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Trends in Antimicrobial Susceptibility of *Campylobacter* Isolates in Poland (2000–2007)

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Abstract

This study analysed the pattern of antibiotic resistance in 251 *Campylobacter* strains isolated from symptomatic children hospitalized in 4 large paediatric hospitals in Poland from 2000 through 2007. The highest resistance was found for ciprofloxacin (49.5% for *C. jejuni* and 51.3% for *C. coli*), followed by tetracycline (17.5% and 18.0%, respectively), and ampicillin (13.2% and 10.2%, respectively). Almost all isolates were susceptible to macrolides. As much as 22.6% of *C. jejuni* and 25.6% of *C. coli* were resistant to more than one class of antimicrobial agents. Multidrug resistance (defined as resistance to at least two classes of antimicrobials) rose significantly from 5.1% in 2000–2003 to 34.6% in 2004–2007.

Key words: *Campylobacter* sp., children resistance

Introduction

Campylobacter jejuni and *C. coli* are common causes of bacterial food-borne gastroenteritis in the world (Allos, 2001). Although surveillance data on human campylobacteriosis in Poland are limited (only 157 cases were registered by the National Institute of Hygiene in 2006) (Sadkowska-Todys and Wardak, 2008), the results of a 6-year study in children revealed that *Campylobacter* infections were more prevalent than salmonellosis (Rożynek and Dzierżanowska, 1994). A lot of evidence suggests that campylobacteriosis is often associated with a consumption of contaminated chicken meat (Park *et al*., 1991). However, our earlier study indicated that children may have additional sources of this infection (Rożynek *et al*., 2005).

Although campylobacteriosis is generally a self-limiting disease, antibiotics maybe required in severe cases or in immunocompromised patients. Erythromycin is often used as a first-line therapy in *Campylobacter* infections (Allos, 2001). However, fluoroquinolones, tetracycline, β-lactams or aminoglycosides can be used as alternatives (Coker *et al*., 2002; Moore *et al*., 2005). In many countries, a significant increase of antibiotic resistance in *Campylobacter* sp. has been reported recently (Gupta *et al*., 2004; Randall *et al*., 2003; Luber *et al*., 2003). This can probably be attributed to the wide use of antimicrobial agents in veterinary medicine and agriculture (Engberg *et al*., 2004; Threlfall *et al*., 2000). In Poland the use of flavomycin and avilamycin as growth promoters in poultry was banned in 2006, but other antimicrobials such as tylosin, lincomycin, amoxicillin, ampicillin and tetracycline can be used in medicated animal feed (Bednarek and Szymańska-Czerwińska, 2006; Gronowicz and Kujawiak, 1999).

The most common mechanism of high-level quinolone resistance in *Campylobacter* sp. is associated with a mutation in the quinolone resistance-determining region (QRDR) at position 86 in the *gyr*A gene (Zirnstein *et al*., 1999; Alonso *et al*., 2004). The high-level resistance to erythromycin is mediated by

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mutations in domain V of the 23S rRNA gene at positions 2074 or 2075 (Vacher *et al*., 2003). The most frequently observed mechanism of tetracycline resistance is the production of Tet(O), a ribosomal protection protein encoded by the *tet*(O) gene (Gibreel *et al*., 2004).

The aim of this study was to analyse trends in antimicrobial resistance among *C. jejuni* and *C. coli* strains isolated from symptomatic children in Poland within eight years.

Experimental

Materials and methods

In the period from January 2000 to December 2007, stool samples from children with diarrhoea were collected from 4 large hospitals in Poland: the Children's Memorial Health Institute in Warsaw, Infectious Diseases Hospital in Dziekanów Leśny, Paediatric Hospital of Warsaw Medical University, and Infectious Diseases Hospital in Bydgoszcz.

Isolation and identification of *Campylobacter* strains were performed according to WHO recommendations and confirmed by the PCR assay, as described previously (Rożynek *et al*., 2005). Briefly, isolation of *Campylobacter* sp. from stool samples was performed by use of selective media: modified Preston *Campylobacter* agar, Skirrow agar with 5% horse blood and CCDA agar. The plates with Preston and Skirrow media were incubated at 42°C, and CCDA plates were incubated at 37°C for 48 hrs under microaerophilic conditions. *Campylobacter* identification and discrimination among thermophilic *Campylobacter* species were based on colony morphology, Gram's staining and biochemical reactions (Hendriksen *et al.*, 2003). Species identification was confirmed by PCR with species specific primers: HipOR2 and HipOF2 for *C. jejuni*, and CC1 and CC2 for *C. coli* (Linton *et al*., 1997).

C. jejuni and *C. coli* susceptibility to gentamicin, tetracycline, ciprofloxacin, ampicillin and erythromycin was determined by the E-test (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar containing 5% sheep blood. E-tests were used in accordance with the manufacturer's instructions. The plates were incubated at 37°C for 48 hrs under microaerophilic conditions. The following CLSI (Clinical and Laboratory Standards Institute 2008) interpretative criteria for the *Enterobacteriaceae* family were used as breakpoints for *Campylobacter* resistance: gentamicin and tetracycline 16 mg/L, ciprofloxacin 4 mg/L, and ampicillin 32 mg/L. For erythromycin an interpretative breakpoint for *Staphylococcus* sp. of 8 mg/L was used (CLSI, 2008). *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 were used as reference strains. The resistance rates were compared using the χ^2 test.

Additionally, all *Campylobacter* strains were screened for molecular mechanisms of resistance to tetracycline, erythromycin, and ciprofloxacin by PCR-based methods. The presence of the *tet*(O) gene was detected by PCR (Gibreel *et al*., 2004). Thr-86-Ile mutations in the *gyr*A gene were identified by PCR-RFLP (Alonso *et al*., 2004). The PCR-RFLP method was also used for the detection of A2074C and A2075G mutations in the 23S rRNA gene (Vacher *et al*., 2003).

Results and Discussion

During an 8-year study, 251 *Campylobacter* sp. strains were isolated from stool samples from children with diarrhoea. The following number of isolates were obtained from participating centeres: the Children's Memorial Health Institute, n = 141; Infectious Diseases Hospital in Dziekanów Leśny, n = 70; Paediatric Hospital of Warsaw Medical University, n = 10; Infectious Diseases Hospital in Bydgoszcz, n = 30. Two hundred and twelve isolates were identified as *C. jejuni* and 39 as *C. coli* by both the classical method and PCR.

The results of antimicrobial susceptibility testing are summarized in Table I. As many as 118 (55.7%) *C. jejuni* and 20 (51.3%) *C. coli* isolates were resistant to at least one antimicrobial agent tested. The highest resistance rates were noted for ciprofloxacin (49.5% for *C. jejuni* and 51.3% for *C. coli*), tetracycline (17.5% for *C. jejuni* and 18.0% for *C. coli*), and ampicillin (13.2% for *C. jejuni* and 10.2% for *C. coli*). Only one (0.4 %) and four (1.8%) *C. jejuni* isolates were resistant to erythromycin and gentamicin, respectively, whereas all *C. coli* strains were susceptible to these agents. The results of phenotypic and genetic analyses of resistance to tetracycline and ciprofloxacin were fully concordant, whereas one isolate resistant to erythromycin by the E-test had no detectable mutations in the 23S rRNA gene (Table II). The latter phenomenon could result from the efflux pump mediated resistance to macrolides. Such a mechanism has been described in multidrug-resistant *Campylobacter* isolates with low-level macrolide resistance (Pumbwe *et al*., 2004). These results indicate that macrolides remain useful drugs for the empirical therapy of campylobacteriosis in our population, if such a treatment is required. On the contrary, due to the high resistance to fluoroquinolones observed both in children and adults in Poland (Wardak *et al*., 2007), these agents can no longer be considered as an alternative empirical therapy in *Campylobacter* infections, a finding which is consistent with reports from other countries (Hakanen *et al*., 2003; Gaudreau and Gilbert, 2003; Quinn *et al*., 2007). The high prevalence of ciprofloxacin and tetracycline resistance in isolates

Table I
MICs of five antimicrobials for *C. jejuni* (n = 212) and *C. coli* (n = 39) strains isolated from Polish children between 2000 and 2007

Antimicrobial agent	Resistance breakpoint (mg/L)	MIC_{50}	MIC_{90}	MIC range	No (%) of resistant strains
Ciprofloxacin	≥ 4				
C. jejuni		3	> 32	0.012 –>32	105 (49.5)
C. coli		> 32	> 32	0.016 – >32	20 (51.3)
Erythromycin	≥ 8				
C. jejuni		0.75	2	<0.016 – 24	1 (0.4)
C. coli		0.50	2	0.016 – 4	0
Ampicillin	≥ 32				
C. jejuni		2	48	0.032 – > 256	28 (13.2)
C. coli		3	48	0.064 – > 256	4 (10.2)
Gentamicin	≥ 16				
C. jejuni		0.75	2	0.016 – 128	4 (1.8)
C. coli		0.75	1.5	0.125 – 4	0
Tetracycline	≥ 16				
C. jejuni		0.125	64	0.012 – > 256	37 (17.5)
C. coli		0.125	64	0.012 – 128	7 (18.0)

Table II
Comparison of phenotypic and genotypic resistance to erythromycin, ciprofloxacin, and tetracycline in *C. jejuni* and *C. coli* isolates

Species	No of strains resistant to erythromycin		No of strains resistant to ciprofloxacin		No of strains resistant to tetracycline	
	A2074C/A2075G mutation	E-test	Thr-86-Ile mutations	E-test	*tet*(O) gene	E-test
C. jejuni	0	1	105	105	37	37
C. coli	0	0	20	20	7	7

obtained from children may result from transmission of animal strains, since these antimicrobials are only occasionally used in paediatric patients. Both quinolones and tetracyclines are approved for therapeutic use in veterinary medicine in Poland, and high resistance to these agents was detected in *Campylobacter* sp., *Salmonella* sp. and *Escherichia coli* strains of poultry origin in our country (Hoszowski and Wasyl, 2005). The possibility that resistant strains are selected and transmitted from animals is also indirectly

Table III
Resistance phenotypes of *Campylobacter* strains isolated from children during 2000–2007

Resistance pattern	No (%) of resistant strains		
	C. jejuni (n = 118)	*C. coli* (n = 20)	Total (n = 138)
CI^R	61 (51.7)	10 (50.0)	71 (51.4)
TC^R	4 (3.4)	0	4 (2.9)
AM^R	5 (4.2)	0	5 (3.6)
$TC^R + CI^R$	25 (21.1)	6 (30.0)	31 (22.4)
$CI^R + AM^R$	12 (10.1)	3 (15.0)	15 (10.8)
$TC^R + AM^R$	1 (0.8)	0	1 (0.7)
$GM^R + AM^R$	3 (2.5)	0	3 (2.2)
$TC^R + CI^R + AM^R$	5 (4.2)	1 (5.0)	6 (4.3)
$EM^R + TC^R + CI^R + AM^R$	1 (0.8)	0	1 (0.7)
$TC^R + CI^R + AM^R + GM^R$	1 (0.8)	0	1 (0.7)

CI^R, resistance to ciprofloxacin; TC^R, resistance to tetracycline; AM^R, resistance to ampicillin; GM^R, resistance to gentamicin; EM^R, resistance to erythromycin.

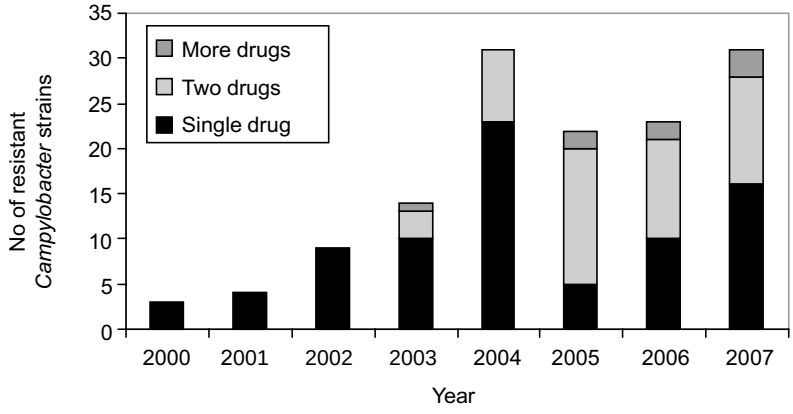

Fig. 1. Single- and multi-drug resistance in *Campylobacter* sp. isolated from symptomatic children in subsequent years.

confirmed by the fact that virtually no macrolide resistance was found in *Campylobacter* strains from children, although macrolides are frequently used in paediatrics. In contrast to *Campylobacter*, an increasing macrolide-resistance in other species such as *Streptococcus pneumoniae* or *Helicobacter pylori* was reported in children (Semczuk *et al*., 2004; Dzierżanowska-Fangrat *et al*., 2005).

Resistance phenotypes are shown in Table III. Resistance to more than one class of antimicrobial agents (multidrug resistance) was detected in 48/212 (22.6%) *C. jejuni* and 10/39 (25.6%) *C. coli* strains. The most common combined resistance phenotype was a double resistance to ciprofloxacin and tetracycline found in 21.1% of *C. jejuni* and 30.0% of *C. coli* resistant isolates, followed by co-resistance to ciprofloxacin and ampicillin detected in 10.1% of *C. jejuni* and 15.0% of *C. coli* resistant strains. Eight *Campylobacter* isolates were simultaneously resistant to three or more antimicrobial agents. The prevalence of multidrug resistance in *Campylobacter* sp. in subsequent years is shown in Fig. 1. Multidrug resistance rose significantly from 5.1% in 2000–2003 to 34.6% in 2004–2007 ($p<0.0001$). This rise was mainly associated with an increasing frequency of simultaneous resistance to ciprofloxacin and tetracycline (3 strains in 2000–2003 versus 28 strains in 2004–2007). The reasons for such a rise have not been elucidated. Some authors reported significantly higher resistance in *Campylobacter* strains acquired abroad than locally, but a travel history for our patients was not available (Hakanen *et al*., 2003).

To our knowledge, this is the first study showing trends in antimicrobial susceptibility of clinical *Campylobacter* isolates in Poland within several years. Increasing resistance is an alarming trend, limiting the number of therapeutic options and making an empirical treatment more difficult. Therefore, constant monitoring of *Campylobacter* susceptibility to antimicrobial agents is mandatory.

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