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Evolution of Susceptibilities of *Campylobacter* spp. to Quinolones and Macrolides

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Erythromycin, new macrolides, and quinolones are alternatives for the treatment of *Campylobacter* infections. Concerns related to the emergence of resistance to both groups of drugs have been raised. We studied the evolution of antimicrobial susceptibilities of 275 clinical isolates of microorganisms of the genus *Campylobacter* isolated in our institution during a 5-year period (1988 to 1992). The microorganisms studied were *C. jejuni* ($n = 230$), *C. coli* ($n = 42$), and *C. fetus* ($n = 3$). The overall resistance rates (determined by the agar dilution method and the recommendations of the National Committee for Clinical Laboratory Standards) were as follows: erythromycin, 2.3%; clarithromycin, 2.3%; azithromycin, 1.9%; ciprofloxacin, 28.5%; norfloxacin, 31%; ofloxacin, 26.3%; and nalidixic acid, 36.8%. The evolution of resistance (percent resistance in 1988 versus percent resistance in 1992) was as follows: erythromycin, 2.6 versus 3.1; clarithromycin, 2.6 versus 3.1; azithromycin, 2.6 versus 3.1; ciprofloxacin, 0 versus 49.5; norfloxacin, 2.6 versus 55.5; ofloxacin, 0 versus 45.6; nalidixic acid, 2.6 versus 56.8. Our data show stable macrolide activity against *Campylobacter* spp. and the rapid development of quinolone resistance over the last 5 years.

Microorganisms of the genus *Campylobacter* are increasingly common enteric pathogens, usually causing a noninvasive diarrhea that occasionally requires antimicrobial therapy (10). *Campylobacter* spp. may also be responsible for a few nonenteric invasive infections. Classically, erythromycin and, more recently, the new macrolides and quinolones are considered the drugs of choice for the treatment of infections caused by *Campylobacter* spp. (2). However, the recent broad use of these drugs has raised concerns related to the development of antimicrobial resistance (6, 18, 20, 22, 23).

We studied the evolution of the antimicrobial susceptibility patterns to macrolides and quinolones of 275 clinical isolates of the genus *Campylobacter* recovered in our laboratory during the last 5 years.

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MATERIALS AND METHODS

Stool samples collected from human sources during the period from January 1988 to December 1992 were routinely plated on Campy-BAP selective medium (Becton-Dickinson) and were incubated for 48 h at 37°C in an atmosphere consisting of 5% O_2–10% CO_2–85% N_2 (Campy-Pack; BBL). Identification of the genus and species of *Campylobacter* was performed by standard methods (10, 15, 16).

Antimicrobial susceptibility tests. One strain from each patient was tested. Isolates (stored at −70°C in skim milk) were subcultured three times onto Columbia agar containing 5% sheep blood prior to study. Antimicrobial susceptibility testing was performed by the agar dilution method by using Mueller-Hinton agar supplemented with 5% lysed horse blood.

The antimicrobial agents studied were erythromycin (Abbott Laboratories, North Chicago, Ill.), clarithromycin (Abbott)

TABLE 1. Distribution of isolates and strains tested during the study period

Year of isolation	No. of stool cultures	No. (%) of positive stool cultures	No. of isolates/no. of strains of *C. jejuni* tested	No. of isolates/no. of strains of *C. coli* tested	No. of isolates/no. of strains of *Campylobacter* spp. tested
1988[a]	3,626	375 (10.3)	41/26	14/11	2/1
1989	3,709	467 (12.5)	49/31	20/15	2/1
1990	3,959	585 (14.7)	78/39	10/6	1/1
1991	4,379	659 (18.9)	121/61	10/2	0
1992[a]	5,557	673 (12.1)	131/73	10/8	0
Total	21,230	2,759 (12.9)	420/230	64/42	5/3

[a] $P = 0.001$ for 1988 versus 1992.

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TABLE 2. Evolution of antimicrobial activity and resistance to macrolides and quinolones of *Campylobacter* spp.[a]

Year of isolation	Erythromycin			Clarithromycin			Azithromycin			Roxithromycin			Ciprofloxacin			Norfloxacin			Ofloxacin			Nalidixic acid		
	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% MIC ≥8 μg/ml	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant[b]	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant[b]	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant[b]	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant[b]
1988	0.25	2	2.6	0.5	1	2.6	0.06	0.12	2.6	2	8	13.1	0.25	0.5	0	0.12	2	2.6	0.06	0.5	0	0.06	8	2.6
1989	0.5	1	2	0.5	2	2	0.06	0.25	2	2	4	8.1	0.25	1	6.1	0.5	8	8.1	0.25	1	6.1	4	32	20.4
1990	0.5	2	0	0.5	2	0	0.06	0.25	0	2	4	8.6	0.25	1	8.6	0.5	4	10.8	0.5	2	8.7	8	32	17.4
1991	0.5	2	3.1	0.5	1	3.1	0.06	0.25	1.5	2	8	14.2	2	64	50.7	16	64	52.3	2	16	47.6	16	64	58.7
1992	0.5	2	3.1	0.5	2	3.1	0.12	0.25	3.1	2	8	10.9	1	32	49.5	32	>64	55.5	2	32	45.6	64	>64	56.8

[a] MIC_{50} and MIC_{90}, MICs for 50 and 90% of isolates tested, respectively. Breakpoints were as follows: erythromycin, ≥8 μg/ml; clarithromycin, ≥8 μg/ml; azithromycin, ≥8 μg/ml; ciprofloxacin, ≥4 μg/ml; norfloxacin, ≥16 μg/ml; ofloxacin, ≥8 μg/ml; nalidixic acid, ≥32 μg/ml.

[b] $P < 0.001$ (1990 versus 1991).

azithromycin (Pfizer Inc., New York, N.Y.), roxithromycin (Glaxo, Greenford, United Kingdom), ciprofloxacin (Bayer AG, Wuppertal, Germany), norfloxacin (Merck Sharp & Dohme, West Point, Pa.), ofloxacin (Hoechst AG, Somerville, N.J.), and nalidixic acid (Sterling Winthrop). All antimicrobial agents were kindly supplied by their respective manufacturers.

The range of concentrations for all antimicrobial agents tested was 0.03 to 64 μg/ml. A final inoculum of 10^4 CFU was applied to agar plates containing twofold dilutions of each drug. Plates were incubated for 48 h at 37°C in the atmosphere described above.

Because the microaerobic atmosphere (CO_2) used in the study may decrease the pH of the test medium and thus increase the MICs of macrolides (8, 9), we performed the susceptibility test for these antimicrobial agents using pH 8, while quinolone MICs were determined at pH 7.2 to 7.4.

Quality control was done by using *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. The respective MIC ranges for these strains were as follows: erythromycin, 0.25 to 0.5 and 32 to 64 μg/ml; clarithromycin, 0.12 to 0.5, and 16 to 64 μg/ml; azithromycin, 0.25 to 1 and 1 to 4 μg/ml; roxithromycin, 0.25 to 1 and 32 to ≥64 μg/ml; ciprofloxacin, 0.12 to 0.25 and ≤0.03 μg/ml; norfloxacin, 0.5 to 1 and 0.03 to 0.12 μg/ml; ofloxacin, 0.12 to 1 and ≤0.03 to 0.06 μg/ml; and nalidixic acid, 8 to 32 and 1 to 4 μg/ml.

The breakpoints were defined according to the criteria of the National Committee for Clinical Laboratory Standards (12). However, the roxithromycin breakpoint has not yet been defined by the National Committee for Clinical Laboratory Standards, and therefore the percent resistance could not be determined.

Statistical analysis. The significance of quantitative variables was analyzed by the chi-square test, considering significant values of P of <0.05 (two-tailed) (21).

RESULTS

During the study period, 21,230 stool samples were submitted for culture. Of these, 2,759 (13%) had a significant bacterial pathogen. *Campylobacter* spp. were the second most frequently isolated microorganisms (2.3% of all samples and 17.7% of positive samples). Of 489 *Campylobacter* species isolated, we tested 275 viable strains after selecting a single isolate from each patient. The species included were 230 *C. jejuni* (84.4%), 42 *C. coli* (15.3%), and 3 *C. fetus*.

During the study period a significant increase in the number of isolates of *Campylobacter* species ($P = 0.001$) was observed (Table 1). The 275 strains selected for the present study were proportionally distributed over the 5-year period. The majority were from pediatric patients (63.2%).

Overall resistance rates were as follows: erythromycin, 2.3%; clarithromycin, 2.3%; azithromycin, 1.9%; ciprofloxacin, 28.5%; norfloxacin, 31%; ofloxacin, 26.3%; and nalidixic acid, 36.8%.

The evolution of resistance to both groups of antimicrobial agents from 1988 to 1992 is summarized in Table 2. Susceptibility patterns to macrolides remained low and stable. The activities of azithromycin and clarithromycin were similar to that of erythromycin. Despite its superior pharmacokinetics, roxithromycin showed the worst in vitro activity (MIC for 90% of strains tested, 8 μg/ml) among the macrolide group.

Regarding the quinolones, we found a striking increase in the level of quinolone resistance in the *Campylobacter* strains isolated in 1991 in comparison with that among those isolated previously (Table 2); the percentages of resistance in 1990 and 1991 were as follows: ciprofloxacin, 8.6 to 50.7%; norfloxacin,

TABLE 3. Evolution of resistance to macrolides and quinolones of different species of *Campylobacter*

Antimicrobial agent	% Resistance in the following yr of isolation											
	1988		1989		1990		1991		1992		Total	
	C. jejuni (n = 26)[a]	*C. coli* (n = 11)	*C. jejuni* (n = 31)	*C. coli* (n = 15)	*C. jejuni* (n = 39)	*C. coli* (n = 6)	*C. jejuni* (n = 61)	*C. coli* (n = 2)	*C. jejuni* (n = 73)	*C. coli* (n = 8)	*C. jejuni* (n = 230)	*C. coli* (n = 42)
Erythromycin	0	9	3	0	0	0	3.3	0	0	25	1.4	7.1
Clarithromycin	0	9	3	0	0	0	3.3	0	0	25	1.4	7.1
Azithromycin	0	9	3	0	0	0	1.6	0	0	25	0.9	7.1
Ciprofloxacin	0	0	9	0	7.5	16.6	51.6	50	51.3	25	31.8	9.5
Norfloxacin	0	9	12.1	0	7.5	33.3	53.3	50	58.3	25	34.9	14.2
Ofloxacin	0	0	9	0	7.5	0	48.3	50	45.8	25	29.3	9.5
Nalidixic acid	0	9	18.2	26.6	15	33.3	60	50	61.1	25	39.6	23.8

[a] n indicates number of strains tested.

10.8 to 52.3%; ofloxacin, 8.7 to 47.6%; and nalidixic acid, 17.4 to 58.7% ($P < 0.001$).

No relationship between macrolide and quinolone susceptibilities was found.

We observed differences in antimicrobial susceptibilities according to the species of *Campylobacter*, with *C. coli* being more resistant to macrolides and *C. jejuni* being more resistant to quinolones (Table 3).

DISCUSSION

Campylobacter spp. are becoming the most common cause of bacterial diarrhea in some developed countries (24). In Spain it ranks second, after *Salmonella* spp. (3, 11).

In Spain macrolides continue to be the drugs of choice for the treatment of many infections caused by *Campylobacter* spp. because the prevalence of erythromycin-resistant strains remains low (2.3%) and stable. This figure varies between 1 and 23% in different references (4, 17, 18, 20, 23, 26, 27). Our study and others (1, 5, 25) show that macrolides, like clarithromycin and azithromycin, have in vitro activities similar to that of erythromycin and superior to that of roxithromycin.

The situation for quinolones is totally different. In our hospital, study of the in vitro susceptibilities of *Campylobacter* species has shown a remarkable increase in the level of resistance since 1991. Ciprofloxacin resistance went from 8.6% in 1990 to 50.7% in 1991, a phenomenon also observed by others (4, 13, 20, 22).

The reasons for this are not well known, but the use of large amounts of quinolones in humans and animals (6, 7, 18, 19, 23, 27) is a possible explanation. It is of interest that in our study 63.2% of patients were from pediatric wards, but quinolones are not recommended for use in the pediatric population.

The increasing prevalence of *Campylobacter* resistance to quinolones may threaten the future of this group of drugs as empiric agents for traveler's diarrhea, as described previously (14).

Susceptibility to nalidixic acid, which so far has been used as a differential criterion for distinguishing *Campylobacter* species (*C. jejuni* susceptible and *C. coli* resistant), can no longer be used as a reliable criterion for such differentiation.

In summary, erythromycin and other macrolides remain the drugs of choice for the treatment of severe *Campylobacter* infections (18). The increase in the level of quinolone resistance is an additional caution and motive against the indiscriminate use of these drugs.

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