ISOLATION OF *Campylobacter jejuni* AND *C. coli* FROM DOMESTIC AND EXPERIMENTAL ANIMALS AND THEIR DRUG SUSCEPTIBILITY

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Summary

A total of 526 domestic and experimental animals in Miyagi prefecture, Japan were investigated for fecal carriage of *Campylobacter jejuni* and *Campylobacter coli*. *C. jejuni* was detected in chickens (8.2%), dogs (6.3%), pigs (4.3%), cattle (1.8%) and hamsters (1.4%). *C. coli* was only detected from pigs (20.7%). Drug susceptibility test was performed on 5 strains of *C. jejuni* isolated from chickens and 13 strains of *C. coli* isolated from pigs to tylosin (TS), thianphenicol (TP), carbadox (CDX), chreltetracyclin (CTC), vancomycin (VCM), cefoperazone (CPZ), latamoxef (LMOX), chloramphenicol (CP), gentamycin (GM), benzylpenicillin (PCG), polymyxin B (PL), trimethoprim (TMP). CDX and GM were highly effective and CTC, CP and PL were moderately effective against both *C. jejuni* and *C. coli*. TS and TPH were moderately effective against *C. jejuni*; however, they were less effective to *C. coli*. One strain of *C. jejuni* against CTC considered to be drug resistant. The results suggest that *C. jejuni* and *C. coli* can be controlled by several drugs effectively, although a drug resistant strain exists.

(Key Words : *Campylobacter coli*, *Campylobacter jejuni*, Drug Susceptibility)

Introduction

Campylobacter jejuni and *Campylobacter coli* are important etiological agents of acute enteritis in humans (Butzler and Skirrow, 1979; Skirrow, 1982) and also cause enteritis in several animal species (Fox, 1982; Prescott and Munroe, 1982; Rosef et al., 1983). Most of animals harboring these bacteria live healthy and act as carriers. These carrier animals and their food products are the main sources for human infection (Skirrow, 1982).

We conducted the present study to know the distribution of *C. jejuni* and *C. coli* among domestic and experimental animals in Miyagi prefecture and drug susceptibility of these isolates.

Materials and Methods

Animals

Sampling was conducted in the fall to the winter during the period of 1987 to 1988 in Miyagi prefecture, Japan. Animals were chosen randomly in following facilities; 69 golden hamsters in animal centers of the Faculty of Agriculture and the Faculty of Medicine, Tohoku University, 32 dogs in the Faculty of Medicine, 22 layer chickens in the Faculty of Agriculture and the Miyagi Prefectural Center for Health and Circumstance, 121 broilers in the Kawasaki Farm and poultry processing centers at Iwanuma, Watari and Sennan, 116 pigs in meat processing centers at Sendai, Iwanuma and Senpoku, 163 beef cattle (Japanese short horn) in the Kawatabi Farm of Tohoku University and meat processing centers in Sendai and Senpoku.

Specimen collection, isolation and identification of *Campylobacter*

Campylobacter was isolated and identified by the methods of Morris and Pattonu (1985). Briefly, rectal swabs, fresh stools or cecal contents were collected and kept in Cary-Blair medium at 5-10 ℃ before culture. The specimens were inoculated on Modified Skirrow Agar Plate (Nissui) and incubated at 42℃ for 48 hr in an atmosphere of approximately 5% O_2, 10% CO_2, 85% N_2 using Campy Pak kit (BBL). Strains which showed the following characters were isolated; positivity in catalase production and nitrate reduction, negativity

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in H_2S and urease production, susceptibility to nalidixic acid, resistance to cephalotin and growth at both 37℃ and 42℃. Among these isolates, strains positive in hippurate hydrolysis were defined as *C. jejuni*, and negative strains were defined as *C. coli*.

Antibiotic susceptibility test

Five strains of *C. jejuni* isolated from chickens and one type strain (CIP702), and 13 strains of *C. coli* isolated from pigs and one type strain (CIP7080) were investigated. Drug susceptibility test was performed on these strains to tylosin (TS), thianphenicol (TP), carbadox (CDX), chroltetracyclin (CTC), vancomycin (VCM), cefoperazone (CPZ), latamoxef (LMOX), chloramphenicol (CP), gentamycin (GM), benzylpenicillin (PCG), polymyxin B (PL), trimethoprim (TMP). Minimal inhibitory concentration (MIC) values were determined by the agar dilution methed of the Japan Society of Chemotherapy (Mitsuhashi et al., 1981). Serial twofold dilution of the test drugs ranging from 100 μg/ml to 0.025 μg/ml were used. The lowest concentration of the drug inhibiting macroscopic growth was regarded as MIC of the drug.

Results

C. jejuni was detected in all animal species examined (table 1). The highest rate of isolation was seen among chickens (8.2%), followed by dogs (6.3%), pigs (4.3%), cattle (1.8%) and hamsters (1.4%). *C. coli* was detected only in pigs with relatively high positive rate.

TABLE 1. ISOLATION OF *CAMPYLOBACTER JEJUNI* AND *C. COLI* FROM EXPERIMENTAL AND DOMESTIC ANIMALS

Animal	Number of animals examined	Number of positive animals (%)	
		C. jejuni	*C. coli*
Hamster	69	1 (1.4)	0
Dog	32	2 (6.3)	0
Chicken	146	12 (8.2)	0
Pig	116	5 (4.3)	24 (20.7)
Cattle	163	3 (1.8)	0

C. jejuni (5 isolates and 1 type strain) were inhibited by <25 μg/ml of TS, TP, CDX, CP, GM and PL, which indicated that all strains examined were highly sensitive to these drugs. On the other hand, all strains tested had MICs of ≧50 μg/ml against VCM, CPZ, LMOX and TMP. Four strains had MICs of ≧50 μg/ml against PCG and other one strain had MICs of 12.5 μg/ml. Although 5 strains had MICs of ≦ 6.25 μg/ml against CTC, one strain had an MIC of 100 μg/ml.

C. coli (13 isolates and 1 type strain) were inhibited by ≤25 μg/ml of CDX, CTC, CP, GM and PL. In these drugs MICs of CDX were ≦0.025 μg/ml, which indicated that all strains examined were especially highly sensitive to CDX. On the other hand, all strains tested had MICs of ≧50 μg/ml against VCM, CPZ, and TMP. Four to seven strains had MICs of ≧50 μg/ml against TS, TP, LMOX, and PCG, which indicated that these drugs were moderate to less effective to *C. coli* strains.

Discussion

Campylobacter was identified as a causative agent in a mass outbreak of a food borne disease in a kindergarten in Tokyo in 1979 (Ito et al., 1993). Since then many mass outbreaks were reported, and *Campylobacter* was one of the impotant causative agent of food borne diseases in Japan. In Miyagi prefectue, 3 mass outbreaks were reported from 1982 to 1983. In 1985, 867 of 3,945 elementary schoolchildren who ate a lunch supplied from a cooking facility had clinical signs, and *Campylobacter* was isolated from feces of the patients (Takahashi et al., 1988). By random sampling of patients with infectious diarrhea in Miyagi prefecture, *C. jejuni* was most frequently isolated. Causative agents of 28 of 33 cases in which causative agents were determined in 1988 were *C. jejuni*, and those of 44 of 47 in 1989 were also *C. jejuni* (Yamamoto, 1988, 1989). Therefore, *Campylobacter* has been considered to be the most important causative agent of infectious diarrhea in Miyagi prefecture.

Although the survey in the present study was a small scale, pigs showed high carriage rates of *Campylobacter*. The results coincide with those of Rosef et al. (1983), who showed highest carriage rates of swine among domestic mammals (swine, sheep, cows, goats and horses). In the present study, not only *C. jejuni* but also *C. coli* were

detected from pigs. *C. coli* have been reported to be detected from healthy pigs, and considered to be normal component of the intestinal flora of pigs (Prescott and Munroe, 1982; Sticht-Groh, 1982). *C. jejuni* is the main causative agent of human *Campylobacter* enteritis; however, *C. coli* also the cause of human infection (Skirrow, 1982; Rosef et al., 1983). Pork meat contaminated during the dressing process is a considerably important source of human infection.

C. jejuni was detected from dogs and hamsters in experimental facilities. The rates of carriage were low; however, they can be a potential source of infection to workers or researchers in those facility.

C. jejuni was also detected from chickens and cattle. Eggs and raw or insufficiently cooked chicken meat and beef are potential sources of infection of man.

With a limited number of strains, our data suggested that against both *C. jejuni* and *C. coli* CDX and GM were highly effective and CTC, CP and PL were moderately effective. TS and TPH were moderately effective against *C. jejuni*; however, they were less effective to *C. coli*. One strain which had an MIC of 100 $\mu g/ml$ against CTC considered to be drug resistant. The results suggest that *C. jejuni* and *C. coli* can be controlled by several drugs effectively; however, in order to prevent the appearance of drug resistant strains antibiotics should be used carefully.

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