Resistance in *Campylobacter* Species: Increased Resistance to Fluoroquinolones and Seasonal Variation


To identify epidemiological features of culture-proven campylobacter infections and to determine resistance rates, we conducted a 4-year demographic survey of culture-proven campylobacteriosis in one Dutch region. Examination of 24,435 fecal specimens revealed 1,315 cases of campylobacteriosis (5.4%). The ofloxacin-resistance rate among *Campylobacter* isolates increased from 11% to 29%. Resistance against tetracycline fluctuated between 7% and 15%, and resistance against erythromycin remained low. Resistance against fluoroquinolones was seasonally influenced, with relatively high rates during winter. We conclude that resistance of *Campylobacter* isolates to fluoroquinolones is still rising, probably because of the use of fluoroquinolones (enrofloxacin) in animal husbandry.

Resistance of *Campylobacter* species against fluoroquinolones developed in the Netherlands [1–3] and other countries in Europe in the late 1980s and early 1990s [4–8]. This development has been related to the introduction of fluoroquinolones in veterinary medicine in the late 1980s and early 1990s [1, 6, 9]. At present, it is unknown whether resistance has further spread.

Patients and Methods

**Database.** The National Institute of Public Health and the Environment developed a computer-based centralized data system called the Infectious Disease Surveillance Information System (ISIS) [10]. We used the ISIS to determine epidemiological trends of campylobacteriosis in a region in the Netherlands, as well as resistance rates against four antibiotics routinely tested against *Campylobacter* species.

**Population served.** We used data from one regional laboratory participating in the ISIS (Streeklaboratorium Arnhem), located in the eastern part of the Netherlands, serving ~1 million inhabitants in both rural and urban areas. This laboratory serves a general patient population, both inpatients and outpatients, and subscribes to a quality assurance system established by the Dutch Society for Medical Microbiology.

**Laboratory analyses.** All stools submitted to the laboratory for bacterial examination were cultured for *Campylobacter* species with use of charcoal cefoperazone deoxycholate plates, incubated at 42°C for 48 hours in 5% O₂, 10% CO₂, and 85% N₂. Antimicrobial resistance testing was done by a standard agar diffusion method with use of New York City (NYC) medium (Regional Laboratory, Arnhem, The Netherlands) and commercial antibiotic tablets. (Neo-sensitabs; A/S Rosco Diagnostica, Taastrup, Denmark) [2]. Susceptibility testing was performed against erythromycin (78 μg), amoxicillin/clavulanic acid (30 + 15 μg), tetracycline (80 μg), and ofloxacin (10 μg). The plates were incubated for 24 hours at 42°C.

Zones were measured to the nearest mm, and the zone diameters were interpreted as either susceptible or resistant on the basis of guidelines provided by the manufacturer (Rosco Diagnostica, in accordance with Dutch criteria) [11, 12]. The strains were considered susceptible at the following MIC values: for erythromycin, ±1 μg/mL; for amoxicillin + clavulanic acid, ±2.5 μg/mL; for tetracycline, ±1 μg/mL; and for ofloxacin, ±1 μg/mL. The technique of susceptibility testing remained the same during the study period.

**Data analysis.** Analyses were performed with SAS software, version 6.12 (SAS Institute, Cary, NC) [13]. Figures were made with use of SAS GRAPH and Microsoft Excel (Redmond, WA) software [14]. Figure 1 was made with a smoothing option of PROC GPLOT in SAS [13]. This figure shows the 91-day centered moving averages, representing results obtained for isolates from the period spanning 45 days before and 45 days after the day on which the resistance rate is plotted.

Both negative and positive results of *Campylobacter* species cultures performed from 1 January 1994 to 1 January 1998 were included in this study. The first positive specimen obtained from a patient identified a case of campylobacteriosis (numerator). All positive isolates until 6 months after the first positive specimen from the same patient were then ignored. The denominator defines the number of specimens tested for *Campylobacter*. Differences in proportions are expressed in absolute differences as well as in 95% confidence intervals.

Results

Examination of a total of 24,435 fecal specimens revealed 1,315 cases of campylobacteriosis. The yearly number of cases...
and the denominator remained stable over the study period, with an isolation rate of 5.4% and from 320 to 335 cases diagnosed each year. The incidence of proven campylobacter infections was \( \sim 35 \) cases per 100,000 person-years and remained unchanged over the surveillance period. Expressed by 91-day moving averages, the incidence of campylobacteriosis showed a clear seasonal pattern, with \( \sim 20 \) cases per 100,000 person-years occurring during the northern-hemisphere winter months and \( \geq 50 \) cases per 100,000 person-years during July, August, and September (figure 1). The incidence by sex and age group is summarized in figure 2; among males, incidence was highest in infants, young children, and the elderly, while in the young-adult age group (20–29 years) the higher incidence was among females.

Resistance to tetracycline fluctuated between 7% and 15%, without a clear trend (table 1). Resistance against erythromycin was not detected until the second year of the study period; it persisted, although it remained below 2%. Resistance against amoxicillin/clavulanic acid was not detected in the surveillance period. However, there was an upward trend with regard to ofloxacin-resistant isolates: the 1994 resistance rate of 11% increased significantly to 29% in 1997 (change, 18%; 95% CI, 12–24) (table 1).

A more detailed analysis of fluoroquinolone resistance in Campylobacter isolates demonstrated an inverse seasonal pattern with lower resistance rates in the summer months, when incidence was higher, in contrast with higher resistance rates in winter and early spring, when incidence was lower (figure 1). The age-group distribution of ofloxacin resistance is shown in figure 3. The clinically most important type of cross-resistance, that between ofloxacin and erythromycin, was rare (0.2%). The cross-resistance between tetracycline and ofloxacin was greater than expected by chance alone \( (\chi^2 = 34.1; \text{df} = 1; P < .001) \) (table 2).

**Discussion**

We demonstrated significantly increased resistance against ofloxacin. Increased resistance against fluoroquinolones in Campylobacter species in the Netherlands was reported earlier

<table>
<thead>
<tr>
<th>Year</th>
<th>Em</th>
<th>Tet</th>
<th>Ofx</th>
<th>Amox/Clv</th>
<th>Total no. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>0</td>
<td>11.3</td>
<td>10.9</td>
<td>0</td>
<td>322</td>
</tr>
<tr>
<td>1995</td>
<td>0.9</td>
<td>13.6</td>
<td>21.0</td>
<td>0</td>
<td>325</td>
</tr>
<tr>
<td>1996</td>
<td>1.2</td>
<td>7.5</td>
<td>24.0</td>
<td>0</td>
<td>333</td>
</tr>
<tr>
<td>1997</td>
<td>0.3</td>
<td>16.1</td>
<td>28.8</td>
<td>0</td>
<td>335</td>
</tr>
</tbody>
</table>

**Table 1.** The resistance rates against erythromycin, tetracycline, amoxicillin/clavulanic acid, and ofloxacin among Campylobacter isolates, stratified by year.

**NOTE.** Amox/clv = amoxicillin + clavulanic acid; Em = erythromycin; Ofx = ofloxacin; Tet = tetracycline.
by Endtz et al. [1–3]. While they did not detect fluoroquinolone resistance in humans in 1982–1983, they found a resistance prevalence of 11% among Campylobacter isolates in 1989. Resistance of Campylobacter species to fluoroquinolones has emerged in many countries [4–8]; in Spain, for instance, rates up to 50% have been reported [15–17].

However, although with our laboratory-based surveillance system we did not have clinical or epidemiological data available to permit assessment of factors responsible for this trend, several factors have been implicated in the increasing resistance of Campylobacter species against fluoroquinolones. Their use in human medicine as treatment and prophylaxis for diarrhea is one such factor, though its impact is still debated [5].

Analyses of incidence by age and sex do not provide epidemiological explanations for the emerging resistance. The higher incidence of campylobacteriosis in young adult women could be the result of contact with young children. Stratification of resistance rates by age group showed 16.5% resistance to ofloxacin in children below the age of 10 years. This cannot be the result of human use, because for patients below the age of 16 years the use of fluoroquinolones is contraindicated (because of the risk of irreversible cartilage erosions).

In the United Kingdom a recent history of foreign travel has been identified as an independent risk factor for fluoroquinolone resistance of Campylobacter isolates [6, 8]. The seasonal variation of fluoroquinolone resistance in the Netherlands noted in our study does not support this observation: in summer, when most trips abroad are undertaken, resistance to fluoroquinolones was substantially lower than in winter.

The results demonstrated an inverse relationship between the rate of resistance to fluoroquinolones and the incidence of culture-proven campylobacteriosis. An explanation could be that during the winter months poultry is the most important source for Campylobacter species isolated from humans, while during summer, isolates from other sources associated with a low rate of fluoroquinolone resistance become more important.

Probably the most important factor may be the use of enrofloxacin in veterinary medicine. Enrofloxacin was introduced for veterinary use in the Netherlands in 1987, but it was not intensively used until the early 1990s. Endtz et al. found 14% resistance among Campylobacter isolates recovered from poultry products in 1989 [1]. In a study in 1992 and 1993 performed by Jacobs-Reitsma et al., the resistance rate was 29% among 617 isolates of Campylobacter from broilers [9].

Enrofloxacin was not licensed for use with poultry until 1993 in the United Kingdom. Fluoroquinolone-resistant Campylobacter jejuni was isolated from 14% of chicken carcasses imported from the Netherlands but only 1% of animals bred in the United Kingdom in 1993 [6].

During the study period, resistance against erythromycin appeared, but it remained below 2%. However, in a number of other countries greater resistance against macrolides has emerged in Campylobacter species [4–6, 18].

The most important type of cross-resistance in Campylobacter species, that between fluoroquinolone and macrolides, was rare in our study (0.2%). We did not find increased resistance against erythromycin in ofloxacin-susceptible vs. ofloxacin-resistant strains. This is in agreement with the findings in an earlier study in the Netherlands, in 1993: there was no difference in macrolide resistance between fluoroquinolone-susceptible and fluoroquinolone-resistant C. jejuni isolates [2]. The cross-resistance between tetracycline and ofloxacin was higher than expected by chance alone. Strains that were resistant to both antibiotics had a selection advantage, probably as the result of antibiotic pressure in their ecological niche.

The continuous increase in the rate of resistance against fluoroquinolones stresses the impact of antibiotic use in veterinary medicine on the resistance rates of isolates in human medicine. Therefore, continuous surveillance of resistance against antibiotics used in humans and animals for campylobacteriosis remains essential. Further investigation into the source of Campylobacter species susceptible to fluoroquinolones during the summer months in Holland is needed.

Table 2. Resistance and cross-resistance to erythromycin, ofloxacin, and tetracycline among Campylobacter isolates (no. of tested strains, 1,241).

<table>
<thead>
<tr>
<th>Antibiotic(s) resistant to</th>
<th>No. (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Em</td>
<td>4 (0.3)</td>
</tr>
<tr>
<td>Tet</td>
<td>87 (7.0)</td>
</tr>
<tr>
<td>Ofx</td>
<td>209 (16.8)</td>
</tr>
<tr>
<td>Em, Tet</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>Em, Ofx</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Tet, Ofx</td>
<td>59 (4.8)</td>
</tr>
<tr>
<td>Em, Tet, Ofx</td>
<td>1 (0.1)</td>
</tr>
</tbody>
</table>

NOTE. Em = erythromycin; Ofx = ofloxacin; Tet = tetracycline.

Figure 3. The rate of resistance of Campylobacter isolates to ofloxacin, stratified by age groups of patients from whom they were recovered.
References


