Antimicrobial susceptibility of *Campylobacter* spp. isolated from broiler chickens in Northern Ireland

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Between February 2000 and October 2001, cloacal swabs were collected from 387 broiler chicken flocks in Northern Ireland. *Campylobacter* isolates from the 262 positive flocks were tested with common antimicrobial agents using a disc diffusion method and by Etests. Resistance to erythromycin, gentamicin and chloramphenicol was <1%, whereas for ampicillin, nalidixic acid and tetracycline, resistance was 33%, 10% and 13%, respectively. Ciprofloxacin resistance was 3%, one of the lowest in recent reports from studies on human or poultry isolates. Sequence data of the quinolone resistance-determining region of the *gyrA* gene showed a mutation leading to Thr-86 to Ile substitution among highly resistant ciprofloxacin isolates. Only 0.8% of the isolates studied were resistant to four or more antibiotics.

Keywords: resistance, poultry, multiresistance, ciprofloxacin resistance, *Campylobacter*, susceptibility

Introduction

*Campylobacter* species infection is the commonest reported cause of acute bacterial enteritis in humans in the UK.1 Epidemiological studies have identified preparation of chicken and consumption of undercooked chicken as important risk factors in acquiring *Campylobacter* infection.2,3 Illness caused by *Campylobacter* is usually self-limiting and therapy is not required, except in severe episodes of the disease, or in immunocompromised patients, when erythromycin is recommended. In moderately severe cases of non-confirmed gastroenteritis and for travellers’ diarrhoea, ciprofloxacin (Cp) is advised.4 Resistance to Cp, which may result in treatment failure, has been rising to alarmingly high rates in *Campylobacter* isolates from humans and animals (62% in Belgium, 99% in Spain).5,6 This rise has been attributed to the increase in veterinary use of fluoroquinolones,7–9 and experimentally resistance has been shown to develop rapidly, and persisted in *Campylobacter jejuni*-infected chickens treated with enrofloxacin or sarafloxacin.10 However, a variety of mutations in the target topoisomerases, in particular the Thr-86→Ile mutations in the quinolone-determining region (QRDR) of the *gyrA* gene, are described more commonly.12,13

Over 95% of all commercial broiler production in Northern Ireland (NI) is coordinated through three independent companies, representing nearly 300 farms. Antimicrobial susceptibility and prevalence data on isolates from pre-slaughter poultry flocks from NI are limited. The aim of this study was to determine a representative baseline for resistance to antimicrobial agents in *Campylobacter* from broiler chicken flocks in NI.

Method

Strains, culture condition and identification

Between February 2000 and October 2001, commercial broiler flocks (*n* = 387) from NI were sampled by cloacal swabbing (14 birds per flock) using Amies charcoal swabs (IASA, Rubí, Spain). Samples were held at +4°C until transfer to the laboratory. Modified charcoal–cefoperazone–deoxycholate agar (Oxoid, Basingstoke, UK) was the medium onto which all swabs were plated, followed by incubation for 48 h under microaerobic conditions (10% CO2, 5% O2, 85% N2) at 37°C. Phenotypic tests were used to confirm and identify positive isolates to species level. The tests involved growth under microaerobic conditions, hippurate hydrolysis and indoxyl acetate.14 A flock was considered positive if at least one sample out of the 14 was confirmed as *Campylobacter* from broiler chicken flocks in NI.

Antibiotic testing

Antimicrobial sensitivity testing was carried out by the disc diffusion method, as previously described.15,16 Test isolates (*n* = 262, one isolate...
Antimicrobial susceptibility of *Campylobacter* spp.

Table 1. Antimicrobial susceptibility of 262 *Campylobacter* isolates from NI poultry during 2000–2001

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc concentration (µg)</th>
<th>C. jejuni (%)</th>
<th>C. coli (%)</th>
<th>Total (%)</th>
<th>95% CIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>79 (33.2)</td>
<td>7 (29.2)</td>
<td>86 (32.8)</td>
<td>27.2, 38.9</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>10</td>
<td>1 (0.4)</td>
<td>0 (0.0)</td>
<td>1 (0.4)</td>
<td>0.0, 2.1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>7 (2.9)</td>
<td>0 (0.0)</td>
<td>7 (2.7)</td>
<td>1.1, 5.4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>1 (0.4)</td>
<td>0 (0.0)</td>
<td>1 (0.4)</td>
<td>0.0, 2.1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>5</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0.0, 1.4</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30</td>
<td>24 (10.1)</td>
<td>1 (4.2)</td>
<td>25 (9.5)</td>
<td>6.3, 13.8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>10</td>
<td>31 (13.0)</td>
<td>2 (8.3)</td>
<td>33 (12.6)</td>
<td>8.8, 17.2</td>
</tr>
<tr>
<td>Drug resistance&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Not applicable</td>
<td>100 (42.0)</td>
<td>10 (41.7)</td>
<td>110 (42.0)</td>
<td>35.9, 48.2</td>
</tr>
<tr>
<td>Multiresistance&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Not applicable</td>
<td>2 (0.8)</td>
<td>0 (0)</td>
<td>2 (0.8)</td>
<td>0.1, 2.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Resistance to one or more antimicrobial drugs.

<sup>b</sup>Resistance to four or more antimicrobial drugs.

Table 2. Presence or absence of the Thr86-Ile mutation detected in the QRDR of two control (NCTC12505 and C8436) and six field *C. jejuni* isolates from NI poultry

<table>
<thead>
<tr>
<th>MIC</th>
<th>Tetracycline</th>
<th>Other changes leading to amino acid substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thr86-Ile</td>
<td></td>
</tr>
<tr>
<td>NCTC12505</td>
<td>1 (ACA-ATA)</td>
<td>Ser22-Gly (AGT-GGT) Asn203-Ser (AAT-AGT) Ala206-Val (GCA-GTA)</td>
</tr>
<tr>
<td>18A</td>
<td>≥256 (ACA-ATA)</td>
<td>No</td>
</tr>
<tr>
<td>137A</td>
<td>1 (ACA-ATA)</td>
<td>No</td>
</tr>
<tr>
<td>C8436</td>
<td>≥256 (ACA-ATA)</td>
<td>Yes</td>
</tr>
<tr>
<td>1I</td>
<td>≥256 (ACA-ATA)</td>
<td>Yes</td>
</tr>
<tr>
<td>2A</td>
<td>≥256 (ACA-ATA)</td>
<td>Yes</td>
</tr>
<tr>
<td>508A</td>
<td>≥256 (ACA-ATA)</td>
<td>Yes</td>
</tr>
<tr>
<td>631F</td>
<td>16 (ACA-ATA)</td>
<td>No</td>
</tr>
<tr>
<td>669Mi</td>
<td>≥256 (ACA-ATA)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

QRDR PCR protocol

The QRDR of the gyrA genes of nine *Campylobacter* isolates (Table 2) was amplified by PCR, principally as described by Zirnstein et al.<sup>18</sup> The forward primer GZgyrA5 (ATTTTTAGCAAAAGATTTCTGAT) and the reverse primer GZgyrA6 (CCATAAATTATCCACTGTT), designed to generate a 673 bp product from *C. jejuni* isolates (MWG-Biotech AG, Ebersberg, Germany), were used in this study. Heat-inactivated cell suspensions (5 µL aliquots) were transferred to a PCR master mix (50 µL final volume) containing 2.5 U of HotStarTag polymerase (Qiagen, Crawley, UK), 20 pmol of each primer, 250 µM dNTP and 1.75 mM MgCl₂. PCR cycling conditions were as follows: denaturation at 94°C for 15 min, followed by 30 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, with a final step of 72°C for 5 min. Electrophoresis was carried out on 5 µL aliquots of each sample on a 2% agarose E-gel (Invitrogen, Paisley, UK). Samples exhibiting the expected 673 bp product were subjected to commercial nucleotide sequence analysis (MWG-Biotech AG).

For these nine isolates, Etests (Cambridge Diagnostics, Cambridge, UK) were carried out for nalidixic acid (Nx) and Cp, and the MIC read as per manufacturer’s instructions.

Data analysis

EpiInfo2000 (Centres for Disease Control and Prevention, Atlanta, GA, USA) was used for statistical analysis. Confidence intervals (CIs) were calculated by the exact binomial method. Statistical significance determination using the χ² test was measured at an α value of 0.05.

Results

Of the 387 flocks tested during the sampling period, 262 (67.7%) were positive. Taking one isolate from each flock, the total species composition was 238 isolates of *C. jejuni* (90.8%) and 24 isolates of *C. coli* (8.0%).
Campylobacter coli (9.2%). Table 1 shows the resistance levels against the antibiotics tested by Campylobacter species.

Ampicillin resistance was high, at 33.2% for C. jejuni and 29.2% for C. coli, as was tetracycline resistance, at 13.0% for C. jejuni and 8.3% for C. coli. There were no isolates resistant to erythromycin; one C. jejuni isolate was resistant to gentamicin (0.4%) and one C. jejuni isolate was resistant to chloramphenicol (0.4%).

Nx resistance was found in 10.1% of C. jejuni and 4.2% of C. coli isolates. No C. coli isolate and 2.9% of C. jejuni isolates were resistant to Cp. QRDR sequences of nine isolates were compared with the sequence data of the same region of strain NCTC11168 (GenBank, see Table 2). The two Cp-sensitive field isolates and one sensitive control isolate showed no mutation leading to an amino acid change at codon 86. The four field isolates and one control isolate that were highly resistant (Nx ≥ 256 mg/L and Cp ≥ 32 mg/L) had the ACA to ATA mutation leading to Thr-86→Ile substitution. One low-resistant field isolate (Nx 16 mg/L and Cp 1 mg/L) did not demonstrate this mutation.

Resistance to one or more antibiotic tested was 42.0% overall, with no significant difference between C. jejuni (42.0%) and C. coli (41.7%). Multiresistance, defined as resistance to four or more antibiotics tested, was very low at 0% for C. coli and 0.8% for C. jejuni. For those isolates that were resistant to more than one antibiotic (n=110), the number resistant to both ampicillin and tetracycline was the highest at 22 (20%).

Discussion
The farms sampled for this study represent over 60% of all the broiler production sites in NI. Therefore, the isolates tested could be considered representative of the prevalence of antimicrobial susceptibility in pre-slaughter broiler chickens from NI. Multiresistance, which poses a threat to humans by limiting therapeutic choice, was very low in the study (0.8% for C. jejuni and 0% for C. coli). This contrasts favourably with other studies where multiresistance data are presented. For example, human isolates in England and Wales19 (11% for C. jejuni and 20% for C. coli) and from food animal isolates from Belgium5 (7.6% for C. jejuni and 17% for C. coli) indicated much higher levels. The low levels in this study could indicate a lack of a source of multiresistant isolates associated with the poultry farms surveyed, or of low usage of veterinary antimicrobial agents. However, the high level of simultaneous resistance to ampicillin and tetracycline may originate from isolates in reservoirs traceable through further typing and epidemiological studies.

No significant difference was found in the overall resistance rates between C. jejuni and C. coli isolates, in contrast to other studies reporting higher resistance among C. coli isolates.5,6,19 Whereas phenotypic discrimination between these two species is based solely on hippurate hydrolysis, species identification was cross-checked with molecular typing and serotyping data generated from a project of which this work forms a small part. The uncommon occurrence of pig husbandry on poultry farms in NI (<4% of the farms tested) may account for lower rates of resistant C. coli isolates.

The Thr-86→Ile mutation in the QRDR of gyrA has been implicated as one of the mechanisms by which resistance to quinolones can develop, particularly in strains with high levels of resistance.12 This mutation was present in the four field isolates with a high quinolone MIC, and absent from the quinolone-resistant isolate with a low quinolone MIC and the two quinolone-sensitive field isolates (Table 2). Therefore, at least two classes of quinolone-resistance mechanisms exist in the strains studied. The Thr-86→Ile mutation accounts for the high level of resistance, which can also be detected with MICs ≥256 mg/L of Nx and ≥32 mg/L of Cp. Although Cp resistance rates remain low in the series of isolates examined, MIC testing would be advantageous should a more detailed understanding of highly resistant isolates be undertaken.

In the UK, fluoroquinolone usage in poultry and cattle started on a trial basis in 1990, and enrofloxacin became licensed for veterinary use in 1993.20 This usage of fluoroquinolones has been correlated with a steady rise in resistance in human Campylobacter isolates7. Whereas more recent data from pre-slaughter chicken isolates from elsewhere in the UK are limited, the findings reported in this study can form a baseline from which to investigate the current contribution of Cp-resistant isolates from NI poultry to human infection. In a report on isolates from NI, only two of the 44 isolates (4.5%) from retail chickens were resistant to Cp.16 The level of Cp resistance in the present study (2.7%, 95% CIs 1.1 and 5.4) encompasses the level reported by Moore et al.,16 who used a similar drug-resistance testing method. During the same period, resistance to Cp in human isolates from NI was much higher, at 17.5% (58 out of 333).16 Similar observations have been made in Denmark, where Cp resistance was seen in 6% of broiler isolates, 13% of broiler meat isolates and 21% of human isolates.21 In other European countries, however, the level remains very high: 62% of food animal isolates in Belgium4 and 99% of human isolates in Spain4 are Cp resistant.

In conclusion, Cp resistance and multiresistance in NI poultry isolates were one of the lowest in recent reports from studies on human and poultry Campylobacter isolates.

Acknowledgements
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References
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