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# COMPARATIVE STUDY BETWEEN THE ANTIBIORESISTANCE OF CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI

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***Abstract***

*The spread of antibiotic resistances and the appearance of multiple-antibiotic-resistant pathogenic bacteria has been recognized by the WHO as a serious problem that complicates medical treatment of bacterial infections. In last years, when campylobacteriosis became the most frequently disease the antibioresistance of this microorganism represents a serious problem. Campylobacter's antibioresistance was carried out through the determination of minimal inhibitory concentration using Sensititre system. This analysis were performed in panels dedicated for these microorganisms. There were tested 132 Campylobacter jejuni and Campylobacter coli strains previously isolated from chicken meat. The species of Campylobacter was perform according ISO 10272/2006.Of the 132 analyzed Campylobacter strains, 39 strains were susceptible to all antimicrobial substances tested, and 93 strains showed resistance to at least one antimicrobial agent; 4 strains were resistant to 6 antimicrobial substances. C. coli showed a higher degree of resistance than C. jejuni to all antimicrobial substances that were tested.*

***Key words****:* antibioresistance, antimicrobials, *Campylobacter*.

## INTRODUCTION

*Campylobacter* is one of the most common causes of diarrheal illness in Europe and the United States. Campylobacteriosis is generally associated with sporadic diarrhea linked with the consumption of improperly handled or cooked food. Animals such as swine, cattle, and poultry are potential reservoirs for the bacteria. Although *C. jejuni* is predominant in broiler chickens and cattle, it is infrequent in pigs, in which *C. coli* predominate (Nielsen et. al, 1997). Transfer of *Campylobacter* from animals to humans has been demonstrated (Blaser, 1997; Nadeau et. al, 2002). Some authors have suggested that the use of antimicrobial agents in animal production plays a key role in the dissemination of antimicrobial resistances genes from animals to the human population (Swartz, 2002). Most cases of campylobacteriosis occur as isolated, sporadic events, not as part of recognized outbreaks. Nevertheless the number of reported confirmed cases of human campylobacteriosis in the EU in 2011 was 220.209, which was an increase of 2.2 % compared to 2010 (EFSA and ECDC, 2013). The interest in campylobacteriosis in Romania started very recently, and its laboratory diagnostics, followed by recording, began in 2006. Whether in 2006 and 2007 in our country did not recognize any case, in 2011 there were recorded 149 disease induced by Campylobacter. (EFSA and ECDC, 2013) Having in consideration the high number campylobacteriosis reported in EU, the low number of isolates recorded in Romania suggests that only a small number of infections are diagnosed and recorded. In addition to expanding of campylobacteriosis, another worried subject is the antibioresistance of this microorganism. The spread of antibiotic resistances and the appearance of multiple-antibiotic-resistant pathogenic bacteria has been recognized by the WHO as a serious problem that complicates medical treatment of bacterial infections. Transmission of antimicrobial resistance from food animals to humans can occur via the food chain. It is difficult to determine the precise extend of the

risk posed to human health (Harada et. al, 2006). The aim of this study was to evaluate the incidence and the distribution of antimicrobial resistance in *Campylobacter* isolates from chicken meat samples. We present the results on antimicrobial susceptibility measured by MIC assay of *Campylobacter jejuni* and *Campylobacter coli* isolated in Romania. Also, the aim of this study was to realize a comparison between the antibioresistance of *Campylobacter jejuni* and *Campylobacter coli.*

## MATERIALS AND METHOD

In our study we tested 72 *Campylobacter jejuni* and 60 *Campylobacter coli* strains isolated from from retail. All strains were stored until use at -80°C and after that, they were cultivated on Columbia agar with 5% horse blood in microaerobic conditions (5% $O_2$, 10% $CO_2$, 85% $N_2$). The species identification was performed using the biochemical tests accordingly ISO 10272/2006. (ISO 10272-1, 2006) Of each strain were obtained a bacterial suspension, from which it was got an amount so that the final inoculum to be 5 x $10^5$ cfu/ml.
The minimum inhibitory concentrations (MICs) of tetracycline, ciprofloxacin, nalidixic acid, erythromycin, chloramphenicol, gentamicin, and streptomycin were carried out by the microdilution method acoordingly ISO 20776-1 and CLSI (CLSI M31-A3, 2008). The MIC represent the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in microdilution wells as detected by the unaided eye. (Piddock et. al, 2003) Growth appears as turbidity or as a deposit of cells at the bottom of a well (CLSI M45-A; M100-S16, 2006). *C. jejuni* ATCC 33560 was used as a control. The range of antimicrobials concentrations used for determining MICs were different depending of antibiotic substances (Andrews, 2001).
The epidemiological cut-off values are established by EUCAST (the European Committee on Antimicrobial Susceptibility Testing); in case of *Campylobacter*, the cut-off points are different for *Campylobacter jejuni* and *Campylobacter coli*. These values and the range tested for each antimicrobial agent are presented in table 1 (CLSI M45-A; M100-S16, 2006). For quantitative MIC data, an isolate is defined as 'resistant' for a selected antimicrobial when its MIC value is above the epidemiological cut-off value as indicated in table 1.

Table 1
Antibacterial substances that were tested, their concentration range and cut-off values of Campylobacter jejuni and Campylobacter coli (microdilution method)

| Antimicrobial substance | Abbreviation | Range of antibiotics' concentrations tested (μg/ml) | The cut-off values (μg/ml) R > | |
|---|---|---|---|---|
| | | | *Campylobacter jejuni* | *Campylobacter coli* |
| Tetracycline | TET | 0,06 - 64 | 2 | 2 |
| Erythromycin | ERY | 0,03 - 64 | 4 | 16 |
| Gentamicin | GEN | 0,12 – 32 | 1 | 2 |
| Ciprofloxacin | CIP | 0,015 – 64 | 1 | 1 |
| Nalidixic acid | NAL | 4 - 64 | 16 | 32 |
| Chloramphenicol | CHL | 2 - 32 | 16 | 16 |
| Streptomycin | STR | 1 - 16 | 2 | 4 |

## RESULTS AND DISCUSSIONS

Of the 132 analyzed *Campylobacter* strains, 39 strains were susceptible to all antimicrobial substances tested, and 93 strains showed resistance to at least one antimicrobial agent; 4 strains were resistant to 6 antimicrobial substances. The results of *Campylobacter jejuni* and *Campylobacter coli* antibioresistance and the percent of antibioresistance depending of antibiotic concentration are presented in table 2 and 3.
The most common the strains were resistant to ciprofloxacin and nalidixic acid. Generally the resistant strains to ciprofloxacin were, also, resistant to nalidixic acid. This aspect is explicable through the appearance of the mutations at *gyr*A and *par*C gene, which determine the resistence of strains both the quinolone of first generation (nalidixic acid) and the fluoroquinolone (ciprofloxacin) (Minihan, 2004; Yan, 2006). Ciprofloxacin is the second choice drug for treatment of campylobacteriosis in humans although resistance rapidly evolves (EFSA, 2011). Also, o quite high resistance was recorded to tetracycline both for C. jejuni (30,6%) and C.

coli (48,0%). Regarding to erythromycin, gentamicin and streptomycin the level of antibioresistance was low. Erythromycin or another suitable macrolide is the first choice drug for the treatment of campylobacteriosis in humans (EFSA, 2011).

Table 2

Antimicrobial resistance in *Campylobacter jejuni* (n = 72) from broiler skin

Distribution (%) of MIC values (µg/ml)

| Antimicrobial | Resistance (%) | The procentual (%) distribution of minimal inhibitory concentration (µg/ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.064 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 |
| TET | 30,6 | | | 14,5 | 0,0 | 25,8 | 29,0 | 14,5 | 8,0 | 4,8 | 3,2 | |
| ERY | 6,4 | | | | 9,6 | 11,3 | 50,0 | 22,5 | 3,2 | 3,2 | 0,0 | |
| STR | 3,2 | | | | | 50,0 | 46,7 | 0,0 | 3,2 | 0,0 | | |
| GEN | 4,8 | | 0,0 | 53,2 | 41,9 | 0,0 | 3,2 | 1,6 | 0,0 | 0,0 | | |
| CIP | 58,0 | 0,0 | 12,9 | 14,5 | 0,0 | 14,5 | 30,6 | 24,1 | 3,2 | | | |
| NAL | 56,4 | | | | | | 17,7 | 0,0 | 19,3 | 6,4 | 35,5 | 20,9 |
| CHL | 11,2 | | | | | | 25,8 | 27,4 | 35,4 | 0,0 | 6,4 | 4,8 |

The upright bolded lines represent the cut-off values (the value above which the strain is considered resistant) and white fields represent the range of tested antimicrobials.

Table 3

Antimicrobial resistance in *Campylobacter coli* (n = 60) from broiler skin

Distribution (%) of MIC values (µg/ml)

| Antimicrobial | Resistance (%) | The procentual (%) distribution of minimal inhibitory concentration (µg/ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.064 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 |
| TET | 48,0 | | | 6,0 | 0,0 | 28,0 | 18,0 | 4,0 | 32,0 | 12,0 | | |
| ERY | 12,0 | | | | 20,0 | 10,0 | 10,0 | 12,0 | 24,0 | 12,0 | 12,0 | |
| STR | 4,0 | | | | | 20,0 | 30,0 | 46,0 | 2,0 | 2,0 | | |
| GEN | 6,0 | | 12,0 | 16,0 | 18,0 | 44,0 | 4,0 | 4,0 | 2,0 | 0,0 | | |
| CIP | 68,0 | 4,0 | 0,0 | 0,0 | 10,0 | 18,0 | 24,0 | 34,0 | 10,0 | | | |
| NAL | 66,0 | | | | | | 2,0 | 8,0 | 6,0 | 12,0 | 6,0 | 66,0 |
| CHL | 16,0 | | | | | | 24,0 | 16,0 | 24,0 | 20,0 | 16,0 | |

The most worrying aspect is represented by the multidrug resistance of some strains. Thus, from all strains tested 4 (3%) were resistant at 6 antimicrobial substances and 6 were resistant at 5 antimicrobial substances (4,5%). About 70 % of tested strains were resistant at less than 3 antimicrobial substances. A resume of these is presented in table no. 4.

Table 4

Multiple resistance to antibacterial substances of the tested Campylobacter strains

| Resistant to: | *C. jejuni* | *C. coli* | Total *Campylobacter* | % |
|---|---|---|---|---|
| 3 antimicrobial substances | 7 | 9 | 16 | 12,1 |
| 4 antimicrobial substances | 5 | 6 | 11 | 8,3 |
| 5 antimicrobial substances | 2 | 4 | 6 | 4,5 |
| 6 antimicrobial substances | 1 | 3 | 4 | 3,0 |

Regarding to the differences between the *C. jejuni* and *C. coli* antibioresistance it is distinguished that *C. coli* strains are more resistant to antimicrobials compared to *C. jejuni* strains. Thus, *C. coli* strains were more likely to be erythromycin-resistant compared to *C. jejuni* (12 % compared with 6,4 %). This aspect is a potential result of the treatment with erythromycin applied to pigs, knowing that *C. coli* is a specie frequently meet to these. (Harada et. al, 2006; Lin, 2009) *C. coli* were also more likely to be tetracycline, streptomycin and ciprofloxacin-resistant compared to *C. jejuni* (48 % compared with 30.6 % for tetracycline, 4 % compared 2 % for streptomycin and 68 % compared with 58 % for ciprofloxacin). The lowest levels of resistance were found to gentamicin (*C. jejuni* 6 % and *C. coli* 4.8 %), streptomycin (*C. jejuni* 2 %) and erythromycin (*C. jejuni* 6,4 %).

## CONCLUSIONS

Of the 132 analyzed *Campylobacter* strains, 39 strains were susceptible to all antimicrobial substances tested, and 93 strains showed resistance to at least one antimicrobial agent; 4 strains were resistant to 6 antimicrobial substances.

*C. coli* showed a higher degree of resistance than *C. jejuni* to all antimicrobial substances that were tested and especially to tetracycline, nalidixic acid, ciprofloxacin, erythromycin and chloramfenicol.

The highest degree of antibioresistance of *C. jejuni* and *C. coli* strains was recorded to

ciprofloxacin and nalidixic acid (58 %, respectively 68 %), while the resistance against streptomycin and gentamicin was low (2 %, respectively 4,8 %). These findings suggest the use of aminoglycosides and the avoidance of quinolones in the treatment of pathological conditions caused by bacteria of the genus *Campylobacter* in human patients.

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