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

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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. MIC90/MIC50 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*

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.

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Campylobacter spp. are known as Gram-negative, microaerophilic/anaerophilic, oxidase positive, non-sporing, spiral-shaped and motile bacteria. 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 worldwide. C. jejuni and C. coli are responsible for the majority (80-90%) of enteric Campylobacter infections. Microaerophilic organisms with a respiratory type of metabolism, such as Campylobacter and Helicobacter spp., grow in an atmosphere of 5-10% oxygen. 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. Furthermore, being considered as an indicator of metabolic activity, metronidazole resistance has been used in a biotyping scheme.

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 microaerobic 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.

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 (10^4 CFU/ml). Point inoculations of 10 µL (10^4 CFU) were made from this suspension onto each series of 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.

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 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 X²=133.928; P<0.001. (SPSS 15.0, statistical software programme). Skirrow and Benjamin, 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 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.

**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 X²=133.928; P<0.001. (SPSS 15.0, statistical software programme). Skirrow and Benjamin, 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 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.
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 tetramodally 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. 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. 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. 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. 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 gastrointestinal 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