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Antimicrobial Susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* Isolated in Sweden: a 10-Year Follow-Up Report

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Resistance to erythromycin and doxycycline and more recently to fluoroquinolones has been reported to occur in *Campylobacter* spp. both in vitro and in patients treated with these antibiotics. The frequency of resistance to 14 antimicrobial agents in *Campylobacter jejuni* and *Campylobacter coli* isolated from patients infected in Sweden or abroad is described. For some agents, a comparison of susceptibility in strains of *Campylobacter* spp. isolated in 1978 with those isolated in 1988 is made. No general increase in in vitro resistance to antibiotics commonly used for the treatment of human gastroenteritis caused by *C. jejuni* or *C. coli* has occurred during the last 10 years in Sweden, which might be a consequence of strict antibiotic control. The numbers of strains from 1988 to 1989 resistant to ciprofloxacin and to norfloxacin included in this study (0.7 and 1.4%, respectively) are still fewer than those that were resistant to erythromycin (7.3%) or doxycycline (12.4%). There is, however, since 1989 to 1990 an indication of increasing resistance to these antibiotics.

Acute diarrheal diseases are major health problems throughout the world, with *Campylobacter* enterocolitis being the most common bacterial source of these infections. Enterocolitis due to *Campylobacter* species is usually a mild to moderate self-limited diarrheal disease; however, patients with severe, prolonged, or relapsing *Campylobacter* enterocolitis are recommended for treatment with antibiotics. Erythromycin, doxycycline, and, more recently, the new quinolones have been utilized as therapy for these infections (8, 11, 23).

An increasing number of antibiotic-resistant strains of different enteropathogenic bacteria have been identified in developed as well as in developing countries (6, 8). Resistance to erythromycin (19, 26) or tetracycline in *Campylobacter* spp. (21) has been reported earlier in up to 15% of strains, and resistance to the newer quinolones has recently been reported (5, 9). Therefore, continued concern about antimicrobial resistance is warranted, as are studies of the activity of new agents used for treatment of diarrheal diseases (18). In an investigation reported 10 years ago, we studied the antibiotic susceptibility of *Campylobacter jejuni* and *Campylobacter coli* from Swedish patients (17). The current study compares the susceptibilities of those isolates with those of isolates obtained in 1988 to 1989.

Antimicrobial agents. The antimicrobial agents tested were amikacin and ampicillin (Bristol Laboratories); gentamicin (Schering Corp.); chloramphenicol (Dumex); cephalothin and tobramycin (Eli Lilly & Co.); cefuroxime (Glaxo Op. Ltd. UK); doxycycline (Pfizer); erythromycin, benzylpenicillin, and norfloxacin (Astra); cefotaxime (Hoechst AG); clindamycin (Upjohn); and ciprofloxacin (Bayer Pharmaceuticals).

Bacterial isolates. One hundred thirty-seven *C. jejuni* or *C. coli* consecutive isolates from stool specimens of patients suffering from acute diarrheal infections during May 1988 to May 1989 were included. The strains were isolated from inpatients and outpatients and were sent from different clinics in Sweden. The cultivations were performed at the Bacteriological Diagnostic Laboratories, Sahlgren Hospital, Göteborg, Sweden. Seventy percent of the patients appeared to have been infected abroad, of which one-half had been visiting Mediterranean countries. Strains were stored by lyophilization after being identified according to established criteria for *C. jejuni* and *C. coli* (10, 13, 16).

Bacterial inoculum. The bacterial strains were plated on nonselective blood agar (Oxoid no. 2) and were cultivated for 48 h in a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2). Colonies from these plates were then suspended in nutrient-glucose broth and adjusted to a final concentration of 10^6 to 10^7 CFU/ml. The antimicrobial susceptibility tests were conducted in a Dynatech 2000 in which a final inoculum of 1.5×10^5 CFU/ml was used in each well of the microtiter trays. The viability of the bacteria was verified in drug-free control wells. Susceptibility tests were controlled by using *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 27922), and *Staphylococcus aureus* (ATCC 25923) as reference strains. The MIC for each strain and of each antimicrobial agent tested was recorded after 48 h in a microaerophilic atmosphere of 5% O_2, 10% CO_2, and 85% N_2. The MIC was defined as the lowest concentration of antibiotic producing no visible growth.

The MICs of the 14 antibiotics inhibiting 50 and 90% (MIC_{50} and MIC_{90}) of the *C. jejuni* ($n = 110$) and *C. coli* ($n = 27$) strains tested are illustrated in Table 1. Significant differences in susceptibility to ampicillin ($P < 0.01$), benzylpenicillin ($P < 0.001$), and cefotaxime ($P < 0.01$) were observed between the two species. The national breakpoints recommended by the Swedish Reference Group for Antibiotics according to the SIR system (S, sensitive; I, intermediate sensitivity; R, resistant) valid for 1988 to 1989 were used for comparison of these isolates (1). In Table 2 the comparative numbers of resistant strains for *C. jejuni* and *C. coli* in 1978 ($n = 78$) and 1988 ($n = 137$) are illustrated. For this comparison the national breakpoints recommended by

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TABLE 1. MICs for *C. jejuni* and *C. coli* isolated from Swedish patients in 1988 and 1989

Antibiotic	*C. jejuni* (n = 110) results			*C. coli* (n = 27) results			P value[a]	Breakpoint[b] (μg/ml)
	MIC_{50}/MIC_{90} (μg/ml)	% Resistant	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	% Resistant	MIC range (μg/ml)		
Amikacin	0.25/1	0	<0.063–2	0.5/1	3.7	0.25–64		≥16
Gentamicin	0.125/0.5	0.9	<0.063–8	0.25/0.5	0	0.125–4		≥8
Tobramycin	0.5/1	0	<0.063–2	1/2	0	0.5–2		≥8
Ampicillin	4/16	20.9	<0.125–>128	8/16	25.9	2–16	<0.01	≥16
Benzylpenicillin	8/16	60.0	<0.125–32	16/32	96.3	4–32	<0.001	≥8
Cephalotin	128/>128	98.2	4–>128	128/>128	100.0	64–128		≥16
Cefotaxime	8/32	44.5	<0.125–64	16/32	66.7	4–32	<0.01	≥16
Cefuroxime	64/128	95.5	<0.125–>256	64/128	100.0	64–256		≥16
Chloramphenicol	2/8	2.7	<0.125–32	2/4	0	2–4		≥16
Ciprofloxacin	0.125/0.25	0.9	<0.063–8	0.125/0.25	0	<0.063–1		≥8
Norfloxacin	0.25/1	1.8	0.125–32	0.25/1	0	0.125–4		≥8
Clindamycin	0.25/0.5	3.6	<0.125–32	0.25/0.5	0	<0.125–1		≥8
Doxycycline	0.25/8	12.7	<0.063–64	0.125/16	11.1	<0.063–32		≥4
Erythromycin	1/4	6.4	<0.125–>256	1/8	11.1	<0.125–>256		≥8

[a] Where no value is given, no significance was present.
[b] By recommendation of the Swedish Reference Group for Antibiotics, valid for 1988 to 1989.

the Swedish Reference Group for Antibiotics valid for 1978 were used. Of the antimicrobial agents tested, eight could be compared with those also tested 10 years ago. The MICs of benzylpenicillin, clindamycin, doxycycline, and erythromycin show a significant difference in the number of strains resistant in 1978 to 1979 and 1988 to 1989.

The frequent use of doxycycline or erythromycin over the past 10 years may have led to an increased number of resistant strains observed in several studies (11, 12, 20, 26). All the strains resistant to doxycycline and 90% of the strains resistant to erythromycin in this study were isolated from patients who were infected abroad. This was also the case with all of the *C. coli* strains isolated. The use of the newer quinolones for treatment of gastroenteritis has been recommended in several investigations (7, 8). Furthermore, interest in the use of fluoroquinolones in the prophylaxis of acute bacterial diarrhea due to organisms such as *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* spp. has increased (5, 14, 18, 24). Most recently (1989 to 1990) we have found an increasing number of nalidixic acid-resistant *Campylobacter* strains, including *C. jejuni* and *C. coli* of different serogroups, which have also been cross resistant to ciprofloxacin and norfloxacin. Cross-resistance is an expected phenomenon due to the close structural relationship of the fluorinated 4-quinolones and the parent compound nalidixic acid (2, 4, 15, 18, 19, 25, 26). Even if the upper MIC range of both norfloxacin and ciprofloxacin for enteropathogens is increasing, the numbers of strains resistant to ciprofloxacin (0.7%) or norfloxacin (1.4%) in this study are still fewer than those that were resistant to erythromycin (7.3%) or doxycycline (12.4%). However, in one of our later studies there is an indication of increasing resistance to quinolones during treatment of acute diarrhea (25). The results of the current study may indicate that the overuse of quinolones for the treatment of *Campylobacter* enteritis, at least in this geographic area, is not common. Nor is the use of these antibiotics in the poultry industry in Sweden common, in contrast to their use for prophylactic purposes in other countries (5).

TABLE 2. Comparative number of resistant strains for *C. jejuni* and *C. coli* in 1978 and 1988

Antibiotic	% Resistant strains in:		Breakpoint[a] (μg/ml)	P value[b]
	1978 (n = 78)	1988 (n = 137)		
Gentamycin	3.9	0	>16	NS
Ampicillin	10.4	3.6	>16	NS
Benzylpenicillin	68.8	32.8	>8	<0.001
Cefuroxime	86.0	93.4	>16	NS
Chloramphenicol	8.0	2.2	>8	NS
Clindamycin	18.2	3.0[c]	>4	<0.01
Doxycycline	22.7	10.9	>4	<0.05
Erythromycin	16.6	7.3	>4	<0.05

[a] By recommendation of the Swedish Reference Group for Antibiotics, valid for 1978 and 1988.
[b] NS, not significant.
[c] 67 strains tested.

Several studies have emphasized that *C. coli* isolates are more likely to acquire antibiotic resistance than are *C. jejuni* strains (3, 6, 19, 22). In this study, the number of *C. coli* strains that were significantly more resistant to ampicillin, benzylpenicillin, and cefotaxime was greater than in the case with *C. jejuni*.

We conclude that no general increase in resistance in *C. jejuni* or *C. coli* to antibiotics commonly used for the treatment of human gastroenteritis has occurred during the last 10 years in Sweden. The quinolones, for which in the present study it was not possible to compare susceptibilities over the last 10 years, have been suggested as alternatives to erythromycin or doxycycline when treatment is indicated. The susceptibility patterns of these antibiotics have to be progressively studied in order to rule out any future increase in the frequency of resistance (9, 25).

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