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Short communication

A five-year study on prevalence and antimicrobial resistance of *Campylobacter* from poultry carcasses in Poland

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ABSTRACT

During 2009–2013 a total of 2114 swab samples collected from broiler carcasses in all 16 voivodeships (administrative districts) of Poland were examined for the presence of *Campylobacter jejuni* and *Campylobacter coli*. The antimicrobial resistance of the isolates to ciprofloxacin, tetracycline and erythromycin using the MIC method was also tested. It was found that 1151 (54.4%) carcasses were contaminated with *Campylobacter*, with 50% of *C. jejuni* and *C. coli* species isolated from positive samples. The temporal trend in the prevalence of *Campylobacter*-positive samples demonstrated that the highest percentage of carcasses was contaminated during the first year of the survey (70.5%) whereas in the last year (2013) only 36.3% of broilers contained these bacteria. Antimicrobial resistance analysis showed that overall 939 (81.6%) of isolates were resistant to ciprofloxacin, 646 (56.1%) to tetracycline but only 28 (2.4%) to erythromycin. Significant differences in resistance profiles between *C. jejuni* and *C. coli* were observed with greater resistance level observed in the latter species. Furthermore, a significant increase in the percentage of *C. jejuni* resistant to ciprofloxacin (from 59.6% in 2009 to 85.9% in 2014) and to tetracycline (from 23.2% to 70.4%, respectively) was identified. Only 20 (1.7%) *Campylobacter* isolates displayed a multiresistance pattern.

1. Introduction

Campylobacter is recognized as one of the most common causes of food-related gastroenteritis in humans (Epps et al., 2013; Anon, 2014a). The pathogen is present as a commensal in many food-producing animals, especially in poultry, constituting an important food-borne source of human infections. According to the recent European Food Safety Authority (EFSA) report, campylobacteriosis in the European Union (EU) is still the most common foodborne bacterial disease with 214,268 laboratory confirmed cases and a notification rate of 55.49 per 100,000 population in 2012 (Anon, 2014a). In Poland just 431 human infections (crude incidence rate of 1.12 per 100,000 population) were notified during a concurrent time period and this low number of reported cases may be due to inadequacies in current surveillance systems (Anon, 2014a).

Poultry meat is considered the most important source of *Campylobacter* infections, especially those due to *Campylobacter jejuni* (Anon., 2010a; Damjanova et al., 2011). It has been estimated that 50–80% of human campylobacteriosis cases can be attributed to public health challenge to reduce human foodborne infections (Anon., 2010a, 2014a; Damjanova et al., 2011; Hakkinen et al., 2009). According to the EFSA report, the proportions of *Campylobacter*-positive broiler meat at the slaughterhouse level varied from 10% in Belgium to 60.0% in the Czech Republic although these results are difficult to compare due to different samples tested and methods used for analyses (Anon., 2014a).

Increased levels of antimicrobial resistance, including multidrug resistance, among *Campylobacter* isolates has been observed within the food chain (Aarestrup and Engberg, 2001; Anon., 2014b). Resistance to certain classes of antimicrobials such as quinolones and macrolides, considered the greatest concern because of their significance in human medicine (Piddock, 1995; Smole Možina et al., 2011). The resistance of *Campylobacter* to these antimicrobials has increased in recent years, raising the possibility of lack of effective antibiotics in the future (Ge et al., 2013). As chickens are one of the most important sources of *Campylobacter*, resistance to antimicrobials in these microorganisms isolated from poultry is a matter of serious concern. The use of antimicrobials in veterinary practice may be responsible for resistance in human isolates,

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particularly to quinolones (Garcia-Migura et al., 2014; Ge et al., 2013). After flouroquinolone use in poultry was approved in Europe, resistant *C. jejuni* strains emerged rapidly in humans during the early 1990s (Piddock, 1995).

The aim of the present study was to determine the prevalence and antimicrobial resistance of *Campylobacter* in poultry carcasses in Poland during 2009–2013. These data may give insight into the epidemiology of reservoirs, sources of infection and potential for transmission of *Campylobacter* resistant strains to humans.

2. Materials and methods

2.1. Sample collection

The survey was conducted and financed under the Polish governmental annual monitoring program. From July 2009 to December 2013 a total of 2114 swab samples collected from poultry broiler carcasses in all 16 voivodeships (administrative districts) of Poland were examined for the presence of *Campylobacter*. The samples were taken at slaughterhouses directly after immersion chilling (0–4 °C) but before further processing such as freezing, cutting, or packaging. The neck skin and the skin surface under the wings of broiler carcasses were wiped 10 times with sterile swabs which were immediately transported to the laboratory in Amies transport medium with charcoal (Medlab, Raszyn, Poland).

2.2. Campylobacter isolation and species identification

Campylobacter bacteria were isolated and identified as described previously (Wieczorek et al., 2013b). Briefly, the swabs were placed in 5 ml of Bolton enrichment broth (Oxoid, Basingstoke, UK) supplemented with 5% lysed horse blood and modified Bolton broth supplement with vancomycin, cefoperazone, trimethoprim, and amphotericin B to prevent growth of non-target microorganisms. The cultures were incubated at 41.5 °C for 48 h under microaerobic conditions using the CampyGen kit (Oxoid). *Campylobacter* strains were isolated and identified according to the ISO 10272-1:2006 standard (International Organization for Standardization, Geneva, Switzerland). After the enrichment step, the cultures were plated onto Karmali agar (Oxoid) and *Campylobacter* blood-free agar (Oxoid) with CCDA selective supplement (Oxoid) and incubated at 41.5 °C for 48 h under microaerobic conditions. From each sample, one presumptive *Campylobacter* isolate was confirmed using a PCR assay as previously described and stored at −80 °C for further analysis (Wang et al., 2002).

2.3. Antimicrobial resistance

A microbroth dilution method was used to establish the minimum inhibitory concentrations (MICs) of *Campylobacter* isolates to three antimicrobials (ciprofloxacin, tetracycline, erythromycin) with the Sensititre® custom susceptibility plates, EUCAMP (Trek Diagnostics, United Kingdom) as described previously (Wieczorek et al., 2013a). The strains were subcultured twice on Columbia agar (Oxoid) at 41.5 °C for 48 h under microaerobic conditions. The MIC of the antimicrobial agents was determined using Mueller-Hinton broth (Oxoid) supplemented with 2–2.5% horse blood (Trek). The plates were incubated at 37 °C for 48 h under microaerophilic conditions and read using the Vision® system (Trek). The cut off values used for the interpretation of the MIC results were in accordance with EUCAST (www.eucast.org) and the European Union Reference Laboratory for Antimicrobial Resistance.

2.4. Statistical analysis

Statistical differences in the prevalence and antimicrobial resistance between *C. jejuni* and *C. coli* isolates and years of the analyses were performed using chi-square test (StatSoft, Krakow, Poland). P values <0.05 were considered as significantly different.

3. Results

3.1. Prevalence of Campylobacter

In total, 1151 (54.4%) samples were positive for *Campylobacter*, either *C. jejuni* (576 isolates, 50.0%) or *C. coli* (575, 50.0% strains) (Table 1). To present the data, the 16 voivodeships were grouped into four geographical regions with four voivodeships in each, i.e. west-northern (I), north-eastern (II), south-western (III), and south-eastern (IV), respectively (Fig. 1). The number of birds examined in each voivodeship was calculated on the percentage of broilers slaughtered in respect to the chickens slaughtered in the whole country. The highest percentage of *Campylobacter* positive samples originated from the south-eastern part (IV) of Poland (61.0%), whereas at the other geographical regions, i.e. west-northern (I), east-northern (II) and south-western (III) the poultry carcasses were less frequently contaminated with these microorganisms. Comparison of the numbers of *Campylobacter* positive samples in each region showed geographical differences in prevalences between regions I and IV ($P < 0.01$) as well as in regions II and IV ($P < 0.05$) but not between the remaining regions (Table 1).

The temporal trend (Fig. 2A) demonstrated that the highest percentage of positive carcasses was observed during the first year of the survey (277 positive/393 tested; 70.5%) whereas in the last year (2013) only 36.3% (149/411) of samples contained these bacteria ($P < 0.0001$).

Identification of *Campylobacter* species revealed differences in prevalences of *C. jejuni* and *C. coli* isolated from samples during years 2010 and 2012 ($P < 0.05$); such differences were not observed in years 2009, 2011 and 2013 (Fig. 2A).

3.2. Antimicrobial resistance

Analysis of antimicrobial resistance showed that 939 of 1151 isolates (81.6%) were resistant to ciprofloxacin, 646 (56.1%) to tetracycline and only 28 (2.4%) to erythromycin (Table 1). Strong differences in resistance among *C. jejuni* and *C. coli* isolates were observed for ciprofloxacin where 431 (74.8%) and 508 (88.3%) resistant strains were identified, respectively ($P < 0.0001$). Furthermore, 268 (46.5%) *C. jejuni* and 378 (65.7%) *C. coli* isolates displayed resistance to tetracycline ($P < 0.0001$). Only 5 (0.9%) *C. jejuni* and 23 (4.0%) *C. coli* were resistant to erythromycin ($P < 0.001$).

Temporal trends in resistance to the three antimicrobials among *C. jejuni* and *C. coli* revealed (Fig. 2B and C) a significant increase in resistance of *C. jejuni* between the first (2009) and the last (2013) years of the study for ciprofloxacin (59.6% and 85.9% isolates, respectively; $P < 0.001$) and for tetracycline (23.2% and 70.4%, respectively; $P < 0.0001$). Such differences in the prevalence of the resistant strains were not observed for the same years among *C. coli* (81.0% and 88.5% for ciprofloxacin and 67.5% and 61.5% for tetracycline).

3.3. Antimicrobial multiresistance

It was found that 20 (1.7%) of the 1151 *Campylobacter* isolates were multi-drug resistant (resistant to all 3 antimicrobials). This multi-drug resistance was observed predominantly in *C. coli* (19/20