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Full Length Research Paper

Resistance to erythromycin of *Campylobacter jejuni* and *Campylobacter coli* isolated from animals and humans

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The sensititivity of thermophilic *Campylobacter* strains isolated from caecum of broiler chickens as well as caecum and colon of pigs and human stools, were tested against erythromycin. In 16 strains isolated in broiler chickens, resistance rate was found to be 12.50%. Three of 10 strains of *Campylobacter jejuni* and one of 6 strains of *Campylobacter coli* isolated from broiler chickens were resistant to erythromycin. In 15 strains of thermophilic *Campylobacters* isolated from pigs, resistance rate to erythromycin was 40.00%. Resistance was exhibited more often in *C. coli* (50.00%) as compared to *C. jejuni* (20.00%). In 24 strains isolated from humans, resistance was demonstrated at the rate of 12.50%. Out of 17 strains of *C. jejuni* isolated from humans, resistance was exhibited in 17.65% strains. None of 7 strains of *C. coli* isolated from humans exhibited resistance to erythromycin. Thermophilic campylobacters, especially *C. coli* isolated from pigs were more resistant to erythromycin than strains isolated from humans and broiler chickens. Therefore, a great attention should be directed to the macrolides monitoring in swine farming in order to prevent resistance in animals and its subsequent spread to human.

Key words: *Campylobacter* spp., resistance, erythromycin.

INTRODUCTION

Campylobacteriosis is the most frequent intestinal bacterial infection in humans, more frequent than salmonellosis, shigellosis, infections caused by enterohaemorrhagic *Escherichia coli* strains and yersiniosis. Intestinal campylobacteriosis in humans is the result of infection due to thermophilic *Campylobacter* spp., mostly *Campylobacter jejuni* and *Campylobacter coli*, resulting in 400 million bacterial intestinal infections around the world every year (Putnam et al., 2003; Zimmer et al., 2003). A very important factor in intestinal campylobacteriosis development is a very low infective doses of only 500 bacteria (Walker et al., 1986). *Campylobacter* spp. is a major cause of bacterial enterocolitis and travelers' diarrhoea (Vlieghe et al., 2008).

Poultry in age of two to three weeks are 50 to 90% colonized by thermophilic *Campylobacter* spp. (Hariharan et al., 2009; Newel, 2002). Swines are less colonized by *Campylobacters* spp. than poultry. A similar result has been obtained in a study performed in Serbia (Tambur et al., 2008).

Drugs which are generally used in human campylobacteriosis treatment are erythromycin, quinolones, tetracycline, ampicillin, chloramphenicol and gentamycin. The actual increase of resistance to erythromycin (drug of the therapeutic choice) in thermophilic *Campylobacter* spp. isolated from humans has become allarming (Bywater et al., 2004; Cardinale et al., 2002). Emergence of the resistant strains coincided with the beginning of macrolides use, for the most part thylosine, in veterinary medicine, mainly in swine farming

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Table 1. Resistance of *Campylobacter* isolates from broiler chickens, pigs and humans as determined by E test.

Source of strains	No. of strains	*MIC range	**MIC_{50}	***MIC_{90}	Resistance (%)
Broiler chickens	16	0.016 - >256	0.125	>256	12.50
Pigs	15	0.016 - >256	1.5	>256	40.00
Humans	24	0.016 - >256	0.125	>256	12.50

* Minimum inhibitory concentration, ** Minimum inhibitory concentration required to inhibit the growth of 50% of organisms,*** Minimum inhibitory concentration required to inhibit the growth of 90% of organisms.

Table 2. Results of erythromycin MIC determination by E-test for *C. jejuni* isolated from broiler chickens, pigs and humans

Source of strains	No. of strains	*MIC range	**MIC_{50}	***MIC_{90}	Resistance (%)
Broiler chickens	10	0.016 - 64	0.25	2	33.33
Pigs	5	0.016 - 64	0.016	64	20.00
Humans	17	0.023 - >256	0.25	>256	17.65

* Minimum inhibitory concentration,** Minimum inhibitory concentration required to inhibit the growth of 50% of organisms, *** Minimum inhibitory concentration required to inhibit the growth of 90% of organisms.

(Aarestrup and Engberg, 2001; Luber et al., 2003).

Since there was little published work on thermophilic *Campylobacter* spp. isolated from animals and humans available in Serbia, it is our objective to determine the sensitivity to erythromycin of thermophilic *Campylobacter* spp. isolated from humans and animals.

MATERIALS AND METHODS

Strains of thermophilic *Campylobacter* spp. were obtained from caecum of broiler chickens as well as caecum and colon of pigs and human stools. Four hundred and four animal specimens were investigated: 24 obtained by scrapping of broiler chickens caecum surfaces, and 31 obtained by scrapping of pigs caecum and colon surfaces. The following procedures were used for the isolation of thermophilic *Campylobacter* in animals.

Immediately after collection, samples were, in aim to obtain individual colonies, diluted and inoculated on Karmali and Skirrow agar. Inoculated plates were placed in pots for anaerobes and then, by Campy Pak, BBL bags, microaerophilic conditions were created. In the laboratory, transported pots were transferred in thermostats and incubated in microaerophilic conditions at 42°C for 48 h.

After incubation, suspicious colonies were picked and dyed with 2% carbol fuchsin for the morphology visualization of *Campylobacter* ("gull wings", S or spiral shaped bacteria). To obtain pure culture of thermophilic *Campylobacter* spp. individual colonies were subcultured on Karmali or blood agar plates.

For further characterization, isolated bacteria were kept in brain heart infusion (BHI) broth with 30% glycerol at temperature of -70°C (Chan et al., 2001). A total of 24 human strains were isolated at the Institute for Public Health in Niš, Serbia. Strains from human feces 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), followed by incubation under microaerobic conditions (bioMérieux, Marcy l'Etoile, France), at 42°C for 48 h. Identification at the genus level was made using colony morphology, Gram staining ("gull wings", S or spiral shaped bacteria), oxidase and catalase tests. For identification and erythromycin susceptibility testing, strains were sent to the Military Medical Academy, Belgrade, Serbia in BHI broth with 30% glycerol at +4°C (Chan et al., 2001).

Isolated bacteria were identified by catalase test, oxidase test, hippurate hydrolysis test, indoxyl acetate hydrolysis, fast H_2S test, susceptibility to cephalotine and nalidixic acid (Aarestrup et al., 1997; Jain et al., 2005). Final identification of *Campylobacter* spp. was performed by automatic identification system API Campy produced by Bio Mérieux, (France). Assessment of thermophilic *C. jejuni* and *C. coli* susceptibility to erythromycin was performed by E-test (AB BIODISK, Solna, Sweden) where breakpoints between ≤0.5 µg/ml and ≥8 µg/ml were interpreted according to the recommendation of manufacturer.

RESULTS

In total, fifty-five *Campylobacter* spp. strains were studied: 10 *C. jejuni* and 6 *C. coli* strains from broiler chickens, 5 *C. jejuni* and 10 *C. coli* from pigs as well as 17 *C. jejuni* and 7 *C. coli* strains from humans. Six out of 15 *Campylobacter* strains isolated from pigs showed the highest level of resistance against erythromycin. For isolates from broiler chickens and humans, resistance to erythromycin was 12.50% (Table 1).

Three of 17 *C. jejuni* strains isolated from humans were resistant to erythromycin. Three of 10 *C. jejuni* strains isolated from broiler chickens and 1 of 5 strains isolated from pigs were resistant to erythromycin (Table 2). None of 7 *C. coli* strains isolated from humans were resistant to erythromycin. One of 6 *C. coli* strains isolated from broiler chickens and 5 of 10 *C. coli* strains isolated from pigs were resistant to erythromycin (Table 3).

DISCUSSION

Based on the MIC values obtained by E-test, resistance to erythromycin of *Campylobacter* spp. isolated from humans was 12.5%. Our results are in accordance to the results of others (Burch, 2002; Ge et al., 2002; Pezzotti et

Table 3. Results of erythromycin MIC determination by E-test for *C. coli* isolated from broiler chickens, pigs and humans.

Source of strains	No. of strains	*MIC range	**MIC_{50}	***MIC_{90}	Resistance (%)
Broiler chickens	6	0.016 - >256	0.50	>256	16.67
Pigs	10	0.016 - >256	48.00	>256	50.00
Humans	7	0.016 – 0.50	0.125	0.50	0.00

*Minimum inhibitory concentration,** Minimum inhibitory concentration required to inhibit the growth of 50% of organisms,*** Minimum inhibitory concentration required to inhibit the growth of 90% of organisms.

al., 2003; Sáenz et al., 2000). High percentage (24.1%) of thermophilic *Campylobacter* strains resistant to erythromycin isolated from humans in developed countries is a surprising fact, having in mind a strict control of antibiotic use in human and veterinary medicine in these countries.

In some countries, for example Chile and Egypt, have not documented the resistance to erythromycin in strains isolated from humans (Fernández et al., 2000; Putnam et al., 2003). Lower level of resistance to erythromycin, ranging from 3.4 to 9.1% was reported by authors from Australia, India, USA, Brasil and Belgium (Alfredson et al., 2003; Aquino et al., 2002; Gupta et al., 2004; Jain et al., 2005; Vlieghe et al., 2008).

There is a tendency of increase in resistance of *Campylobacter* to erythromycin. In 1998, 3% *C. jejuni/coli* resistant strains were reported in Canada, but the percentage rose to 12% in 2001 (Gaudreau and Michaud, 2003). In Germany, 7.1 and 29.4% *C. coli* resistant to erythromycin were reported in 1991 and 2001, respectively (Luber et al., 2003). Besides, a rising trend of resistance to erythromycin was reported in Northern Ireland, from 0.6% in 1996 to 4.2% in 2000 (Moore et al., 2001).

In our study even 12.5% *Campylobacter* strains resistant to erythromycin were detected, in spite of the fact that this antibiotic has not been used in poultry. Our results are in accordance with results of other authors (Aarestrup et al., 1997; Burch, 2002; Avrain et al., 2003).

A low level of resistance to erythromycin was registered in Great Britain (0 to 8%), USA (3.1%) and Czech Republic (6%) (Bardon et al., 2009; Bywater et al., 2004; Hariharan et al., 2009). Engberg et al. (2004) reported 2% *C. jejuni* and 18.5% *C. coli* strains resistant to erythromycin. Data concerning such a high level of resistance to erythromycin in *Campylobacter* strains isolated from poultry are surprising having in mind that Denmark is a developed country in which the use of antibiotics is limited to therapeutic indications only.

A high percentage of *C. coli* strains resistant to erythromycin isolated from broiler chickens and eggs-laying hens (35 and 50%) was registered in Japan (Ishihara et al., 2004), South Africa (Bester and Essack, 2008) and USA (Han et al., 2009). Authors in Italy reported a high level of resistance to erythromycin, up to 45%, in *C. coli* strains isolated from poultry faeces (Pezzotti et al., 2003).

Macrolides are widely used in swine farming and, as a consequence of intensive pressure of drugs included in this thylosine group a rise of *Campylobacter* strains resistant to erythromycin originating from swines occured.

In our study, 40% resistant *Campylobacter* strains were detected from pigs with a higher degree of resistance (50%) detected in *C. coli* strains. Several authors cited significantly higher percentage of resistant *Campylobacter* strains, while some others reported results in accordance to the results obtained in this investigation.

A high percentage (74 and 71%) of resistant *C. coli* strains isolated from swine was reported in Denmark (Aarestrup et al., 1997) and Canada, respectively (Rosengren et al., 2009). A high percentage of resistance in *C. coli* originating from swines was reported in Spain (81%), and in Denmark this percentage in *C. jejuni* strains was 33%, and in *C. coli* strains was 74% (Burch, 2002). The investigators in Denmark detected in swines 35% *C. jejuni* and 57% *C. coli* resistant to erythromycin. Bywater et al. (2004), reported that 41.8% strains of *C. jejuni/coli* in Holand and 36.7% strains in Denmark were resistant to erythromycin. Resistance to erythromycin is detected in 39.7% *C. jejuni/coli* strains isolated from swines in USA (Gebreyes et al., 2005). Hart et al. (2004) reported on 75.5% strains isolated in Australia from swines before evisceration and 86.8% after evisceration resistant to erythromycin. Even 81.1% *C. jejuni/coli* strains isolated from swines in Spain were resistant to erythromycin (Sáenz et al., 2000). A high percentage of resistance to erythromycin is registered in thermophilic *Campylobacter* strains isolated from swines in France (55%) (Payot et al., 2004). These results are nearly identical to results obtained in this investigation.

Schuppers et al. (2005) in Switzerland reported a level of 19.2% *C. jejuni/coli* strains resistant to erythromycin, significantly lower than in data reported by authors in other countries.

Continuous surveillance of resistance to erythromycin in thermophilic campylobacters is necessary, since resistance occurs in both humans and animals strains. The strains isolated from pigs were more often resistant to erythromycin than the strains isolated from human and broiler chickens. Therefore, a great attention should be directed to the macrolydes application monitoring in swine farming in order to prevent resistance apperance in animal strains and its subsequent spread to human strains.

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