Campylobacter species and their antimicrobial resistance in Latvian broiler chicken production

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ABSTRACT

In the present study Campylobacter species and their antimicrobial resistance in Latvian broiler chicken production was determined. Furthermore, this is the first report on the antimicrobial resistance patterns for Campylobacter isolates from broiler chickens at slaughterhouse and retail level in Latvia. Two biggest Latvian broiler chicken meat producing company products were included in the study. Altogether, 74 randomly selected broiler chicken Campylobacter spp. isolates were analysed for species identification. Campylobacter isolates were obtained during a 12-month period within the Latvian Campylobacter prevalence study in 2010. Colony multiplex PCR was used for all isolates to identify Campylobacter species. Minimal inhibitory concentration (MIC) was determined for 58 Campylobacter spp. isolates. Resistance to one or more antimicrobials was detected in all 58 isolates (100%). A high proportion of the isolates were resistant to ciprofloxacin (100%) and nalidixic acid (87.9%). Multidrug resistance, which was determined as resistance to three or more unrelated antimicrobials, was detected in 39 isolates (67.2%). Moreover, all multiresistant isolates were resistant to ciprofloxacin and nalidixic acid. Analyses of Campylobacter isolates from two Latvian broiler chicken meat producing companies resulted with significant differences in Campylobacter species; from the company A mainly Campylobacter coli were found, while in the company B Campylobacter jejuni.

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1. Introduction

Campylobacteriosis in humans is caused by thermotolerant Campylobacter spp. From which Campylobacter jejuni and Campylobacter coli are the most commonly reported species associated with human intestinal infections in European Union (EU). In average, 55.49 confirmed campylobacteriosis cases per 100,000 EU inhabitants were reported in 2012 (EFSA, 2014). In most cases Campylobacter spp. cause gastroenteritis in humans, but may also result in post-infection complications such as Miller–Fisher and Guillain–Barré syndrome (Fica et al., 2011; Kuwabara, 2011). In severe campylobacteriosis cases the antibiotic may be needed to treat the disease, but as the resistance of Campylobacter against antibiotics is increasing, the success of the treatment may be compromised (EFSA, 2012; Lehtopolku et al., 2010).

It is well documented that fluoroquinolone resistance among Campylobacter isolates from human infections increased after the approval of fluoroquinolones in agriculture, especially in poultry farming (Jacobs-Reitsma, Kan, & Bolder, 1994; McDermott et al., 2002).

The most important source of Campylobacter is poultry meat (Friedman et al., 2004). Therefore, the effective control of Campylobacter at the farm level and control of the antimicrobial resistance in poultry meat production chain is the major public health strategy (EFSA, 2011). In 2010, the proportions of Campylobacter-positive broiler meat samples varied widely in EU member states, from 3.1% to 90%. In accordance with EU-wide
baseline survey, the average *Campylobacter* prevalence for fresh broiler chicken carcasses was 75.8% (EFSA, 2012). The prevalence of *Campylobacter* in Latvian broiler chicken samples in 2010 was 59.2% (Kovalenko, Roasto, Liepiņš, Mäesaar, & Hörman, 2013).

The aim of the present study was to determine the *Campylobacter* species and their antimicrobial resistance in broiler chicken production at slaughterhouse and retail level in Latvia.

2. Sampling

A total of 240 fresh broiler chicken neck skin, 240 fresh broiler chicken carcass samples and 2400 whole broiler chicken intact intestines were collected during 2010. Samples were collected monthly at a random basis among products of two biggest companies in Latvia, representing the production of more than 75% of all commercial broilers in Latvia.

Every month, 10 neck skin samples were collected from each of the two investigated slaughterhouse. The neck skin samples were placed separately in sterile plastic bags for transportation to the laboratory. Additionally, every month 10 fresh broiler chicken carcasses from the production of the same broiler meat producers were collected at retail level in Latvia from two biggest supermarket chains. All broiler carcasses were packed individually in plastic bags prior shipping to stores. Carcass samples were collected at the same day as the sampling in slaughterhouses was performed, but they did not represent the same slaughter batch as the neck skin samples and cecal samples. Broiler chicken carcasses from slaughterhouse A were sold in lose, unsealed plastic bags whereas from slaughterhouse B broiler chicken carcasses were sold in tight, sealed plastic bags. From total of 502 *Campylobacter* isolates randomly 74 isolates were selected for *Campylobacter* species identification. All isolates were tested with colony multiplex PCR according to the Wang et al., 2002. By PCR 23s rRNA, hyp O, gly A genes were determined. After thawing and incubation, deoxyribonucleic acid (DNA) extraction and PCR was performed followed by the electrophoresis in agarose gel for electrophoretic evaluation.

2.2. Determination of antimicrobial susceptibility

The determination of antimicrobial resistance was performed for 28 *C. jejuni* and 30 *C. coli* isolates. Only broiler chicken meat origin isolates were studied for antimicrobial susceptibility. These isolates were tested for the minimal inhibitory concentration (MIC) by a broth microdilution method (National Veterinary Institute, Uppsala, Sweden) against erythromycin, ciprofloxacin, tetracycline, gentamicin, streptomycin and nalidixic acid. After frozen storage at –70 °C the *Campylobacter* isolates were cultured on Columbia blood agar (Oxoid, Basingstoke, Hampshire, England) and incubated at 41.5 ± 0.5 °C for 48 h. After incubation, a loopful (1 μl) of bacterial growth was transferred to 2 ml of 0.9% saline and then 100 μl of suspension were added to 10 ml of cation-adjusted Mueller–Hinton broth (CAMHB, Oxoid, Basingstoke, Hampshire, England) with 2.5–5% lysed horse blood to get final inoculum of 10⁶ CFU/ml each well of MIC panel (VetMIC Camp Ver.2, SVA, Uppsala, Sweden). Inoculated with 100 μl of 10⁶ CFU/ml bacterial suspension. After filling of the panel, 10 μl of inoculums were streaked to Columbia blood agar plates for purity control. The density of the bacterial suspension was controlled and colony counts from 50 to 250 per plate were accepted. *C. jejuni* ATCC 33560 was used as a control strain. The plates were incubated at 37 ± 1 °C for 40–48 h in microaerobic conditions. After incubation the MIC was read as the lowest concentration completely inhibiting visible growth of *Campylobacter* in accordance with the instructions given by the test manufacturer (SVA, Uppsala, Sweden).

According to the Eucast (2014a) epidemiological cut-off values *C. jejuni* was considered to be resistant when the MIC values were for: erythromycin >4 μg/ml, ciprofloxacin >1 μg/ml, tetracycline >2 μg/ml, streptomycin >2 μg/ml, nalidixic acid >16 μg/ml and gentamicin >1 μg/ml.

According to Eucast (2014b) epidemiological cut-off values *C. coli* was considered to be resistant when the MIC values were for: erythromycin >16 μg/ml, ciprofloxacin >1 μg/ml, tetracycline >2 μg/ml, streptomycin >4 μg/ml, nalidixic acid >32 μg/ml and gentamicin >2 μg/ml.

2.3. Statistical analysis

Statistical analysis were performed with the Statistical Package R in order to determine statistically significant differences at 95% confidence level in the antimicrobial resistance and species of the *Campylobacter* spp. samples between the two slaughterhouses and between the antimicrobials by Pearson correlation test.
3. Results

All 74 isolates belonged to the genus Campylobacter, 37.8% (n = 28) were C. jejuni, 60.8% (n = 45) C. coli, and 1.3% (n = 1) remained unidentified.

Analyses of Campylobacter isolates originating from two Latvian broiler chicken meat producing companies showed significant differences at species level. Campylobacter isolates obtained from broiler chicken neck skin samples were all (100%) C. coli from the slaughterhouse A, while all (100%) neck skin isolates were identified as C. jejuni from the slaughterhouse B. It was found (Table 1) that most of the Latvian origin Campylobacter isolates were resistant to ciprofloxacin (100%) and nalidixic acid (87.9%). The least observed resistance was observed against streptomycin (39.6%). Among fluoroquinolone and aminoglycoside antimicrobial group significant differences in resistance of Campylobacter was not observed (p > 0.05). Study showed that 44.8% (n = 26) of tested isolates were resistant to erythromycin and 77.6% (n = 45) to tetracycline.

Overall, 67.2% (n = 39) of Campylobacter isolates were resistant to three or more unrelated antimicrobials (Fig. 1), of which 37.9% (n = 22) were resistant to all six studied antimicrobials.

The most common antimicrobial resistance pattern was the combination of nalidixic acid, ciprofloxacin, tetracycline and erythromycin.

Statistically significant difference was found in erythromycin (p = 0.03) and streptomycin resistance level between C. jejuni and C. coli (Table 1). Among the other antimicrobials there were no significant (p > 0.05) differences in Campylobacter antimicrobial resistance when comparing the two species. For Campylobacter isolates originating from slaughterhouse A antimicrobial resistance when comparing the two species. For Campylobacter isolates originating from slaughterhouse A significant difference (p ≤ 0.05) between erythromycin resistance and ciprofloxacin, nalidixic acid, streptomycin and gentamicin resistance was found. Campylobacter resistance to fluoroquinolones for slaughterhouse A was significantly (p < 0.05) higher than the resistance to erythromycin, tetracycline, gentamicin and streptomycin.

Also the resistance of Campylobacter isolates from slaughterhouse A to tetracycline was significantly (p < 0.05) higher than the resistance against streptomycin and erythromycin.

For Campylobacter isolates from the slaughterhouse B significant difference (p < 0.05) in resistance was found between fluoroquinolone group of antimicrobials and erythromycin, tetracycline and aminoglycoside group of antimicrobials.

Higher proportion of multiresistant Campylobacter isolates was obtained from slaughterhouse A.

4. Discussion

In the present study we have found that farm birds in one Latvian broiler chicken farm were colonized mostly by C. jejuni while in another farm by C. coli.

Table 1
Antimicrobial MIC levels of Campylobacter spp. isolates\(^a\) from slaughterhouse A and B.

<table>
<thead>
<tr>
<th>Species</th>
<th>µg/ml(^b) Antimicrobials</th>
<th>Ery</th>
<th>Cip</th>
<th>Tet</th>
<th>Str</th>
<th>Gen</th>
<th>Nal</th>
<th>No. of isolates (%)</th>
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<tr>
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<td>N</td>
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</tr>
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<td>6 (20)</td>
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<tr>
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</table>

\(^a\) Campylobacter isolates obtained from neck skin or broiler chicken carcasses.

\(^b\) Concentration of the studied antimicrobials.

\(^c\) The resistance breakpoint (EUCAST, 2014a; EUCAST, 2014b).
The results of the present study confirmed that Latvian broiler chicken carcasses and neck skins contain two most prevalent species of thermophilic *Campylobacter* which are also related with the most campylobacteriosis cases in humans (EFSA, 2011). Because of the chicken/carcasses hanging position during the slaughter and further processing, *Campylobacter* can be transmitted from the intestines via the fecal contamination onto the carcass surface. Furthermore, the rinsing water during the processing drains the contamination along the carcass to the cranial end direction (EFSA, 2011; Ugarte-Ruiz et al., 2012). It also explains the uniformity of *Campylobacter* species in the skin neck samples.

In our study high *C. coli* occurrence in the samples of the company A was found. Only in a few other studies *C. coli* was found to dominate in certain flocks of the same farm but the dominant *Campylobacter* species was *C. jejuni* (Cesare, Parisi, Bondioli, Normanno, & Manfreda, 2008; Manfreda, Cesare, Bondioli, Stern, & Franchini, 2006). Previous studies also showed that *C. jejuni* was mainly found on farm environment and on broilers chickens at farm level, and only in few cases *C. coli* (Melero, Juntunen, Hänninen, Jaime, & Rovira, 2012; Newell & Fearlmy, 2003; Tram, Cao, Högberg, Wolff, & Bang, 2012). It is proven that broiler chicken house may be colonized either by one or with several *Campylobacter* species (Reich, Atanassova, Haunhorst, & Klein, 2007). While discussing the differences on *Campylobacter* species distribution between the company A and B, we cannot exclude the impact of the wildlife and environment on the chicken houses *Campylobacter* contamination despite that recent findings suggesting that *Campylobacter* strains may be more host species specific (Giantspooor et al., 2013). Wild animals, birds and rodents are the potential *Campylobacter* vectors to the broiler houses (Hald, Skovgård, Pedersen, & Bankenburg, 2008). There is need for further research to explain the possible reasons on *Campylobacter* species distribution differences among studied company farms.

In our study the most commonly determined resistance of the tested *Campylobacter* isolates from both the two slaughterhouses was to fluoroquinolone group of antibiotics. *C. coli* strains showed high resistance to macrolide group antimicrobials, when 56.7% of the examined strains showed resistance to erythromycin. Macrolides and fluoroquinolones are first and second line medicines recommended for human campylobacteriosis antibiotic treatment (Zhao et al., 2010), and increasing resistance against these antimicrobials has been observed since their use in meat production started (Hiroi et al., 2012; Serichantalergs et al., 2007). Comparing the resistance rates of *C. coli* to erythromycin in other EU countries is lower than in our study. In Poland it was 9.3% (Mackiw, Korsak, Rzewuska, Tomczuk, & Rozynek, 2012), in Austria 9%, in Netherlands 5%, in France 10%, and in Spain 35%, while *C. jejuni* resistance against erythromycin in EU during the year 2010 was no higher than 6% (EFSA, 2012). At the same time, the *Campylobacter* isolated from humans in EU during the year 2010 showed in average 11% for *C. coli* and 1.1% for *C. jejuni* resistance to erythromycin (EFSA, 2012). In Finland, 50% of human *C. coli* isolates obtained from 2003 to 2005 were resistant to erythromycin (Lehtopolku et al., 2010).

Resistance to fluoroquinolones group of antimicrobials among *Campylobacter* species also tend to differ. In a number of studies it has been found that *C. jejuni* resistance to ciprofloxacin and nalidixic acid is less common compared with *C. coli* (EFSA, 2012; Gallay et al., 2007; Wieczorek, Kania, & Osek, 2013). In other studies *C. jejuni* resistance against fluoroquinolones were higher than for *C. coli* (Chen et al., 2010; Piddock et al., 2008; Salihu, Nunaidu, Magaji, & Yakubu, 2012; Son, Englen, Berrang, Fedorka-Cray, & Harrison, 2007) Present study showed that *Campylobacter* isolates from the slaughterhouse A were less resistant to fluoroquinolones, compared with *Campylobacter* isolates from the slaughterhouse B. In Latvian border country Estonia, formerly high proportions of the isolates were resistant to enrofloxacin (73.3%) and nalidixic acid (75.6%). The same Estonian study revealed that multidrug resistance was detected in 27.5% of isolates, all of which were resistant to enrofloxacin (Roasto et al., 2007). According to recent data the proportion of *Campylobacter* isolates resistant to fluoroquinolones (nalidixic acid and ciprofloxacin) was 60% in Estonia (Roasto, 2014). In other Latvian border country Lithuania, the resistance of *C. coli* and *C. jejuni* human isolates against ciprofloxacin was 74.4% and 83.1%, respectively (EFSA, 2013).

*Campylobacter* resistance against tetracycline in isolates from slaughterhouse A and B was not significantly different (p < 0.05) and it was similar to the EU countries in general. In year 2010, nine of the EU countries were notifying *C. jejuni* isolate resistance against tetracycline and it was 32% in average. The resistance of *C. coli* against tetracycline was higher, respectively 73% (EFSA, 2012). In the past ten years the lowest resistance rate of *Campylobacter* against tetracycline was reported in Denmark and the highest in Spain, respectively 10% and 94% (EFSA, 2013).

The lowest resistance of *Campylobacter* isolates from both slaughterhouses was observed against aminoglycoside antimicrobials for which the resistance rate in average was 42%. In 2010, among the EU countries 25% of *C. coli* isolates were resistant to gentamicin and in average only 0.8% of the *C. jejuni* isolates showed resistance to these antimicrobials in EU (EFSA, 2012).

5. Conclusion

It was found that company A broiler chicken samples were significantly more often (p < 0.05) contaminated with *C. coli* compared to the company B in which samples had a higher prevalence of *C. jejuni*. *Campylobacter* spp. isolates from two biggest Latvian broiler chicken slaughterhouse production had a high level of antimicrobial resistance, especially against fluoroquinolones. Also high level of multiresistance was observed for *Campylobacter* isolates of Latvian broiler chicken origin. High level of resistance and multiresistance among Latvian origin *Campylobacter* isolates may compromise the antimicrobial treatment of campylobacteriosis cases in humans. Therefore, constant monitoring of *Campylobacter* resistance patterns is required, and the use of antibiotics in poultry meat production should be restricted and systematically controlled in Latvia.

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