Bull Vet Inst Pulawy 55, 51-54, 2011

TETRACYCLINE, ERYTHROMYCIN, AND GENTAMICIN RESISTANCE OF *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI* ISOLATED FROM POULTRY IN POLAND

ANNA WOŹNIAK AND ALINA WIELICZKO

Department of Epizootiology with Clinic of Birds and Exotic Animals,
Faculty of Veterinary Medicine,
Wroclaw University of Environmental and Life Sciences, 50-366 Wroclaw, Poland
alina.wieliczko@up.wroc.pl

Received: August 12, 2010 *Accepted: January 20, 2011*

Abstract

The aim of the research was the determination of resistance of 387 *Campylobacter* sp. strains isolated between 1994 and 1996 (broilers, laying hens) and 2005-2008 (broilers, laying hens, turkeys) to tetracycline, erythromycin, and gentamicin. The resistance was determined with the reference to minimum inhibitory concentration on the agar plates (MIC). Limit concentration value for tetracycline and gentamicin was measured at ≥8 μg/mL, while for erythromycin it was obtained at ≥16 μg/mL. Among the *Campylobacter* sp. isolates obtained in 2005-2008, tetracycline resistant strains were found, while the isolates obtained between 1994 and 1996 were tetracycline susceptible. An increase in the percentage of erythromycin intermediate susceptible strains from 2005-2008 was reported in comparison to the isolates obtained in 1994-1996. Gentamicin susceptibility was found in 99.5% of all the *Campylobacter* sp. strains isolated between 1994 and 2008. These findings reveal a broad extent of antimicrobial resistance in *Campylobacter* isolates from poultry in Poland and underline the need for prudent use of antibiotics in poultry production to minimise the spread of antibiotic resistant *Campylobacter* strains.

Key words: poultry, *Campylobacter jejuni*, *Campylobacter coli,* resistance to antibiotics.

Both *Campylobacter jejuni* and *Campylobacter coli* usually belong to the microorganisms isolated from cases of intoxication and alimentary infections in humans (13, 25). According to the Food and Drug Administration Agency (FDA), *C. jejuni* infections are reported more often than infections caused by *Salmonella* sp. or *Shigella* sp. (23). With accordance to EFSA report, the number of *Campylobacter* infections in humans in 2005 was higher in comparison to the number of *Salmonella* sp. infections, therefore 2005 was reported as "*Campylobacter* year" (12).

Campylobacter sp. is commonly present in normal intestine microflora of the slaughter animals, particularly often isolated from poultry. Human infections are usually a result of *Campylobacter* sp. contamination of raw poultry meat (8). The most important symptoms of human campylobacteriosis are diarrhoea, dizziness, and vomits. Most of the patients recover without treatment; however, acute or chronic symptoms require therapy (1, 4).

For modern poultry production, antimicrobial agents have been widely used for growth promotion and disease control. Many of the antimicrobials used for animal agriculture are also used for human medicine. Thus, agricultural use of antibiotics poses a risk of selecting antibiotic resistant pathogens that can be potentially transmitted to humans and may compromise clinical treatment. Indeed, previous studies have shown that use of certain antimicrobials in chickens, especially fluoroquinolones, rapidly select antibiotic-resistant *Campylobacter* sp. (17, 19). Many studies have reported the prevalence of antimicrobial-resistant *Campylobacter* sp. in animal reservoirs in different countries (3, 6, 14-16). However, little information is available on the prevalence and antimicrobial resistance of *Campylobacter* from poultry in Poland, where poultry production represents an important sector of animal husbandry and consumption of poultry meat is significant.

The aim of the following research was the assessment of resistance of *Campylobacter* sp. strains isolated in 1994-1996 and 2005-2008 to tetracycline, erythromycin, and gentamicin.

Material and Methods

Material. The research material covered 314 *Campylobacter* sp. strains isolated from poultry (broilers, laying hens and turkeys) between 2005 and 2008 and 73 *Campylobacter* sp. strains from strain museum in the Department of Birds Diseases at the Wroclaw University of Environmental and Life

Sciences. The museum strains were isolated from broilers and laying hens between 1994 and 1996. The strains were determined as *C. jejuni* and *C. coli*. The isolates were stored in bullion containing glycerol at -70°C.

Methods. The obtained material was inoculated directly onto mCCDA agar plates (modified Charcoal Cefoperazone Deoxycholate Agar, Oxoid, United Kingdom) with an addition of antibiotic such as cafoperazone and amphotericin B (CCDA Selective Suplement, Oxoid, United Kingdom), and incubated under conditions appropriate for *Campylobacter*. Then, the strains were biochemically typified with the usage of oxidase and catalase tests, and hippurate hydrolysis. Gram-stained preparations of the bacterial colonies were done. Detailed isolation, identification, and biochemical typing methods for the *Campylobacter* strains were presented in the earlier paper (26).

Multiplex PCR. Multiplex PCR was used for the *C. jejuni* and *C coli* species affiliation. Genetic material was isolated using Genomic - Mini® kit (A & A Biotechnology, Poland) with the accordance to the producer's recommendation. The examination was performed in 25 µl reaction mixture containing 2.5 µl of 10x reaction buffer (Sigma-Aldrich, Germany); 0.5 µl of 10 mM dNTPs (Fermentas, Lithuania); 1 µl of each of the two primer pairs (Oligo, Poland), 25 pM concentrated (*C. jejuni:* mapA1 5`-CTATTTTATTTTTGAGTGCTTGTG-3` and mapA2 5`-GCTTTATTTGCCATTTGTTTTATTA-3`; *C. coli:* COL3 5`-ATTTGAAAATTGCTCCAACTATG-3` and COL2 5`-TGATTTTATTATTTGTAGCAGCG-3`); 1 µl of Red-Taq DNA polymerase (Sigma-Aldrich, Germany) concentrated 1 U/µL; 1 µl of genomic DNA (about 20 ng per reaction). The PCR programme was performed under the following conditions: 94°C for 5 min, followed by 35 cycles of 95°C for 1 min, 52°C for 1 min, 72°C for 60 s, with a final incubation at 72°C for 10 min to complete the extension.

Products obtained during amplification were separated by electrophoresis in 2% agarose gel. Strains suitability to different species was determined by their length; 589 bp product was appropriate for *C. jejuni* while 462 bp - for *C. coli*.

Assessment of *Campylobacter* strain susceptibility to tetracycline, erythromycin, and gentamicin with the usage of MIC factor. The examination of microorganism susceptibility to selected antibiotics was conducted by determination of the minimal inhibitory concentration (MIC) on the agar plates. This method is recommended by Clinical and Laboratory Standards Institute (10) for *Campylobacter* strains. Limit concentration for tetracycline and gentamicin was accepted at ≥8 µg/mL, while for erythromycin - at the level of ≥16 µg/mL.

Statistical analysis. The relation between microorganism susceptibility and the time of the strain sampling were determined using Fisher`s test. All the calculations were done in the R statistical packet. The significance level was defined at $P<0.05$.

Results

Microbiological examinations. Among all the isolates taken between 1994 and 1996, the percentage of *C. jejuni* was 68.5% (n=50) and *C. coli* was 31.5% (n=23). The percentage of *C. jejuni* and *C. coli* strains isolated between 2005 and 2008 was 54.5% (n=171) and 45.5% (n=143), respectively.

***Campylobacter* sp. susceptibility to tetracycline, erythromycin, and gentamicin.** *Campylobacter* sp. recovered between 2005 and 2008 revealed statistically significant increase in the percentage of strains resistant to tetracycline in comparison to the isolates gathered between 1994 and 1996 (Table 1). Particularly high percentage of tetracycline resistant strains was found among isolates from laying hens and turkeys obtained between 2005 and 2008 (95.7% and 86.9%, respectively).

All *Campylobacter* sp. strains (100%) isolated from turkeys in 2005-2008 showed medium susceptibility to erythromycin (Table 1).

Table 1

Susceptibility of *Campylobacter* sp. strains, isolated from poultry in 1994-1996 (broilers, laying hens) and in 2005-2008 (broilers, laying hens, turkeys), to tetracycline, erythromycin, and gentamicin determined by MIC factor

Strains collection	Tetracycline			Erythromycin			Gentamicin		
	S	I	R	S	I	R	S	I	R
	n (%)			n (%)			n (%)		
1994-1996:									
Broilers (n=55)	55 (100.0)	0	0	29 (52.7)	26 (47.3)	0	55 (100.0)	0	0
Laying hens (n=18)	18 (100.0)	0	0	8 (44.4)	10 (55.6)	0	18 (100.0)	0	0
Total	73 (100.0)	0	0	37 (50.7)	36 (49.3)	0	73 (100.0)	0	0
2005-2008:									
Broilers (n=192)	123 (64.1)	33 (17.2)	36 (18.7)*	20 (10.4)	169 (88.0)*	3 (1.6)	191 (99.5)	1 (0.5)	0
Laying hens (n=23)	1 (4.3)	0	22 (95.7)*	12 (52.2)	11 (47.8)	0	23 (100.0)	0	0
Turkeys (n=99)	13 (13.1)	0	86 (86.9)	0	99 (100.0)	0	98 (99.0)	0	1 (1.0)
Total	137 (43.6)	33 (10.5)	144 (45.9)	32 (10.2)	279 (88.9)	3 (0.9)	312 (99.4)	1 (0.3)	1 (0.3)

S – susceptible, I – medium susceptible, R – resistant, n –number of strains, *- statistically significant difference.

In comparison to the strains obtained between 1994 and 1996, statistically significant ($P<0.05$) increase in the percentage of medium susceptible to erythromycin strains was observed among the isolates obtained from broilers in 2005-2008. Among the isolates obtained in 1994-1996, the percentage was 47.3%, while it was 88% in the isolates from 2005-2008. Only 1.6% of the isolates obtained from broilers between 2005 and 2008 revealed resistance to erythromycin.

All isolates obtained in 1994-1996 and 99.4% *Campylobacter* strains obtained in 2005-2008 displayed full susceptibility to gentamicin (Table 1). Only one strain (0.3%) isolated from turkeys was found to be gentamicin resistant.

Discussion

Tetracyclines, macrolides belong to antibiotics often used in the treatment of poultry diseases which may be a result of their broad activity spectrum. Additionally, these antibiotics have been used in poultry production as antibiotic growth promoters for over 30 years now. The role of these antibiotics was the inhibition of pathogenic microflora expansion and creation of unfavourable metabolites– cadaverine, putrescine, and an increase in production effects. Unfortunately, antibiotics originating from the same chemical group are often used in both human and veterinary medicine. Tetracyclines in humans are used in the treatment of respiratory diseases (2), erythromycin is used in campylobacteriosis treatment (4), while aminoglycosides due to their oto- and nephrotoxic activity are rarely used in general therapy. The treatment of sepsis, urinary tract infections caused by *Pseudomonas* sp. and *Klebsiella* sp., and tuberculosis are the most common indications for their usage.

The analysis of the obtained results indicates that the percentage of tetracycline resistant strains between 2005 and 2008 was 45.9%, while all the *Campylobacter* sp. isolates obtained from laying hens and broilers in 1994-1996 showed full susceptibility to tetracycline.

Mazi *et al.* (18) observed an increase in the percentage of tetracycline resistant *Campylobacter* sp. strains isolated from poultry in 2002-2003 and 2005-2006 in the Kingdom of Bahrain. In 2002-2003, the percentage was 80.9% while in 2005-2006 it was 100%. Equally high percentage (77.3%) of the strains isolated from poultry and resistant to tetracycline was obtained by the authors from Turkey (11). Such a high increase in the percentage of resistant strains may result from the fact that these antibiotics are widely, permanently, and uncontrollably used in poultry diseases treatment and, moreover, tetracyclines were applied in poultry production as growth promoters. Additional characteristic feature in *Campylobacter* sp. resistance may be the *tet*O gene transferring the ability (responsible for tetracyclines resistance) among bacteria in the bird intestine tract (5, 7).

In the presented research, very low percentage of erythromycin resistant strains among the strains isolated between 2005 and 2008 was observed. The most strains isolated in 2005-2008 showed low erythromycin susceptibility and its percentage was 10.2%. Among the isolates obtained from broilers and turkeys in 2005-2008, the percentage indicating medium susceptibility to erythromycin was 88% and 100%, respectively. Such a high percentage may be a result of the fact that macrolides are often used in turkeys and broilers flocks (22).

Campylobacter sp. strain susceptibility to erythromycin was also examined by Cokal *et al.* (11) and Mazi *et al.* (18). Research conducted by Mazi *et al.* concerned strains isolated from the Kingdom of Bahrain in poultry in 2002-2003 and 2005-2006. None of the authors claimed erythromycin resistant strains in the investigated periods. Results obtained by these authors are consistent with the results of the presented research. However, it was reported that in Portugal, the percentage of resistant *Campylobacter* sp. strains was growing during the last 15 years (9, 20). In the mid-1990s, the percentage of resistant strains was 5.1%, while between 2005 and 2006 erythromycin resistance was reported in over 90% of all the isolates. The presented research, as well as the studies performed by the authors mentioned above, indicate a tendency to increase the resistance to macrolides in *Campylobacter* sp. strains. High percentage of medium susceptible strains found in the following research may reflect that trend.

In the presented study, the strains obtained between 1994 and 1996 showed higher percentage of erythromycin fully susceptible isolates (50.7%) in comparison to the strains isolated in 2005-2008 (10.2%). Museum strains also revealed almost two-fold lower percentage (49.3%) of erythromycin medium susceptible strains. Among the isolates obtained between 2005 and 2008, the percentage of fully susceptible strains was 10.2%, while the percentage of medium susceptible strains was 88.9%. Additionally, all the isolates obtained from laying hens and broilers between 1994 and 1996 showed medium and full susceptibility to erythromycin. This indicates that the ratio in erythromycin medium and fully susceptible strains underwent transformation during past 14 years. Comparing the results obtained among the isolates collected in 1994-1996 and 2005-2008, it seems clear that the percentage of erythromycin fully susceptible strains showed falling tendency while medium susceptible strains revealed growing tendency. The results obtained by Van Looveren *et al.* (24) in Belgium in 1998 are consistent with the results of our research. The authors reported that the percentage of fully and medium susceptible to erythromycin isolates ranged between 91.4% (laying hens) and 93.7% (broilers).

The investigation presented in this paper and by other authors indicate a very low percentage of gentamicin resistant *Campylobacter* sp. strains. In our study, only one *C. jejuni* strain collected in 2005-2008 was found gentamicin resistant which amounted to 0.3%. All the strains collected between 1994 and 1996 showed full gentamicin susceptibility. Similarly, in Portugal, Cabrita *et al.* (9) found no isolates collected from

broilers resistant to gentamicin. Avrain *et al.* (5) obtained the same results in France.

The latest research conducted in Turkey in 2008 confirms high *Campylobacter* sp. strain susceptibility to gentamicin (11). The authors demonstrated that 126 strains collected from poultry showed full susceptibility toward the antibiotic. Rodrigo *et al.* (21) claim the lowest percentage (5.4%) of resistant to gentamicin *Campylobacter* sp. strains isolated from poultry in Trinidad and Tobago.

In conclusion, it should be underlined that growing resistance to tetracycline and erythromycin among *Campylobacter* sp. strains collected from poultry is a very current issue. Broad usage of chemotherapy in poultry and increasing consumption of antibiotics in human medicine contribute to a high percentage of the strains resistant to one or several chemotherapeutics (multidrug resistance). Resistant *Campylobacter* sp. strains are zoonotic pathogens connected with food, and they can be transmitted easily to humans by products of animal origin. Therefore, it is of the utmost importance to use chemotherapeutics in animal production rationally and to monitor foodborne pathogen susceptibility to broadly used chemotherapeutics in poultry production.

References

1. Aarestrup F.M., Nielsen E.M., Madsen M., Engberg J.: Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. from human, pigs, cattle and broilers in Denmark. Antimicrob Agents Chemother 1997, **41**, 2244-2250.
2. Aarestrup F.M., Engberg J.: Antimicrobial resistance of thermophilic *Campylobacter.* Vet Res 2001, **32**, 311-321.
3. Alfredson D.A., Korolik V.: Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli*. FEMS Microbiol Lett 2007, **277**, 123–132.
4. Alterkruse S.F., Stern N.J., Fields P.I., Swerdlof D.L.: *Campylobacter jejuni* – an emerging foodborne pathogen. Emerg Infect Dis 1999, **5**, 28-35.
5. Avrain L., Vernozy-Rozand C., Kempf I.: Evidence for natural horizontal transfer of tetO gene between *Campylobacter jejuni* strains in chicken. J Appl Microbiol 2004, **97**, 134-140.
6. Bachoual R., Ouabdesselam S., Mory F., Lascols C., Soussy C.J., Tankovic J.: Single or double mutational alterations of gyrA associated with fluoroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli*. Microb Drug Resist 2001**,** **7**, 257–261.
7. Barlow R.S., Pemberton J.M., Desmarchelier P.M., Gobius K.S.: Isolation and characterization of integron-containing bacteria without antibiotic selection. Antimicrob Agents Chemother 2004, **48**, 838-842.
8. Blaser M.: Epidemiologic and clinical features of *Campylobacter jejuni* infections. J Infect Dis 1997, **176**, 103–105.
9. Cabrita J., Rodrigues J., Braganca F., Morgado C., Pires I., Goncalves A.P.: Prevalence, biotypes, plasmid profile and antimicrobial resistance of *Campylobacter* isolated from wild and domestic animals from Northeast Portugal. J Appl Bacteriol 1992, **73**, 279-285.
10. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard-third edition. M31-A3, Vol. **28**, No. 8, 2008.
11. Cokal Y., Caner V., Sen A., Cetin C., Karagenc N.: *Campylobacter* spp. and their antimicrobial resistance patterns in poultry: an epidemiological survey study in Turkey. Zoonoses Public Health 2009, **56**, 105-110.
12. EFSA, 2006. The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2005, http://www.efsa.europa.eu /en/scdocs/scdoc/310ar.htm
13. Frost J.: Current epidemiological issues in human campylobacteriosis. J Appl Microbiol 2001, **90**, 85–95.
14. Gibreel A., Taylor D.E.: Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. J Antimicrob Chemother 2006, **58**, 243–255.
15. Hariharan H., Sharma S., Chikweto A., Matthew V., DeAllie C.: Antimicrobial drug resistance as determined by the E-test in *Campylobacter jejuni, C. coli,* and *C. lari* isolates from the caeca of broiler and layer chickens in Grenada. Comp Immunol Microbiol Infect Dis 2009, **32**, 21–28.
16. Luangtongkum T., Jeon B., Han J., Plummer P., Logue C.M., Zhang Q.: Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiol 2009, **4**, 189–200.
17. Luo N., Sahin O., Lin J., Michel L.O., Zhang Q.: *In vivo* selection of *Campylobacter* isolates with high levels of fluoroquinolone resistance associated with *gyrA* mutations and the function of the CmeABC efflux pump. Antimicrob Agents Chemother 2003, **47**, 390–394.
18. Mazi W., Senok A., Al-Mahmeed A., Arzese A., Bindayna K., Botta G.: Trends in antibiotic sensitivity pattern and molecular detection of tet(O)-mediated tetracycline resistance in *Campyloabcter jejuni* isolates from human and poultry sources. Jpn J Infect Dis 2008, **61**, 82-84.
19. McDermott P.F., Bodeis S.M., English L.L., White D.G., Walker R.D., Zhao S., Simjee S., Wagner D.D.: Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. J Infect Dis 2002, **185**, 837–840.
20. Mena C., Rodrigues D., Silva J., Gibbs P., Teixeira P.: Occurrence, identification and characterization of *Campylobacter* species isolated from Portuguese poultry samples collected from retail establishments. Poultry Sci 2008, **87**, 187-190.
21. Rodrigo S., Adesiyun A., Asgarali Z., Swanston W.: Antimicrobial resistance of *Campylobacter* spp. isolated from broilers in small poultry processing operations in Trinidad. Food Control 2007, **18**, 321-325.
22. Saif Y. M.: Diseases of Poultry. Blackwell Publishing Professional, 12th edition, Iowa State Press, Ames, United States of America 2008, pp. 675-682.
23. U.S. Department of Heath and Human Services, BBB - *Campylobacter jejuni*, Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. http://www.fda.gov/Food/FoodSafety/ucm070024.htm
24. Van Looveren M., Daube G., De Zutter L., Dumont J.-M., Lammens C., Wijdooghe M., Vandamme P., Jouret M., Cornelis M., Goossens H.: Antimicrobial susceptibilities of *Campylobacter* strain isolated from food animals in Belgium. J Antimicrob Chemother 2001, **48**, 235-240.
25. Van Vliet A.H.M., Ketley J.M.: Pathogenesis of enteric *Campylobacter* infection. J Appl Microbiol 2001, **90**, 45–56.
26. Woźniak A.: Fluoroquinolones resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in 1994-1996 and 2005-2008 in Poland. Bull Vet Inst Pulawy – in press.

Many strains isolated from poultry and responsible for food poisoning in humans demonstrate resistance to selected antibiotics. For that reason, much attention is drawn to eggs and poultry meat as sources of Salmonella bacteria [124]. Infected birds are the primary source of Salmonella spp. infection in the production environment.Â Lin, J. Novel Approaches for Campylobacter Control in Poultry. Foodborne Pathog. Dis. Campylobacter coli is a Gram-negative, microaerophilic, non-endospore-forming, S-shaped bacterial species within genus Campylobacter. In humans, it C. coli can cause campylobacteriosis, a diarrhoeal disease which is the most frequently reported foodborne illness in the European Union. C. coli grows slowly with an optimum temperature of 42 Â°C. When exposed to air for long periods, they become spherical or coccoid shaped.