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ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI* ISOLATES FROM BROILER FLOCKS

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ABSTRACT

In this study 176 isolates of *Campylobacter jejuni* and 69 isolates of *Campylobacter coli* detected in samples from broiler flocks were tested for their antimicrobial susceptibility. The antimicrobial resistance of each isolate was determined for the following antimicrobial agents: erythromycin, ciprofloxacin, tetracycline, streptomycin and gentamicin. The parameters used for the determination of the antimicrobial resistance are given in the *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A4* (Clinical and Laboratory Standards Institute, USA).

The results of the testing showed that *C. jejuni* isolates expressed highest antimicrobial resistance towards ciprofloxacine (42,4% of resistant isolates) and tetracycline (39,77%). Much lower antimicrobial resistance was detected towards erythromycin (5,11%), streptomycin (3,4%) and gentamicin (1,7%). Similar conclusions were made after the analysis of the *C. coli* isolates. Highest resistance was detected towards ciprofloxacin (62,3%) and tetracycline (57,97%). Only 12 isolates of *C. coli* (17,39%) showed resistance to streptomycin, while the resistance detected towards erythromycin (11,59%) and gentamicin (1,44%) was similar to the resistance detected in *C. jejuni* isolates.

Overall, higher antimicrobial resistance was detected to quinolones and tetracyclines and lower resistance towards macrolides and aminoglycosides. Observing the results it can be noticed that *C. jejuni* and *C. coli* showed low resistance to erythromycin and gentamicin. The results emphasize the higher resistance of *C. coli* versus *C. jejuni* to ciprofloxacin, tetracycline, streptomycin and erythromycin.

Key words: *broiler flocks, Campylobacter jejuni, Campylobacter coli, antimicrobial resistance*

INTRODUCTION

Campylobacteriosis is a significant public health problem in many developed countries. In Europe alone more than 200,000 confirmed cases were reported to the European Food Safety Authority in 2007 (1). Inadequately cooked meat, particularly poultry, unpasteurized milk and contaminated drinking water are the most common sources for epidemic and sporadic food borne cases (2, 3). Poultry products are known as an important source of *Campylobacter* spp. At the age of two to three weeks, 50-90% of the poultry in the flock is colonized with termophilic *Campylobacter* spp. (4). Two Campylobacter species, *Campylobacter jejuni* and *Campylobacter coli*, are responsible for the majority of human infections, among which 80–90% are due to *C. jejuni* (5).

Most Campylobacter infections are self-limiting (3-5 days), but in very young individuals, the elderly persons and people with chronic

diseases prolonged or severe campylobacteriosis can occur (6). For treating such Campylobacter infections, fluoroquinolones (e.g., ciprofloxacin) and macrolides (e.g., erythromycin) are the drugs of choice (Skirrow et al., 2000). There is growing concern that the use of antibiotics in food animals can lead to the development of resistant pathogenic bacteria that can affect humans through the food chain. In this study the antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler flocks in Macedonia was examined.

MATERIALS AND METHODS

Specimen isolation

All of the analyzed isolates of *C. jejuni* ($n = 176$) and *C. coli* ($n = 69$) were detected in samples taken from broiler flocks on farm and at the slaughterhouse (caecal swabs, chicken faeces, caeca, carcass swabs).

The samples were enriched in Preston selective broth (Fluka) and incubated microaerophilically on 42°C for 24 hours (7).

The enrichment was streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid) and incubated under microaerobic conditions at 37°C for 48 h.

Identification of isolates

Isolates were identified using standard parameters including Gram staining, oxidase and catalase testing, temperature tolerance, morphology, indoxyl acetate test, hippurate hydrolysis, growth and production of H_2S on triple sugar iron agar.

Testing of the antimicrobial resistance

For the antimicrobial susceptibility testing of the isolates disk diffusion method (Kirby Bauer method) was used. The inoculums were prepared with density adjusted to 0,5 McFarland turbidity standard. The inoculum was delivered with sterile swabs on Columbia blood agar (Biomerieux) and the following antimicrobial agents were used: erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin (Oxoid). The following CLSI breakpoints (8) were used for the classification of the isolates:

Table 1. CLSI (former NCCLS) breakpoints used for determination of the antimicrobial resistance of *C. jejuni* and *C. coli*

	Antimicrobial agent	concentration (µg)	Zone of inhibition (mm)		
			resistant	intermediate	susceptible
1.	Ciprofloxacin	5	≤15	16-20	21≥
2.	Erythromycin	15	≤13	14-22	23≥
3.	Gentamicin	10	≤12	13-14	15≥
4.	Tetracycline	30	≤14	15-18	19≥
5.	Streptomycin	10	≤12	13-14	15≥

RESULTS

The results of antimicrobial susceptibility testing for *C. coli* and *C. jejuni* are shown in Table 2 and 3.

The results of the the tests identify that *C. jejuni* isolates expressed highest antimicrobial resistance towards ciprofloxacin (42,4% of resistant isolates) and tetracycline (39,77%). Much lower antimicrobial resistance was detected towards erythromycin (5,11%), streptomycin (3,4%) and gentamicin (1,7%). Similar conclusions were made after the analysis with the *C. coli* isolates. Highest resistance was detected towards ciprofloxacin (62,3%) and tetracycline (57,97%).

Only 12 isolates of *C. coli* (17,39%) showed resistance to streptomycin, while the resistance detected towards erythromycin (11,59%) and gentamicin (1,44%) was similar to the resistance detected in *C. jejuni* isolates.

Table 2. Antimicrobial susceptibility of *C. jejuni* isolates

Antimicrobial used	Erythromycin	Ciprofloxacin	Tetracycline	Streptomycin	Gentamicin
Susceptibility	R	R	R	R	R
Total No. of isolates = 176	9 (5,11%)	75 (42,6%)	70 (39,7%)	6 (3,4%)	3 (1,7%)

Table 3. Antimicrobial susceptibility of *C. coli* isolates

Antimicrobial used	Erythromycin	Ciprofloxacin	Tetracycline	Streptomycin	Gentamicin
Susceptibility	**R**	**R**	**R**	**R**	**R**
Total No. of isolates = 69	8 (11,59%)	43 (62,31%)	40 (57,97%)	12 (17,39%)	1 (1,44%)

DISCUSSION

This study is the first to highlight the importance of antibiotic susceptibility of *Campylobacter* spp. isolated from broilers in Macedonia. The principal purpose of monitoring antimicrobial resistance trends in enteric pathogens is to provide clinicians with data that can be used to select appropriate treatment regimens. Surveillance should emphasize antibiotics that are being used routinely to treat diarrhea, as well as any alternatives, such as fluoroquinolones, macrolides, and gentamicin. Equally important is the accessibility of the data to those providing primary care.

However, as everywhere else in the world, the increase of Campylobacter-resistant strains seems to be related to the amounts of antibiotics used in animals (9). Thus, to prevent transfer of resistant bacteria or resistance genes from animals to humans via the food chain (10), measures that should be implemented are: reduction of the use of antibiotics, encourage narrow-spectrum specific antibiotic therapy instead of broad spectrum antimicrobials (11), and replacement of antibiotics with improvements in hygiene and flock management. Consequently, it would be useful to set up an surveillance network to monitor antimicrobial resistance in bacteria of animal origin likewise the countries in the European Union.

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