Public Health England

FSA Project FS241044

Antibiotic Resistance Report for FS241044 - Sept 2016 (Final)

Forming part of the project: A Microbiological survey of campylobacter contamination in fresh whole UK produced chilled chickens at retail sale (2014-15)

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ABBREVIATIONS

BPW	Buffered Peptone Water
C	Chloramphenicol
Cp	Ciprofloxacin
cfu	Colony forming units
CI	Confidence Interval
ºC	Degrees Celsius
Ery	Erythromycin
EQA	External Quality Assurance
FSA	Food Standards Agency
g	Gram
GBRU	Gastrointestinal Bacteria Reference Unit
G	Gentamicin
IQA	Internal Quality Assurance
ISO	International Standard Organisation
K	Kanamycin
l	Litre
mCCDA	Modified Charcoal Cefoperazone Deoxycholate Agar
mg	Milligram
MRD	Maximum Recovery Diluent
n	Number
Nal	Nalidixic Acid
Ne	Neomycin
PHE	Public Health England
SOP	Standard Operating Procedures
spp.	Species
Tet	Tetracycline
T	Trimethoprim
S	Streptomycin
UK	United Kingdom
UKAS	United Kingdom Accreditation Service

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EXECUTIVE SUMMARY

This report presents antimicrobial resistance data for isolates collected as part of FSA study FS241044: A Microbiological survey of campylobacter contamination in fresh whole UK produced chilled chickens at retail sale (2014-15).
A proportion of *Campylobacter jejuni* and *C. coli* strains that were isolated from retail chicken using the EN/TS/ISO 10272-2 standard enumeration method (applied with a detection limit of 10 cfu per gram of skin or per outer packaging swab sample tested) were tested to determine the antimicrobial resistance profiles of the strains.

Ciprofloxacin resistance was identified in 49 % of the 230 *C. jejuni* isolates and 55 % of the 53 *C. coli* isolates tested. All isolates tested were sensitive to gentamycin, neomycin and kanamycin and 55 *C. jejuni* and 15 *C. coli* isolates were susceptible to all antimicrobials tested. The proportion of multi-resistant isolates was higher within *C. coli* (21 %) than within *C. jejuni* (0.9 %). The data suggest that the proportion of ciprofloxacin resistant *C. jejuni* and *C. coli* strains has increased since 2007-08 while the proportion of erythromycin resistant *C. coli* appears to be un-changed and erythromycin resistance may be decreasing in *C. jejuni*. In comparison with the previous 2007-08 survey there was no significant change in the level of resistance found to aminoglycosides, chloramphenicol or tetracycline.

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 food-borne 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* strains are more likely to exhibit resistance to antimicrobials than *C. jejuni* strains and it is therefore important to determine trends for *C. coli* and *C. jejuni* as separate species. Antimicrobial resistance 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 infection warrants treatment with an antibiotic, the drugs of choice are usually macrolides and fluoroquinolones (Skirrow and Blaser 2000). It is therefore 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 humans. 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 strains are emerging and disseminating from animal production to humans.

The work presented here aimed to ascertain what proportion of *Campylobacter jejuni* and *C. coli* strains examined were resistant to a range of antimicrobial agents relevant to public health using an established standard method.

2. METHODS

The survey protocol agreed with the FSA was used for sampling and testing procedures (FSA 2014). Sampling methods were described in the report published (PHE 2015).

2.1 Microbiological methods

Campylobacter isolates recovered and confirmed during project FS241044 (A Microbiological survey of campylobacter contamination in fresh whole UK produced chilled chickens at retail sale (2014-15)) were sent to the PHE Gastrointestinal Bacteria Reference Unit (GBRU) for speciation and archiving. A proportion of isolates (funding was available for recovering 300 strains) 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 adequate representation of producer premises and retailers. If the tenth isolate did not meet the criteria, the 11^{th}, then 12^{th} etc. isolate was reviewed and used to ensure fair representation. A total of 283 isolates were tested. All recoverable organic and free range chicken isolates were included.
Iso-Sensitest Agar with the addition of 5 % horse blood containing specified breakpoint concentrations of antimicrobials were used to determine resistance. Control strains are used with known minimum inhibitory concentration results to determine that the agar is working and allow growth of the test isolates to be assessed against. A suspension of an isolate is made in brain heart infusion broth to McFarland 0.5 turbidity and is inoculated onto the surface of each of the antimicrobial containing agars. An isolate is considered resistant if it grows on the agar and is scored sensitive if there is no growth and the corresponding antimicrobial free plate shows pure growth from the suspension. Antimicrobial resistance profiles were determined using the following antimicrobials and concentrations as described in Thwaites & Frost (1999):

Chloramphenicol	8 mg/l, 16 mg/l
Ciprofloxacin	1 mg/l (CpL), 5 mg/l (CpH)
Erythromycin	4 mg/l (EryL), 16 mg/l (EryH)
Gentamicin	1 mg/l, 2 mg/l, 4 mg/l (GH)
Kanamycin	16 mg/l (K)
Nalidixic Acid	16 mg/l (NalL), 32 mg/l (NalH)
Neomycin	8 mg/l (Ne)
Tetracycline	2 mg/l (TetL), 8 mg/l (TetII), 128 mg/l (TetH)
Trimethoprim	2 mg/l
Streptomycin	2 mg/l (SL), 4 mg/l (SH)

Multi-resistance was defined in accordance with that used in the 2013 antimicrobial resistance report for the EU (EFSA 2015) based on non-susceptibility to at least three different antimicrobial classes as listed in Table 1. 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 epidemiological cut-off (ECOFF) values which separate the naive, susceptible wild-type bacterial populations from isolates that have developed reduced susceptibility to a given antimicrobial agent.

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

Table 1. Antimicrobial groups and the compounds within them

Antimicrobial Group	Antimicrobial(s) included
Aminoglycosides	Gentamicin, kanamycin, neomycin, streptomycin
Chloramphenicols	Chloramphenicol
Macrolides	Erythromycin
Quinolones	Ciprofloxacin, nalidixic acid
Tetracyclines	Tetracycline

The breakpoints used in this report are similar to EUCAST ECOFF interpretative threshold for antimicrobial resistance in *C. jejuni* and *C. coli* (Table 2).

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

Antimicrobial	Species	ECOFF threshold (mg/l)
Erythromycin	*C. jejuni*	> 4
	C. coli	> 8
Ciprofloxacin	*C. jejuni*	> 0.5
	C. coli	> 0.5
Tetracycline	*C. jejuni*	> 1
	C. coli	> 2
Gentamicin	*C. jejuni*	> 2
	C. coli	> 2
Nalidixic acid	*C. jejuni*	> 16
	C. coli	> 16
Streptomycin	*C. jejuni*	> 4
	C. coli	> 4

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 antimicrobial resistance were published in the first project report (PHE 2015). The antimicrobial susceptibility testing results are presented in detail in Appendix I.

3.1 Antimicrobial resistance in *C. jejuni* and *C. coli* isolates from chicken

Of the *C. jejuni* isolates examined (n = 230), 49 % were resistant to ciprofloxacin and 0.9 % were resistant to erythromycin (Table 3). For *C. coli* over half of the strains were resistant to ciprofloxacin while 11 % were resistant to erythromycin. All isolates tested were sensitive to gentamycin, neomycin and kanamycin.

Table 3. Antimicrobial resistance in *C. jejuni* and *C. coli* strains (n = 283) isolated from retail chickens.

Antimicrobial (mg/l breakpoint applied)	No. of resistant isolates (%) [binomial 95% CI]	
	***C. jejuni* (230)**	***C. coli* (53)**
Chloramphenicol (8)	1 (0.4) [0.01 to 2.4]	0 (< 1.9) [0 to 6.7]
Ciprofloxacin (1)	113 (49) [42.5 to 55.8]	29 (55) [40.4 to 68.4]
Erythromycin (4)	2 (0.9) [0.1 to 3.1]	6 (11) [4.3 to 23.0]
Nalidixic Acid (16)	118 (51) [44.6 to 57.9]	29 (55) [40.4 to 68.4]
Streptomycin (4)	1 (0.4) [0.01 to 2.4]	8 (15) [6.7 to 27.6]
Tetracycline (2)	144 (63) [56.0 to 68.9]	36 (68) [53.7 to 80.1]

The proportion of multi-resistant isolates was higher within *C. coli* (11/53) than within *C. jejuni* (2/230) (Table 4). There were 55 *C. jejuni* isolates and 15 *C. coli* isolates were susceptible to all antimicrobials (i.e. susceptible to the lowest breakpoint tested for all antibiotics). Detailed antimicrobial susceptibility profiles for all isolates tested are presented in Appendix I. No ampicillin resistance data has been reported for these isolates due to concerns that arose during testing regarding the performance of the ampicillin plates in the study.

Table 4. Multi-resistance profiles of *C. coli* and *C. jejuni* isolates

Antimicrobial resistance profile	No. of isolates with antimicrobial resistance profile	
	C. jejuni	***C. coli***
TetI, NalH, CipH, SH	0	5
TetI, NalH, CipH, EryH	0	2
TetI, NalH, CipH, EryL	0	1
TetH, NalH, CipH, EryL	0	1
TetH, NalH, CipH, SH	0	1
TetH, NalH, CipH, EryH	0	1
TetL, CipH, EryH	1	0
TetL, NalH, CipH, EryL	1	0
Total no. (with any of the profiles above)	2 (1 %)	11 (21 %)

While there was no significant difference in the level of antimicrobial resistance observed in strains from standard compared to non-housed chickens (Table 5; Fishers exact test), the sample size, especially for organic chickens, was small limiting the ability to make conclusive inferences as to whether important differences actually do exist.

Table 5. Levels of antimicrobial resistance associated with *C. jejuni* and *C. coli* in relation to bird type.

Chicken type	*Campylobacter* spp. (no. of isolates)	Number of strains (%) resistant to antimicrobial indicated			
		CpL	**EryL**	**TetL**	**SH**
Standard	*C. jejuni* (198)	99 (50)	2 (1)	127 (64)	1 (0.5)
	C. coli (34)	15 (44)	4 (12)	21 (62)	4 (12)
Free-range	*C. jejuni (27)*	11(41)	0 (< 4)	15 (56)	0 (< 4)
	C. coli (13)	9 (69)	1 (8)	9 (69)	4 (31)
Organic	*C. jejuni* (5)	3 (60)	0 (< 17)	2 (40)	0 (< 17)
	C. coli (6)	5 (83)	1 (17)	6 (100)	0 (< 17)

4 DISCUSSION

In this UK survey on campylobacters in retail chickens just under half of the *C. jejuni* isolates were resistant to ciprofloxacin. This proportion is significantly ($p < 0.001$; Fisher's Exact Test) higher than the proportion of resistant strains found in the 2007/2008 FSA survey (Table 6), where 19 % of *C. jejuni* strains from whole fresh UK produced chicken were resistant and the CLASSP survey[1] (CLASSP 2010), where 18 % of all UK *C. jejuni* isolates were reported as resistant. The proportion of ciprofloxacin resistant *C. coli* isolates in this survey was also significantly higher compared to that reported in the 2007/2008 FSA survey (34 % ciprofloxacin resistant *C. coli*) and the CLASSP survey (30 % ciprofloxacin resistant *C. coli* in UK samples). Interestingly, the EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013, observed increasing trends in ciprofloxacin resistance in campylobacters from broilers in several EU member states (EFSA 2015). It has been suggested that an increased level of fluoroquinolone resistant bacteria may relate to increased consumption of fluoroquinolones possibly exacerbated by a fitness advantage of resistant strains (Redgrave et al. 2014). 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. coli* isolates in this survey was not significantly different to that found in the 2007/2008 FSA survey, where 21 % were resistant. However, for *C. jejuni*, this study found a lower percentage of erythromycin resistant strains compared to the previous survey (Table 6). A similar picture appears when comparing to the CLASSP survey findings, where 3 and 13 % of *C. jejuni* and *C. coli*, respectively, were erythromycin resistant. Resistance to erythromycin is associated with resistance to other macrolides including clarithromycin, which is often used in preference to erythromycin to treat infections.

[1]The Coordinated Local Authority Sentinel Surveillance of Pathogens (CLASSP) study undertaken by local authorities and the Health Protection Agency in England between 2004 and 2007 of campylobacters on 2000 raw whole chickens were tested using the enrichment culture method

Together, these data suggest that the proportion of ciprofloxacin resistant *C. jejuni* and *C. coli* strains has increased since 2007-08 while the proportion of erythromycin resistant *C. coli* appears to be un-changed and erythromycin resistance may be decreasing in *C. jejuni*.
The proportions of antimicrobial resistant isolates found in this study were similar to that reported by EFSA for EU member states although a high level of tetracycline resistance within *C. jejuni* was found in this survey compared to the EU data (Table 7). A higher proportion of *C. coli* isolates from this survey were multi-resistant compared to the proportion of multi-resistant *C. jejuni* isolates. This was also observed in the EFSA report (EFSA 2015) where 1.5 % of *C. jejuni* and 12.6 % of *C. coli* strains exhibited multi-resistance. 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. Direct comparison of the proportion of multi-resistant isolates from this survey with the proportion of multi-resistant strains found in the previous UK surveys is hampered by differences in the range of antimicrobials tested for and breakpoint levels applied.

Table 6. Comparison of percentage resistant isolates in UK retail chickens from 2007/08 and this survey.

Antimicrobial (> mg/l breakpoint)	Species	% of strains resistant in dataset		
		This survey	2007/08 survey (all chicken)	2007/08 survey (fresh whole UK chicken only)
Cp (1)	*C. jejuni*	49	21	19
	C. coli	55	35	34
Ery (4)	*C. jejuni*	0.9	5	4
	C. coli	11	23	21
Tet (8)	*C. jejuni*	57	47	45
	C. coli	62	53	53
C (8)	*C. jejuni*	0.4	0.7	"0" (< 0.3)
	C. coli	"0" (< 1.9)	2	1.2
Nal (16)	*C. jejuni*	51	22	20
	C. coli	55	36	34
G (4)	*C. jejuni*	"0" (< 0.4)	"0" (< 0.1)	"0" (< 0.3)
	C. coli	"0" (< 1.9)	"0" (< 0.1)	"0" (< 0.3)
K (16)	*C. jejuni*	"0" (< 0.4)	0.2	0.3
	C. coli	"0" (< 1.9)	0.6	0.6
Ne (8)	*C. jejuni*	"0" (< 0.4)	0.2	0.3
	C. coli	"0" (< 1.9)	0.6	0.6

Given the high levels of resistance to fluoroquinolones, and the assessment that a large proportion of human campylobacteriosis 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, co-resistance to the critically important ciprofloxacin and erythromycin was very low.
It is recommended that trends in antimicrobial resistance 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.

Table 7. Comparison of the percentage antimicrobial resistant *Campylobacter jejuni* and *C. coli* isolates from chicken meat.

Antimicrobial (> mg/l breakpoint used for comparison)	Species	% of strains resistant in dataset	
		This survey	EU data from 2013
Cp (1)	*C. jejuni*	49	53
	C. coli	55	76
Ery (4)	*C. jejuni*	0.9	0.9
	C. coli	11	11*
Tet (2)	*C. jejuni*	63	33
	C. coli	68	58

* A break point of 8 was applied for *C. coli* in the EU dataset (EFSA 2015)

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