Journal of Antimicrobial Chemotherapy (2003) **52**, 1035–1039
DOI: 10.1093/jac/dkg489
Advance Access publication 12 November 2003

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Multidrug resistance in *Campylobacter jejuni* strains collected from Finnish patients during 1995–2000

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Received 16 July 2003; returned 15 August 2003; revised 25 September 2003; accepted 27 September 2003

***Objectives*: The resistance of *Campylobacter jejuni* to fluoroquinolones is increasing globally. This study was performed to delineate those antimicrobial agents that are effective *in vitro* against ciprofloxacin-resistant *C. jejuni* isolates and potentially suitable for the treatment of severe disease when fluoroquinolone resistance or multidrug resistance is known or suspected.**

***Methods*: During 1995–2000 we collected 376 *C. jejuni* strains, of which 354 were of foreign origin from multiple countries and 22 were of domestic origin. The MICs of 12 antimicrobial agents against the isolates were determined.**

***Results*: Of the 376 strains, 174 (46%) were resistant to ciprofloxacin. Among other antimicrobials, resistance was most common to tetracycline (46%) and ampicillin (17%). Of the ciprofloxacin-resistant strains, 68% and 25%, respectively, were resistant to tetracycline and ampicillin, and 3% were resistant to erythromycin, gentamicin or clindamycin. One (0.6%) ciprofloxacin-resistant isolate was resistant to co-amoxiclav and none was resistant to imipenem. Resistance to three or more antimicrobial groups was detected in 22% of the isolates. Multidrug resistance was significantly associated with ciprofloxacin resistance (33% versus 12%; $P < 0.01$). Eight (2%) strains were resistant to macrolides, of which 75% were also resistant to ciprofloxacin, but none was resistant to co-amoxiclav or imipenem.**

***Conclusions*: Macrolides still appear to be the first-choice alternative for suspected *C. jejuni* enteritis, if antimicrobial treatment is needed. The *in vitro* susceptibilities suggest that clinical trials to treat enteritis caused by multidrug-resistant *C. jejuni* with co-amoxiclav, and life-threatening infections with a carbapenem, may be valuable.**

Keywords: drug resistance, fluoroquinolones, macrolides, diarrhoea

Introduction

Campylobacter jejuni is a major causative agent of bacterial diarrhoea. Although campylobacters are naturally susceptible to fluoroquinolones, the resistance to these antimicrobials has increased rapidly in several countries during the 1990s.[1] This increasing resistance has complicated the empirical treatment of bacterial diarrhoea, as well as of severe *Campylobacter* infections in countries where fluoroquinolone-resistant strains predominate.[2] Although most *C. jejuni* infections do not require antimicrobial treatment, some may be fatal, especially if the patient is immunocompromised. The aim of this study was to determine the susceptibilities of 376 *C. jejuni* strains to 12 antimicrobial agents. These strains were recovered from Finnish patients during 1995–2000.

Materials and methods

Campylobacter strains and susceptibility testing

A total of 376 clinical human faecal *C. jejuni* strains were isolated from Finnish patients (one isolate per patient). The strains were isolated in the laboratory of a large private hospital in Helsinki, Finland, over two distinct time periods between 1995 and 2000. Subjects were treated as

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outpatients and no data on antimicrobial usage prior to faecal sampling were available. Two hundred and sixteen consecutive strains were isolated between January 1995 and November 1997, and 160 between October 1998 and January 2000. The strains were identified by standard microbiological methods. The strains isolated from patients travelling abroad within 2 weeks preceding their symptoms were classified as foreign isolates, all other strains were classified as domestic isolates. The countries of origin for the foreign isolates have been described previously.[1]

The MICs of antibiotics for the isolates were determined by the agar plate dilution method. Mueller–Hinton II agar (BBL, Becton Dickinson and Company, Cockeysville, MD, USA) supplemented with 5% sheep blood was used as the culture medium. The plates were incubated at 35°C for 48 h in a microaerobic atmosphere (CampyPak; BBL). The antimicrobials evaluated were nalidixic acid, ciprofloxacin, erythromycin, azithromycin, clindamycin, ampicillin, co-amoxiclav, cefotaxime, imipenem, tetracycline, gentamicin and chloramphenicol. *C. jejuni* RH 3583 (a local control strain, originally isolated in Edinburgh, UK as *C. jejuni* 143483) was used as a control in susceptibility testing and also as a growth control strain.[3] The MIC breakpoints used for resistance to ciprofloxacin, cefotaxime, imipenem, tetracycline, gentamicin and chloramphenicol were those recommended by the NCCLS for non-Enterobacteriaceae.[4] For nalidixic acid, ampicillin and co-amoxiclav, which lack breakpoints for non-Enterobacteriaceae, we used those recommended by the NCCLS for Enterobacteriaceae.[4] The resistance breakpoints for erythromycin, azithromycin and clindamycin were chosen on the basis of earlier publications and histogram analysis (Figure 1). They were ≥16 mg/L for erythromycin,[5] ≥4 mg/L for azithromycin[2] and ≥8 mg/L for clindamycin.[6] For the isolates exhibiting MICs ≥ 16 mg/L of erythromycin, we also determined MICs of clarithromycin and telithromycin. Multidrug resistance was defined as resistance to three or more antimicrobial groups. The antimicrobial groups were as follows: (i) quinolones (ciprofloxacin and nalidixic acid); (ii) macrolides (erythromycin and azithromycin) and clindamycin; (iii) tetracycline; (iv) β-lactams; (v) gentamicin; and (vi) chloramphenicol.

Data analysis

The susceptibility data were analysed using the WHONET5 computer program (available from www.who.int/emc/WHONET/WHONET.html).

Statistical analysis was made using the χ^2-test and the Fisher's exact test. The statistical data were analysed using the SAS (v. 8.2) program.

Results

Of the 376 *C. jejuni* isolates, 354 were collected from travellers returning to Finland and 22 were from patients with no travel history within the preceding 3 weeks.

Of all 376 isolates, 174 (46%) were resistant to ciprofloxacin (Table 1). Among the other antimicrobial groups studied, resistance was most common to tetracycline (46%) and ampicillin (17%). Only 2% of the isolates were resistant to erythromycin, azithromycin or clindamycin. Similarly, 2% were resistant to cefotaxime, but the proportion of intermediately cefotaxime-resistant isolates was 40%. Resistance to chloramphenicol was 3% and to gentamicin 2%. There was no resistance to imipenem, and only one (0.3%) isolate was resistant to co-amoxiclav (Table 1). The histograms illustrating the MICs are presented in Figure 1.

Of the 354 isolates of foreign origin, 172 (49%) were resistant to ciprofloxacin, compared with only two (9%) of the 22 domestic isolates ($P < 0.01$). Of the 174 ciprofloxacin-resistant strains tested with 10 non-quinolone antimicrobial agents, 68% and 25%, respectively, were resistant to tetracycline and ampicillin, as compared with the resistance among the ciprofloxacin-susceptible strains (27% and 9%; $P < 0.01$). Of the ciprofloxacin-resistant isolates, 4% were resistant to chloramphenicol, and 3% to erythromycin, gentamicin or clindamycin. Resistance to cefotaxime was also 3%, but the proportion of intermediately cefotaxime-resistant isolates was 48%. One (0.6%) ciprofloxacin-resistant isolate was resistant to co-amoxiclav and none was resistant to imipenem.

Multidrug resistance was detected in 81 (22%) isolates. While 57 (33%) of the 174 ciprofloxacin-resistant isolates had three or more additional resistance properties, 24 (12%) of the 202 quinolone-susceptible isolates were resistant to three or more antimicrobial agents ($P < 0.01$). Cefotaxime was excluded from the multidrug-resistance profile analysis because of the high number of intermediately cefotaxime-resistant isolates.

All of the eight erythromycin-resistant *C. jejuni* isolates were multidrug resistant, six (75%) of them being resistant to ciprofloxacin. None was resistant to co-amoxiclav or imipenem. The five isolates for which the erythromycin MICs were between 16 and 32 mg/L had MICs of clarithromycin between 8 and 32 mg/L and of telithromycin between 4 and 32 mg/L. The three isolates for which the erythromycin MICs were >256 mg/L had MICs of clarithromycin ≥128 mg/L and of telithromycin between 16 and >128 mg/L.

Discussion

Campylobacter infections are often self-limiting, thus requiring no antimicrobial treatment. However, therapy may be needed, for example in severe and prolonged cases of diarrhoea, in septicaemia or when the patient is immunocompromised. At the present time, fluoroquinolones may be unsatisfactory in the empirical treatment of *Campylobacter* infections or severe community-acquired bacterial diarrhoea in countries where fluoroquinolone-resistant strains are prevailing, or for tourists returning from those areas. The high fluoroquinolone resistance rate (49%) among our foreign *C. jejuni* isolates suggests that most international holiday destinations popular among Finns now belong to such a category.

In this study, *C. jejuni* resistance to ciprofloxacin was found to be significantly associated with resistance to three or more antimicrobial groups. Multidrug resistance is problematic, but a number of drugs are still effective against these fluoroquinolone-resistant and multidrug-resistant *C. jejuni* strains. Macrolides are currently the first-choice antimicrobials for the empirical treatment of suspected *C. jejuni* enteritis in many countries.[2,7,8] Fortunately, macrolide resistance has so far remained relatively uncommon, with only 2% of all our isolates and 3% of the ciprofloxacin-resistant isolates classified as macrolide resistant. Thus, also in Finland, macrolides still appear to be the best alternative in suspected *C. jejuni* enteritis, if antimicrobial therapy is needed.

Higher macrolide resistance rates have been reported from some other countries. According to a recent survey, the rate of erythromycin resistance among *C. jejuni* was 17% in both Spain and Taiwan.[7,8] In another study, the azithromycin resistance rate in Thailand was only 6%, but it was alarming that all azithromycin-resistant isolates were also fluoroquinolone resistant.[9] A similar finding has been made by another group.[2] In the present work, as many as 75% of the erythromycin-resistant isolates were ciprofloxacin resistant, and all erythromycin-resistant isolates were multidrug resistant. Based on *in vitro* results, no apparent benefits are afforded by the use of newer macrolides or ketolides in the treatment of erythromycin-resistant *C. jejuni* infections, since all isolates with elevated erythro-

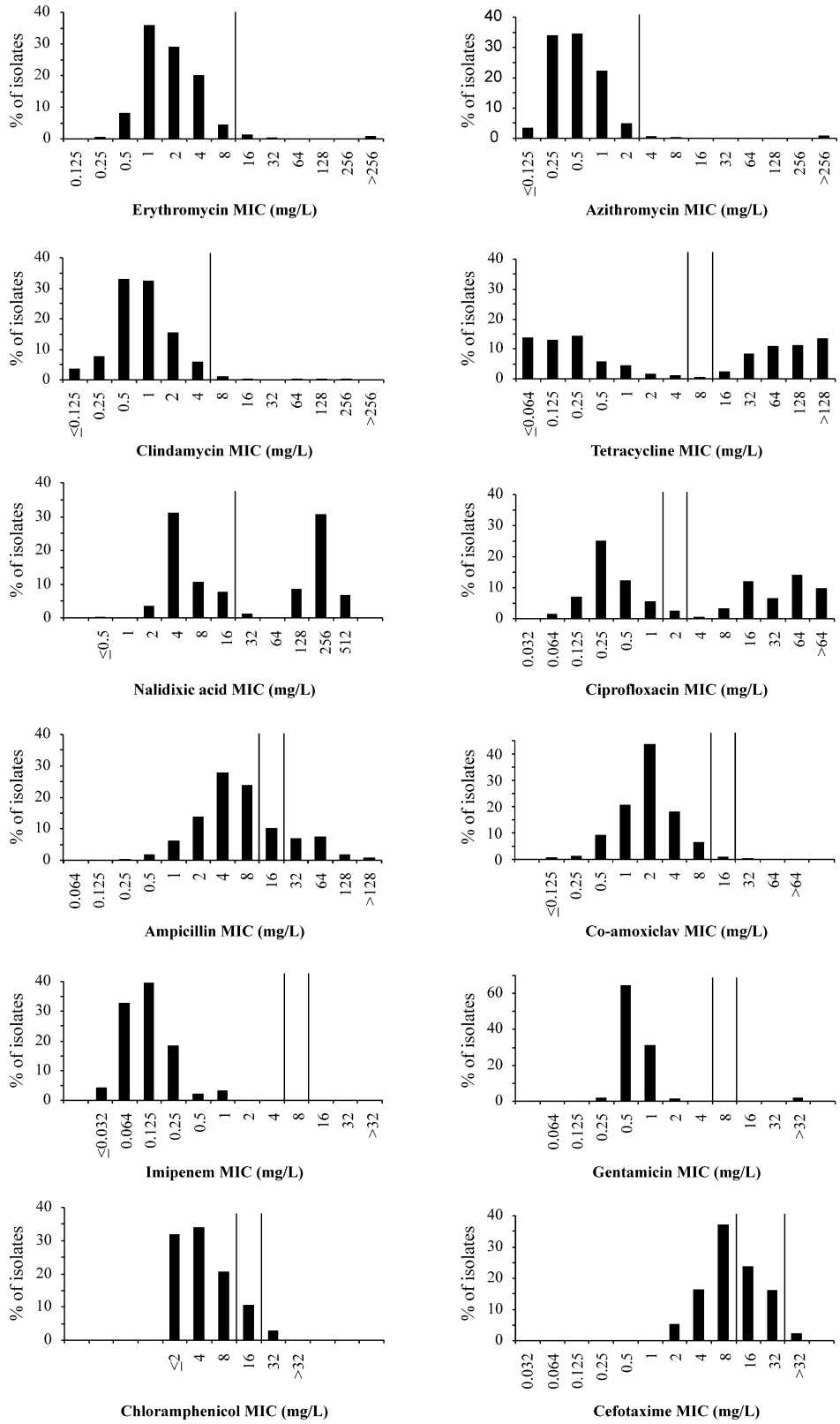

Figure 1. MIC histograms of 12 antimicrobials for 376 *C. jejuni* isolates collected from Finnish patients between January 1995 and January 2000. The vertical lines represent the breakpoint of resistance for erythromycin, azithromycin and clindamycin, and the breakpoints of resistance and susceptibility for other antimicrobials.

Table 1. MICs of 12 antimicrobials for 376 *C. jejuni* strains collected from Finnish patients between 1995 and 2000

Antimicrobial agent	MIC (mg/L)			Resistance breakpoint (mg/L)	% Resistant
	MIC_{50}	MIC_{90}	range		
Ciprofloxacin	1	64	0.064–>64	≥4	46.3
Nalidixic acid	16	256	0.5–512	≥32	46.8
Erythromycin	2	4	0.25–>256	≥16	2.1
Azithromycin	0.5	1	<0.125–>256	≥4	1.6
Clindamycin	1	2	<0.125–256	≥8	2.1
Ampicillin	8	32	0.25–>128	≥32	16.8
Co-amoxiclav[a]	2	4	<0.125–32	≥32	0.3
Cefotaxime	8	32	2–>32	≥64	2.1[b]
Imipenem	0.125	0.25	<0.032–1	≥16	0
Gentamicin	0.5	1	0.25–>32	≥16	1.6
Tetracycline	1	>128	0.064–>128	≥16	46.0
Chloramphenicol	4	16	<2–32	≥32	2.7

[a]Values indicate the concentration of amoxicillin. Amoxicillin and clavulanic acid were used in a 2:1 (w/w) ratio.
[b]The percentage of intermediately cefotaxime-resistant isolates was 39.6%.

mycin MICs exhibited elevated MICs of clarithromycin and telithromycin. Among our entire *C. jejuni* collection, however, only one isolate was resistant to co-amoxiclav, and none was resistant to imipenem. These *in vitro* susceptibilities suggest that co-amoxiclav might be a candidate for clinical trials in enteritis caused by multidrug-resistant *C. jejuni*, and if the situation is life-threatening, a carbapenem may be the drug of choice. Nevertheless, it must be kept in mind that very few data exist on the clinical efficacy of co-amoxiclav or carbapenems,[10] or of the other β-lactams,[11] for the treatment of *C. jejuni* infections.

In Finland, ciprofloxacin-resistant strains that had not existed in 1980 composed 9% of the strains isolated in 1990 and tested by Rautelin *et al*.[5] In that study, no efforts were made to determine the origin of all ciprofloxacin-resistant *C. jejuni* isolates identified. Yet, the authors regarded it as plausible that the majority, if not all, of their resistant strains were derived from abroad. This assumption is consistent with our finding that ciprofloxacin resistance is still significantly more common among the foreign isolates than among those domestic in Finland, with only 9% of the domestic isolates resistant to ciprofloxacin. Despite the low rate of ciprofloxacin resistance among our domestic *C. jejuni* isolates, fluoroquinolones are of limited usefulness in the treatment of campylobacteriosis in Finland, since at least 80% of the clinical strains in our country are acquired abroad.[5] This may be the reason for the discrepancy between the numbers of the foreign and domestic isolates included in the present study: when consecutive *C. jejuni* isolates are collected in one hospital, domestic isolates inevitably remain in the minority in Finland.

In conclusion, multidrug resistance was found to be significantly associated with resistance to ciprofloxacin. Macrolides still appear to be the first-choice alternative for suspected *C. jejuni* enteritis. The *in vitro* susceptibilities found suggest that co-amoxiclav might be a candidate for clinical trials in enteritis caused by multidrug-resistant *C. jejuni*, and if the situation is life-threatening, a carbapenem may be the drug of choice. The widespread emergence of multidrug resistance among *C. jejuni* is of great concern.

Acknowledgements

We are indebted to Jari Ahvenainen for statistical assistance, and to Liisa Immonen, Minna Lamppu, Tarja Laustola, Marja-Liisa Lindman, Erkki Nieminen, Saija Nylander and all the staff members at the laboratories of the study for expert technical assistance. This study was supported by grants from the Maud Kuistila Memorial Foundation, the Finnish Medical Foundation Duodecim, the Research Foundation of Orion Corporation and a special government grant (EVO grant) from Turku University Central Hospital (all to A.J.H.).

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