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

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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 occurred more frequent in C. coli strains (66.67%). In 15 strains of thermophilic 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 campylobacteriosis. Campylobacter genus consists of 18 species, of which thermophilic campylobacters: Campylobacter jejuni, Campylobacter coli, Campylobacter lari, Campylobacter upsaliensis 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, microaerophilic environment (5% O₂, 10% CO₂ and 85% N₂) 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 three weeks is 50 - 90% colonized with thermophilic 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 400,000,000 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 Campylobacter 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 morphology were visualised. Some formed a confluent 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 stored in BHI (brain-heart -infusion- media) with 30% glycerol 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 10⁵ 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 H₂S 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 scrapping 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 tetracycline. The lowest frequency of Campylobacter strains resistant to tetracycline was found in strains isolated from humans 29.17%. In strains isolated from broilers resistance 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 thermophilic Campylobacter spp. in humans occurred. This phenomenon has been attributed to uncontrolled antibiotics (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 therapeutical 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 %).

<table>
<thead>
<tr>
<th>Host</th>
<th>Number of strains isolated</th>
<th><em>C. jejuni</em></th>
<th><em>C. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Broilers chickens</td>
<td>16</td>
<td>10 (62.50%)</td>
<td>6 (37.50%)</td>
</tr>
<tr>
<td>Pigs</td>
<td>15</td>
<td>5 (33.33%)</td>
<td>10 (66.67%)</td>
</tr>
<tr>
<td>Humans</td>
<td>24</td>
<td>17 (70.83%)</td>
<td>7 (29.17%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Source of strains</th>
<th>N° of strains</th>
<th>*MIC range</th>
<th>**MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>***MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler chickens</td>
<td>10</td>
<td>0.016 - &gt;256</td>
<td>24.00</td>
<td>&gt;256</td>
<td>50.00</td>
</tr>
<tr>
<td>Pigs</td>
<td>5</td>
<td>0.016 - &gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>60.00</td>
</tr>
<tr>
<td>Humans</td>
<td>17</td>
<td>0.006 - &gt;256</td>
<td>0.25</td>
<td>&gt;256</td>
<td>29.41</td>
</tr>
</tbody>
</table>

* 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.

<table>
<thead>
<tr>
<th>Source of strains</th>
<th>N° of strains</th>
<th>*MIC range</th>
<th>**MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>***MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler chickens</td>
<td>6</td>
<td>&lt; 0.016 - &gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>66.66</td>
</tr>
<tr>
<td>Pigs</td>
<td>10</td>
<td>3.00 - &gt;256</td>
<td>48.00</td>
<td>&gt;256</td>
<td>90.00</td>
</tr>
<tr>
<td>Humans</td>
<td>7</td>
<td>0.032 - &gt;256</td>
<td>4.00</td>
<td>&gt;256</td>
<td>28.57</td>
</tr>
</tbody>
</table>

* 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.

<table>
<thead>
<tr>
<th>Source of strains</th>
<th>N° of strains</th>
<th>*MIC range</th>
<th>**MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>***MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler chickens</td>
<td>16</td>
<td>&lt; 0.016 - &gt;256</td>
<td>24.00</td>
<td>&gt;256</td>
<td>56.25</td>
</tr>
<tr>
<td>Pigs</td>
<td>15</td>
<td>0.016 - &gt;256</td>
<td>14.00</td>
<td>&gt;256</td>
<td>80.00</td>
</tr>
<tr>
<td>Humans</td>
<td>24</td>
<td>0.006 - &gt;256</td>
<td>0.06</td>
<td>&gt;256</td>
<td>29.16</td>
</tr>
</tbody>
</table>

* 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 tetracycline 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).

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REFERENCES


