Antimicrobial Agents Resistance in Campylobacter coli from Swine and Humans

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Summary: C. coli from slaughtered pigs and from human patients were examined for resistance to quinolones and tetracycline. Detection of tetO was performed. Gyrase A gene (gyrA) was amplified and sequenced and tested by an alternative method. Tetracycline resistance levels were respectively of 67.7 % and 56.3 % in swine and human isolates. In C. coli of human origin, all resistant isolates had the tetO determinant while 82.8 % of resistant C. coli from swine possessed it. Among the susceptible swine isolates, 17.2 % possessed this gene. Resistance to enrofloxacin (7.3 %) and ciprofloxacin (11.4 %) was observed in swine isolates and resistance for enrofloxacin (12.5 %) and ciprofloxacin (18.8 %) were observed in C. coli from humans. In addition, 72.7 % of swine resistant isolates and all isolates from humans had a mutation at position 86. Results were similar with MAMA-PCR which can thus be considered as a good alternative to sequencing.

Keywords: MAMA-PCR, tetO, gyrA, agar dilution, sequencing.

Introduction: An increase in antimicrobial agents resistance have been reported in many countries for Campylobacter, specially for tetracycline, fluoroquinolones and erythromycin (Engberg et al., 2001). Campylobacteriosis is generally associated with sporadic episodes of diarrhea linked with consumption of improperly handled or cooked food. Animal productions such as swine are potential reservoirs for this bacteria. Some authors suggested that the usage of antimicrobial agents in animal productions play a key role in the dissemination of antimicrobial resistance genes from animals to human population (Swartz 2002). To evaluate this possible link it is important to verify the distribution of antimicrobial resistance determinants in various populations. The aim of this study was to evaluate the incidence and the distribution of antimicrobial resistance in Campylobacter isolates from humans and swine recovered within a restricted geographical area in order to increase chances to establish links. The detection of some genetic determinants of resistance was performed in order to characterize the resistance.

Materials and Methods: A total of 16 human clinical cases isolates and 96 isolates from cecal content of slaughtered swine were included in the study. Resistance tetracycline and fluoroquinolones was evaluated by the agar dilution technique following guidelines of the NCCLS for veterinary pathogens.
Following DNA extraction, *tetO* gene was amplified by PCR using primers previously described (Widdowson et al., 1996). A PCR product containing the quinolone resistance-determining region (QRDR) was generated to be further sequenced (Zirnstein et al., 2000) at Sheldon Biotechnology Center, McGill University, Montreal, Canada. The Mismatch amplification mutation assay-PCR (MAMA-PCR) was used to evaluate the reliability of this technique as an alternative to sequencing. A conserved forward primer and a reverse mutation detection primer were used for *C. coli*. As expected, a 192-bp PCR product for *C. coli* was a positive indication of the presence of the Thr-86 to Ile mutation in *gyrA* gene.

**Results:** There was no significant difference between resistance levels to ciprofloxacin and enrofloxacin from swine and humans isolates and the highest resistance level observed was for tetracycline (Table 1). The genetic determinant *tetO* was recovered from 82.8 % of swine resistant isolates and from 100 % of the resistant human isolates. On the other hand, 17.2 % (5/29) of the swine susceptible isolates had the *tetO* determinant. After the sequencing of quinolones resistant *C. coli*, all human isolates and 7 out of 11 (63.6 %) of swine isolates had the transition of one nucleotide associated with a change from a threonine to an isoleucine at position 86 of GyrA. These findings were in accordance with results obtained by MAMA PCR since the wild-type amino acid 86 codon was not amplified with the reverse mutation primer. No other amino acid substitution was observed.

**Table 1. Antimicrobial agents susceptibility of *C. coli* isolated from swine and humans**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Pigs (n = 96)</th>
<th>Human (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&lt;0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>&lt;0.25</td>
<td>&lt;0.25-16</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>32</td>
<td>64</td>
</tr>
</tbody>
</table>

<sup>a</sup>MIC in µg/ml  
<sup>b</sup>%R, percentage resistant

**Discussion:** Tetracyclines are relatively inexpensive drugs with a broad spectrum of activity and have been widely used. The highest levels of resistance observed were for this antimicrobial agent. Similar results were observed in studies conducted in Belgium (Van Looveren et al., 2001) and Italy (Pezzotti et al., 2003). So far, *tetO* is the only gene reported to be responsible for the resistance in *Campylobacter*. In this study, 17.2 % of the tetracycline susceptible swine isolates possessed the determinant but did not express it. A required DNA sequence upstream of this gene (Wang & Taylor, 1991) might have been altered in those isolates, affecting their expression. Other isolates, found resistant by the agar dilution technique, did not possess *tetO* suggesting that other genetic determinant might be present. Resistance to fluoroquinolones in *Campylobacter* from food animal origin is recognized as an important emerging public health threat (Engberg et al., 2001). Mutations in *gyrA* at positions Thr-86, were reported as mainly responsible for quinolone resistance. In this study, only the Thr-86 mutation have been observed. Since some isolates that did not possessed the mutation in *gyrA* had high MIC, it suggest that the resistance could be linked to others genes, such as *gyrB*, topoisomerase IV *parC* and *parE*, or to efflux pumps or permeability factors.

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**References:**


FAECAL SHEDDING OF ARCOBACTER SPECIES IN BELGIAN PIGS

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Summary: The prevalence of Arcobacter was determined in porcine faecal samples collected at slaughterhouse and two unrelated finishing barns (A and B) using the previously developed Arcobacter isolation procedure. In 43.9% of the slaughterhouse samples tested (n=82) arcobacters were detected, and identified as A. butzleri and A. cryaerophilus. Two pigs shedded both species simultaneously. On farm A (n=98), arcobacters were isolated from 16.3% of the samples and identified as A. cryaerophilus and A. skirrowii. In samples (n=118) collected at farm B, arcobacters were detected in 45.1% of the samples. A. butzleri was the most frequently occurring species. Co-infections were found in 11 animals. Arcobacters were detected in clinically healthy pigs at contamination levels up to 10^3 cfu/g faeces.

Keywords: Porcine faeces, Arcobacter prevalence, Slaughterhouse, Farm level, Belgium.

Introduction: The genus Arcobacter includes bacteria formerly known as aerotolerant campylobacters. They are Gram-negative non-spore-forming rods with a single polar flagellum and differ from the closely related campylobacters in their ability to grow aerobically from 15 up to 42 °C. Within the genus Arcobacter, four species are presently recognized: Arcobacter nitrofigilis, a nitrogen-fixing plant associated species (McClung et al., 1983) and the animal and human related species Arcobacter butzleri, Arcobacter cryaerophilus and Arcobacter skirrowii. Arcobacters are frequently present on foods of animal origin, including pork (Collins et al.,1996). Contamination probably occurs by faecal contamination during slaughter. Foods of animal origin are considered as a main source of Arcobacter infection in humans and therefore, arcobacters are established as an emerging foodborne pathogen (Wesley, 1996). In pigs, arcobacters are associated with reproductive problems and are found in stomachs of pigs with gastric ulcers. Moreover, arcobacters can be isolated from faeces of clinically healthy animals. The aim of the present study was to determine the prevalence and contamination level of Arcobacter in Belgian pigs at slaughter age and during raising.