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THE COMPARISON OF IN VITRO ANTIMICROBIAL SUSCEPTIBILITY OF CAMPYLOBACTER JEJUNI FROM AVIAN AND BOVINE-OVINE ORIGIN

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Kanatlı ve sığır-koyun orijinli Campylobacter jejuni suşlarının in vitro antimikrobiyal duyarlılıklarının karşılaştırılması

Özet: *Kanatlı (100 suş) ve sığır-koyun (100 suş) orijinli Campylobacter jejuni suşlarının antimikrobiyal duyarlılığı disk diffuzyon tekniği ile incelenerek karşılaştırıldı. Genel olarak, tavuk izolatları beş antimikrobiyal ajana sığır-koyun izolatlarına göre çok duyarlı bulundu. Campylobacter jejuni'nin bütün kanatlı suşlarının penisilin ve ampisiline dirençli tetrasiklin, eritromisin, kanamisin, neomisin ve streptomisine duyarlı olduğu saptandı. Sığır-koyun izolatlarının terasikline % 15 ve eritromisine % 4 oranında dirençli olduğu belirlendi. İstatistiksel olarak, kanatlı ve sığır-koyun izolatlarının antimikrobiyal duyarlılıkları arasında, penisilin dışında, değişik derecelerde olmak üzere belirgin farklar saptandı.*

Summary: *The antimicrobial susceptibility of Campylobacter jejuni strains obtained from avian (100 strains) and bovine-ovine (100 strains) origins was investigated by disk diffusion technique. In general, chicken isolates were more susceptible to five antimicrobial agents than cattle-sheep isolates. All avian strains of C. jejuni were resistant to penicillin and ampicillin and sensitive to tetracycline, erythromycin, kanamycin, neomycin and streptomycin. The frequency of tetracycline and erythromycin resistance among bovine-ovine isolates was % 15 and % 4, respectively. The differences between the antimicrobial susceptibility of avian and bovine-ovine isolates except penicillin were considered as significant in varying degrees, statistically.*

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Introduction

During the past decade, several reports have dealt with the susceptibility of *Campylobacter jejuni* to antimicrobial agents. Some of these reports indicate the differences in the resistance frequency of *C. jejuni* from human and animals to several antimicrobial agents (11, 12). Studies have also shown geographical differences in the antibiotic sensitivity patterns of this organism to some drugs, especially tetracycline and erythromycin (6, 8). Since *C. jejuni* strains showed such differences and possessed plasmid DNA (3), these variations present a new filed of epidemiological value. Because animals can function as a reservoir of antibiotic resistant plasmids which could be transferred to humans (4), it is important to know the susceptibility patterns of *C. jejuni* from animals to different antibiotics to recognize possible natural sources of antibiotic-resistant strains.

C. jejuni strains recently isolated in Turkey from cattle-sheep and poultry were examined for the purpose of finding possible differences in antimicrobial susceptibility patterns of *C. jejuni* from two different groups of hosts.

Materials and Methods

Two hundred strains of *C. jejuni* consisted of 100 isolated from faeces of cattle and sheep (60 from sheep and 40 from cattle), and 100 isolated from intestinal tract of chickens were tested for antimicrobial susceptibility. The chicken isolates came from seven different flocks at the different locations and cattle-sheep isolates from more than twenty herds. All Campylobacter organisms were isolated by using blood agar with Preston Selective Supplement (Oxoid, SR 117). *C. jejuni* strains were identified according to the criteria of Smibert (9) and stored frozen at – 70 °C in Brucela broth (Difco) containing 15 % (v/v) glycerol.

Susceptibility testing was performed by standart disk diffusion method (2). All strains were cultured onto blood agar plates which were then incubated for 48 h at 37 °C in microaerophilic atmosphere. This condition was obtained by evacuating two-thirds of the air from an anaerobic jar (without catalyst) and replacing the evacuated air with a carbon dioxide-nitrogen mixture. After incubation, colonies were suspended in saline (0.85 %) and adjusted to a density approximating a McFarland no. 2 turbidity standard. Mueller-Hinton agar (Oxoid) contanining 7 % defibrinated sheep blood were swabbed for confluent growth with each of the test organisms. Antibiotic disks were placed on each plate; plates were incubated for 36 h at 37 °C in microaerophilic condition, with the exception of tetracycline which required a reduced carbon dioxide tension. This was achiveed by evacuting two-thirds of the air from an anaerobic jar and replacing the evacuated air with nitrogen.

The following eight antibiotics were chosen for testing the susceptibility of *C. jejuni* strains: penicillin (10 ui), ampicillin (10 μg), tetracycline (30 μg), kanamycin (10 μg), neomycin (10 μg), streptomycin (10 μg), chloramphenicol (30 μg) and erythromycin (15 μg) (Oxoid-Susceptibility Test Disks).

Differences between the susceptibility of avian and bovine-ovine isolates to antibiotics were compared using a chi-square test.

Results

Since antimicrobial susceptibility patterns of cattle and sheep isolates were almost same, during the evaluation of results, these two *C. jejuni* sources were considered as a single group. The susceptibility of *C. jejuni* strains to the 8 antimicrobial agents is shown in Table 1. Among 100 chicken isolates, seven strains were resistant to chloramphenicol and all of the isolates were resistant to penicillin and ampicillin. All avian isolates of *C. jejuni* were sensitive to the other antibiotics studied. Intermedier zone formation against chloramphenicol was observed in 13 % of these strains.

Table 1. Susceptibility pattern of *C. jejuni* from avian and bovine-ovine origins to eight antimicrobial agents

Antibiotics tested	Avian strains (n = 100)			Bovine-ovine strains (n = 100)			
	[a]R	S	I	R	S	I	
Ampicillin	100	0	0	25	61	14	$P < 0.001$[b]
Chloramphenicol	7	80	13	2	91	7	$P < 0.01$
Erythromycin	0	100	0	4	90	6	$P < 0.05$
Kanamycin	0	100	0	11	73	16	$P < 0.001$
Neomycin	0	100	0	15	68	17	$P < 0.001$
Penicillin	100	0	0	100	0	0	
Streptomycin	0	100	0	36	35	29	$P < 0.001$
Tetracycline	0	100	0	15	69	16	$P < 0.001$

[a](R = resistant, S = sensitive, I = intermedier)

[b]Significance of difference between resistant and sensitive-intermedier strains

All of the bovine-ovine isolates of *C. jejuni* were resistant to penicillin. The prevalence of resistance among these strains against chloramphenicol, erythromycin, kanamycin, neomycin, tetracycline, amplicillin and streptomycin were 2, 4, 11, 15, 15, 25 and 36 per cent, respectively. In bovine-ovine group, intermedier zone against all antibiotics except penicillin occured in varying incidence.

Discussion and Conclusion

Our results showed considerable differences between the antibiotic suscebtibility of chicken *C. jejuni* and bovine-ovine *C. jejuni.* Differences between the resistance frequency of avian and bovine-ovine isolates were significant ($P < 0.001$) for kanamycin, neomycin, streptomycine, tetracycline and ampicillin. It was also significant, in the lesser degrees, for erythromycin ($P < 0.05$) and for chloramphenicol ($P < 0.01$) The chicken *C. jejuni* strains behaved more uniformly although they came from seven different flocks. Although several studies which compared the antimicrobial susceptibility patterns of human and animal strains (mostly pig strains) have conducted (11, 12), information on the comparison of antibiotic sensitivity of *C. jejuni* from different animal species is rather scarce (3). In these studies, it has been generally accepted that human isolates were more susceptible than animal isolates. Vanhoof et al. (12) indicated that animal *C. jejuni* (including avian, bovine and ovine strains) had a bimodal distribution for ampicillin and tetracycline. Bradbury and Munroe (3) have also found considerable differences between the antibiotic senisitivity of chicken and mammalian strains.

The frequency of resistant *C. jejuni* to certain antibiotics is not uniform in the different studies published. When we compared our findings with those of other studies as considering each antibiotic and origin of strain, the differences in the frequency of resistance were apparent. In contrast to our results, it has been reported that chicken *C. jejuni* strains were especially sensitive to penicillin. Vanhoof et al. (12) have indicated that chicken isolates were susceptible to ampicillin and cattle isolates to tetracycline. Bradbury and Munroe (3) have also reported that most chicken isolates were susceptible to ampicillin and few isolates to tetracycline and streptomycin. These observed variations may be due in part to methodology, as differences in technique affect antibiotic activity under microaerophilic conditions or as the threshold used to classify a strain as resistant. Although most workers used dilution techniques when testing campylobacters, the reason why we chose disk diffusion technique was its agreement with dilution techniques which had been also established by Barrett, Kaplan and Goodman (1).

Geographical differences may contribute to the variations in the resistance frequency of *C. jejuni* to some drugs. This is apparent in the case of tetracycline resistance: 37.9 % in Israel (6), 14.5 % in Canada (5) and none in Indonesia (8). Our chicken isolates showed an unusual resistance frequency (100 %) to ampicillin and bovine-ovine isolates to tetractracycline (15 %). The results indicate that resistance to ampicillin among avian isolates of *C. jejuni* and resistance to tetracycline among bovine-ovine isolates of *C. jejuni* seem to be more common in Turkey than those of other countries. The data obtained by the comparison of antimicrobial susceptibility of *C. jejuni* from different origins and different countries can be used for epidemiological purposes.

There is concern that the widespread use of antibiotics in food producing animals, as theraputic agents and feed additives, may lead the development of antibiotic resistant bacteria (10). In view of epidemiological findings (7), it must be kept in mind that the increased level of antibiotic resistance in *C. jejuni* from animals may represent a human health concern.

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