Erythromycin-resistant *Campylobacter coli* from slaughtered animals as a potential public health risk

K. Wieczorek, I. Kania, J. Osek

National Veterinary Research Institute, Pulawy, Poland

ABSTRACT: Erythromycin-resistant *Campylobacter* were isolated from pig, cattle, and poultry carcasses slaughtered in Poland between 2008 and 2011. A total of 1335 strains were examined and among them 20 (1.5%) showed a high level of erythromycin resistance (≥ 32 mg/l) as determined by the microbroth dilution method. All these isolates were *C. coli* and mainly originated from poultry (15 strains). PCR amplification or DNA sequencing identified the mutation A2075G in the *23S rRNA* gene in all strains tested. The vast majority of such *C. coli* were also resistant to quinolones, tetracyclines, and streptomycin whereas none of them revealed resistance to gentamycin. Furthermore, several isolates (14; 70.0%) displayed multi-resistance pattern against quinolones, aminoglycosides, and tetracyclines. PCR analysis identified several putative virulence genes such as *cadF*, *flaA*, and *iam* (present in all erythromycin resistant isolates) as well as the *cdtA* and *flhA* markers (19 and 16 strains, respectively) among *C. coli* tested. On the other hand, only two out of 20 isolates were positive for the *ciaB* and *docA* genes. Furthermore, none of the analysed strains had the *virB11* and *wlaN* markers. A molecular relationship determination of the erythromycin-resistant *C. coli* performed by pulsed field gel electrophoresis (PFGE) revealed 17 different types. This reflects the high genetic diversity among the examined isolates. The results obtained suggest that erythromycin-resistant *C. coli* from food-producing animals may represent an underestimated potential health risk for consumers.

Keywords: *Campylobacter*; carcasses; erythromycin resistance; molecular characteristics; public health

Campylobacter is recognised as a major cause of bacterial gastroenteritis in humans worldwide and has been isolated from a diverse range of domestic and wild animals, especially poultry (Scallan et al. 2011; Anonymous 2012a). Macrolides, such as erythromycin, are regarded as drugs of choice for the treatment of severe human intestinal infections or immuno-compromised patients (Gibreel and Taylor 2006). Resistance of *Campylobacter* clinical isolates to this antimicrobial group is relatively rare as compared to other antimicrobials and it is mainly found among *C. jejuni*; however, some strains, especially *C. coli* originating from food, show a high resistance rate (Anonymous 2012b). Several molecular mechanisms have been described as being responsible for macrolide resistance in *Campylobacter* and among them the point mutations in domain V of the *23S rRNA* target gene at positions 2074 and 2075 are the most common ones. It has also been shown that the mutation A2075G is usually responsible for a high level of resistance to erythromycin (Alonso et al. 2005; Gibreel and Taylor 2006).

The increasing resistance among *Campylobacter* to macrolides, especially combined with resistance to quinolones, is recognised as an emerging public health problem. Moreover, it was shown that human infections caused by macrolide or quinolone resistant *Campylobacter* are associated with increased risk of adverse events or development of the invasive form of the disease compared to infections with susceptible isolates (Gibreel and Taylor 2006).

Several putative virulence markers have been described in *Campylobacter*; however, the pathogenesis of infection is still not well defined (Tam et al. 2003). Virulence properties such as flagella-mediated motility, adherence to intestinal epithelial

Supported by the National Science Centre, Krakow, Poland (Grant No. NN 308 237636).

cells, invasion and survival in the host cells as well as the ability to produce toxins are important in the development of human campylobacteriosis (Tam et al. 2003).

The aim of the present study was to investigate the prevalence of erythromycin-resistant *Campylobacter* among strains isolated from food producing animals. Furthermore, the molecular background of such resistance and genetic relationship of the strains were also investigated. Moreover, the potential risk for public health of such isolates was characterised by investigating antibiotic resistance profiles and the presence of genes putatively associated with virulence.

MATERIAL AND METHODS

Sample collection. A total of 1802 swabs were collected from poultry carcasses after cooling at the slaughter level all over Poland between 2008 and 2011 and immediately transported to the laboratory in Amies transport medium with charcoal (Medlab, Szczecin, Poland). The swabs were put into 5 ml of Bolton broth plus 5% leaked horse blood and modified Bolton broth-selective supplement containing the following antimicrobials: vancomycin, cefoperazone, trimethoprim, and amphotericin B to prevent non-target microbials (Oxoid, Basingstoke, UK) and then incubated for 48 h at 41.5 °C under microaerobic conditions using the CampyGen kit (Oxoid). *Campylobacter* isolation and identification were performed according to the ISO 10272-1: 2006 standard. From each sample one isolate classified as potentially *Campylobacter* was further identified using PCR methods as described earlier (Wieczorek 2010).

The cattle (n = 624) and pig (n = 187) carcasses tested in the same period as poultry samples were swabbed at the brisket area using sterile sponges. To each swab, 200 ml of maximum recovery dilution (Oxoid) was added followed by stomaching for 3 min. After centrifugation at 1000 × g for 15 min, pellets were re-suspended in 100 ml of selective enrichment Bolton broth. The enrichment cultures were grown for 48 h at 41.5 °C under microaerobic conditions and then plated onto Karmali agar (Oxoid) and *Campylobacter* blood free agar (Oxoid) with CCDA-selective supplement (Oxoid) followed by re-incubation under the previously described conditions for 48 h. The bacterial isolates were stored at –80 °C until further analysis.

PCR assays. One bacterial isolate from each positive sample was tested using PCR. A bacterial colony was suspended in 1 ml of sterile water and centrifuged at 13 000 × g for 1 min. DNA was extracted using the Genomic-Mini kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instruction. *Campylobacter* species were identified using multiplex PCR (m-PCR) with three sets of primers specific for the simultaneous detection of the *C. jejuni* (the *mapA* gene target), *C. coli* (*ceuE* gene), and *Campylobacter*-specific *16S rRNA* gene as described previously (Wieczorek 2010). Furthermore, in cases of doubtful results, a second m-PCR was applied to identify the species-specific *hipO* and *23S rRNA* (*C. jejuni*), *glyA* (*C. coli*, *C. lari*, and *C. upsaliensis*), and *sapB2* genes (*C. fetus* subsp. *fetus*), respectively (Wang et al. 2002).

Campylobacter isolates were also tested for the presence of the most often described putative virulence genes: *flaA*, *flhA*, *cadF*, *docA*, *cdtA*, *cdtB*, *cdtC*, *ciaB*, *iam*, *wlaN*, and *virB11*. The PCR conditions for all amplification reactions were exactly the same as previously described (Wieczorek 2010).

Antimicrobial susceptibility. A microbroth dilution method using the Sensititre® custom susceptibility plates, EUCAMP (Trek Diagnostics, East Grinstead, UK) was applied to establish the

Table 1. Antimicrobials, dilution ranges and cut-off values used for MIC

Antimicrobial class	Antimicrobials	Dilution range (mg/l)	Cut off values (mg/l)	
			C. jejuni	*C. coli*
Aminoglycosides	gentamycin (GEN)	0.12–16	1	2
	streptomycin (STR)	1–16	2	4
Macrolides	erythromycin (ERY)	0.5–32	4	16
Quinolones and Fluoroquinolones	ciprofloxacin (CIP)	0.06–4	1	1
	nalidixic acid (NAL)	2–64	16	32
Tetracyclines	tetracycline (TET)	0.25–16	2	2

minimum inhibitory concentrations (MICs) of six antimicrobial agents for *Campylobacter* isolates. Antimicrobials, dilution ranges and cut-off values used for MIC determination are described in Table 1. The strains were sub-cultured 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 antimicrobials and cut-off values used for the interpretation of the MIC results were in accordance with the values provided by EUCAST (www.eucast.org) and the European Union Reference Laboratory for Antimicrobial Resistance.

PFGE analysis. All *Campylobacter* isolates were typed by pulsed field gel electrophoresis (PFGE) using the standard operating procedure of PulseNet (Ribot et al. 2001). Briefly, the plugs were prepared from 400 µl of bacterial suspensions to which 20 µl proteinase K (20 mg/ml) (Sigma-Aldrich, St. Louis, MO, USA) and 400 µl of Seakem Gold Agarose (Lonza, Allendale, NJ, USA) in TE buffer (10mM Tris, 1mM EDTA, pH 8.0) were added. Bacterial cell lysis was performed with 50mM Tris, 50mM EDTA, pH 8.0 + 1% Sarcosyl and 0.1 mg proteinase K per ml. The DNA was digested with *Sma*I enzyme (Fermentas, Vilnius, EU), 40 IU at 25 °C for 4 h. *Salmonella* Braenderup H9812 was used as the molecular weight standard. PFGE was performed using the CHEF DR II System (Bio-Rad, Hercules, CA, USA) with the following parameters: initial switch time of 6.8 s, final switch time of 35.4 s for 18 h at 6 V and 14 °C in 0.5 × TBE buffer (Sigma). The gels were stained with ethidium bromide (5 µg/ml) for 15–20 min and the DNA banding pattern was captured with the Gel Doc 2000 system (Bio-Rad).

Reference strains. The following reference strains were included in the study: *C. jejuni* ATCC 33560, *C. coli* ATCC 43478, and *S.* Braenderup H9812 ATCC BAA-664.

Data analysis. BioNumerics software version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium) was used for analysis of PFGE fingerprinting profiles. Dendrograms were generated based on the Dice correlation co-efficient for similarity and the unweighted-pair group method with arithmetic means (UPGMA) was employed for cluster analysis.

RESULTS

A total of 2613 swabs from poultry, cattle, and pig carcasses were screened for the presence of *Campylobacter*. Altogether, 1335 samples (51.1%) were positive for *Campylobacter*, 716 for *C. jejuni* and 619 for *C. coli* (Table 2). It was found that 20 (1.5%) strains, all of them *C. coli*, were resistant to erythromycin as determined by the microbroth dilution method. The isolates were mainly recovered from poultry carcasses (15 strains, 75.0%) followed by pigs (four isolates) and cattle (one strain). All isolates displayed a high level of resistance, i.e., the MIC value was ≥32 mg/l. PCR amplification identified the mutation A2075G in the *23S rRNA* gene in all but one erythromycin-resistant *Campylobacter* tested. However, the PCR-negative isolate was also confirmed to possess this mutation by DNA sequencing.

The vast majority of *C. coli* strains examined (19 out of 20 isolates; 95.0%) were also resistant to quinolones (ciprofloxacin and nalidixic acid), tetracyclines (17 strains; 85.0%), and streptomycin (15 isolates; 75.0%) whereas none of these strains revealed resistance to gentamycin (Table 3). The most common (14 isolates; 70.0%) multi-resistance

Table 2. Erythromycin-resistant *Campylobacter* isolates identified in the study

Source*	Number of strains tested		Number (%) of strains resistant to erythromycin	
	C. jejuni	*C. coli*	*C. jejuni*	*C. coli*
Poultry	623	519	0	15 (2.9)
Cattle	83	61	0	1 (1.6)
Pig	10	39	0	4 (10.2)
Total	716	619	0	20 (3.2)
Altogether	1335		(1.5)	

*the isolates were recovered from carcasses during the slaughter process

Table 3. Characteristics of erythromycin-resistant *C. coli* strains

Isolate number	Origin	Pulsotype	Virulence gene markers											Antimicrobial resistance				
			cadF	*cdtA*	*cdtB*	*cdtC*	*ciaB*	*docA*	*flaA*	*flhA*	*iam*	*virB11*	*wlaN*	CIP	NAL	GEN	STR	TET
82R	cattle	14	+	+	+	+	+	+	+	+	+	–	–	R*	R	S	R	R
68P	pigs	1	+	+	+	+	–	–	+	+	+	–	–	R	R	S	R	R
86P		5	+	+	+	+	–	–	+	+	+	–	–	R	R	S	R	R
88P		1	+	+	+	+	–	–	+	+	+	–	–	R	R	S	R	R
1233P		11	+	+	+	–	–	–	+	+	+	–	–	R	R	S	S	S
174G	poultry	16	+	+	+	+	+	+	+	+	+	–	–	R	R	S	R	R
175G		17	+	–	–	–	–	–	+	+	+	–	–	R	R	S	R	R
76P		4	+	+	+	–	–	–	+	–	+	–	–	R	R	S	R	R
609P		6	+	+	+	–	–	–	+	+	+	–	–	R	R	S	S	R
646P		3	+	+	+	–	–	–	+	+	+	–	–	R	R	S	R	R
800P		7	+	+	+	–	–	–	+	+	+	–	–	R	R	S	R	R
801P		3	+	+	+	–	–	–	+	–	+	–	–	R	R	S	R	R
802P		8	+	+	+	–	–	–	+	+	+	–	–	R	R	S	R	R
964P		9	+	+	+	+	–	–	+	+	+	–	–	R	R	S	R	S
1107P		10	+	+	+	–	–	–	+	+	+	–	–	R	R	S	R	R
1361P		12	+	+	+	–	–	–	+	+	+	–	–	R	R	S	R	R
1515P		13	+	+	+	–	–	–	+	–	+	–	–	S	S	S	S	S
1518P		2	+	+	–	–	–	–	+	–	+	–	–	R	R	S	S	R
1519P		2	+	+	–	–	–	–	+	+	+	–	–	R	R	S	S	R
123C		15	+	+	+	+	–	–	+	+	+	–	–	R	R	S	R	R

CIP – ciprofloxacin, GEN – gentamycin, NAL – nalidixic acid, STR – streptomycin, TET – tetracycline
*R = resistant, S = sensitive

pattern observed was against quinolones, streptomycin, and tetracycline (Table 3).

The PCR analysis revealed that all erythromycin-resistant *Campylobacter* strains had the *cadF*, *flaA*, and *iam* markers. Most of them also possessed the *cdtA*, *cdtB*, and *flhA* genes. On the other hand, only two out of 20 isolates were positive for the *ciaB* and *docA* genes. Furthermore, none of these strains had the *virB11* and *wlaN* genes (Table 3). It was also shown that the most common virulence gene pattern among erythromycin-resistant *C. coli* was: *cadF*$^{+}$, *cdtA*$^{+}$, *cdtB*$^{+}$, *cdtC*$^{-}$, *ciaB*$^{-}$, *docA*$^{-}$, *flaA*$^{+}$, *flhA*$^{+}$, *iam*$^{+}$, *virB11*$^{-}$, *wlaN*$^{-}$, identified in seven (35.0%) of the isolates (Table 3).

Determination of the molecular relationship of the erythromycin-resistant *C. coli* performed by PFGE with *Sma*I identified 17 different clonal types (with 95% similarity). This reflects the high genetic diversity among the examined isolates. Only three PFGE pulsotypes (numbers 1, 2, and 3) appeared more than once (Table 3). The strains with the common genetic profiles were isolated from the same sources (pig or poultry carcasses) and had the same resistance profile but slightly different virulence gene patterns (Table 3).

DISCUSSION

Several reports have documented the isolation and genetic characterisation of campylobacters from humans and food-producing animals (Van Deun et al. 2007; Wang et al. 2011; Egger et al. 2012; Mattheus et al. 2012). However, limited information is available on the drug resistance profiles and genotypic characterisations of erythromycin-resistant *Campylobacter* isolates. Such studies are important because drug-resistant strains from

food-producing animals may be a source of antibiotic resistance genes to other campylobacters, also those involved in human infections. The results of the present study demonstrated that only 1.5% of the 1335 *Campylobacter* isolates tested were resistant to erythromycin and the resistance mechanism was due to the point mutation in the domain V of the *23S rRNA* gene (Alonso et al. 2005; Gibreel and Taylor 2006). As described in a recent EFSA report, between 0.2% and 2% of *C. jejuni* and as many as 12% to 25% of *C. coli* isolates were resistant to erythromycin (Anonymous 2012b). The highest level of resistance to this antimicrobial was in *C. coli* from pigs, whereas only a small number of erythromycin-resistant *C. jejuni* of bovine origin were detected (Anonymous 2012b). Similar findings were made in the present study where only *C. coli* were resistant to erythromycin and a higher percentage of the resistant isolates were of porcine origin (10.2%) as compared to cattle (1.6%) and poultry (2.9%) (Table 2). Other studies also confirmed that more *C. coli* were resistant to macrolides, including erythromycin, as compared to *C. jejuni*, especially those recovered from pigs (Smole Mozina et al. 2011).

Recently, Quintana-Hayashi and Thakur (2012) performed a longitudinal study on the persistence of antimicrobial-resistant *Campylobacter* in pigs and found that as much as 20.9% of the isolates recovered from porcine carcasses were resistant to erythromycin. However, the bacterial species were not identified. Qin et al. (2011) analysed 190 *C. coli* of porcine origin and found that from 37.9% to 54.7% of the isolates were erythromycin-resistant. A similar resistance rate (46.5%) of *C. coli* recovered from porcine carcasses was identified in Korea (Shin and Lee 2007). In Europe, Pezzotti et al. (2003) isolated 45% out of 47 *C. coli* from pig carcasses that were resistant to erythromycin. Egger et al. (2012) identified 28 (10.9%) strains with the point mutation A2075G in the *23S rRNA* gene responsible for macrolide resistance. However, two of these isolates were sensitive to erythromycin when tested using the MIC method. As shown in the present study all 20 *C. coli* possessing the A2075G mutation were also phenotypically resistant in the MIC test. Moreover, further characterisation of erythromycin-resistant *C. coli* strains indicated that most of them (14 out of 20 isolates; 70.0%) displayed a multidrug resistant pattern, i.e., resistance to other antimicrobial classes – quinolones, aminoglycosides and tetracyclines. Other authors have also isolated several multidrug-resistant *Campylobacter* strains which confirms that such isolates, mainly *C. coli*, are common, especially among pigs but also poultry and cattle (Wang et al. 2011; Sanad et al. 2011; Schweitzer et al. 2011; Anonymous 2012b; Mattheus et al. 2012). Such data are important from a public health point of view since antimicrobial resistant *Campylobacter* in food-producing animals may act as a reservoir of resistance genes that may be disseminated to human strains.

The *Campylobacter* strains isolated in the present study were characterised for the presence of virulence and toxin genes. Despite the large amount of available data in this area it is difficult to compare the results due to the different genes examined, PCR primers used, number and origin of the samples. Generally, the prevalence of putative virulence genes among erythromycin-resistant *C. coli* identified in the present study was similar to other data. All strains tested possessed the *cadF*, *flaA*, and *iam* markers, which are involved in the adherence, motility, and invasiveness processes of *Campylobacter* infection, respectively. Furthermore, most isolates were also positive for the *cdtA* and *cdtB* markers responsible for toxin production as well as for the *flhA* gene involved in the motility of bacterial cells (Tam et al. 2003). On the other hand, none of the isolates possessed the *virB11* and *wlaN* markers that are possibly responsible for the invasion process of the host cells (Tam et al. 2003). These results are in agreement with several other studies in which virulence markers were identified in *Campylobacter* isolates of poultry, pig, cattle or human origin (Tam et al. 2003; Ripabelli et al. 2010; Wieczorek 2010; Egger et al. 2012; Quintana-Hayashi and Thakur 2012). It should be mentioned that the presence of particular virulence gene factors is not direct evidence that such strains are pathogenic for humans; however, it strongly suggests that they may be potentially able to induce disease.

The PFGE results from these studies showed that the *Sma*I PFGE patterns of erythromycin-resistant *C. coli* were the same only in the case of three strains, whereas the remaining 17 isolates displayed unique molecular profiles. A good correlation between PFGE patterns and virulence as well as antimicrobial resistance profiles was observed; i.e., *C. coli* with the same macrorestriction profile had identical drug resistance patterns and very similar virulence gene profiles (Table 3). On the other hand, such correlation among *Campylobacter* strains of different origin was less pronounced in

the studies of other authors (Thakur and Gebreyes 2005; Van Deun et al. 2007; Wang et al. 2011). It should be underlined that in the present study a limited number of isolated was tested. Since the PFGE technique has a high discriminatory power it may be less valuable for epidemiological studies among *Campylobacter* isolates when used alone. Thus, a combination of macrorestriction, antimicrobial resistant and virulence gene profiles as performed in the present study may be a valuable tool for determination of the origin and subsequent spread of *Campylobacter* isolates as well as their antimicrobial resistance genes.

In conclusion, a low percentage (1.5%) of *Campylobacter* among the strains recovered from slaughtered animals was resistant to erythromycin. However, the majority of these isolates revealed multi-antimicrobial resistance properties and possessed several virulence and toxin genes. Molecular analysis revealed that the mutation A2075G in the *23S rRNA* gene was responsible for erythromycin resistance in all isolates tested. Moreover, comparisons of the PFGE and virulence marker profiles of the strains reflected a high genetic diversity among the tested *Campylobacter*. All these findings suggest that *C. coli* from food-producing animals may represent an underestimated potential health risk for consumers.

Acknowledgement

The authors thank Dr. Kerstin Stingl (Federal Institute for Risk Assessment, Berlin, Germany) for DNA sequencing and valuable comments concerning the manuscript. financially

REFERENCES

Alonso R, Mateo E, Churruca E, Martinez I, Girbau C, Fernandez-Astorga A (2005): MAMA-PCR assay for the detection of point mutations associated with high-level erythromycin resistance in Campylobacter jejuni and Campylobacter coli strains. Journal of Microbiological Methods 63, 99–103.

Anonymous (2012a): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. The EFSA Journal 10, 2597.

Anonymous (2012b): The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010. The EFSA Journal 10, 2598.

Egger R, Korczak BM, Niederer I, Overesch G, Kuhnert P (2012): Genotypes and antibiotic resistance of Campylobacter coli in fattening pigs. Veterinary Microbiology 155, 272–278.

Gibreel A, Taylor DE (2006): Macrolide resistance in Campylobacter jejuni and Campylobacter coli. Journal of Antimicrobial Chemotherapy 58, 243–255.

Mattheus W, Botteldoorn N, Heylen K, Pochet B, Dierick K (2012): Analysis of antimicrobial resistance in Campylobacter jejuni and Campylobacter coli isolated from Belgian pork and poultry meat products using surveillance data of 2004–2009. Foodborne Pathogens and Disease 9, 465–472.

Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M, Perin R (2003): Occurrence and resistance to antimicrobials of Campylobacter jejuni and Campylobacter coli in animals and meat in northeastern Italy. International Journal of Food Microbiology 82, 281–287.

Qin SS, Wu CM, Wang Y, Jeon B, Shen ZQ, Wang Y, Zhang Q, Shen JZ (2011): Antimicrobial resistance in Campylobacter coli isolated from pigs in two provinces in China. International Journal of Food Microbiology 146, 94–98.

Quintana-Hayashi M, Thakur S (2012): Longitudinal study of the persistence of antimicrobial-resistant Campylobacter strains in distinct swine production systems on farms, at slaughter, and in the environment. Applied and Environmental Microbiology 78, 2698–2705.

Ribot EM, Fitzgerald C, Kubota K, Swaminathan B, Barret TJ (2001): Rapid pulsed-field gel electrophoresis protocol for subtyping of Campylobacter jejuni. Journal of Clinical Microbiology 39, 1889–1894.

Ripabelli G, Tamburro M, Minelli F, Leone A, Sammarco ML (2010): Prevalence of virulence – associated genes and cytolethal distending toxin production in Campylobacter spp. isolated in Italy. Comparative Immunolology, Microbiology and Infectious Diseases 33, 355–364.

Sanad YM, Kassem II, Abley M, Gebreyes W, LeJeune JT, Rajashekara G (2011): Genotypic and phenotypic properties of cattle-associated Campylobacter and their implications to public health in the USA. PLoS One, 10, e25778.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011): Foodborne illness acquired in the United States – major pathogens. Emerging Infectious Diseases 17, 7–15.

Schweitzer N, Dan A, Kaszanyitzky E, Samu P, Toth AG, Varga J, Damjanova I (2011): Molecular epidemiology and antimicrobial susceptibility of Campylobacter jejuni and Campylobacter coli isolates of poultry, swine,

and cattle origin collected from slaughterhouses in Hungary. Journal of Food Protection 74, 905–911.

Shin E, Lee Y (2007): Antimicrobial resistance of 114 porcine isolates of Campylobacter coli. International Journal of Food Microbiology 118, 223–227.

Smole Mozina S, Kurincic M, Klancnik A, Mavri A (2011): Campylobacter and its multi-resistance in the food chain. Trends in Food Science and Technology 22, 391–398.

Tam CC, O'Brien SJ, Adak GK, Meakins SM, Frost JA (2003): Campylobacter coli – an important foodborne pathogen. Journal of Infection 47, 28–32.

Thakur S, Gebreyes WA (2005). Campylobacter coli in swine production: antimicrobial resistance mechanisms and molecular epidemiology. Journal of Clinical Microbiology 43, 5705–5714.

Van Deun K, Haesebrouck F, Heyndrickx M, Favoreel H, Dewulf J, Ceelen L, Dumez L, Messens W, Leleu S, Van Immerseel F, Ducatelle R, Pasmans F (2007): Virulence properties of Campylobacter jejuni isolates of poultry and human origin. Journal of Medical Microbiology 56, 1284–1289.

Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Woodward DL, Rodgers FG (2002): Colony multiplex PCR assay for identification and differentiation of Campylobacter jejuni, C. coli, C. lari, C. upsaliensis, and C. fetus subsp. fetus. Journal of Clinical Microbiology 40, 4744–4747.

Wang X, Zhao S, Harbottle H, Tran T, Blickenstaff K, Abbott J, Meng J (2011): Antimicrobial resistance and molecular subtyping of Campylobacter jejuni and Campylobacter coli from retail meats. Journal of Food Protection 74, 616–621.

Wieczorek K (2010): Antimicrobial resistance and virulence markers of Campylobacter jejuni and Campylobacter coli isolated from retail poultry meat in Poland. Bulletin of the Veterinary Institute in Pulawy 54, 563–569.

Received: 2013–03–22
Accepted after corrections: 2013–07–07

Corresponding Author:

Jacek Osek, Department of Hygiene of Food of Animal Origin, National Veterinary Research Institute, Partyzantow 57, 24-100 Pulawy, Poland
Tel. +48 81 889 3182, E-mail: josek@piwet.pulawy.pl