jejuni* (0.4%) and none of the *C. coli* isolates were resistant to erythromycin (Table 3 and Figure 1). The resistance of the *C. coli* strains to the 4 mg erythromycin per l cut-off was also tested and four *C. coli* isolates were able to grow at this level (but not at the ECOFF limit of 8 mg/l). Of all the isolates tested, 55% were resistant to tetracycline and 3% to streptomycin, but all isolates tested were sensitive to gentamicin.

**Table 3. Antimicrobial resistance in *C. jejuni* and *C. coli* (n = 585) isolated from retail chickens from 2016 – 2017.**

Antimicrobial	No. of resistant isolates (% of isolates resistant; 95% confidence interval)		
	<i>C. jejuni</i> <sup>a</sup> (n = 489)	<i>C. coli</i> <sup>b</sup> (n = 96)	Total (n = 585)
<b>Ery</b>	2 (0.4; 0.05-1.5)	0 (0; 0-4)	2 (0.3; 0-1)
<b>Cp</b>	201 (41; 36-46)	50 (52; 42-62)	251 (43; 39-47)
<b>Tet</b>	263 (54; 49-58]	59 (62; 51-71)	322 (55; 51-59)
<b>G</b>	0 (0; 0-1)	0 (0; 0-4)	0 (0; 0-1)
<b>Nal</b>	199 (41; 36-45)	48 (50; 40-60)	247 (42;38-46)
<b>S</b>	7 (1; 1-3)	11 (11; 6-20)	18 (3; 2-5)
<b>Any tested</b>	305 (62; 58-67)	69 (72; 62-81)	374 (64; 60-68)

Multidrug resistance defined as reduced susceptibility to at least three unrelated antimicrobial classes (according to the ECDC definition<sup>1</sup>) was found in nine *C. coli* (9.4%) and eight *C. jejuni* (1.6%) isolates examined (Table 4). The proportion of MDR isolates was significantly higher within *C. coli* compared to within *C. jejuni* (p 0.005; Fishers exact test). In total, 211 were fully sensitive with 184 (38%) *C. jejuni* isolates and 27 (28%) *C. coli* isolates susceptible to all antimicrobials tested.

<sup>1</sup>ECDC definition of MDR for *Campylobacters* taken from EFSA and ECDC 2016  
PHE Antimicrobial resistance report for FSA Project FS102121 Year 3

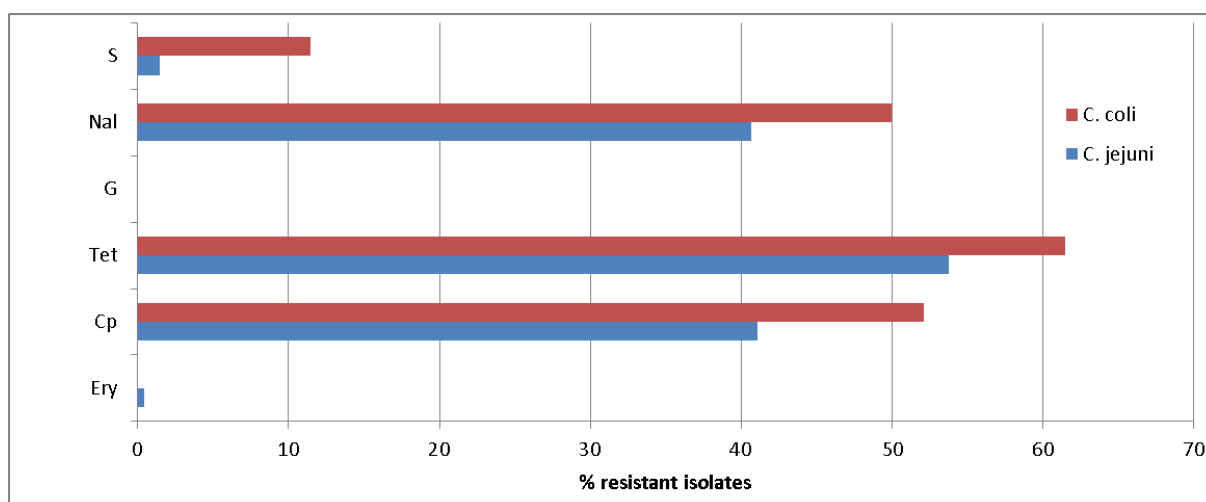


Figure 1. Comparison of the percentages of drug-resistant *C. jejuni* or *C. coli* isolates from retail chickens for survey year 2016 - 2017.

Table 4. Multi-drug resistance profiles in *C. jejuni* and *C. coli* isolates from retail chickens examined from 2016 - 2017.

AMR profile	No. of isolates with the given AMR profile (% of isolates; 95% CI)		
	<i>C. jejuni</i> (n = 489)	<i>C. coli</i> (n = 96)	All isolates (n = 585)
Tet, Nal and/or Cip, S	7	9	16
Tet, Nal and/or Cip, Ery	1	0	1
<b>Total for all profiles</b>	8 (1.6; 0.7-3.2)	9 (9.4; 4.4-17.1)	17 (2.9; 1.7-4.6)

Table 5. Antimicrobial resistance in *C. jejuni* and *C. coli* isolates in relation to chicken type (from retail chickens on sale 2016 - 2017)

Antimicrobial	Chicken type					
	Standard (n = 484)		Free-range (n = 83)		Organic (n = 18)	
	<i>C. jejuni</i> (n=433)	<i>C. coli</i> (n=51)	<i>C. jejuni</i> (n=44)	<i>C. coli</i> (n=39)	<i>C. jejuni</i> (n=12)	<i>C. coli</i> (n=6)
	No. of resistant isolates (Percentage of isolates resistant)					
Ery	2 (0.5)	0	0	0	0	0
Cp	178 (41.1)	17 (33.3)	18 (40.9)	28 (71.8)	5 (41.7)	5 (83.3)
Tet	235 (54.3)	25 (49.0)	24 (54.5)	30 (76.9)	4 (33.3)	4 (66.7)
Nal	177 (40.9)	16 (31.4)	17 (38.6)	27 (69.2)	5 (41.7)	5 (83.3)
S	7 (1.6)	6 (11.8)	0	4 (10.3)	0	1 (16.7)
<b>Any tested</b>	269 (62.1)	31 (60.8)	30 (68.2)	32 (82.1)	6 (50.0)	6 (100)

\*According to ECOFF threshold as described in section 2.1

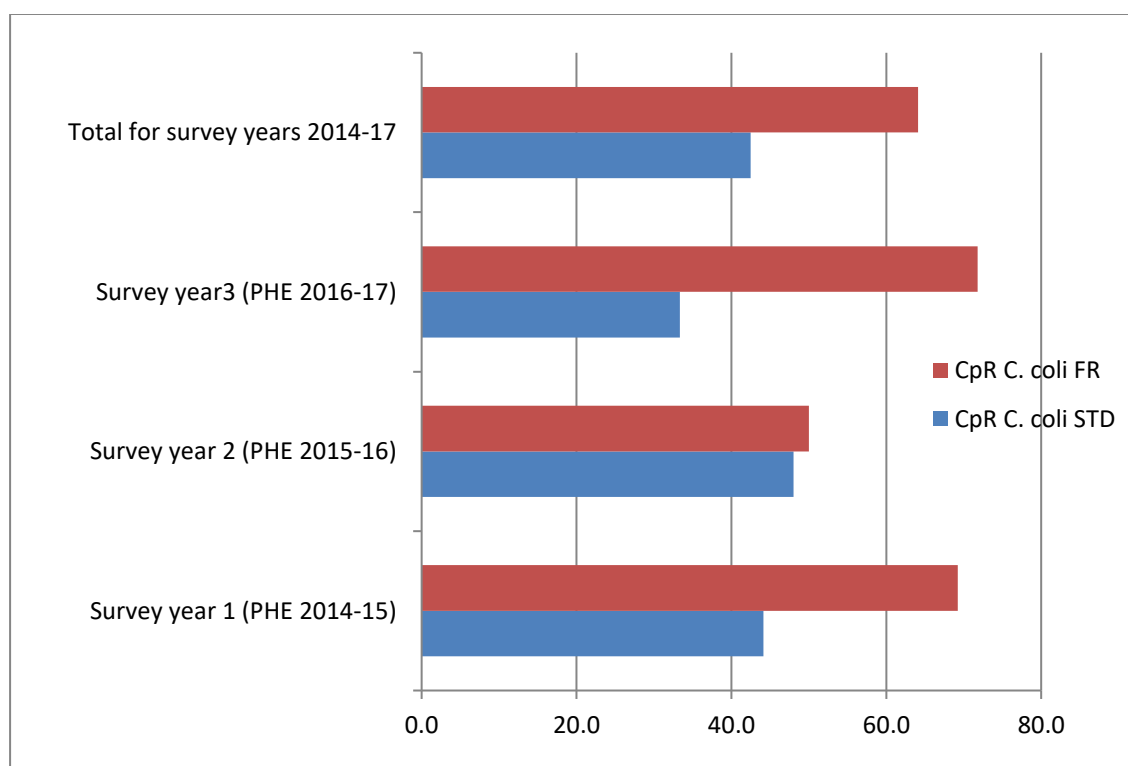
Differences in levels of ciprofloxacin- and tetracycline-resistance for isolates from standard and free-range birds were examined (Table 5). There were no differences

within *C. jejuni* isolates but a higher proportion of *C. coli* isolates from free-range chickens were resistant to ciprofloxacin or tetracycline compared to *C. coli* isolates recovered from standard chickens ( $p < 0.001$  and  $p < 0.01$ , respectively; Fishers exact test). Differences in levels of ciprofloxacin- and tetracycline-resistance in isolates from standard and organic birds were also examined. No significant differences were found; the small sample size for organic chickens, may have limited the ability to detect important differences should they exist.

## 4. Discussion

In agreement with recent EFSA data, this study found that (fluoro)quinolone (ciprofloxacin and nalidixic acid) and tetracycline-resistance was common in campylobacters isolated from chicken meat (EFSA and ECDC, 2018). In comparison, resistance to erythromycin, streptomycin and gentamicin was much rarer in the *Campylobacter* spp. isolates examined.

The proportion of ciprofloxacin-resistant *C. coli* isolates was higher in both free-range and organic although this difference was only statistically significant for free-range compared to standards but this finding must be interpreted with caution as this was not reflected in all three survey years (Figure 2). Also low numbers of isolates were examined and bias in the isolate sample may not be ruled out. Nevertheless, while it is uncertain whether fluoroquinolone usage is higher for chickens reared as free-range it is possible that they are exposed to a more persistent population of campylobacters in their environment and possibly are more likely to be colonised with ciprofloxacin resistant lineages (Webb *et al.*, 2018; Wimalarathna *et al.*, 2013). For standard chickens a higher turnover and more frequent clean-down of their housing environment may mean they are more likely to be exposed to more recently evolved and possibly less resistant *C. coli* types. This hypothesis would require further investigation.



**Figure 2. Comparison of the percentages of ciprofloxacin resistant (CpR) *C. coli* isolates from standard and free-range chickens for survey years 2014 - 2017.**

Although the proportions of tetracycline and ciprofloxacin-resistant isolates were broadly similar to that reported by EFSA on average for all MS, the survey year data presented in this report found a slightly higher proportion of tetracycline-resistant *C. jejuni* isolates but a lower proportion of ciprofloxacin-resistant *C. coli* compared to the EFSA data (Table 6). This study also found a lower proportion of erythromycin resistant *C. coli* isolates (95% CI = 0-4%) compared to the EFSA data (13% in 2016).

The reasons for this are not known but unlikely to reflect method differences as the AST was designed to be compliant with EFSA recommended protocols using identical cut-off values.

A significantly higher proportion of *C. coli* isolates exhibited MDR compared to the proportion of MDR *C. jejuni* isolates. This was also observed in the 2015-16 survey year, where 6.7% and 1.6% of *C. coli* and *C. jejuni*, respectively were classed as MDR. EFSA data from 2016 also reported a higher proportion of MDR *C. coli* (1.9%) compared to within *C. jejuni* (1.1%) in isolates recovered from chickens (EFSA and ECDC, 2018). The reason for this is not well understood but may relate to intrinsic factors e.g. differences in micro-membrane structures in the two species.

This data from *Campylobacter* spp. isolates obtained from retail chickens from August 2016 to July 2017 showed similar results for AMR compared to the data from isolates obtained from survey years one and two (covering February 2014 to March 2015 and July 2015 to May 2016, respectively; Table 6). The proportion of ciprofloxacin- and tetracycline-resistant *C. jejuni* was, significantly lower in this study compared to that found in the previous survey year (Fishers exact test;  $p < 0.001$ ; Table 6). Otherwise, there was no significant difference in the proportion of drug-resistant *C. jejuni* for any of the other antimicrobials compared to those found in the previous survey year (PHE, 2016). The proportions of MDR *C. jejuni* or *C. coli* found in this study were not significantly different to those found in the previous survey year (where 0 and 7.4% were MDR, respectively).

**Table 6. Comparison of occurrence of resistance to selected antimicrobials in *C. jejuni* and *C. coli* isolates from chicken meat between this survey and across EU member states.**

Anti-microbial	Break point (mg/l)	Species	% of isolates resistant in dataset				
			This survey year	2015-16 survey year	2014 -15 survey year	EU data	
						2014 <sup>c</sup>	2016 <sup>d</sup>
Cp	> 0.5	<i>C. jejuni</i>	41	54	49 <sup>a</sup>	66	65
		<i>C. coli</i>	52	48	55 <sup>a</sup>	86	81
Ery	> 4	<i>C. jejuni</i>	0.4	0 [95% CI = 0-0.8]	0.9	1.6	2.2
	> 8	<i>C. coli</i>	0	1.9 [95% CI = 0.2-6.5]	7.5	17	13
Tet	> 2	<i>C. jejuni</i>	54	68	63 <sup>b</sup>	36	49
		<i>C. coli</i>	62	67	68	74	73

<sup>a</sup>Data for break point of 1 mg Cp/l; <sup>b</sup>Data for break point of 2 mg Tet/l; <sup>c</sup>Data taken from EFSA and ECDC, 2015 (submitted by all MSs); <sup>d</sup>Data (submitted by all MSs) taken from EFSA and ECDC, 2018.

Taken together the datasets from the first, second and third survey year demonstrate significantly higher proportions of ciprofloxacin-resistance than found in earlier studies including the 2007/2008 FSA retail chicken survey and in the 2010 CLASSP survey (Figure 3). This finding must be treated with caution, as the isolates obtained

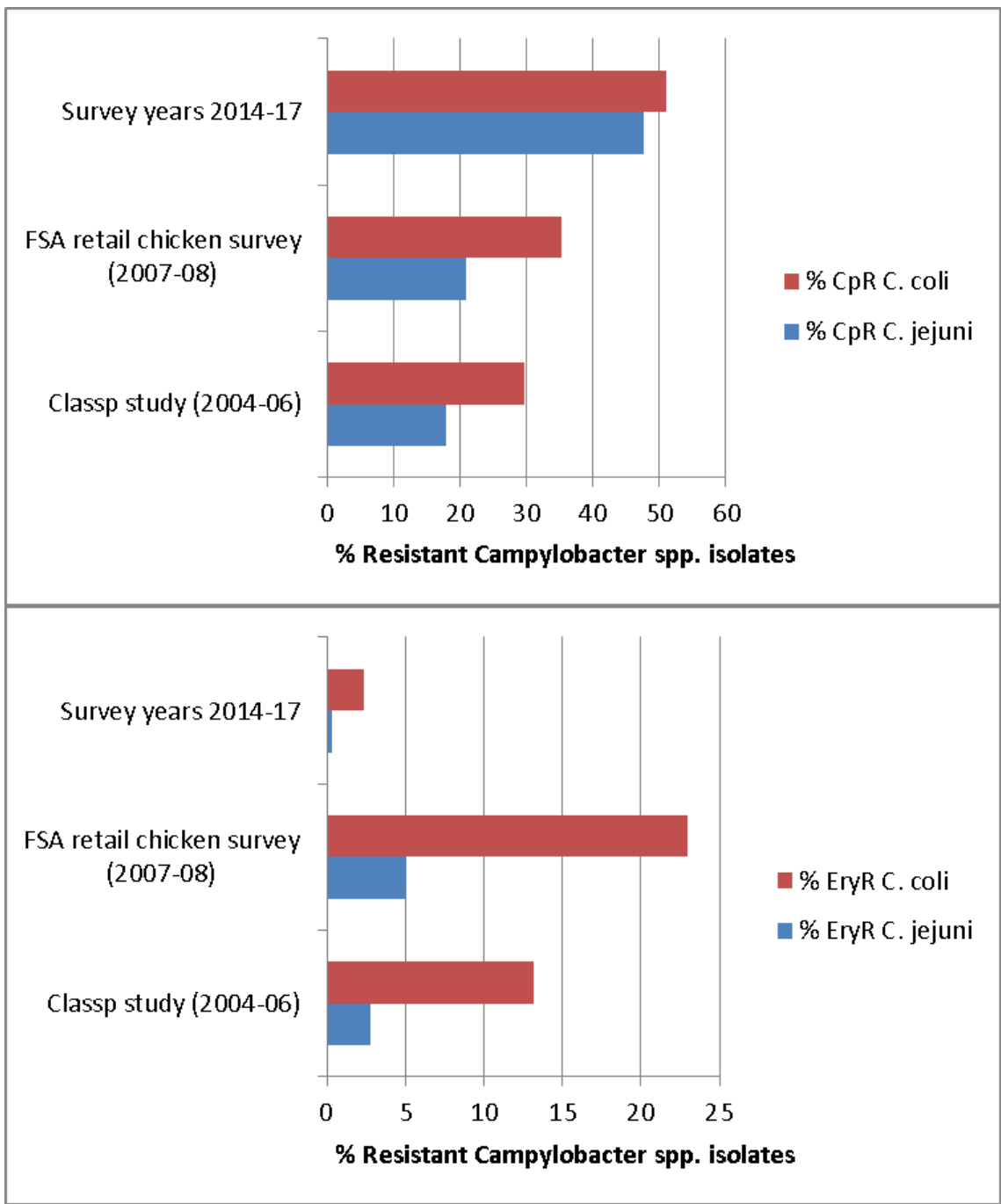
in the earlier surveys were derived using enrichment followed by plating, while direct plating (without prior enrichment) was used in the recent survey years. Nevertheless, the actual plating medium was the same in all survey years (mCCDA) and to our knowledge there is no evidence to suggest that enrichment in Bolton broth would or would not be any less likely to select for ciprofloxacin resistant campylobacters compared to direct plating.

Interestingly, the EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014, reported that over the 2008 - 2014 period, statistically significant increasing trends in resistance to ciprofloxacin and erythromycin were observed in several MS for both *C. jejuni* and *C. coli* isolates from chicken meats (EFSA and ECDC, 2016). In 2017, the 2<sup>nd</sup> JIACRA report found that consumption of fluoroquinolones in animals was significantly associated with increased percentages of resistance to fluoroquinolones in campylobacters isolated from animals and humans (ECDC, EFSA and EMA, 2017). Similar levels of ciprofloxacin- and erythromycin-resistance has been observed in isolates from human cases (Nichols *et al.*, 2012; Cody *et al.*, 2010).

The proportion of erythromycin resistant *C. jejuni* and *C. coli* isolates from UK chicken in this survey was lower compared to that found in the FSA 2007/2008 UK retail chicken survey suggesting a decreasing trend in erythromycin resistance especially in *C. coli* isolates (PHE, 2016). It is important to ascertain any changes in erythromycin-resistance as resistance to erythromycin is associated with resistance to other macrolides including clarithromycin, which is often used in preference to erythromycin to treat infections.

In summary, the average data from all three survey years suggest that the proportion of ciprofloxacin resistant *C. jejuni* and *C. coli* isolates has increased since 2004 - 2008 while the proportion of erythromycin-resistant *C. jejuni* and *C. coli*, maybe decreasing. Given the high percentage of isolates that are resistant to fluoroquinolones, and the assessment that a large proportion of human campylobacter infections probably relate to handling, preparation and consumption of chicken meat, this raises concern about the availability of effective antimicrobial agents for the treatment of severe human campylobacter infections. Nevertheless, there was a significant recent decrease in the proportion of ciprofloxacin-resistant *C. jejuni* and co-resistance to the critically important ciprofloxacin and erythromycin was very low (0.2%).

It is recommended that trends in AMR in *Campylobacter* isolates from retail chickens continue to be monitored. It would also be useful to examine more isolates from organic birds to enable a more robust comparison with isolates from chicken not reared to organic standards. This study has shown that fresh retail chicken can be contaminated with campylobacters with reduced antimicrobial susceptibilities and it remains important to ensure hygienic handling and adequate cooking of raw poultry.



**Figure 3. Comparison of the proportion of ciprofloxacin-resistant (CpR) and erythromycin-resistant (EryR) *C. jejuni* and *C. coli* isolates from survey years 2014 - 2017 with results from prior surveys.**



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