

# 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, phenotypic 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.

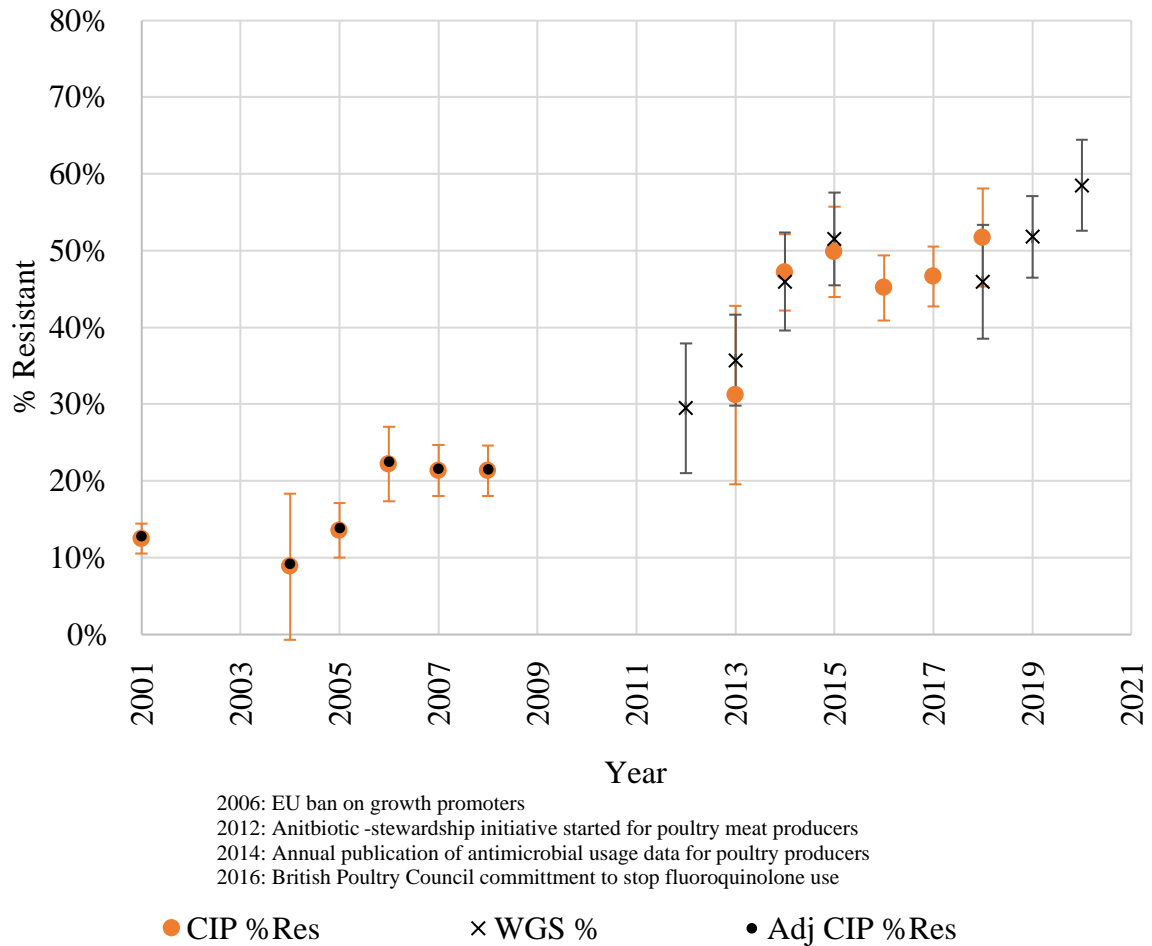
Results from two datasets, containing additional isolates collected from carcasses between 2012-2015 and 2018 to 2020 with AMR predicted from genome sequence data were also examined and results presented.

## 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 these analyses, however the impact of the adjustment was negligible 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

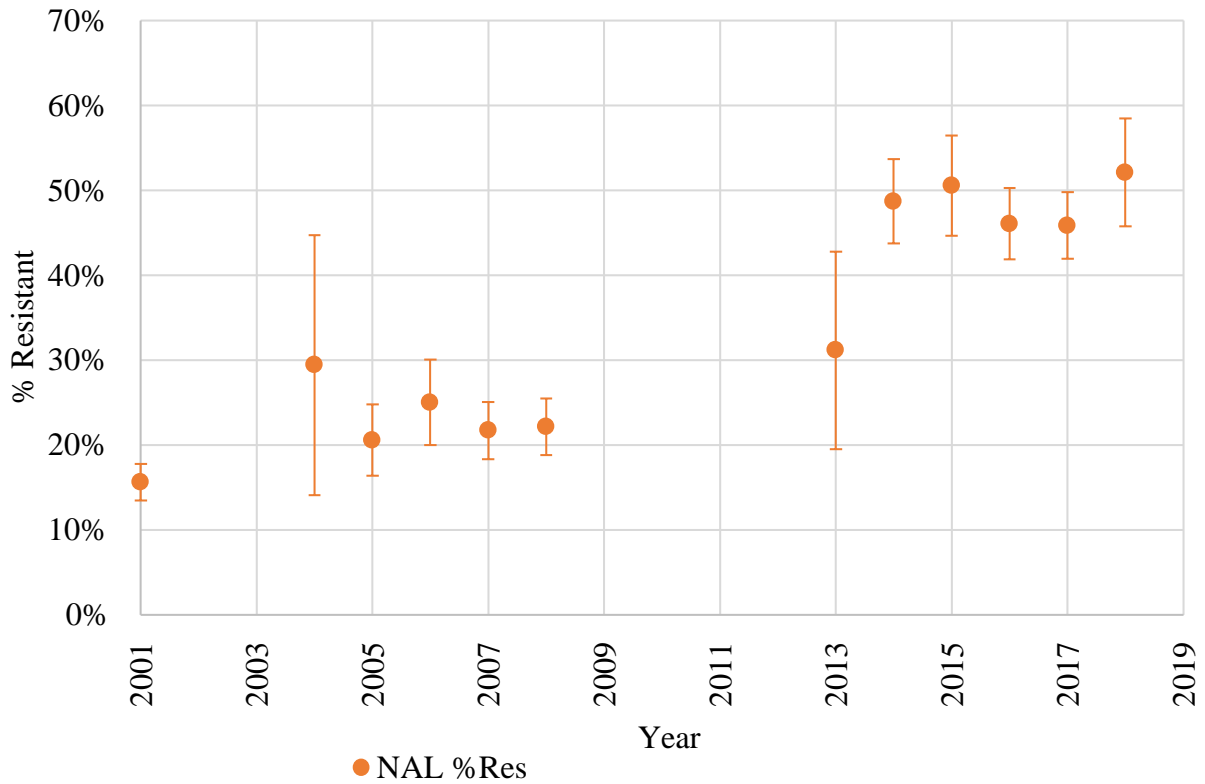
CIP category factors	Number of isolates	Percentage resistant	Unadjusted OR (95%CI)	p value	Adjusted OR (95% CI)	p value
Frozen sample	348	20%	0.6 (0.5-0.8)	<0.001	1.5 (1.1-1.9)	0.010
unknown	2	0%	NA	NA	NA	NA
Total	5,269	29%	NA	NA	NA	NA

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

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

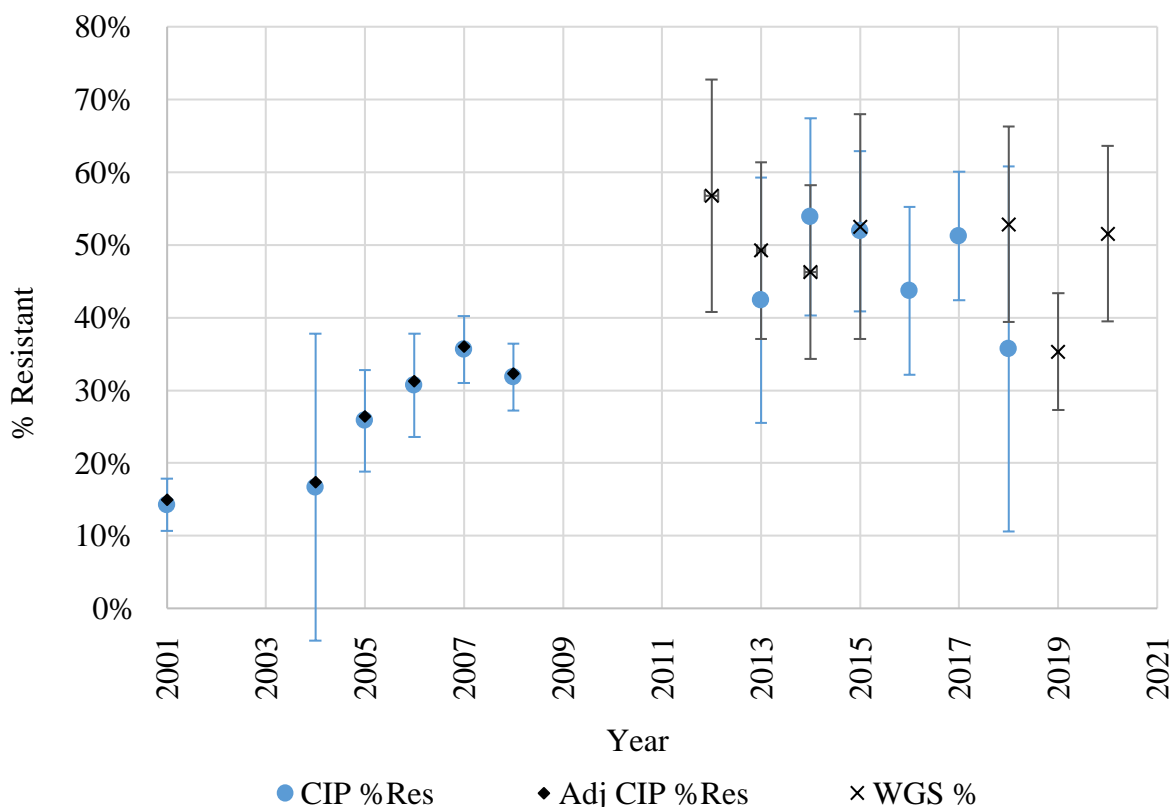
Including the sample type and state categories did not improve the overall fit of the multivariable model to the data, however the results of the univariate analysis for both variables were significant. Isolates from portions were less likely to have NAL resistance than isolates from whole chicken (OR = 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

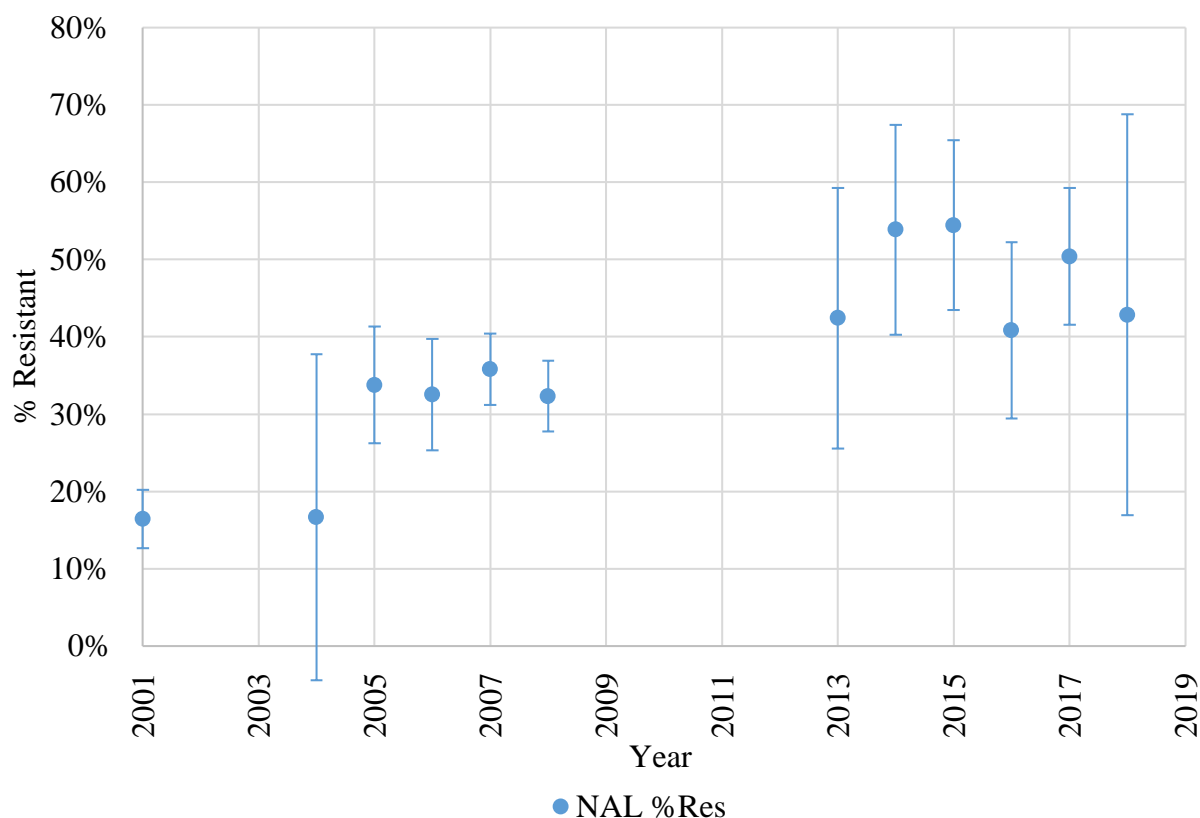


CIP category factors	Number of isolates	Percentage resistant	Unadjusted OR (95%CI)	p value	Adjusted OR (95% CI)	p value
Unknown	27	26%	0.7 (0.3-1.8)	0.506	1.2 (0.5-3.0)	0.691
Total	1,997	32%	NA	NA	NA	NA

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 carcasses 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
2006-2010	1,034	35%	2.0 (1.6-2.5)	<0.001	1.8 (1.4-2.3)	<0.001

<b>NAL category factors</b>	<b>Number of isolates</b>	<b>Percentage resistant</b>	<b>Unadjusted OR (95%CI)</b>	<b>p value</b>	<b>Adjusted OR (95% CI)</b>	<b>p value</b>
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

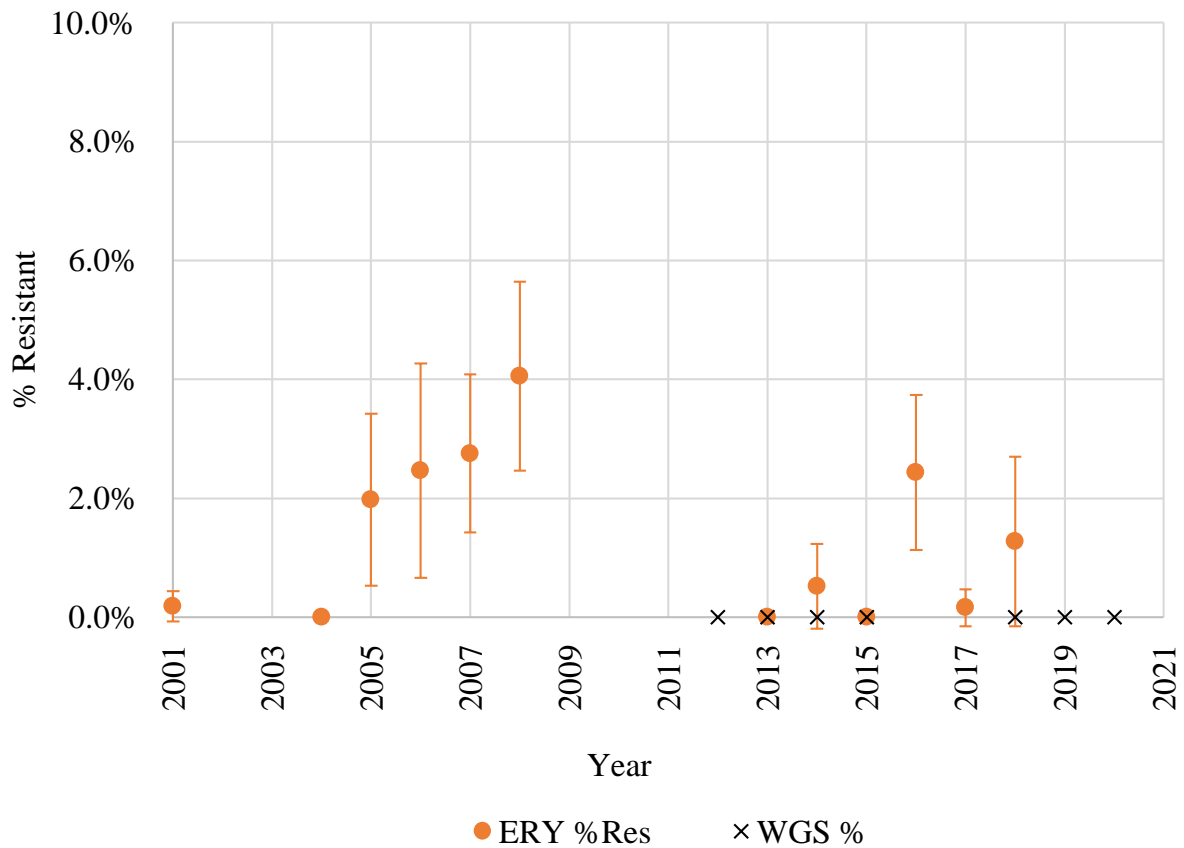
NAL category factors	Number of isolates	Percentage resistant	Unadjusted OR (95%CI)	p value	Adjusted OR (95% CI)	p value
UK	1,878	33%	1 (ref)	NA	1 (ref)	NA
Non-UK	92	42%	1.5 (1.0-2.2)	0.074	2.0 (1.3-3.0)	0.003
Unknown	27	26%	0.7 (0.3-1.7)	0.420	1.0 (0.4-2.5)	1
Total	1,997	34%	NA	NA	NA	NA

## 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

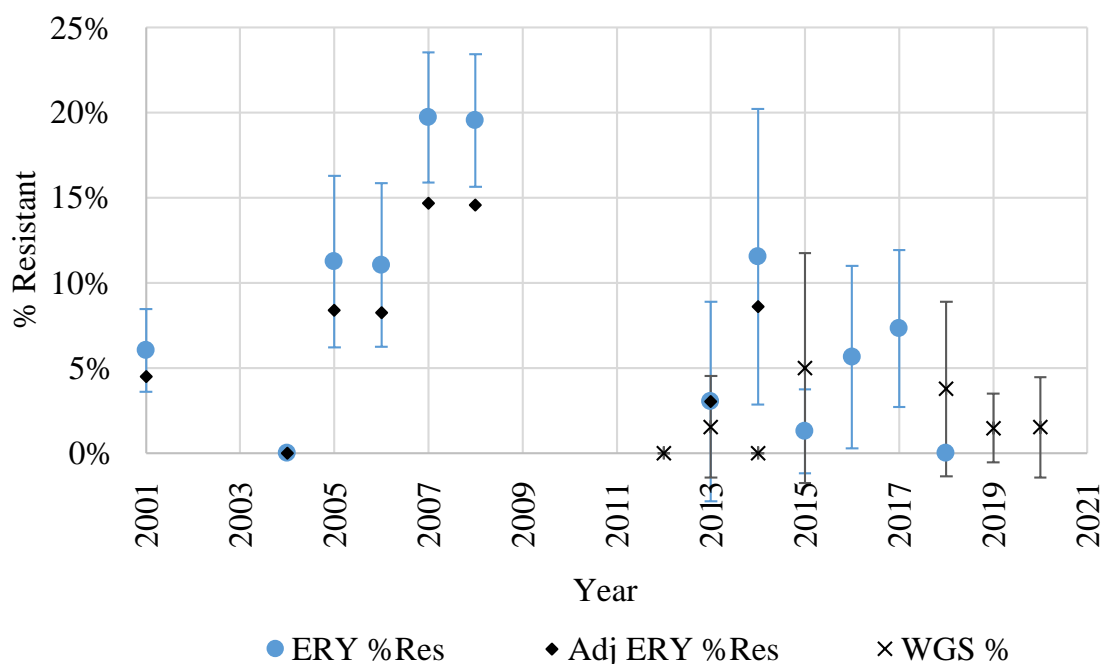
2001-2005	1,612	0.6%	1 (ref)	NA	1 (ref)	NA
206-2010	1,530	3.6%	6.0 (3.0-11.8)	<0.001	8.6 (4.2-17.5)	<0.001
2011-2015	726	0.3%	0.4 (0.1-2.0)	0.293	0.9 (4.2-17.5)	0.914
2016-2018	1,401	1.2%	2.0 (0.9-4.3)	0.091	3.6 (1.6-8.5)	0.003
Fresh	4,919	1.4%	1 (ref)	NA	1 (ref)	NA
Frozen	348	4.3%	3.2 (1.8-5.6)	<0.001	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

ERY category factors	Number of isolates	Percentage resistant	Unadjusted OR (95%CI)	p value	Adjusted OR (95% CI)	p value
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 (0.2-0.6)	<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



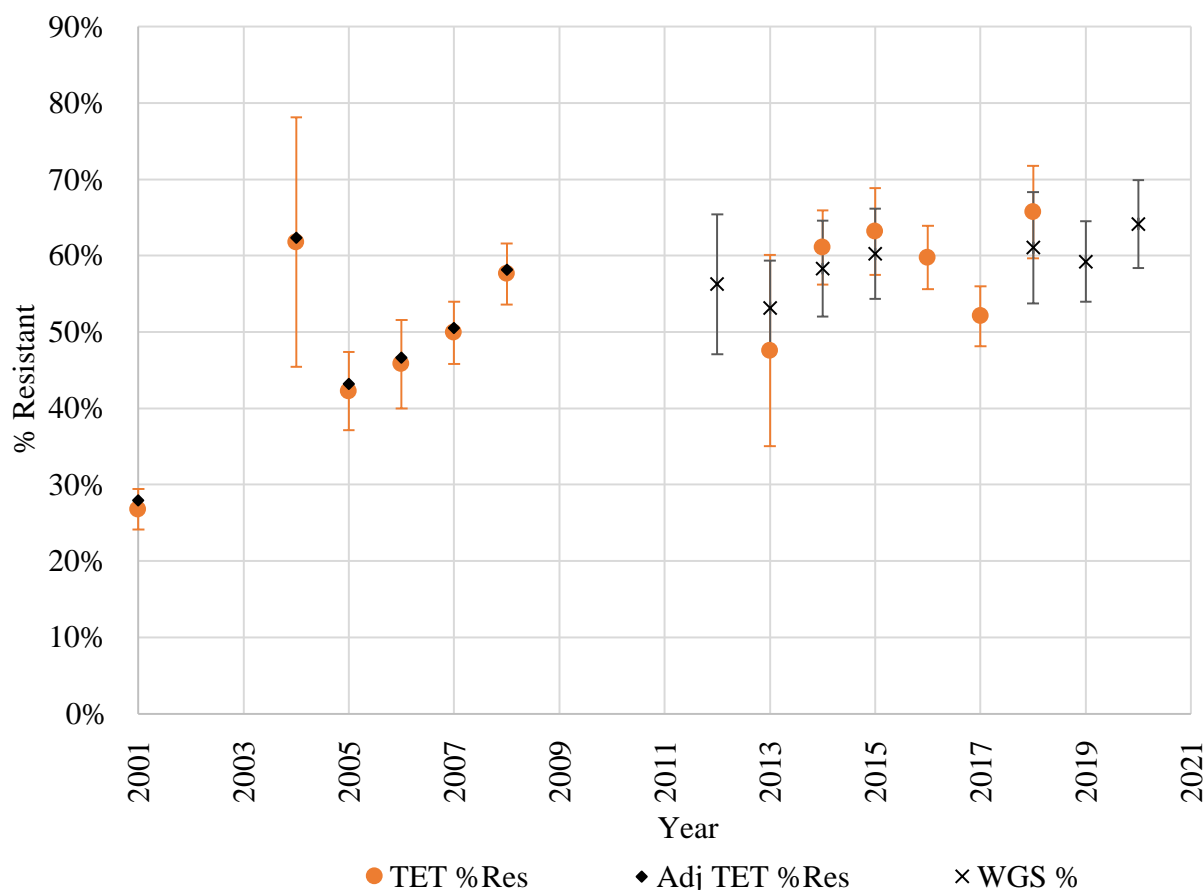
non-UK birds were significantly more likely to have ERY resistance than UK birds (OR = 1.78, 95% CI 1.05-3.00; p=0.031). However, after adjusting for sample category (which was included in the multivariable model), origin was not significantly associated with ERY resistance (OR = 1.65, 95% CI 0.98-2.78; 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

TET category factors	Number of isolates	Percentage resistant	Unadjusted OR (95%CI)	p value	Adjusted OR (95% CI)	p value
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

TET category factors	Number of isolates	Percentage resistant	Unadjusted OR (95%, CI)	p value	Adjusted OR (95% CI)	p value
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
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

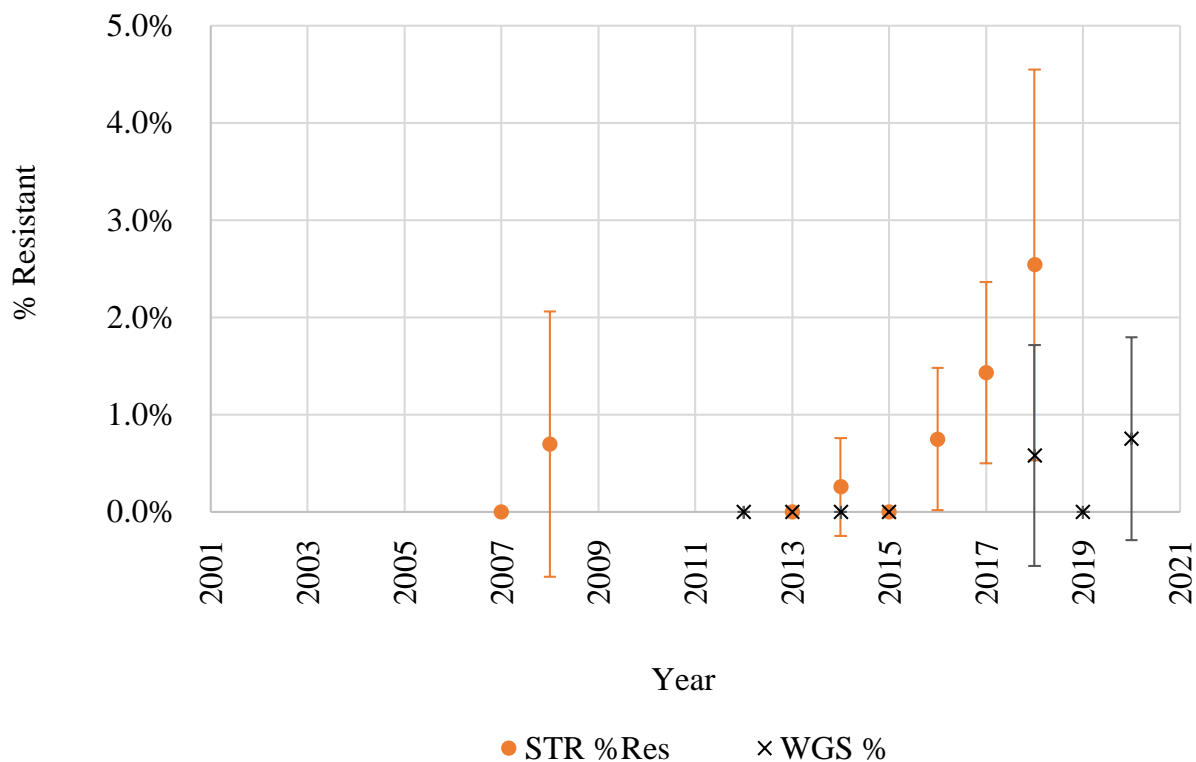
Resistance to gentamicin (GEN) was detected in two (0.04%; 95%CI: 0.00-0.14%) of the 5,269 *C. jejuni* isolates from 2001-2018 based on phenotypic data. One of these isolates originated from a whole frozen chicken collected at retail in 2005, the other from a caecal sample collected from a slaughterhouse 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%).

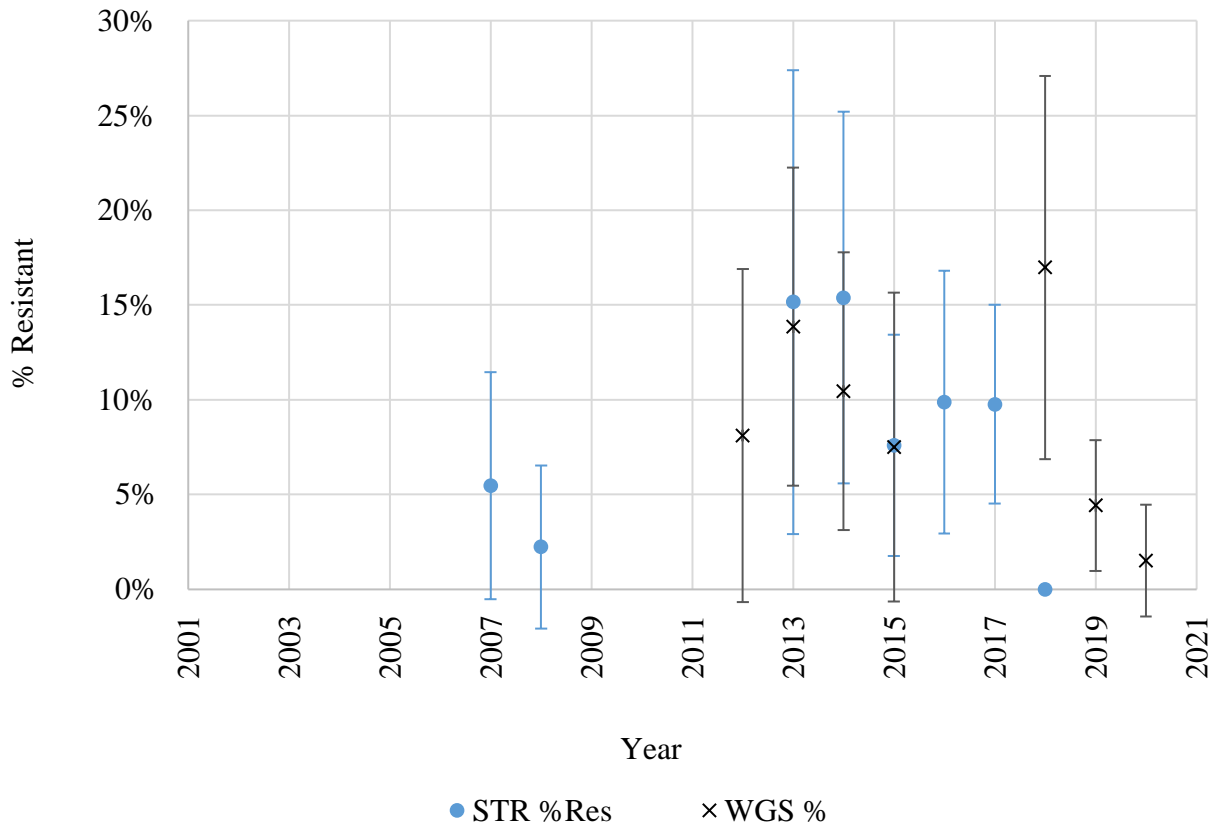
**Figure 9. Trend in the percentages of streptomycin (STR) resistant *C. jejuni* isolates from chicken sampled at slaughterhouses and at the point of retail in UK between 2001 and 2020.** Orange circles are based on phenotypic data. The crosses represent percentages of STR resistant isolates based on 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)
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, 0-0.4)	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.