



INVESTIGATIONS ON THE SENSITIVITY OF AVIAN *CAMPYLOBACTER SPP.* ISOLATES TO ANTIMICROBIAL DRUGS

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ABSTRACT

The sensitivity of 39 *Campylobacter spp.* strains, isolated from broiler chickens and mule ducks to 5 antimicrobial drugs was tested. The samples originated from two poultry slaughterhouses in South Bulgaria.

The inhibition zones were interpreted by the three-degree system of Bauer- Kirby's disk diffusion method according to NCCLS requirements.

The highest resistance percentage (66.7%) was observed to ampicillin. The resistance of studied isolates to erythromycin was 10.3%, and to tetracycline – 5.1%. Against fluorinated quinolones, a resistance of 15.4% against each of norfloxacin and enrofloxacin has been observed.

A resistance pattern characterized with (A Nor Enr) profile was encountered in 3 strains (7.6%).

The profile (A Ery Tet) was also seen in three tested isolates. The commonest pattern of resistance (43.5%) was determined in campylobacteria, resistant only to ampicillin (A), followed by isolates, sensitive to all five tested chemotherapeutics (33.3%).

Key words: *Campylobacter jejuni*, *Campylobacter coli*, antimicrobial resistance, birds

INTRODUCTION

Campylobacteria are involved in the etiology of human gastroenteritis (1,2,3). In industrial countries such as the USA, Canada, Australia and New Zealand, the incidence of campylobacterial infections increased during the last 20 years, but this fact could be hardly explained. Campylobacteria are widely distributed in nature, among both wild and domestic animals. Birds in particular, are one of the essential reservoirs of campylobacteria allowing transfer of strains to humans (1,2,4). In Denmark, data about increased incidence of campylobacterial infections in men are reported and the consumption of poultry meat is incriminated as a risk factor (5). The prevalence of campylobacteria in broiler chickens flocks in Europe is estimated to be 35-37% (6,7,8,9). The cases of campylobacterial gastroenteritis in the USA were 20.1 per 100 000 in 2000 and in North European countries – 60 to 90 per 100 000 (2,10). From all members of the *Campylobacter*

genus, *C. jejuni* is more frequently implicated in human gastroenteritis (11). The epidemic outbreaks in European countries is attributed to the consumption of raw milk, whereas the presence of sporadic cases – to poultry meat consumption (12, 13).

In Thailand, *Campylobacter* and *Salmonella* are the most commonly isolated strains from neonates and children under the age of 5 years (14,15). Unlike the reports from South Asia, in the USA and Western Europe, peaks of campylobacterial infections were reported both in children younger than 4 years and in adults at the aged of 15 to 44 years (2). Also, seasonal peaks during the hot months are observed (2).

The direct comparison of data related to the increasing prevalence of campylobacterial isolates in poultry farms and their resistance to fluorinated quinolones in the different countries could be hardly made in an objective manner because of the variations in factors related to rearing systems and the methods of sampling and analysis of specimens. Yet, a considerable proportion of

poultry flocks are negative with regard to resistance of *Campylobacter* isolates. In some European countries: Denmark (16), Norway (17), Sweden (18) and Holland (19), a seasonal prevalence of avian *Campylobacter* strains has been reported.

On the other side, fluorinated quinolones are most frequently used for treatment of human salmonellosis, shigellosis, campylobacteriosis and non-diagnosed gastroenteritis (20,21,23). The clinical efficacy of applied fluorinated quinolones in non-typhoid salmonellosis and campylobacteriosis is not consistent. Macrolide chemotherapeutics are a good alternative (20,21,22,23). Therefore, the information about the rapidly emerging resistance of campylobacteria to fluoroquinolones is an important issue influencing the therapeutic choice (22,23,24). The first communication about resistance to quinolones is made in 1980 (25, 26). In domestic animals, the cases of campylobacterial enteritis are rare but nevertheless, the resistance to this group of chemotherapeutics is widely prevalent among animal isolates (4). This fact could be interpreted in the light of the use of fluorinated quinolones for treatment of *E. coli* infections in animals.

Barrow et al. (27) discussed the common application of fluoroquinolones in numerous countries in the therapy of *Salmonella* infections in domestic fowl that, on its part influences the emergence of resistance against quinolones in septicemic avian *E. coli* and *Campylobacter* strains.

The mechanism of the resistance to fluoroquinolones in *Campylobacter jejuni* is chromosomally determined and is realized via mutations in *gyr A* and *par C* genes (28). The correlation between the resistance to azithromycin and that fluoroquinolones is proved (29).

During the last years, analyses of the prevalence of campylobacterial avian isolates and their resistance to quinolones in both conventional and organic poultry flocks has been studied (30). In their studies, the authors determined that the prevalence of campylobacteria in the birds from the organic flocks was 100% whereas that in the extensive rearing farms – 49.2%. In Sweden, the prevalence of *Campylobacter* in poultry slaughterhouses was 27 % and this was thought to be closely related to the age of birds (18).

In Kenia, the prevalence of campylobacterial isolates was estimated at 77% in poultry

products and 60–100% in poultry-rearing farms (31, 32).

The sensitivity to nalidixic acid and cefalotin is used as a diagnostic criterion for differentiation of the species of *Campylobacter* isolates (33). Thus, the presence and the rapid spreading of the resistance to quinolones could be problematic with regard to diagnostic criteria utilized for determination of isolates.

The resistance exhibited by thermophilic *Campylobacter* to beta-lactam chemotherapeutics is also intriguing. *Campylobacter coli* and *Campylobacter jejuni* possess a natural resistance to penicillin G and to some broad-spectrum cephalosporins, primarily related to the presence of penicillin-binding proteins with decreased affinity to beta-lactam antimicrobial drugs. With regard to the acquired resistance to beta-lactams, the primary mechanism of its presentation remains the production of beta-lactamases (34, 35).

MATERIAL AND METHODS

In the period from April 2006 to March 2007, 40 samples obtained from broiler chickens have been examined, 22 of them from caeca, 12 – from gizzards and 6 – from livers. Moreover, 70 samples from mule ducks were analyzed too (40 from caeca, 10 from the skin surface and 20 – from breast muscles).

Bacterial strains – The total number of *Campylobacter* isolates was 39: 30 *C. jejuni* and 9 *C. coli* isolates.

Nutrient media – The isolation and differentiation of strains was done according to ISO 10272 – horizontal method for the detection and identification of *Campylobacter* spp. in food and feeding stuffs. The cultivation of samples was done in thioglycolate broth (Merck) and on selective *Campylobacter* agar (Merck) supplemented with the combination of vancomycin, polymyxin B, trimethoprim. Horse red blood cells were added to the agar base.

The samples were incubated at 37°C and 42°C for 48 hours in microaerophilic conditions (5% O₂, 10% CO₂, 85% N₂).

Determination of isolates to the species level – The following tests were used for screening of the obtained pure cultures: production of cytochrome oxidase, catalase, hydrolysis of sodium hippurate and hydrolysis of indoxyl acetate. The tests for

determining of isolates' sensitivity to nalidixic acid (30 µg) and cefalotin (30 µg) were performed. The strains were differentiated by means of API Campy® (Bio Mérieux).

Determination of isolates' sensitivity to chemotherapeutics

The sensitivity of isolated campylobacteria to antimicrobial drugs was performed by the disk diffusion method (NCCLS). For this purpose, Mueller-Hinton agar II (Becton Dickinson) was prepared. Campylobacteria were preliminary cultivated in Mueller-Hinton broth (Becton Dickinson) at 37°C for 24 h in microaerophilic conditions. The

inoculum's density used for antibiogrammes was 10⁸ cfu/mL. The strains were incubated in microaerophilic conditions 37°C for 36 hours.

Antibiotic disks – Ampicillin 10 µg, Erythromycin 15 µg, Tetracycline 30 µg (BulBio, National Centre for Infectious and Parasitic Diseases, Sofia); Norfloxacin 5 µg (SEVA, France) and Enrofloxacin 5 µg (BAUER, Germany).

The strains were defined on the three scale system as sensitive, intermediate and resistant. Table 1 presents the diameters of inhibition zones for the respective groups.

Table 1. Classification of the sensitivity of tested isolates.

Chemotherapeutics	Content per disk	S mm	I mm	R mm	Reference
Ampicillin	10 µg,	≥14	12-13	≤11	NCCLS
Erythromycin	15 µg	≥18	14-17	≤13	NCCLS
Tetracycline	30 µg	≥19	15-18	≤14	NCCLS
Norfloxacin	5 µg	≥22	12-21	≤12	SEVA
Enrofloxacin	5 µg	≥22	18-21	≤17	BAUER

RESULTS AND DISCUSSION

The highest percentage of resistance (61.5 %) in studied Campylobacter strains was observed against ampicillin. With regard to erythromycin, the resistance was 10.2 %, and against tetracycline – 5.1%. The percentage

of resistance to each of norfloxacin and enrofloxacin was 15.4 %. The results obtained by means of the disk diffusion method are presented in Table 2. The proportions of resistant isolates are given as (R+I).

Table 2. Percentages of sensitive and resistant campylobacteria, isolated from birds.

Chemotherapeutics	S %	Confidence intervals	R+I %	Confidence intervals
Ampicillin	38.5	23.8 ÷ 54.4	61.5	45.6 ÷ 76.2
Erythromycin	89.7	78.1 ÷ 97.2	10.2%	2.7 ÷ 21.7
Tetracycline	94.9	85.9 ÷ 99.5	5.1%	0.5 ÷ 14.3
Norfloxacin	84.6	71.5 ÷ 94.2	15.4%	6.8 ÷ 28.5
Enrofloxacin	84.6	71.5 ÷ 94.2	15.4%	6.8 ÷ 28.5

The most commonly observed resistance pattern with 43.6% was only against ampicillin (A). In three strains (7.6%) the profile included resistance against 3 of tested antimicrobial drugs: ampicillin and to both fluorinated quinolones. The same percentage

was determined in the resistance pattern characterized with resistance against erythromycin and tetracycline (Ery Tet). Sensitivity to all five chemotherapeutics was exhibited by 33.3% of strains. The pattern of resistance to 2 antimicrobial drugs

(ampicillin and one fluoroquinolone) was observed in 2.5% of strains. Resistance only to erythromycin (E) was also the pattern shown by 2.5% of strains.

Our data for the antimicrobial resistance patterns in avian *Campylobacter* strains differed considerably by those, reported by Erdeger et al. in Turkey (36). They determined 15 multiresistance patterns in avian strains. At the same time, they pointed out the highest resistance to ampicillin (43.6 %) that corresponded to our data about the resistance to this antibiotic.

The results from a National Monitoring Programme in Denmark about the resistance to quinolones in avian *Campylobacter* isolates are published by Pedersen et al. in 2002 г.(38). They observed 11.3% resistance to quinolones in *C. jejuni* strains and 28.7% in *Campylobacter coli* isolates. Our data are similar as the resistance exhibited to either enrofloxacin or norfloxacin was 15.4 %. A differentiation of resistant isolates could be hardly made as the number of *Campylobacter coli* isolates studied by us was very small.

In Senegal, 34% resistance to ciprofloxacin in campylobacterial strains isolated from the skin surface of avian carcasses was reported by Cardinale et al. for 2001–2002 (39). Related to our data about the resistance to enrofloxacin, the reported percentage was almost twice higher.

Ledergerber et al. reported that the resistance incidence to ciprofloxacin in *Campylobacter* isolated from raw poultry meat in Switzerland was 28.7%. Against ampicillin, the resistance was lower – 10.3%. In our study, the percentages of isolates resistant to ampicillin were four times higher (40).

In the communication of Frediani - Wolf et al , data about the resistance to erythromycin, ciprofloxacin in campylobacteria, isolated

from two large poultry slaughterhouses in Switzerland are given. The lack of observed resistance to tetracycline is an interesting finding. In contrast, our results showed resistance to tetracycline in 5.1 % of isolates (41).

The investigations on the resistance of *Campylobacter* isolates in broiler chickens in slaughterhouses in France, reported by Avrain et al. demonstrated 23 % resistance to ampicillin, 17 % to enrofloxacin, 57% to tetracycline and 0.3% to erythromycin. Only the data about campylobacteria resistant to enrofloxacin are similar to ours. With regard to erythromycin, the differences were considerable as according to our results, 10.2 % of strains were determined as resistant. The high resistance percentage to tetracycline is also inconsistent with our findings (5.1%) (42).

In Ireland, Lucey et al. reported comparative data for the resistance in *Campylobacter* isolates from men and poultry referring to two different periods: 1996–1998 and 2000. The resistance to ciprofloxacin in avian strains in 2000 was high (30%) compared to 1998, when it was only 3.1%. In our investigation, this percentage was almost twice higher. The resistance to tetracycline was also higher from a comparative point of view: from 13.9% in 1998 to 18.8% in 2000. These values are 3 times higher than our findings about tetracycline resistance. Nevertheless, the trend towards increase in the number of resistant avian isolates is without any doubt corresponding to the increasing resistance in human strains as well. (43).

Table 3. Resistance patterns, determined in avian campylobacterial isolates

Resistance patterns	Chemotherapeutics					%
	Amp	Ery	Tet	Nor	Enr	
1	R	S	S	S	S	43.6
2	S	R	R	S	S	7.6
3	R	S	S	R	R	7.6
4	R	S	S	R	S	2.5
5	R	S	S	S	R	2.5
6	S	R	S	S	S	2.5

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