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Epidemiology of *Campylobacter jejuni* and *Campylobacter coli* Infections in the Zenica – Doboj Canton, Bosnia and Herzegovina – A Laboratory Based Surveillance in the 1999–2001 Period

Selma Uzunović-Kamberović

Laboratory for Clinical and Sanitary Microbiology, Cantonal Public Health Institution Zenica, Zenica, Bosnia and Herzegovina

A B S T R A C T

Previous studies in the Zenica – Doboj Canton, Bosnia and Herzegovina, indicated some different epidemiological features of Campylobacter *infections and high degree of antimicrobial resistance. Therefore, it was important to investigate epidemiology of* Campylobacter jejuni *and* Campylobacter coli *infections by demographic features and antimicrobial resistance in the 1999–2001 period. A total number of 40 (75.5%)* C. jejuni *and 13 (24.5%)* C. coli *non-repeated clinical isolates were analyzed. More than half of isolates, 30 (56.6%) were from urban dwellers.* Campylobacter *isolates mainly obtained from children under 6 years of age, 42 (79.2%), resulting in far off highest incidence rate of 41.4/100,000/year in this age group. There was noted high degree of resistance to ciprofloxacin in children less than 6 years of age (14.3%), and extremely high overall erythromycin-resistance rate (30%). Campylobacteriosis in this region is a public health concern not in the term of the number reported cases, but of distinctive epidemiologic features.*

Key words: Campylobacter jejuni, Campylobacter coli, *epidemiology, antimicrobial resistance, Zenica, Bosnia and Herzegovina*

Introduction

Campylobacter jejuni and *Campylobacter coli* are still one of the most important enteropathogens in both industrialized and developing countries with the number of cases often exceeding those of salmonellosis and shigellosis[1–3]. In developing countries, *Campylobacter* is the most commonly isolated bacterial pathogen from children under two years of age with diarrhea, and disease does not appear to be important in adults[3,4]. In contrast, infection occurs in adults and children in industrialized countries[5].

Campylobacteriosis is usually self-limited disease. Antimicrobial therapy was used more common in patients with *C. jejuni* than in *C. coli* diarrhoea[6]. Erythromycin and fluoroquinolones consider the drugs of choice for the treatment of *Campylobacter* infections. However, recently broad use of these drugs in human and veterinary medicine has led to the development of antimicrobial resistance[7–12].

Some interesting epidemiological features of *Campylobacter jejuni/coli* infections in this region were reported in the previous reports: high carriage rate of *C. coli* in both, pre- and post-war periods, by far the highest incidence rate of *Campylobacter* infections in the children below six years of age in the urban zone and high ciprofloxacin and erythromycin resistance rates[13–15].

This prompted as to follow up the epidemiology *of Campylobacter jejuni* and *Campylobacter coli* causing diarrhea in this region in the1999–2001 period, distribution among patients by demographic features and antibiotic susceptibility.

Methods

Availability of national surveillance programs in industrialized countries has facilitated monitoring of *Campylobacter* and other gastrointestinal infections[5,16].

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National surveillance programs for campylobacteriosis generally do not exist in the most developing countries, despite substantial burden of the disease. The estimate of incidence campylobacter infections in developing countries originates mostly from laboratory-based surveillance[3]. Campylobacteriosis does not notified disease in Bosnia and Herzegovina. Gastrointestinal infections in the Zenica-Doboj Canton are under laboratory-based surveillance in the Laboratory for Clinical and Sanitary Microbiology of the Canton Public Health Institution in Zenica started from 1998. The age, gender, zone of residence and susceptibility to antimicrobials of all outpatients presenting to the Laboratory with acute diarrhea were recorded.

The Zenica-Doboj Canton has a total population of 331,229 in two distinct residential zones: an urban zone where most of the 149,053 inhabitants live in apartments, and farming and agricultural rural zone where 218,758 inhabitants mainly live in farmhouses, rising domestic animals and working on the land. The Laboratory of Clinical and Sanitary Microbiology of the Canton Public Health Institute in Zenica serves all outpatients of the Zenica-Doboj Canton. From January 1, 1999 to December 31, 2001 stool specimens were received from 5,426 consecutive outpatients with diarrhea. There were 2,000 specimens from children up to 6 years of age, 732 from elementary school students, 530 from high school students, and 1,856 from adults.

The samples were cultured on modified Preston medium (Oxoid, Basingstoke, UK) and incubated in a micro-aerophilic atmosphere[17,18] at 42°C for 48 hours. *Campylobacter jejuni* and *Campylobacter coli* were identified using standard microbiological methods[19]. Forty isolates of *C. jejuni* and thirteen of *C. coli* (one isolate from each patient) were tested by a disc diffusion method using Mueller-Hinton agar supplemented with 5% sheep blood and eight antimicrobials (Oxoid): ampicillin (10 µg), erythromycin (15 µg), azithromycin (15 µg) gentamicin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg). *E. coli* (ATCC 25922), and *Staphylococcus aureus* (ATCC 25923) control strains were used. For *Campylobacter* spp. no internationally accepted criteria for susceptibility testing, including assessments of breakpoints for susceptible versus resistant strains are available, so the zones of growth inhibition were evaluated in accordance with standards published by the National Committee for Clinical Laboratory Standards (NCCLS) for aerobic bacteria[20]. The following cutoff values were used: ampicillin ≤13 mm, erythromycin ≤13 mm azithromycin ≤13 mm, gentamicin ≤12 mm, tetracycline ≤14 mm, nalidixic acid ≤13 mm, ciprofloxacin ≤15 mm, nitrofurantoin ≤14 mm.

Results

A total number of 40 (75.5%) *C. jejuni* and 13 (24.5%) *C. coli* strains were isolated in the study period. Twenty-five strains were isolated during the year of 1999. 6 in 2000 and 22 in 2001. Corresponding incidence values were 7.5, 1.8 and 6.6 per 100 000 per year, respectively (Table 1). More than half of isolates in this period, 30 (56.6%), were from urban dwellers, resulting in the average incidence rate of 6.7 cases/100,000/year in urban population, compared with 4.2/100,000/year in rural one. The most isolations were obtained from children under 6 years of age, 42 (79.2%), resulting in far off highest incidence rate of 41.4/100,000/year in this age group. Corresponding (average) age-specific incidence values for other age groups were in the range 1.1, to 1.6/100,000/year. In the present study, the age and zone of residence distributions of the population from whom specimens were received is not representative of these distributions in the Zenica-Doboj Canton population as a whole. Analyzing the percentage of faecal specimens positive within each age group eliminates sampling bias; highest rate was again in the youngest age group, in whom 1.9% of faecal specimens were positive. Isolation rates in other age groups were from 0.2% in the 7–14 age group to 0.8% in the patients over the age 65 years.

Twenty-eight isolates obtained from male (52.8%), and 25 (47.2%) from female patients, resulting in gender-specific average incidence of 8.3/100 000/year in male, and 3.8/100,000/year in women, respectively. Accordingly, the combined male/female ratio for the incidence of campylobacter diarrhea was 2.2:1.

Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* human isolates has shown in Figure 1. *C. coli* strains have shown higher resistance rates for all antibiotics tested than *C. jejuni*, except for gentamycin and nitrofurantoin, for which they were almost equal in both species.

TABLE 1
THE COMPARISION OF *CAMPYLOBACTER* spp. AND *SALMONELLA* spp. GASTROINTESTINAL INFECTIONS IN THE ZENICA-DOBOJ CANTON

Year (number of samples examined)	Incidence per 100,000/year (number of isolates)		
	Campylobacter spp.	*Salmonella* spp.[21]	Total
1998 (1703)	18.3 (78)[14]	12.7 (55)	40.2 (133)
1999 (1700)	7.5 (25)	16.9 (73)	29.6 (98)
2000 (1862)	1.8 (6)	25.5 (110)	35.0 (116)
2001 (1867)	6.6 (22)	NA	

NA – data not analyzed (data sources are from laboratory and hospital records)

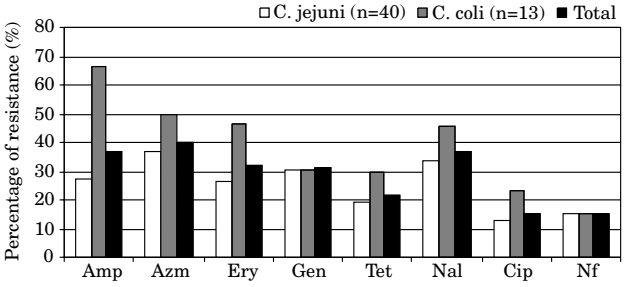

Fig. 1. Antimicrobial resistance in Campylobacter jejuni and C. coli human isolates in the 1999–2001 period (antimicrobial agents: Amp – ampicilin, Azm – azithromycin, Ery – erythromycin, Gen – gentamycin, Tet – tetracycline, Nal – nalidixic acid, Cip – ciprofloxacin, Nf – nitrofurantoin)

The highest resistance rates for all antibiotic tested were noted in the 20–64 age group. The high resistance rates to nalidixic acid and ciprofloxacin in children under 6 years of age, 27.3% and 14.3%, respectively, have deserved full attention. The extents of nalidixic acid- and ciprofloxacin-resistance were higher in the urban zone, 41.7% and 24.1%, respectively, than in rural zone, 29.4% and 4.3%, respectively. Resistance rates for all antibiotic tested were much lower in female comparing to male patients (data not shown).

Discussion

In the 1999–2001 period surprisingly low number of isolates, and accordingly, significant decrease of campylobacter infection incidences in this region were recorded, comparing to 1998[14], given that the data source were from the same laboratory (the same method and staff). The similar situation was reported for a decrease in campylobacter carriage rate after the war comparing the pre-war period in this region[13]. In the same period, the number of *Salmonella* infections increased, so than the overall incidences of these two microorganisms were relatively constant during 1998–2000 period[21] (Table 1). Decreasing number of *Campylobacter* infections was also observed in some other reports[1,22]. It might be some kind of natural counterbalance of these pathogens[1]. Different reasons were attributed to that: disease prevention efforts[1], or changes in nutritional habit of the population[22].

There were two possible explanations for that. The Institute of Statistics of Federation of Bosnia and Herzegovina reported that a 75% to 90% decrease in livestock resources was recorded between 1990 and 1998. (Statistical yearbook of R/F B&H, Sarajevo, 2000) After the war the meat and poultry for consumption mainly imported from other countries: importation of poultry increased from 43% in 1998 to 70% in 1999. Obviously that the reason for decreasing incidence of *Campylobacter* infections in this report was not disease prevention efforts (because of restricted available resources after the war), but rather it was a reflection of its incidence in food animals from other countries. Besides, humanitarian aid after the war was stopped, standard have declined and nutritional habits of the population have changed: far less milk, meat and product derived from them consumed during this period. As it was stated before, some year-to-year variation in incidence of food borne disease can be attributed to the prevalence of pathogens in their respective important animal reservoirs and the foods derived from them[1]. The reason for the variation in incidence of the two pathogens in the humans might be different epidemiology, origin and transmission cycle of the two microorganisms. All *Campylobacter* infections were sporadic in our region, but 20% of *Salmonella* infections appeared in small-family outbreaks[21]. While epidemiology, natural reservoir and transmission of the most important *Salmonella* spp. are well established[23,24], for *Campylobacter* spp. they are still poorly understood[25]. It is because of the complicated epidemiology of this zoonosis, by sporadic nature of the disease along with the organism's wide distribution, high level of genetic and antigenic diversity, and lack of representative population samples[26]. Unfortunately, epidemiological data in this study obtained from only 18 patients (34%). The four of which (22.2%) connected their illness with particular food items (poultry and beef meat were involved in the two cases for each), and nine (50%) of them have had a contact with an animals (five with pets, three with chicken and one patient with cows). Therefore, strong epidemiological link between the cases and potential sources of infection missed.

Although campylobacter isolation rate was low, as in industrialized countries, both, the highest incidence and isolation rate of infections in children fewer than 6 years of age are epidemiological features similar to that of developing countries[3]. However, irregularities of sampling have influenced the isolation rate. This bias is inherent in all laboratory-based surveillance studies, and reflects actual clinical practice. Most surveys in developed countries show bimodal age-distribution of infection[2]. This unimodal age-specific distribution might be also consequence of recent war in this region[14]. Crowded urban community and poor sanitary conditions influence spreading of infections to be highest in young children[4,13]. Although the youngest are also the most affected population in *Salmonella* infections in this region, there was no strictly unimodal age distribution persisted, but incidence of *Salmonella* infections rather have had decreasing trend according to age[21].

C. jejuni and *C. coli* are the two main species isolated in both, industrialized and developing countries. Isolation rate of *C. jejuni* usually exceeds that of *C. coli*[7,8]. In this and previous reports from this region, *C. coli* accounted for 24.5%, 26.5% and 36%[13,14]. It is surpassingly given that the post-war population in the Zenica-Doboj Canton is overwhelmingly Muslim 82.3% (Statistical yearbook of R/F B&H, Sarajevo, 2000), in which consumption of pork, customarily associated with *C. coli* infection, is almost nonexistent. It suggests that primary source of *C. coli* infection might be something other than pigs. Such distribution of *C. jejuni* and *C. coli* infections

were shown only in a few reports. In Croatia, excess of *C. coli* infections (54%) was probably the consequence of the local tradition of home slaughter and processing of pigs at the end of the summer, and the fact that *C. coli* is particularly associated with pigs[27]. In the Central African Republic (39%) an individual well-water supply was identified as a possible source of *C. coli* infection[28].

It was proposed that the single most important route of *Campylobacter* infections (50%–70%) in industrialized countries remains consumption and handling of chicken[8,22]. This should not be surprising in the light of the frequency with which poultry products are consumed and nearly universal contamination of chicken with *Campylobacter* spp.[10,22,29]. As *Campylobacter* may be transferred from animals to humans, the possible development of antimicrobial resistance in *Campylobacter* spp., due to the use of antimicrobial agents in food animals, is a meter of concern[9,12]. Studies from northern Europe have associated fluoroquinolone use in food animals, particularly in poultry, as the source for human infections with fluoroquinolone-resistant *Campylobacter* spp.[12].

It is usual feature of *C. coli* strains to be more resistant than *C. jejuni*[10,30]. High overall erythromycin-resistance rate of *Campylobacter* spp. isolates (32%) persisted in this region since 1998 (25%)[15]. In the most other reports erythromycin resistance is up to 2%[7,11]. *C. coli* strains have usually shown ten times higher resistance rate to erythromycin than *C. jejuni*[7,10]. This difference was much lower in this paper: 46.3% of *C. coli* isolates were erythromycin-resistant and 26.3% of *C. jejuni*, respectively. In the 1998 survey, the erythromycin-resistance in the two species was almost equal[15]. Such extremely high erythromycin-resistance rates could be comparable only with the report from Spain[10].

The pig is the favored host of *C. coli* and historically been shown to have a high resistance rate to macrolides. Macrolide tylosin have been permitted as growth promoter in pigs, but not in broilers, and this could explain the lower proportion of erythromycin-resistant strains observed in broilers than in the pigs[10]. Given that *C. coli* strains in our region probably to not originate from pigs, our observation is unexplained. The differences in isolation rate of the different *Campylobacter* species among different sources may make it difficult to compare levels of resistance between the sources. Thus, it is at present difficult to say whether the higher level of macrolide resistance among *C. coli* isolates is because of their origin (most isolates are from pigs) or is related to true differences among the species[30].

The resistance rate to nalidixic acid of 36% is worried, because the appearance of nalidixic-acid resistance is the first step to future quinolone resistance[10]. The highest rates of fluoroquinolone-resistant *Campylobacter* spp. have been reported from southern Europe, and other regions[7,10–12,15,31,32]. In this study, the frequency of resistance to ciprofloxacin in the youngest for which fluoroquinolone use is not recommended was surpassingly high. Thus, overuse of this drug probably did not the reason for such high frequency of resistance. Some reports indicated that the source of fluoroquinolne-resistant *Campylobacter* infections was consuming poultry colonized with resistant strains, rather than selection for *Campylobacter* resistance in the human gut after clinical fluoroquinolone use to threat diarrheas[11].

In conclusion, existing notification system of infectious diseases in the Zenica – Doboj Canton is not sufficient for the evaluation of campylobacter infections, because of lacking the most important epidemiological data for surveillance. So, the problem of campylobacteriosis remains underestimated, detection of outbreaks is not possible and many important epidemiological data, as a basis for intervention, are not available. The scale of *Campylobacter* infections in the community, the widespread distribution in the environment, and the paucity of linked microbiological and epidemiological data mean that, at present, the epidemiology of *Campylobacter* infections in humans is still poorly understood[9,26].

Campylobacteriosis in this region is a public health concern, but not in the term of the number reported cases, but of distinctive epidemiological features: significant proportion of *C. coli* infections and high erythromycin-resistance rate. Thus, further research is required to identify the reservoir of *Campylobacter* spp. in the environment.

Preliminary results of epidemiological typing work about correlation genotypes of fluoroquinolone- and erythromycin-resistant isolates of *Campylobacter* spp. strains from humans, poultry and farm-animals are promising in the efforts to better understanding *Campylobacter* epidemiology in this region[29,33]. The presence of *Campylobacter jejun and C. coli* in the water supply in the Zenica – Doboj Canton also is under investigation. We hope that they will help us to find sources involved in contamination and transmission of *Campylobacter* infections in this region.

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S. Uzunović-Kamberović

Laboratory for Clinical and Sanitary Microbiology, Cantonal Public Health Institution Zenica, Fra Ivana Jukića 2, 72000 Zenica, Bosnia and Herzegovina
e-mail: selma_kamb@yahoo.com

EPIDEMIOLOGIJA *CAMPYLOBACTER JEJUNI* I *CAMPYLOBACTER COLI* INFEKCIJA U ZENIČKO-DOBOJSKOM KANTONU, BOSNA I HERCEGOVINA, U PERIODU 1999.–2001. GODINE

S A Ž E T A K

Istraživanja infekcija uzrokovanih sa *Campylobacter jejuni* i *Campylobacter coli* na području zeničko-dobojskog kantona u Bosni i Hercegovini sprovedenih do 1998. godine pokazala su neke epidemiološke osobenosti. Zbog toga je bilo važno istražiti epidemiologiju *C. jejuni i C. coli* infekcija kao i rezistenciju na antibiotike u periodu 1991.–2001. godine. Ukupno je izolirano 40 (75.5%) *C. jejuni* i 13 (24.5%) *C. coli* u ovome periodu. Kod pacijenata iz gradske sredine je izolirano 30 (56.6%) kamplobaktera. Većina kampilobaktera je izolirana kod djece mlađe od 6 godina starosti, 42 (79.2%), te je i incidencija u ovoj dobnoj skupini bila daleko najviša, 41.4/100,000/godišnje. U najmlađoj dobnoj skupini je zabilježen visok stupanj rezistancije na ciprofloxacin (14.3%) i erythromycin (30%). Uslijed karakteristične epidemiološke slike te visoke rezistencije na antibiotike, kampilobakterioza u ovome regionu predstavlja javno-zdravstveni problem, te su potrebna daljnja istraživanja.