Kafkas Univ Vet Fak Derg
15 (4): 629-632, 2009
DOI:10.9775/kvfd.2009.510

RESEARCH ARTICLE

Metronidazole Resistance of *Campylobacter jejuni* and *Campylobacter coli* from Different Animal Species

Tuba ICA * Alper CIFTCI ** Serap SAVASAN *** Kadir Serdar DIKER ****

* *Erciyes University, Faculty of Veterinary Medicine, Department of Microbiology, Kayseri - TURKEY*
** *Ondokuz Mayis University, Faculty of Veterinary Medicine, Department of Microbiology, Samsun - TURKEY*
*** *Adnan Menderes University, Faculty of Veterinary Medicine, Department of Microbiology, Aydın - TURKEY*
**** *Ankara University, Faculty of Veterinary Medicine, Department of Microbiology, Ankara - TURKEY*

Makale Kodu (Article Code): KVFD-2009-510

Summary

In this study, we aimed to screen animal isolates of *Campylobacter jejuni/Campylobacter coli* for resistance to metronidazole and compared the resistance of selected groups of isolates. A total of 240 *C. jejuni/C. coli* isolates isolated from chickens, cattle, sheep and dogs were tested by an agar dilution technique to determine the Minimum Inhibitory Concentration (MIC) of metronidazole. The frequency of resistance in 133 avian, 32 bovine, 48 ovine and 27 canine isolates were 91.7%, 18.75%, 16.7% and 18.5%, respectively. Of 170 *C. jejuni* and 70 *C. coli* isolates tested, 52.9% and 72.85% were resistant to metronidazole, respectively. MIC (mcg/ml) ranges of metronidazole for animal isolates were as follows: chicken, 2->64; cattle, 0.25-32; sheep, 0.12-16 and dog, 0.25-32. MIC50/MIC90 values of metronidazole for animal isolates were as follows: chicken 16/64 (mcg/ml), cattle 2/8 (mcg/ml), sheep 1/8 (mcg/ml) and dog 2/8 (mcg/ml). We observed a wide range of sensitivity to metronidazole among campylobacters and frequency of resistance differed in relation to the origin of the isolate. Although it was not clear why the resistant isolates were common in chickens, a host-phenotype relationship for metronidazole resistance was demonstrated.

Keywords: *Campylobacter coli, Campylobacter jejuni, Metronidazole resistance*

Farklı Hayvan Türlerinden İzole Edilen *Campylobacter jejuni* ve *Campylobacter coli* Türlerinin Metronidazol Duyarlılıkları

Özet

Bu çalışmada çeşitli hayvanlardan izole edilen *Campylobacter jejuni* ve *Campylobacter coli* suşlarında metronidazol direnç sıklığının belirlenmesi ve karşılaştırılması amaçlandı. Çalışmada tavuk, sığır, koyun ve köpek kökenli 240 *C. jejuni/C.coli* izolatının metronidazol Minimum İnhibisyon Konsantrasyon (MIC) değerleri agar dilusyon tekniği ile incelendi. Direnç sıklığı 133 tavuk, 32 sığır, 48 koyun ve 27 köpek orijinli izolatlar için sırasıyla %91.7, %18.75, %16.7, ve %18.5 oranlarında bulundu. Yüz yetmiş *C. jejuni* izolatının %52.9'unda ve 70 *C. coli* suşunun %72.85'inde metronidazol dirençliliği saptandı. Farklı hayvan izolatları için metronidazol MIC (mcg/ml) aralıkları şu şekilde bulundu; tavuk, 2->64; sığır, 0.25-32, koyun, 0.12-16 ve köpek, 0.25-32. Farklı hayvan izolatları için metronidazol MIC50/MIC90 değerleri; tavuk 16/64 (mcg/ml), sığır 2/8 (mcg/ml), koyun 1/8 (mcg/ml) ve köpek 2/8 (mcg/ml) olarak tespit edildi. Çalışma sonucunda kampilobakterlerde metronidazol duyarlılığı bakımından çeşitlilik olduğu ve direnç sıklığının suşun kökenine göre değiştiği anlaşıldı. Dirençli suşların tavuklarda neden daha sık görüldüğü açıklanamamakla birlikte metronidazol dirençliliği bakımından bir konak fenotip varlığının olduğu ortaya konuldu.

Anahtar sözcükler: *Campylobacter coli, Campylobacter jejuni, Metronidazole dirençliliği*

INTRODUCTION

Metronidazole is a 5-nitroimidazole-based antibiotic used against a wide range of anaerobic bacteria and protozoa which are of significance to human and veterinary medicine. Metronidazole is relatively inactive until metabolized within the microbial cell, and is activated by a reduction of the nitro group that is attached to the imidazole ring. Products of this reduction step lead to the death of the microorganism

İletişim (Correspondence)
☎ +90 362 3121919/2814
✉ aciftci@omu.edu.tr

as a result of DNA damage. Despite displaying no difference between its diffusion into aerobic and anaerobic bacteria, metronidazole is not effective against aerobic bacteria. The underlying reason for this ineffectiveness is due to the fact that metronidazole has a very low redox potential and being reduced in anaerobic environment, thereby resulting in its conversion into cytotoxic form by anaerobic micro-organisms [1,2].

Campylobacter spp. are known as Gram-negative, microaerophilic/anaerophilic, oxidase positive, non-spore forming, spiral-shaped and motile bacteria [3,4]. *Campylobacter jejuni* and its close relative *Campylobacter coli* are known as the members of thermophilic group of campylobacters. They are characterized by growing at 42°C, but not below 30°C. The thermophilic campylobacters are the most common human enteric pathogens causing acute bacterial diarrhea world-wide [5]. *C. jejuni* and *C. coli* are responsible for the majority (80-90%) of enteric *Campylobacter* infections [6,7]. Microaerophilic organisms with a respiratory type of metabolism, such as *Campylobacter* and *Helicobacter* spp., grow in an atmosphere of 5-10% oxygen [2,8]. Since these bacteria prefer molecular oxygen as an electron acceptor under low oxygen pressure, normally, they are expected to be intrinsically resistant to metronidazole. However, *Campylobacter* and *Helicobacter* species have been determined to be highly sensitive to metronidazole, and the exact mechanism remains unsolved. Sensitivity to metronidazole differs among *Campylobacter* isolates in relation to their growth media and the host from which they are isolated [9]. Furthermore, being considered as an indicator of metabolic activity, metronidazole resistance has been used in a biotyping scheme [10].

The present study was aimed at the screening of thermophilic animal isolates of the *Campylobacter* genus for resistance to metronidazole and the comparison of the resistance of selected groups of isolates.

MATERIAL and METHODS

Campylobacter isolates

In the present study, 133 avian, 32 bovine, 48 ovine and 27 canine thermophilic Campylobacter isolates isolated from animal faeces under micro-aerobic conditions on Preston or Modified CCDA selective media were used *(Table 1)*. Before used in study, all isolates were identified as *C. jejuni* or *C. coli* [11-13]. The isolates were stored at -70°C in Brucella Broth with 15% (v/v) glycerin until use.

Antibiotic sensitivity test

The sensitivity of Campylobacter isolates to metronidazole was determined by the agar dilution technique. The agar dilution technique was employed using Mueller-Hinton Agar supplemented with 5% horse blood containing two-fold concentrations of metronidazole. For the test medium, a 0.64 g/L stock solution of metronidazole was prepared in 0.2 M potassium phosphate buffer (pH 7.4), and was sterilized by filtration. Two-fold serial dilutions of metronidazole in potassium phosphate were added at a rate of 1:10 to melted Mueller-Hinton Agar supplemented with 5% horse blood, thereby obtaining a final concentration range of 0.12 - 64 mcg/L.

For the preparation of the inocula, the *Campylobacter* isolates stored at -70°C were grown on Brucella agar supplemented with 5% defibrinated sheep blood under microaerobic conditions for 48 h. The colonies grown were suspended in Mueller-Hinton Broth to obtain a final bacterium concentration of 10^6 CFU/mL. For adjusting the final concentration, *Campylobacter* isolates were tenfold diluted serially with PBS and 0.1 ml of each dilution was inoculated on CSA. After the incubation period, the colonies were counted and diluted to the desired concentration (10^6 CFU/ml). Point inoculations of 10 µL (10^4 CFU) were made from this suspension onto each series of Mueller-Hinton Agar with 5% horse blood containing metronidazole. The media were incubated at 37°C under microaerobic conditions for 48 h. The lowest dilution showing no growth was recorded as the MIC (minimal inhibitory concentration). In the agar dilution technique, the resistance breakpoint for metronidazole was defined as ≥8 µg/mL. Chi-Square test was used for statistical analyses (SPSS 15.0, statistical software programme).

In all experiments, the microaerobic conditions were generated using a Gas Generating Kit-Anaerobic System (Oxoid BR38) placed into a 5-litre anaerobic jar fitted with a manometer. Standard bacteria strains (*C. jejuni* ATCC 11168 and *C. coli* ATCC 33559) were used for control purposes.

RESULTS

For the 240 *Campylobacter* isolates screened in the present study, MIC (mcg/mL) ranges of metronidazole are given in *Table 1*. The origin and frequency of

resistance of the *Campylobacter* isolates are presented in *Table 2*. Upon the evaluation of the isolates with respect to the animal species from which they were isolated, no significant difference was determined to exist between *C. jejuni* and *C. coli* for resistance to metronidazole. Of all *C. jejuni* and *C. coli* isolates tested, 52.9% and 72.8% were determined to be resistant to metronidazole, respectively.

The cumulative evaluation of the MIC values for metronidazole of the Campylobacter isolates isolated from different animal species *(Fig. 1)* demonstrated chicken isolates to be more resistant in comparison to *Campylobacter* isolates isolated from other animal species.

Table 1. *Campylobacter isolates (C. jejuni and C. coli) of animal origin inhibited by different concentrations of metronidazole (MIC)*

Tablo 1. *Farklı konsantrasyonlarda metronidazol (MIC) tarafından inhibe edilen hayvan kökenli Campylobacter suşları (C. jejuni ve C. coli)*

Origin of isolates	Number of isolates	Metronidazole MIC (mcg/ml)			Resistant isolates (%)
		Range	50%	90%	
Chicken	133	2- ≥64	16	64	91.7
Cattle	32	0.25-32	2	8	18.75
Sheep	48	≤0.12-16	1	8	16.7
Dog	27	0.25-32	2	8	18.5

Table 2. *Numbers and percentage frequency rates of metronidazole-resistant thermophilic Campylobacter isolates isolated from various animal species*

Tablo 2. *Çeşitli hayvan türlerinden izole edilen metronidazol-dirençli Campylobacter suşlarının sayı ve oranları*

Origin of isolates	*C. jejuni* (%)	*C. coli* (%)	Total (%)
Chicken	73/78 (93.5)	49/55 (89.1)	122 (91.7)
Cattle	5/25 (20)	1/7 (14.3)	6 (18.7)
Sheep	7/40 (17.5)	1/8 (12.5)	8 (16.7)
Dog	5/27 (18.5)	-	5 (18.5)
Total (240)	90/170 (52.9)	51/70 (72.8)	141/240 (58.75)

DISCUSSION

The frequencies of metronidazole resistance of the *Campylobacter* isolates screened in the present study differed in relation to the origin of the isolate. The isolates originating from chickens were determined to display a high rate of resistance equivalent to 91.7%, whereas resistance was ascertained to range between 16.7 and 18.8% in bovine and canine isolates. Statistical analysis of the occurrence of metronidazole resistance among chicken isolates indicates significant difference for X^2=133.928; P<0.001. (SPSS 15.0, statistical software programme). Skirrow and Benjamin [14], without reference to avian isolates, have reported that metronidazole resistance may differ in relation to the origin of the isolate, and that this feature may be used as a differential test. In a study carried out on 2157 *C. jejuni* isolates, Stanley and Jones [15] reported frequencies of metronidazole resistance to be 82-100%, 17.3-19.5% and 5.5-9% in avian, bovine and ovine isolates, respectively. Frequency of resistance was determined as 62.8% in *C. jejuni* isolates isolated from animals with diarrhea.

In total, a difference of 19.9% was determined between *C. jejuni* and *C. coli* for metronidazole resistance. However, when evaluated on the basis of animals, *C. jejuni* isolates were determined to be more resistant than *C. coli* isolates. The difference ascertained to exist between *C. jejuni* and *C. coli* isolates in total, is considered to have arisen as a result of an imbalance in the distribution of isolates among animal species and the absence of canine *C. coli* isolates.

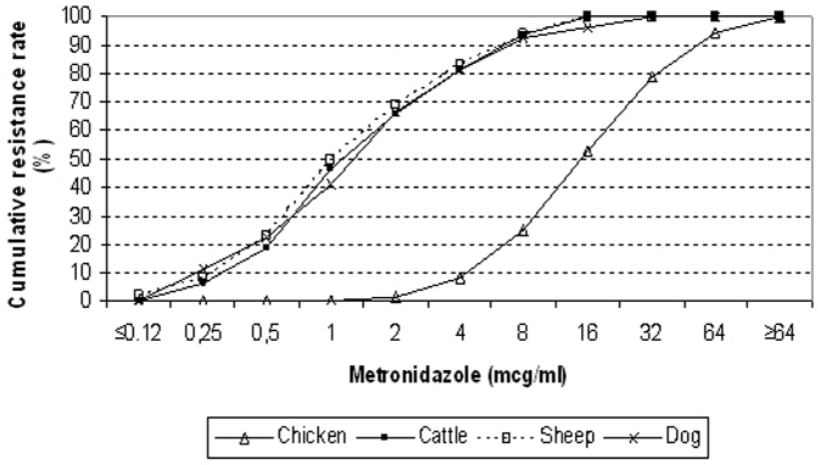

Fig 1. Cumulative percentage rates of thermophilic *Campylobacter* isolates inhibited by increasing concentrations of metronidazole

Şekil 1. Artan konsantrasyonlarda metronidazol tarafından inhibe edilen termofilik *Campylobacter* suşlarının kümülatif oranı

There is no standard metronidazole breakpoint is presented in CLSI and no applicable references for *Campylobacter* spp. On this study, the breakpoint was set as the midpoint between the peaks when the MICs were tetramodelly distributed. Because of the fact that on the 8 µg/ml, similar inhibition rates of *Campylobacter* strains, isolated from four different animal origin, were observed, we chose to use metronidazole breakpoint as ≥8 µg/ml. Hayward et al.[16] reported the MIC range of metronidazole in 53 *C. jejuni* isolates of clinical origin as 0.032->256 mcg/ml, and MIC50 and MIC90 values as 32 and 256 mcg/mL, respectively. Hannah et al.[17] determined the MIC range of metronidazole for anaerobic *Campylobacter* species including *C. gracilis, C. curvus, C. concisus* and *C. rectus* to be 0.25-2 mcg/mL, and reported not to encounter any resistant isolates.

In the present study, as regards thermophilic *Campylobacter* isolates of different origin, MIC ranges were determined to be 2- ≥64 mcg/mL for chickens, 0.12-32 mcg/mL for cattle and dogs and ≤0.12-16 mcg/mL for sheep. Evaluation of the MIC values demonstrated frequency of resistance to differ among thermophilic Campylobacter isolates, which were isolated from four animal species, in relation to the origin of the isolate, and to be highest in chicken isolates.

Campylobacters and Helicobacters are very similar in metabolism. The nitroreductase enzyme of *H. pylori* which is coded by the rdxA gene activates metronidazole. Mutations of the indicated gene are reported to result in metronidazole-sensitive bacteria becoming resistant. However, whether such a pathway is involved in the development of resistance in Campylobacters is yet to be solved [9,18,19].

It is reported that Campylobacters possess anaerobic components including ferrodoxin, flavodoxin, flavoprotein and pyruvat: ferrodoxin oxireductase, and that these components have the redox potential to induce sensitivity to metronidazole [2]. The presence of the hydrogenase enzyme is also closely related to the sensitivity of organisms to metronidazole. The expression of these enzymes may differ in relation to the host of origin [13]. Furthermore, the difference in resistance may arise from the different conditions in the gastrointestinal system of the investigated host species. Furthermore, the difference in resistance may arise from the different conditions in the gastro-intestinal system of the investigated host species. Therefore, it may be suggested that metronidazole resistance can be used as an epidemiological tool for the tracing of the source of the infection.

REFERENCES

1. Samuelson J: Why metronidazole is active against both bacteria and parasites. *Antimicrob Agents Ch,* 43, 1533-1541, 1999.

2. Smith MA, Jorgensen MA, Mendz GL, Hazell SL: Metronidazole resistance and microaerophily in *Campylobacter* species. *Arch Microbiol,* 170, 279-284,1998.

3. Harada K, Asai T, Kojima A, Sameshima T, Takahashi T: Characterization of macrolide-resistant *Campylobacter coli* isolates from food-producing animals on farms across Japan during 2004. *J Vet Med Sci,* 68, 1109-1111, 2006.

4. Kaneene JB, Padungton P: *Campylobacter* spp. in human, chickens, pigs and their antimicrobial resistance. *J Vet Med Sci,* 65, 161-170, 2003.

5. Moreno Y, Hernandez M, Ferrus MA, Alonso JL, Botella S, Montes R, Hernandez J: Direct detection of thermotolerant campylobacters in chicken products by PCR and in situ hybridization. *Res Microbiol,* 152, 577-582, 2001.

6. Bawa EK, Adekeye JO, Oyedipe EO, Umoh JU: Prevalence of bovine campylobacterıosis in indigenous cattle of three states in Nigeria. *Trop Anim Health Prod,* 13, 157-160, 1991.

7. Doyle MP: Association of *Campylobacter jejuni* with laying hens and eggs. *Appl Environ Microbiol,* 47, 533-536, 1948.

8. Uysal H: Glycosylated proteins of the gastric pathogen *Helicobacter pylori*. *Kafkas Univ Vet Fak Derg,* 14 (2): 179-184, 2008.

9. Mendz GL, Mégraud F: Is the molecular basis of metronidazole resistance in microaerophilic organisms understood? *Trends Microbiol,* 10, 370-375, 2002.

10. Bolton FJ, Holt AV, Hutchinson DN: *Campylobacter* biotyping scheme of epidemiological value. *J Clin Pathol,* 37, 677-681, 1984.

11. On SLW: Identification methods for Compylobacters, Helicobacters and related organisms. *Clin Microbiol Rev,* 9, 405-422, 1996.

12. Aydin F, Atabay HI, Akan M: The isolation and characterization of Campylobacter jejuni subsp jejuni from domestic geese *(Anser anser). J Appl Microbiol,* 90, 637-642, 2001.

13. Atabay HI, Corry JEL: The isolation and prevalence of campylobacters from dairy cattle using a variety of methods. *J Appl Microbiol,* 84, 733-740, 1998.

14. Skirrow MB, Benjamin J: *Campylobacter*: Cultural characteristics of intestinal campylobacters from man and animals. *J Hyg,* 85, 427-442, 1980.

15. Stanley KN, Jones K: High frequency of metronidazole resistance among strains of *Campylobacter jejuni* isolated from birds. *Lett Appl Microbiol,* 27, 247-250, 1998.

16. Hayward CL, Erwin ME, Barrett MS, Jones RN: Comparative antimicrobial activity of gatifloxacin tested against *Campylobacter jejuni* including fluoroquinolone-resistant clinical isolates. *Diagn Microbiol Infect Dis,* 34, 99-102, 1999.

17. Hannah MW, Molitoris D, John SS, Vu A, Read EK, Finegold SM: In vitro activities of faropenem against 579 strains of anaerobic bacteria. *Antimicrob Agents Ch,* 46, 3669-3675, 2002.

18. Land KM, Johnson PJ: Molecular basis of metronidazole resistance in pathogenic bacteria and protozoa. *Drug Resist Update,* 2, 289-294, 1999.

19. Tankovic J, Lamarque D, Delchier JC, Soussy CJ, Labigne A, Jenks PJ : Frequent association between alteration of the rdxA gene and metronidazole resistance in French and North African isolates of *Helicobacter pylori*. *Antimicrob Agents Ch,* 44, 608-613, 2000.