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Antimicrobial susceptibility profiles of thermophilic campylobacters isolated from patients in the town of Niš

Profil osetljivosti termofilnih kampilobaktera izolovanih kod obolelih u Nišu

Biljana Miljković-Selimović*, Tatjana Babić†, Branislava Kocić*, Ljiljana Ristić†

University of Niš, *School of Medicine, †Institute of Public Health, **Niš, Serbia**

Abstract

Background/Aim. In some clinical forms of human *Campylobacter* infections, such as prolonged diarrhea or associated with postinfections sequels, antibacterial treatment is necessary. The aim of the present study was to evaluate the antimicrobial susceptibility of thermophilic *Campylobacter* strains isolated from patients with diarrhea, as well as from patients with diarrhea followed by postinfections sequels, to drugs used in the therapy of enterocolitis, and to nalidixic acid used in laboratory identification and differentiation of thermophilic *Campylobacter* spp. **Methods.** We studied the antimicrobial susceptibility profiles of 131 *Campylobacter* strains isolated from patients with diarrhea (122 strains), diarrhea associated with rheumatic disorders (8 strains), and one strain isolated from a patient with Guillain-Barré Syndrome following *Campylobacter* enterocolitis. Susceptibility testing to erythromycin, gentamicin, tetracycline, chloramphenicol, ciprofloxacin and nalidixic acid was performed by the agar dilution method. **Results.** In the strains we investigated, resistance to gentamicin and chloramphenicol was not recorded, whereas a low rate of strains resistant to erythromycin (2.4%), a higher prevalence of strains resistant to tetracycline (9.9%), and a high level of resistance to ciprofloxacin (29.8%) and nalidixic acid (33.3%) were registered. All strains resistant to nalidixic acid were also resistant to ciprofloxacin. In addition, there was no difference in the occurrence of resistance between strains isolated from patients with diarrhea as compared to those isolated from patients with diarrhea followed by postinfection disorders. **Conclusion.** The fact that the most of *Campylobacter* strains were sensitive to erythromycin and all to gentamicin, makes erythromycin an antibiotic of choice in the treatment of *Campylobacter* diarrhea and gentamicin when parenteral therapy should be administered. Resistance to tetracycline and, especially, ciprofloxacin, necessitates antibiotic susceptibility testing.

Key words:
drug resistance, microbial; campylobacter infections; diarrhea; humans; yugoslavia.

Apstrakt

Uvod/Cilj. Kod nekih kliničkih oblika infekcije ljudi termofilnim kampilobakterima, kao što su prolongirana dijareja ili pojava postinfektivnih sekvela, neophodna je primena antibakterijske terapije. Cilj ovog rada bio je ispitivanje osetljivosti termofilnih kampilobaktera na antibiotike koji se primenjuju u terapiji enterokolitisa, kao i na nalidiksinsku kiselinu koja se primenjuje u identifikaciji i diferencijaciji termofilnih kampilobaktera. **Metode.** Ispitivan je profil osetljivosti 131 soja termofilnih kampilobaktera izolovanih kod bolesnika sa dijarejom (122 izolata), kod bolesnika sa dijarejom reumatskim tegobama (8 izolata), kao i kod bolesnika sa Guillain-Barré sindromom posle enterokolitisa izazvanog kampilobakterom (jedan izolat). Osetljivost na eritromicin, gentamicin, tetraciklin, hloramfenikol, ciprofloksacin i nalidiksinsku kiselinu testirana je agar dilucionom metodom. **Rezultati.** Kod ispitivanih sojeva nije zabeležena rezistencija na gentamicin i hloramfenikol, dok je mali procenat sojeva bio rezistentan na eritromicin (2,4%), a nešto viši na tetraciklin (9,9%). Na ciprofloksacin ispoljen je visok procenat rezistencije (29,8%) kao i na nalidiksinsku kiselinu (33,3%). Svi sojevi rezistentni na nalidiksinsku kiselinu bili su istovremeno rezistentni i na ciprofloksacin. Nije bilo razlike u pojavi rezistencije kod izolata koji su doveli samo do dijareje i kod onih koji su izazvali dijareju praćenu postinfektivnim smetnjama. **Zaključak.** Činjenica da je većina sojeva bila osetljiva na eritromicin, a svi sojevi osetljivi na gentamicin, čini eritromicin antibiotikom izbora u lečenju enterokolitisa izazvanog kampilobakterom, a gentamicin antibiotikom koji se može primenjivati kada je neophodna parenteralna terapija. Otpornost na tetraciklin i, naročito, ciprofloksacin ukazuje na neophodnost testiranja osetljivosti kampilobaktera.

Ključne reči:
lekovi, rezistesncija bakterija; kampilobakter infekcije; dijareja; ljudi; srbija

Correspondence to: Biljana Miljković-Selimović, School of Medicine, Niš, Bul. dr Zorana Đinđića 81, 18 000 Niš, Srbija.
Phones: +381 18 558 352, +381 64 4332 238. E-mail: biljams@eunet.rs

Introduction

Although human *Campylobacter* enterocolitis is often a self-limiting disease, treatment is necessary in illness with severe symptoms, prolonged disease, in immunocompromised patients and in patients with chronic sequels, such as Guillain-Barré syndrome (GBS) [1]. In the therapy of *Campylobacter* enterocolitis, macrolides and quinolones are very effective [2, 3]. However, reports on resistance to erythromycin and also increasing *Campylobacter* resistance to quinolones may pose a threat to efficient therapy [4, 5]. In addition, the rate of sensitivity to drugs recommended for therapy differs between different geographic regions [6].

The aim of the present study was to evaluate the antimicrobial susceptibility of thermophilic *Campylobacter* strains isolated from patients with diarrhea, as well as from patients with diarrhea followed by postinfections sequels, against drugs used in the therapy of enterocolitis, and to nalidixic acid used in laboratory identification and differentiation of thermophilic *Campylobacter* spp.

Methods

We investigated antimicrobial susceptibility of thermophilic *Campylobacter* strains isolated at the Institute for Public Health, the town of Niš, Serbia, in 2002 and 2003 from the stool of patients with diarrhea (n = 122) and diarrhea followed by rheumatic disorder (n = 8) in clinic and outclinic patients in Niš. We also included a strain of *Campylobacter jejuni* associated with GBS isolated at the Republic Institute for Public Health, Belgrade. A total of 131 strains was thus included in the study.

Strains were isolated on Columbia agar base supplemented with 5% sheep blood and antibiotics (cefoperazone 1.5 g/L, colistin 10^6 U, vancomycin 1 g/L, amphotericin B 0.2 g/L), (bioMérieux, Marcy l'Etoile, France), following incubation in a jar under microaerobic conditions (Gas generating system "Torlak", Belgrade, Serbia), at 42º C, 48 hours. Identification to the level of genus was made using colony morphology, Gram staining ("gull wings", S- or spiral-shaped bacteria), oxidase and catalase tests. Strains were stored at –20º C in a glucose broth supplemented with 5% horse serum until susceptibility testing was performed.

Strains grown after 48 hours of incubation at 37º C on Columbia agar base (bioMérieux, Marcy l'Etoile, France) with 5% defibrinated horse blood under microaerophilic conditions described above were resuspended in sterile saline to obtain a density of 0.5 on a McFarland scale. Susceptibility testing was performed using the agar dilution method to erythromycin, gentamicin, tetracycline, chloramphenicol, ciprofloxacin and nalidixic acid. Pure substances of antibiotics were purchased from the manufacturer ("Galenika", Belgrade). Erythromycin and chloramphenicol were suspended in 95% ethanol, gentamicin in phosphate-buffered saline (PBS) (pH 8), tetracycline in distilled H_2O, ciprofloxacin in PBS (pH6) and nalidixic acid in 1N NaOH for stock dilutions. They were prepared as serial dilutions, and added to agar base at 50º C in 90 mm agar plates.

One mL of bacterial suspension with a density of 10^5 colony-forming cells (CFU) was cultured in a microaerobic atmosphere for 48–72 hours on Columbia agar supplemented with 5% defibrinated horse blood and the appropriate antibiotic, in serial dilutions, for minimal inhibitory concentration (MIC) determination with the concentrations (mg/L) ranging for erythromycin, gentamicin, ciprofloxacin, tetracyline, chloramphenicol, and nalidixic acid in intervals 0.12–4, 0.25–8, 0.25–16, 0.25–16, 2–16, 4–64, respectively.

A minimal inhibitory concentration was defined as the lowest concentration producing no visible growth.

As no official recommendations for breakpoints exist, we used from the literature data for erythromycin 4 mg/L [7], and for gentamicin and tetracycline, 8 mg/L, cholaramphenicol 16 mg/L, ciprofloxacin 4 mg/L, nalidixic acid 32 mg/L. We used MIC interpretative standards for *Enterobacteriaceae* [8].

Campylobacter jejuni NCCLS 11951 and *Staphylococcus aureus* ATCC 29213 were used as control for growth.

A multiresistant strain was defined as a strain resistant to three or more antibiotics.

In order to determine the difference in frequency of resistant strains occurring in the two groups of patients, Fisher's exact test was performed. Statistical calculation was performed using a standard statistical program (EpiInfo ver 6.04).

Results

By using the agar dilution method, we detected antimicrobial resistance in 47 strains: to one antibiotic in 32 strains, to two in 13 strains and to three in two strains. The results of the susceptibility testing, along with the values of MIC_{50} and MIC_{90}, are presented in Table 1.

The results showing the MIC distribution for the six antibiotics are presented in Table 2.

Table 1

Susceptibility of thermophilic *Campylobacter spp.* strains to selected antibiotics

Antibiotics	No of investigated strains	MIC* range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Resistance (%)
Erythromycin	123	≤0.12–2.0	0.5	1.0	2.4
Gentamicin	126	≤ 0.25–4.0	0.5	1.0	0
Tetracycline	131	≤ 0.25–≥ 16	≤ 0.5	8.0	9.9
Ciprofloxacin	131	≤ 0.25–16	≤ 0.25	8.0	29.8
Nalidixic acid	36	≤ 4.0–≥ 64	8.0	≥ 64	33.3
Chloramphenicol	130	≤ 2–16	≤ 4.0	8.0	0

*minimal inhibitory concentration

Table 2

Minimal inhibitory concentrations (MICs) distribution of antibiotics tested among thermophilic Camylobacter spp. strains

Antibiotics	No of strains	MICs distribution (%)									
		0.12	0.25	0.5	1	2	4	8	16	32	64 (mg/L)
Erythromycin	123	12.2	33.3	37.4	13.8	0.8|	*2.4	–	–	–	–
Gentamicin	126	–	†6.3	57.1	29.4	3.2	4.0	–|	–	–	–
Tetracycline	131	–	†14.5	45	18.3	1.5	6.9	3.8|	‡9.9	–	–
Chloramphenicol	130	–	–	–	–	§40.8	45.4	7.7	6.2 |	–	–
Ciprofloxacin	131	–	†55.0	9.9	1.5	3.1|	13.7	11.5	‡4.6	–	–
Nalidixic acid	36	–	–	–	–	–	‖47.2	19.4	0	0|	¶33.3

*MIC(mg/L) ≥ 4; †MIC ≤ 0.25; ‡ MIC ≥ 16; § MIC ≤ 2; ‖MIC ≤ 4; ¶MIC ≥ 64

Vertical lines in table indicate breakpoints

Overall, resistance was recorded for erythromycin, tetracycline, ciprofloxacin and nalidixic acid activity in 3 (2.4%), 13 (9.9%), 39 (29.8%), and 12 (33.3%) of the investigated strains, respectively.

When strains associated with postinfections sequels were selected, MIC_{50} and MIC_{90} (mg/L) for erythromycin were 0.25 and 0.5, for gentamicin 1 (both values), for tetracycline 0.5 and 4, for chloramphenicol 2 and 4, and for ciprofloxacin 0.25 and 8, respectively.

In strains isolated from patients with diarrhea only, MIC_{50} and MIC_{90} were not changed as compared with values obtained for all investigated strains. Minimal inhibitory concentrations (mg/L) for the strain isolated from the patient with GBS were 0.12 for erythromycin, 2 for chloramphenicol, 0.5 for ciprofloxacin, and 1 for tetracycline and gentamicin.

A closer investigation of strains isolated from diarrhea associated with postinfections sequels (patients with rheumatic disorders and GBS) showed resistance to two antibiotics: one strain was resistant to tetracycline (11%) and three strains to ciprofloxacin (33%). Resistance to erythromycin, chloramphenicol and gentamicin was not recorded. The strain isolated from the patient with GBS was susceptible to all antibiotics tested.

When the frequencies of detected resistance to antibiotics in the group of strains isolated from patients with diarrhea and from patients with diarrhea complicated with rheumatic or neurological disorders were compared, no differences were found (Fisher' exact test $p = 1.00$) for erythromycin, tetracycline and ciprofloxacin. Since there was no recorded resistance to gentamicin and chloramphenicol in both investigated groups, statistical analysis was not performed for those antibiotics.

Discussion

Depending on the geographic localization, the success of treating *Campylobacter spp.* infection with drugs recommended for the therapy may differ considerably. The present susceptibility testing of strains isolated in the town of Niš, Serbia, revealed occurrence of antimicrobial resistance to erythromycin, ciprofloxacin, tetracycline and nalidixic acid. All strains were sensitive to gentamicin and chloramphenicol. Higher percentage of resistant strains was proved in the study conducted in north Indian rural community – antibiotic resistance of *Campylobacter* species was as follows: ciprofloxacin 71.4%, tetracycline 26.5%, furazolidine 14.3%, gentamicin 10.2% and erythromycin 6.1%; 30.6% of strains were multidrug resistant [9]. In the study conducted in Poland, the highest resistance was observed for ciprofloxacin (more than 40%), followed by ampicillin, and tetracycline, with significant resistance increase to tetracycline between 2003 and 2005 [10].

The growth of 50 and 90% of our isolates was inhibited by erythromycin concentrations of 0.5 and 1 mg/L, respectively. In a Finnish study on domestic and foreign strains of thermophilic *Campylobacter* strains, MIC_{50} and MIC_{90} values were 1 and 2 μg/ml for domestic strains, whereas the values for foreign strains were 1 and 4 μg/ml, respectively [11]. At the breakpoint of MIC ≥ 0.4 mg/L, we detected strains resistant to erythromycin in 2.4% of isolates. That fact underlines the possibility of an increasing prevalence of strains resistant to erythromycin in the future.

In a comprehensive study published in Spain in 1994, resistance to erythromycin was detected in only 3.2% of strains, with MIC of ≥ 4 μg/ml, while later studies reported an increase of strains resistant to erythromycin [7, 12]. In the study conducted in the Netherlands, resistance to erythromycin increased from 1.9% (in their wide 2001) to 2.7% (in 2004) [13]. In Crete, 14.9% of thermophilic *Campylobacter spp.* strains were resistant to erythromycin [14]. In some reports, an increasing resistance to macrolides (50%) seems to be a real threat; however, other studies report on quite low or absent resistance rates to erythromycin [15].

Values of MIC_{50} and MIC_{90} for gentamicin in our strains were 0.5 and 1 mg/L, respectively. Our strains did not exhibit resistance to gentamicin (MIC ranged from ≤ 0.25 to 4 mg/L). In strains studied in Germany MIC_{50} and MIC_{90} were 2, without detection of resistant strains at the breakpoint of MIC ≥ 16 mg/L [16]. In the Spanish study referred above, 1% of strains investigated were resistant to gentamicin [7]. In Crete, resistance was detected in 2.3% of *Campylobacter spp.* isolates [14].

In this study, MIC_{50} and MIC_{90} (mg/L) of tetracycline were ≤ 0.5 and 8, respectively, and resistance to tetracycline was seen in 10% of the strains, at the breakpoint of 8 mg/L. For the strains isolated in Germany, MIC_{50} and MIC_{90} (mg/L) were 0.06 and 16, respectively, with resistant strains occurring in 13.5% of isolates at the same breakpoint [16]. Resistance to tetracycline was recorded in the Spanish study in 21.2% of strains [8].

In this study, MIC_{50} and MIC_{90} (mg/L) of ciprofloxacin were ≤ 0.25 and 8.0, respectively. In addition, 29.8% of

strains investigated were resistant to ciprofloxacin. One of the first reports of ciprofloxacin resistance (9%) was in 1991, in Finland [17]. Since then, the prevalence of strains resistant to ciprofloxacin has increased several times [9]. In a new Finnish study, MIC_{50} and MIC_{90} for domestically acquired strains were 0.25 and 0.5 μg/mL, respectively and for imported strains 1 and 64 μg/mL [11]. Those findings suggest a progressively reduced therapeutic value of ciprofloxacin. A resistance rate of 39% was found in human isolates in a study recently conducted in Austria [18]. In another recent study conducted in Thailand, 90% of strains were resistant to ciprofloxacin [15]. In Crete, 42.5% of *Campylobacter spp.* strains were resistant to ciprofloxacin [14].

Resistance to quinolones in *Campylobacter spp.* from human infections may be related to clinical use, or use of fluoroquinolones in animal husbandry, or both [19]. A more thorough investigation of this problem is necessary to prevent its increase. A study conducted in England and Wales [20] recommended that both veterinary and clinical use should be reconsidered and that fluoroquinolone antibiotics should be used only to treat serious infections requiring hospital admission. Also, using antibiotics in a month before the onset is the risk factor for acquering a ciprofloxacin-resistant strain of *Campylobacter* [21]. Resistance rates increased with increasing urbanisation, too [13]. Increased resistance to macrolide and quinolone antibiotics poses major risks for treatment failure [22].

We detected a relatively high proportion of resistance to nalidixic acid. Resistance to nalidixic acid in both *Campilobacter jejuni* and *Campilobacter coli* strains was observed during preliminary identification. All of the strains, which were resistant to nalidixic acid, were simultaneously resistant to ciprofloxacin.

This study did not detect strains resistant to chloramphenicol. In the study conducted in Spain in 1994 resistance to chloramphenicol occurred in 2.6 % of isolates [7]. In strains isolated in Crete, Greece, 7.9% of investigated strains were resistant to that antibiotic [14]. In England and Wales, resistance to chloramphenicol was recorded in 5.4 % of investigated strains at the breakpoint of 8 mg/L [20].

Since we have detected two strains that were simultaneously resistant to quinolones (ciprofloxacin and nalidixic acid) and tetracycline, we can not discuss the presence of multiple resistance in our strains. Multiple resistance in *Campylobacter* can occur, but is usually seen in animal isolates [23]. A relatively high rate of multiple resistant strains (14.8%) was described in Harare, Zimbabwe [24]. In human isolates, multidrug resistance may include antibiotics important for infection treatment, such as erythromycin, tetracycline, and gentamicin or ciprofloxacin, tetracycline, and erythromycin [24, 25]. The appearance of resistant strains may be due to less prudent use of antibiotics in veterinary and/or human practice [26].

Conclusion

Strains isolated from patients with enterocolitis and enterocolitis associated with postinfections sequels expressed a similar pattern of sensitivity. Low levels of resistance to erythromycin makes it as the antibiotic of choice in the treatment of diarrhea or in diarrhea complicated with post infections sequels. When parenteral therapy should be included, gentamicin is also a drug of choice. Resistance to tetracycline and fluoroquinolones, ciprofloxacin, necessitates sensitivity testing. Resistance to nalidixic acid diminished its value in preliminary identification, but in our strains, it was a marker of resistance to ciprofloxacin. Further investigation should be considered in Serbia in the future.

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