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Porcine carcasses as an underestimated source of antimicrobial resistant *Campylobacter coli*

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Abstract

Introduction: Campylobacteriosis is the most common human foodborne bacterial infection worldwide and is caused by bacteria of the *Camplylobacter* genus. The main source of these bacteria is poultry, but other food-producing animals such as pigs are also responsible for human infections. An increasing number of strains with resistance to fluoroquinolones and other antimicrobials such as macrolides were recently noted. The aim of the study was to investigate *Campylobacter* contamination of porcine carcasses and determine the antimicrobial resistance of the obtained isolates. **Material and Methods:** A total of 534 swabs from carcasses of pigs slaughtered in Poland during 2019–2022 were tested for *Campylobacter* spp. **Results:** *Campylobacter* was detected in 164 (30.7%) carcasses; among them 149 (90.8%) were classified as *C. coli* and the remaining 15 (9.2%) samples were *C. jejuni*-positive. Because a low number of *C. jejuni* isolates were identified, only the *C. coli* isolates were subjected to antimicrobial resistance analysis. The majority of these isolates were resistant to streptomycin (94.0%), ciprofloxacin (65.8%) and tetracycline (65.1%). A total of 94 (63.1%) strains displayed antimicrobial multiresistance patterns and were mainly resistant to fluoroquinolones, aminoglycosides and tetracyclines (74; 49.7% of the isolates tested). **Conclusion:** The obtained results showed that pig carcasses may be contaminated with antimicrobial-resistant *C. coli*.

Keywords: *Campylobacter*, *C. coli*, pig carcasses, prevalence, antimicrobial resistance.

Introduction

Campylobacteriosis is the most common human bacterial infection worldwide resulting from ingestion of contaminated food, mainly of animal origin (8). According to the recent European Union (EU) One Health 2021 Zoonoses Report, 127,840 *Campylobacter* infection cases were confirmed in 27 Member States, corresponding to a notification rate of 41.1 per 100,000 population (8). In the same year, there were only 616 cases in Poland; however, it is probable that the majority of infections were not reported. In USA, it is estimated that 2.1–2.4 million cases of human campylobacteriosis occur every year (4).

It has been shown that the main source of *Campylobacter* bacteria is poultry, but other food-producing animals, *i.e.* cattle and pigs, are also responsible for several human infections because they harbour *C. jejuni* and *C. coli*, respectively (8). *Campylobacter jejuni* is the species most often identified as responsible for disease in humans; however, *C. coli* infection cases may be under-reported, and should be also considered for their public health impact (8, 12, 13). These bacteria are transmitted through carcasses contaminated with faeces at slaughter, mainly during evisceration of animals. The presence of *Campylobacter* spp. in pork meat and meat products (33). There is less information on the prevalence of *Campylobacter* in porcine carcasses than in chicken carcasses, although a high incidence of these bacteria in pigs and pig production environments has been documented (14, 20, 25, 29, 30).

The European Union is a significant pig producer, where 148 million pigs were farmed in 2020. Of these, 11.4 million were grown reared in Poland (https://ec.europa.eu/eurostat). In the same year, around 260 million pigs were slaughtered in the EU and the overall production of pig meat was 23.8 million tonnes, including approximately 1.97 million tonnes in Poland (https://ec.europa.eu/eurostat). Since pigs are considered an important reservoir of *Campylobacter* spp. (mainly *C. coli*), with the bacteria's prevalence estimated at 50–100%, it is important to assess the level of porcine carcass contamination as a potential risk for human infection (14, 20, 30).

Campylobacteriosis is usually self-limiting after 3–5 days, but in immunocompromised individuals it can spread into the bloodstream and become potentially lethal (15, 36). The self-limiting nature does not, however, obviate of the need for therapy in very young or elderly patients, in pregnant women, and in infections with bacteraemia, antibiotics and especially macrolides (erythromycin – ERY) or fluoroquinolones (ciprofloxacin – CIP) being the drugs of first choice (27). Tetracyclines have been suggested as an alternative choice for the treatment of clinical campylobacteriosis, but are rarely applied in clinical practice (23, 39).

The emergence of antimicrobial-resistant bacterial pathogens, including *Campylobacter* species, has been attributed to the intensive use of antimicrobials in swine production (1). Several studies noted an increasing number of strains with resistance to fluoroquinolones and macrolides (6, 11, 19, 22, 25, 29, 30). For the resistance of *Campylobacter* spp. to fluoroquinolones, the World Health Organization (WHO) raised them to high priority as antimicrobial-resistant bacterial pathogens (42). It was also shown that antimicrobial resistance (AMR) is very common in *Campylobacter* spp. isolated from food-producing animals in many European countries (7). In particular, a high level of resistance was shown to ciprofloxacin, nalidixic acid and tetracycline, most notably by *C. coli* (7). Moreover, an increasing trend was observed of multidrug resistance (MDR) in these bacteria (7, 23, 27, 28). Thus, resistance to both macrolides and fluoroquinolones is one of the major public health concerns in *Campylobacter* infections.

The aim of the present study was to investigate *Campylobacter* contamination of porcine carcasses and determine the antimicrobial resistance of the obtained isolates to assess their possible threats to public health.

Material and Methods

Porcine carcasses. A total of 534 porcine carcasses were used for the study. The samples were collected during 2019–2022 by official veterinarians in commercial abattoirs located in disparate parts of Poland. The number of samples was calculated based on the number of cattle and pigs slaughtered in each of 16 voivodeships (Polish administrative provinces) according to the monitoring plan for *Campylobacter* prepared by the Polish National Reference Laboratory at the National Veterinary Research Institute in Puławy. The samples were collected from pig carcasses after exsanguination but prior to chilling by swabbing two brisket areas of 100 cm^2 each with two sterile sponges premoistened in 10 mL of buffered peptone water (Thermo Fisher Scientific, Waltham, MA, USA), rubbing 10 times vertically and 10 times horizontally as described previously (38). This sampling process was applied to each pig half carcass. The four sponges from the whole porcine carcass were then placed in a plastic bag, tagged, and immediately transported to the laboratory refrigerated at 1–8°C.

Isolation of *Campylobacter*. Isolation of bacteria was performed using the ISO standard procedure as described previously (38). Briefly, the four sponges used for sampling of one porcine carcass were put into 200 mL of Maximum Recovery Diluent (0.1% peptone, 0.85% NaCl; Oxoid, Basingstoke, UK), stomached for 3 min, and centrifuged at 1,000 g for 15 min at 5°C. The pellet was resuspended in 100 mL of Bolton Selective Enrichment Broth (Oxoid) and cultured for 48 h at 41.5°C under microaerobic conditions in the CampyGen Atmosphere Generation System (Oxoid). The bacteria were subsequently grown on Karmali Agar Base with Skirrow *Campylobacter* Selective Supplement (Oxoid) and *Campylobacter* Blood-Free Selective Agar Base (Oxoid) with *Campylobacter* charcoal differential agar (CCDA) Selective Supplement (Oxoid) under the same conditions. One morphologically typical *Campylobacter* colony from each carcass sample was confirmed by PCR and identified as *C. jejuni* or *C. coli* as described (35). Isolates classified as *C. coli* were selected and stored in cryotubes (Oxoid) at –80°C for antimicrobial resistance analysis.

Antimicrobial resistance. The antimicrobial resistance analysis of the isolated *C. coli* was tested essentially as previously described (38). A microbroth dilution method was used to establish the minimum inhibitory concentrations (MICs) of the isolates to antimicrobial agents using the Sensititre custom susceptibility EUCAMP plates (Trek Diagnostics, East Grinstead, UK). The obtained results were evaluated using a Vizion system (Trek Diagnostics). The following antimicrobials belonging to different antimicrobial classes were used: gentamycin and streptomycin (GEN and STR – aminoglycosides), CIP – (a fluoroquinolone), tetracycline (TET), and ERY (a macrolide), respectively. The cut off values for the interpretation of the MIC results were in accordance with those of the European Committee on Antimicrobial Susceptibility Testing (www.eucast.org) and the EU Reference Laboratory for Antimicrobial Resistance (32). Multidrug resistance of the isolated *C. coli* was defined as resistance to at least three of the classes of antimicrobials used in the study (24).

Statistical analysis. Statistical analysis based on Pearson chi-squared tests with the appropriate correction for group size was performed as described previously (37). The accepted significance level was $P < 0.05$.

Results

Prevalence of *Campylobacter*. Among 534 porcine carcasses tested, 164 (30.7%) were positive for *Campylobacter* spp. as isolated by the ISO method and identified by PCR (16, 35). The vast majority of the samples were classified as *C. coli*-positive (149; 90.8%)

whereas the remaining 15 (9.2%) were *C. jejuni*-positive (Table 1). *Campylobacter* spp.-positive carcasses were identified in each year of the study, although the prevalence of such samples were different in each year, ranging from 21.4% in 2020 to 32.1% in 2019 (Table 1). These differences were not statistically significant ($P > 0.05$).

Antimicrobial resistance of *C. coli*. Only *C. coli* isolates (n = 149) were subjected to antimicrobial resistance analysis because a low number of *C. jejuni* isolates were identified during the study. The results showed that irrespective of the year of isolation, the vast majority of isolates were resistant to STR (94.0%) (Table 2). Most of the *C. coli* isolates also showed resistance to CIP (65.8%) and TET (65.1%). Some of the isolates were resistant to ERY (14.1%), and only one strain, isolated in 2021, was resistant to GEN. Fourteen (9.4%) of the isolates tested were simultaneously resistant to two antimicrobials – ERY and CIP.

Antimicrobial multiresistance of *C. coli*. Among all 149 *C. coli* isolates tested, 94 (63.1%) displayed an AMR pattern, *i.e.* were resistant to at least three of the four classes of antibiotics tested in the study (Table 3). The majority of these isolates were simultaneously resistant to fluoroquinolones (CIP), aminoglycosides (STR) and tetracyclines (TET), there being 74 such (49.7% of the *C. coli* isolates tested and 78.7% of all multiresistant *C. coli* isolates). Such strains were identified during each year of the study and the differences in their prevalence were not statistically significant ($P > 0.05$) (Table 3). The second multiresistant *C. coli* profile, which was much less prevalent, was one of resistance to CIP, ERY, STR and TET. Ten (6.7%) tested strains had this profile, *i.e.* 10.6% of all multiresistant strains. The remaining ten *C. coli* isolates displayed simultaneous resistance to a different grouping of three (CIP, ERY and STR or ERY, STR and TET) or a different combination of four antimicrobials (CIP, ERY, GEN and STR), respectively (Table 3).

Table 1. Prevalence of *Campylobacter* spp. in porcine carcasses tested

Year of sampling	Number of carcasses		Number (%) of carcasses positive by *Campylobacter* spp.	
	Tested	Positive for *Campylobacter* spp. (%)	*C. coli*	*C. jejuni*
2019	53	18 (34.0)	17 (32.1)	1 (1.9)
2020	154	38 (24.7)	33 (21.4)	5 (3.2)
2021	165	55 (33.3)	50 (30.3)	5 (3.0)
2022	162	53 (32.7)	49 (30.2)	4 (2.5)
Total	534	164 (30.7)	149 (27.9)	15 (2.8)

Table 2. Antimicrobial resistance of *C. coli* isolated from porcine carcasses

Antimicrobials		Number (%) of *C. coli* resistant isolates				
		Year of isolation (number of isolates)				
		2019 (n = 17)	2020 (n = 33)	2021 (n = 50)	2022 (n = 49)	Total (n = 149)
Aminoglycosides	gentamycin	0	0	1 (1.7)	0	1 (0.7)
	streptomycin	14 (82.3)	30 (76.9)	49 (86.0)	47 (90.4)	140 (94.0)
Fluoroquinolones	ciprofloxacin	10 (58.8)	22 (66.7)	31 (62.0)	35 (71.4)	98 (65.8)
Tetracyclines	tetracycline	12 (70.6)	26 (78.8)	24 (48.0)	35 (71.4)	97 (65.1)
Macrolides	erythromycin	3 (17.6)	1 (2.6)	8 (14.0)	9 (17.3)	21 (14.1)

Table 3. Antimicrobial multiresistance profiles of *C. coli* isolated from porcine carcasses

Antimicrobial resistance profile	Number (%) of multiresistant isolates				
	Year of isolation (number of isolates)				
	2019 (n = 17)	2020 (n = 33)	2021 (n = 50)	2022 (n = 49)	Total (n = 149)
CIP + STR + TET	6 (35.3)	19 (48.7)	24 (48.0)	25 (51.0)	74 (49.7)
CIP + ERY + STR + TET	0	1 (3.0)	3 (6.0)	6 (12.2)	10 (6.7)
CIP + ERY + STR	2 (11.8)	0	5 (10.0)	0	7 (4.7)
ERY + STR + TET	0	0	1 (1.7)	1 (2.0)	2 (1.3)
CIR + ERY + GEN + STR	0	0	1 (1.7)	0	1 (0.7)

Discussion

Pork and pork meat products are currently coming under consideration more often as potential sources of *Campylobacter* infection in humans (8, 20, 30). Transmission of these bacteria to porcine carcasses is from intestinal waste and usually takes place during slaughtering, particularly in the process of evisceration (14). Pigs are usually carriers of *C. coli* and this *Campylobacter* species has the potential to induce human campylobacteriosis, although its potential seems lower than that of *C. jejuni* (12, 13, 18). As stated in the recent European Food Safety Authority (EFSA)/ European Centre for Disease Prevention and Control (ECDC) zoonotic report, *Campylobacter* genus bacteria isolated from humans in Poland in 2021 were not identified to species level; therefore, how many of them were classified as *C. coli* cannot be known (8).

Investigations to determine the *Campylobacter* prevalence in porcine carcasses were performed by several researchers (14, 20, 25, 29, 30). Previous similar studies were also conducted by our group in Poland and revealed that the percentage of such positive carcasses ranged from 26.0% through 30.4% to 36.3% (38, 40, 41). In the present investigation, 30.7% of pig carcasses were *Campylobacter*-positive as tested with a similar method to that applied in the previous studies. Among the contaminated samples, the vast majority of them were positive for *C. coli* (90.8%), which was a similar finding to that made during the previous studies, where the percentages of such carcasses were from 75.3% to 93.8% (40, 41).

Abley *et al.* (2) investigated the prevalence of *Campylobacter* spp. at different stages of the pig slaughter process in the USA and found that all of the 100 porcine carcasses tested were contaminated with these bacteria. However, species identification of the isolates was not performed. In other studies in the USA, Quintana-Hayashi and Thakur (30) tested the prevalence of bacteria of the *Campylobacter* genus in pig carcasses from conventional and antimicrobial-free production systems and found that 27.9% and 73.1% samples were positive, respectively. Most isolates were classified as *C. coli*; the 98.3% proportion made up by this species was higher than in the present investigation. Furthermore, Thakur and Gebreyes (34) found that among 757 pig carcasses investigated during 2002–2005, 144 (19.0%) were *C. coli*-positive.

A survey performed in China revealed that from 2.8% to 42.5% of samples collected from a pig slaughtering line were positive for *Campylobacter* spp., including 29.4% of samples taken after evisceration and determined to have been contaminated at this stage. Sampling in this study was performed at a similar stage to the stage in the present investigation (14). However, these isolates were not classified into species. A broad investigation of the presence of *Campylobacter* species in pork carcasses in Belgium revealed that during 2004–2009, percentages of contaminated samples ranged from 5.0% to 16.6%, which was much lower that detected in the present study (26). Marotta *et al.* (25) tested 178 pig carcasses in Italy for *Campylobacter* spp. using the ISO 10272-1:2017 method and found that 53.4% of them were contaminated, which was a higher value than the 30.7% identified in the current investigation. At a 96.8% proportion, the vast majority of the positive samples were classified as *C. coli*, Marotta *et al.*'s result being a higher percentage than the 90.8% obtained by us. Scanlon *et al.* (31) investigated 401 swabs from pig carcasses in Ireland using a very similar method to that applied during the present study, and found that only 42 (10.5%) samples were positive for bacteria of the *Campylobacteraceae* family, including 7 classified as *C. coli* (1.7%). This level of prevalence was much lower than that identified in the present study.

These differences in the prevalence of *Campylobacter* spp. in pig carcasses between the current study and previous investigations performed by other groups may be due to different sampling and analysis protocols, study sizes, geographical locations, pig rearing systems and antibiotic use patterns during animal growth.

Several investigations on *Campylobacter* from animals and food of animal origin as well as from humans with campylobacteriosis showed that high percentages of isolates were resistant to several antimicrobials, including those used for treatment of the disease, *e.g.* fluoroquinolones or macrolides (6, 7, 19, 22, 25, 26, 28–30). In the present study, the majority of *C. coli* displayed high resistance to streptomycin (an aminoglycoside) and tetracycline, and lower resistance to ciprofloxacin (a fluoroquinolone). Similar results were observed in our previous investigations performed in Poland (38, 40, 41). This information is important in relation to the effectiveness of the treatment of human infections with *C. coli*, because fluoroquinolones are one of the drugs of choice used in campylobacteriosis (43). According to the recent EFSA/ECDC antimicrobial resistance report, *C. coli* isolates of human origin identified in the EU in 2020 were mainly resistant to TET (74.0% of 1,502 isolates) and CIP (65.8% of 1,566 isolates). Some isolates displayed resistance to ERY (10.0% of 1,567) and GEN (1.3% of 1,069) (7). At the same time, 7.5% of *C. coli* isolates showed a multiresistance pattern (simultaneous resistance to fluoroquinolones, macrolides, tetracyclines and aminoglycosides), whereas 12.5% of strains were susceptible to all these antimicrobials (7). Interestingly, 9.0% of strains displayed combined resistance to two important antibiotics – ERY and CIP (7).

During our previous study on the antimicrobial resistance of *C. coli* of porcine carcass origin, it was shown that most of the isolates were resistant to STR (86.0%), TET (79.3%) and CIP (70.2%) (38). This shows that the level of resistance to streptomycin remained very similar among the isolates recovered during 2014–2018 and 2019–2022, although in the first period the number of samples was lower than the

number currently tested (38). On the other hand, in relation to the other two antimicrobials (TET and CIP), the percentages of resistant *C. coli* decreased over time from 79.3% to 65.1% and from 70.2% to 65.8%, respectively, although in both cases resistance remained high (38). It has been suggested that the prevalence of quinolone- and tetracycline-resistant *Campylobacter* isolates may be due to the broad use of these antimicrobials in food-producing animals (including pigs) for therapeutic purposes rather than for other purposes, because antibiotics have been banned as growth promoters in the EU since 2006 (10, 11). As shown in the recent European Medicines Agency report, in Poland as much as 36.9 mg of tetracyclines per population correction unit (PCU) and 12.7 mg of fluoroquinolones per PCU were sold for veterinary use in 2021 respectively (9). While not all of these antimicrobials were used for pig treatment, such large amounts given to animals might have an influence on the antimicrobial resistance development of *C. coli* tested.

In relation to ERY, another drug of choice for treatment of humans infected with *Campylobacter* spp., much lower percentages of previously and currently tested *C. coli* were resistant, i.e. 9.9% and 14.1%, respectively (38). However, an increasing trend in resistance to this antibiotic emerged and it may have a negative influence on its effectiveness against *Campylobacter* infections in humans and have potential public health implications. As has been described before, there is a clear association between the use of antimicrobials in food animals and resistance rates of *Campylobacter* species infecting humans (3, 17, 21, 24).

Information on the antimicrobial resistance of *C. coli* from porcine carcasses provided by other authors shows various rates depending on the geographical region and the date of the study. Choi *et al.* (6) tested 643 strains from pigs and pig carcasses in Korea and revealed a higher resistance rate (88.8%) to CIP, TET (78.4%), and especially to ERY (39.2%) than identified in the current study. Such a high percentage of erythromycin-resistant *C. coli* may be associated with massive use of this antimicrobial in pig husbandry in Korea (5). In a study performed in Ghana, among 37 isolates from pig carcasses, 35% and 60% were resistant to CIP and TET, respectively (19). Interestingly, all isolates were resistant to ERY. However, the *Campylobacter* species were not disclosed. An investigation conducted in Italy on *C. coli* isolated from pigs and pig carcasses showed that the strains displayed higher resistance rates to antimicrobials than those noted in the present study, especially to ERY (36.5% of isolates with resistance), TET (89.9%) and CIP (72.5%), (25). In the USA in contrast, among 158 *C. coli* from pig carcasses, only 15.2% of isolates were resistant to CIP and 49.9% to TET (30). At the same time, 20.9% of isolates displayed resistance to ERY, which was a higher rate than that identified during our investigation.

European data collated from a broad range of locations on the resistance of 1,174 *C. coli* isolated in 2019 from pigs (but not from porcine carcasses) and provided in the EFSA/ECDC report revealed that the majority of strains were resistant to STR (70.0%), TET (62.8%) and CIP (51.9%), whereas lower resistance rates were found for ERY (11.2%) and GEN (1.8%) (7). Additionally, 8.0% of those *C. coli* isolates showed resistance to both of two important antimicrobials used in human medicine, *i.e.* ERY and CIP. In the present study such simultaneously resistant isolates were 9.4% of the total isolates of the species. It is relevant to note that no relevant data from Poland was provided in this EFSA/ECDC report.

As shown in the present study, 63.1% of *C. coli* isolates showed multiresistance patterns, *i.e.* were resistant to antimicrobials of at least three classes (32). Most of them were CIP + STR + TET-resistant, but some strains (12.1%) were simultaneously resistant to critically important antibiotics for the treatment of *Campylobacter* infections in humans with frequent application in patients with developed campylobacteriosis – ERY and CIP (43). The presence of such isolates along the pig food chain is important from a public health point of view, because pork is considered a potential source of campylobacters and the consumption of such meat in Poland is considerable, being estimated at 43.4 kg *per capita* in 2021 (www.statista.com).

The incidence of *C. coli* isolates with AMR from porcine carcasses was also identified in our previous study. More than half of such strains were resistant to CIP + STR + TET, 63 out of 121 isolates and 52.1% being so, which was a similar rate to that found in the current investigation (38). However, 6.6% of the strains were also resistant to ERY + CIP and TET + STR. This resistance profile was also identified in 10 (6.7%) *C. coli* isolates during the present study.

Campylobacter coli of pork carcass or pig origin resistant to several antimicrobials were also identified by other researchers. Choi *et al.* (6) found 83.3% of such *C. coli* strains to have AMR in Korea, whereas Lopez-Chavarrias *et al.* (22) identified 40% of isolates to be resistant to antibiotics classified to three or more classes, with the most common profile being CIP + TET + STR + ERY, the same as identified in the current investigation. High percentages of multiresistant *C. coli* from pig carcasses were also identified in Italy, where the most common profiles were CIP + STR + TET (56% of strains) and CIP + ERY + STR + TET (29%) (25). Strains with the same antimicrobial profiles were also detected in our study (49.7% and 6.7%, respectively).

The obtained results showed that pig carcasses may be contaminated with a relatively high level of *C. coli*, which may suggest that this kind of food may be an underestimated source of these bacteria for consumers. Although *C. coli* seems to be less virulent than *C. jejuni*, the high incidences of resistance of such isolates to antibiotics used in the treatment of *Campylobacter* infections in humans and the existence of strains with multiple resistance patterns to several classes of antimicrobials, including erythromycin and

fluoroquinolones, may pose a public health risk. Therefore, the findings highlight the need for proper hygienic practices to prevent the spread of antimicrobial-resistant strains of *C. coli* along the food chain. Furthermore, there is a need for prudence in the use of antimicrobials in food animals and monitoring of antimicrobial resistance among *Campylobacter* isolates originating from pigs and swine carcasses.

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