Advanced Journal of Microbiology Research Vol. 2017
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Susceptibility of *Campylobacter jejuni and Campylobacter coli* isolated from animals and humans to tetracycline

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Accepted 31 May, 2016

Fifty five thermophilic *Campylobacter* spp. strains were isolated from cecum of broilers, cecum and colon of pigs and from human feces. The strains were identified as *Campylobacter jejuni* and *Campylobacter coli*. The more prevalent species in broilers and humans was *C. jejuni* and in pigs *C. coli*. In the framework of this study, sensitivity to tetracycline in isolated strains of *C. jejuni* and *C. coli* was tested by E-test. In 16 tested strains isolated from broilers, 56.25% were resistant to tetracycline. Resistance occured more frequent in *C. coli* strains (66.67%). In 15 strains of termophilic *Campylobacter* spp. isolated from pigs the percentage of resistant strains was 80%. Resistance was detected more often in *C. coli* (90.00%) isolates. The percentage of resistant *C. jejuni* strains from pigs was 60.00%. Resistance to tetracycline occurred in 29.17% of 24 thermophilic *Campylobacter* spp. strains isolated from humans. Generally, strains of thermophilic campylobacters, especially *C. coli* isolated in pigs are more frequent resistant to tetracycline than strains isolated in poultry and human. Therefore, attention should be directed to the tetracycline application monitoring in swine farming in order to prevent resistance appearance in animal strains and its subsequent spread to human strains.

Key words: *Campylobacter jejuni*, *Campylobacter coli*, tetracycline, resistance.

INTRODUCTION

One of the most important bacterial zoonosis is campylo-bacteriosis. *Campylobacter* genus consists of 18 species, of which thermophilic campylobacters: *Campylobacter jejuni, Campylobacter coli, Campylobacter lari, Campylo-bacter upsaliensi* are the most frequent causative agents of the disease in humans. Thermophilic *Campylobacter* species are fastidious bacteria which require: special media supplemented with antibiotics inhibiting other gut bacteria growth, microaerophylic environment (5% O_2, 10% CO_2 and 85% N_2) and 42°C temperature for their growth. Animals very rarely develop a disease with very mild symptoms. The disease is a result of an infection with one of virulent strains, an intercurrent infection or low immunity level in infected individuals (López et al., 2002). Animals are colonized by *Campylobacter* spp. in different proportions. Poultry in age of two to tree weeks is 50 - 90% colonized with termophilic *Campylobacter* spp. (Newel, 2002). Pigs are less colonized by the same bacteria than poultry.

C jejuni and *C. coli* are the most important etiologic agents of bacterial intestinal infections actually present in the world, with 400,000000 cases every year. An important factor in the intestinal campylobacteriosis development is a low infective dose of 500 bacteria only (Walker et al., 1986) . Increase of resistance in genus *Campylobacter* to the antibiotics most frequently used in therapy of campylo-bacteriosis in humans, recently becomes alarming. Many authors in the world report on resistance of *Campylobacter* to tetracycline (Aarestrup et al., 1997; Fernández et al., 2000; Aarestrup and

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Engberg, 2001; Aquino et al., 2002; Ge et al., 2002; Avrain et al., 2003; Butzler, 2004). Having in mind the fact that a very small number of investigations on thermophilic *Campylobacter* spp. sensitivity to the drugs were available in our country, we decided to determine the sensitivity to tetracycline of thermophilic *Campylobac-ter* isolated from animals and humans.

MATERIALS AND METHODS

Scrappings of broiler cecum surfaces, scrappings of pigs cecum and colon surfaces, as well as human faeces were used as a material to study. For thermophilic *Campylobacter* isolation from animals, following procedures were used:

1. Immediately after collection, samples were, in aim to obtain individual colonies, diluted and then inoculated on Karmali agar 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 on 42°C for 48 h.
2. After incubation period, individual colonies were picked up and slides dyed with 2% carbol fuchsin for microscopy were made. *Campylobacter* spp. grow forming colonies of characteristic mor-phology were visualised. Some formed a confluental growth with the line of inoculation.
3. To obtain pure culture of thermophilic *Campylobacter* spp. individual colonies were subcultured on Karmali or blood agar plates.

For further investigations isolated bacteria were storred in BHI (brain-heart -infusion- media) with 30% glicerol at temperature of -70°C (Kam et al., 2001). 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), following incubation in a jar under micro aerobic conditions (bioMérieux, Marcy l'Etoile, France), at 42°C, for 48 h. Isolated bacteria were identified by classic and commercial biochemical tests. Classic biochemical tests included 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 (Bio Mérieux, France) following producers's instructions. Assessment of thermophilic *C. jejuni* and *C. coli* susceptibility to tetracycline was performed by E-test (AB BIODISK, Solna, Sweden). E-test was performed by recomendations of the manufacturer.

RESULTS

Four hundred forty-nine specimens obtained by scrapp-ing of broiler caecum surfaces, scrapping of pigs caecum and colon surfaces and human faeces were investigated. Out of the samples collected, fifty five *Campylobacter* spp. strains were isolated, by conven-tional and automa-tised microbiology tests 32 *C. jejuni* strains and 23 *C. coli* strains were identified (Table 1). *C. jejuni* is predominant in humans and broilers and *C. coli* is predominant in pigs. Five of 17 *C. jejuni* strains isolated from humans were resistant to tetracycline. Five of 10 *C. jejuni* strains isolated from broilers and 3 of 5 strains from pigs were resistant to tetracycline (Table 2). Nine of 10 *C. coli* strains isolated from pigs were resistant to tetracyclin (Table 3) . Four of 6 *C. coli* strains isolated from broilers and 2 of 7 strains from humans were resistant to tetra-cycline. The lowest frequency of *Campylobacter* strains resistant to tetracycline was found in strains isolated from humans 29.17%. In strains isolated from broilers resis-tance frequency to tetracycline was 56.25%. Very high frequency of resistance to tetracycline was found in strains isolated from pigs (80.00%) (Table 4).

DISCUSSION

Beginning with eighties of the last century onward, a selection and spreading of resistant strains of thermo-philic *Campylobacter* spp. in humans occurred. This phenomenon has been attributed to uncontrolled antibio-tics (especially tetracycline) use in veterinary medicine as a growth promoters, also in prophylaxis and therapy (Desmonts et al., 2004). In human medicine tetracyclines have been used without control, too (Golub et al., 2008).

Sensitivity to tetracycline was studied in 55 *C. jejuni* and *C. coli* strains isolated from humans, broilers and pigs. Twenty four strains were isolated from humans, 16 from broiler and 15 strains were isolated from pigs. Based on the minimum inhibitory concentration (MIC) values obtained by E-test, resistance to tetracycline of *C. jejuni* and *C. coli* isolated from humans was 29.17%. Our results are in accordance to the results of others (Krause and Ullman, 2003; Boyanova et al., 2004). A lower level of resistance to tetracycline, ranging from 12 to 16%, was reported in Australia, Turkey and India (Alfredson et al., 2003; Oncul et al., 2003; Jain et al., 2005). Very low level of thermophilic *Campylobacter* spp. isolated from human, resistant to tetracyclines, only 1.8%, was registered in Chile (Fernández et al., 2000). High percentage of resistant thermophilic *Campilobacter* strains isolated from humans, ranging from 43 to 85%, report authors in USA, Spain and Finland (Ge et al., 2002; Garcia-Campos et al., 2003; Hakanen et al., 2003; Gupta et al., 2004), a surprising fact having in mind that, developed countries use antibiotics as controlled and based only on thera-peutic indications and antibiogram results. Annoying is the trend of resistance increase to tetracycline in many countries (Gaudreau and Gilbert, 1998; Moore et al., 2005).

The resistance to tetracycline of *C. jejuni* and *C. coli* strains isolated from broilers was 56.25%. The more resistant species was *C. coli* (66.67%). Similar results are reported in other studies (Sáenz et al., 2000; Avrain et al., 2003; Ishihara et al., 2004, Luangtongkum et al., 2008, Baster and Essack, 2008). Great number of authors report lower percentages of resistance (Looveren

Table 1. Thermophilic *Campylobacter* spp in broiler chickens, pigs and humans (N° and %).

Host	Number of strains isolated	*C. jejuni*	*C. coli*
Broilers chickens	16	10 (62.50%)	6 (37.50%)
Pigs	15	5 (33.33%)	10 (66.67%)
Humans	24	17 (70.83%)	7 (29.17%)

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

Source of strains	N° of strains	*MIC range	**MIC_{50}	***MIC_{90}	Resistance (%)
Broiler chickens	10	0.016 - >256	24.00	>256	50.00
Pigs	5	0.016 - >256	>256	>256	60.00
Humans	17	0.006 - >256	0,25	>256	29.41

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

Source of strains	N° of strains	*MIC range	**MIC_{50}	***MIC_{90}	Resistance (%)
Broiler chickens	6	< 0.016 - >256	>256	>256	66.66
Pigs	10	3.00 - >256	48.00	>256	90.00
Humans	7	0.032 – >256	4.00	>256	28.57

* 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 4. Results of tetracycline MIC determination by E-test for *Campylobacter* strains isolated from broiler chickens, pigs and humans.

Source of strains	N° of strains	*MIC range	**MIC_{50}	***MIC_{90}	Resistance (%)
Broiler chickens	16	< 0.016 - >256	24.00	>256	56.25
Pigs	15	0.016 - >256	14.00	>256	80.00
Humans	24	0.006 - >256	0.06	>256	29.16

* 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.

et al., 2001; Fallon et al., 2003; Ledergerber et al., 2003, Han et al., 2009). Aarestrup and Wegener (1999) in Denmark were found low resistance level to tetracyclines in *C. jejuni/coli* strains isolated from poultry (2%). Low resistance level is due to controlled use of tetracycline in poultry farms in this country.The resistance to tetracycline of *C. jejuni* and *C. coli* strains isolated from pigs was 80.00% and it was significantly higher than resistance in strains isolated from humans. The more resistant species was *C. coli* (90.00%) and was significantly higher than the strains isolated in humans. Similar results are reported in other studies (Burch, 2002; Hart et al., 2004). Some authors report lower percentages of resistance (Gebreyes et al., 2005; Schuppers et al., 2005). Aarestrup and Wegener (1999) in Denmark were found a low resistance level to tetracyclin in *C. jejuni/coli* strains isolated from pigs (1%).

Four mechanisms in resistance development are included: efflux system, tetracycline modification, protection of ribosomal coupling point for tetracycline and mutation in 16S rDNA. In *C. jejuni* and *C. coli* the resistance is related to self -transmissive plasmids (Aarestrup and Engberg, 2001). The consequence is a possible transmission of resistance between *Campylobacter* spp. strains and transmission of resistance genes on bacteria of other genera (Speer et al., 1992). The high percentage of resistant *Campylobacter* strains to tetracycline could be explained by uncontrolled use of oxytetracycline in poultry farming and swine farming as a prophylaxis and its use in sub-optimal dosage to growth promotion and in high dosage in therapy of infections generally caused by gram-negative bacteria.

In conclusion, since *C. coli* is more frequent resistant to tetracycline than *C. jejuni*, as well as strains isolated in pigs, attention should be directed to the tetracycline application monitoring in swine farming in order to prevent resistance appearance in animal strains and its subsequent spread to human strains. Therefore, instead of tetracycline and oxytetracycline use, somewhat a better susceptibility should be realized by the use of doxycycline which has better pharmacokinetic properties and distribution in the body (Prescott, 2006).

ACKNOWLEDGEMENT

This study is supported by the Ministry of Science and Technological Development of Republic of Serbia.

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