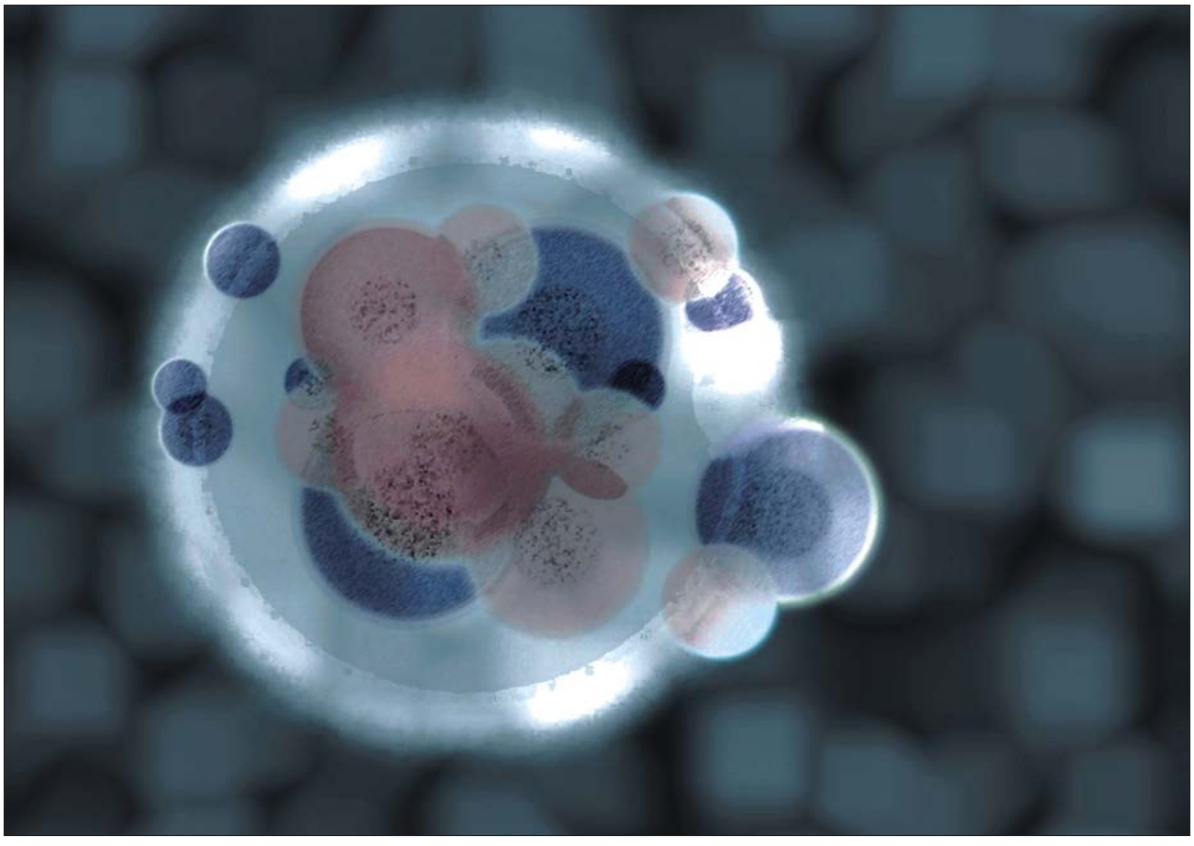

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A Comparison Study on Antimicrobial Susceptibility of *Campylobacter* spp. Isolates from Faecal Samples of Domestic Animals and Poultry in India and Iran

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Abstract: Faecal samples of domestic animals and poultry were subjected to survey frequency of occurrence of pathogenic *Campylobacter* spp. in India (Pune) and Iran (Shiraz). Antimicrobial susceptibility of the isolates was assessed to evaluate the rate of antibiotic resistant campylobacters in both of the areas. The methods for isolation of pathogenic *Campylobacter* spp. was Kapadnis Baseri (prêt- KB) and for antimicrobial susceptibility of the isolates was disc diffusion and E- tests. A total 70 and 37 *Campylobacter* spp. were isolated in India and Iran respectively. All pathogenic *Campylobacter* spp. isolates were sensitive to ciprofloxacin, however, varied responses to the other antibiotics have been observed among the isolates. In addition, lowest MIC values were found for ciprofloxacin and highest MIC values were found for Ampicillin and Choloramphenicol. Overall, based on our observations domestic animals and poultry should be considered as reservoirs of *Campylobacter* spp. in both of the countries. Although, frequency of existence of antibiotic resistance *Campylobacter* in India was relatively high, ciprofloxacin resistant *Campylobacter* were isolated neither from India nor Iran.

Key words: *Campylobacter*, domestic animals, poultry, antibiotics

INTRODUCTION

Campylobacter is the most common cause of bacterial acute gastroenteritis in human beings. The natural habitat of these bacteria is the intestine of birds and other warm-blooded animals, including seagulls and several other wild birds. *Campylobacter* may enter the environment, including water and food through the faeces of animals, birds, or infected humans. These organisms are unable to grow but may survive in the environment for several weeks at temperatures around 4°C (Kapperud and Rosef, 1983). The genus *Campylobacter* comprises 14 species, out of which, *C. jejuni*, *C. coli* and *C. lari* are responsible for cases of gastroenteritis. However, antimicrobial chemotherapy in case of patients with acute *Campylobacter* enteritis involves treatment with erythromycin, tetracyclines and fluoroquinolones (Luber *et al*., 2003; Alfredson *et al*., 2003; Luber *et al*., 2003), but the resistant strains of *Campylobacter* to erythromycin, tetracyclines and fluoroquinolones from developed (Taylor and Courvalin, 1988; Isenbarger *et al*., 2002) and developing countries (Feierl *et al*., 1999) were isolated. For instance, due to increasing fluoroquinolone-resistant campylobacters in Thailand, from 0-84% during 1990-1995 and Austria (Feierl *et al*., 1999) still questions on use of flouroquinolones for treatment of patients suffering from *Campylobacter* enteritis remained. Therefore, based on foregoing evidence and because, investigations on bacteriological, pathological, clinical and epidemiological aspects of campylobacters in India and Iran are relatively recent, the present study was undertaken to determine antimicrobial susceptibility of pathogenic campylobacters isolates from environment in both countries as a comparative study.

MATERIALS AND METHODS

Isolation of *Campylobacter* from environmental samples: In all 246 faecal samples were collected from healthy domestic animals and poultry at different farms of India and Iran. Out of all, 126 samples were collected from buffalo, cow, ox, sheep, goat and poultry in Pune, India and 120 samples were collected from cow, horse and Poultry in Shiraz, Iran. The faecal samples were collected using sterile sticks and polyethylene bags and transferred to the laboratory within one hour of sampling. The samples were subjected for detection of *Campylobacter* immediately upon arrival in the laboratory. The method of *Camplylobacter* detection in this study was

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pre-treatment-Kapadnis Baseri (prêt- KB) method and medium was blood and antibiotic free Kapadnis Baseri (KB) medium (Baserisalehi *et al*., 2004).

To perform this method faecal samples were emulsified at 10% (w/v) in sterile phosphate- buffered saline (0.1 M, pH = 7) to give 10% suspension. The suspension was centrifuged at 8500 rpm for 10 min followed by holding them at room temperature. After 10-15 min, 0.1 mL supernatant from the tube was plated on the KB medium.

All suspected colonies grew on the KB medium were picked up and confirmed by typical morphology, darting motility, Gram staining, oxidase and catalase tests. The isolates exhibiting characteristics of *Campylobacter* were subjected to standard *Campylobacter* phenotypic identification tests (Atabay and Corry, 1997). These tests included H_2S by lead acetate strip, nitrate reduction, growth in 1% glycine and 3.5% NaCl, growth at temperatures 25, 37 and 42°C and resistance to nalidixic acid (30 μg) and cephalothin (30 μg). All thermophilic campylobacters were confirmed using hippurate hydrolysis, indoxyl acetate and urease tests.

Antibiotic susceptibility by disc diffusion method and E-test: Antimicrobial susceptibility of *Campylobacter* spp. isolates in this study was determined by disc diffusion method (Bauer *et al*., 1966) and E-test (Baker, 1992). For disc diffusion test, the antibiotic discs were chloramphenicol 30 μg, co-trimoxazole 25 μg, cefotaxime 30 μg, ampicillin 10 μg, ciprofloxacin 5 μg, tetracycline 30 μg, erythromycin 15 μg, gentamicin 10 μg and cephalexin 30 μg (Hi Media, Mumbai). The disc strengths and the zone size interpretation was in accordance with National Committee for Clinical Laboratory standards (NCCLS, 2002).

The antibiotic strips for E-test were tetracycline, erythromycin, gentamicin, ciprofloxacin, ampicillin and chloramphenicol obtained from AB Biodisk, Sweden.

To perform the disc diffusion test, each culture was grown in 5 mL of Muller-Hinton broth until the turbidity corresponded to 0.5 MacFarland standard tubes (1.5×10^8 cells mL^{-1}). The suspension was spread inoculated using sterile cotton swab onto Muller-Hinton agar plate and various antibiotic discs were placed on it. After incubating the plates at 37°C under microaerophilic conditions for 48h the inhibition zones were recorded.

To perform the E-test three different antibiotic E-test strips were applied on each plate. The plates were incubated at 37°C for 48 h under microaerophilic conditions and inhibitory concentration of each antibiotic was read at the point where the elliptical zone of inhibition intersected the E-test strip.

RESULTS

Isolation and identification of *Campylobacter* spp.: Seventy and thirty seven *Campylobacter* spp. were isolated from faecal samples of domestic animal and poultry in India and Iran respectively. Out of seventy *Campylobacter* isolates in India 27 were belonged to *C. jejuni*, 18 to *C. coli* and 25 to *C. lari* and Out of thirty seven isolates in Iran 15 were belonged to *C. jejuni*, 10 to *C. coli* and 12 to *C. lari* species.

Antibiotic susceptibility of *Campylobacter* isolates: The results on antibiotic susceptibility of *Campylobacter* isolates from faecal samples of domestic animal and poultry by disc diffusion method indicated that all *Campylobacter* isolates were sensitive to ciprofloxacin whilst, different responses to the other antibiotics have been observed among the *Campylobacter* isolates from both of the countries. In addition, present finding showed that frequency of existence of antibiotic sensitive strains of *Campylobacter* in Iran was relatively high. For instance, all *Campylobacter* strains isolates in India were resistant to Cephalexin and Cefotaxime whereas, the sensitive strains of *Campylobacter* to these antibiotics were found among the isolates in Iran. Furthermore, the rate of existence of Ampicillin resistant strains of *Campylobacter* in India was relatively high (Table 1).

Minimal Inhibitory Concentration (MIC) of antibiotics against environmental isolates of *Campylobacter* from domestic animals and poultry in India and Iran by E-test: Minimal inhibitory concentrations of six important antibiotics against *Campylobacter* spp. isolates from domestic animals and poultry were determined by E-test. Swarming of some *Campylobacter* isolates coupled with hazy growth at the edge of the inhibition zone affected precise reading of the E-test results.

As shown in Table 2 and 3, varied ranges of MIC values were observed for different antibiotics due to varied responses of the *Campylobacter* isolates. The lowest MIC values against the *Campylobacter* isolates from both of the areas were found for ciprofloxacin (2 μg mL^{-1}) and highest MIC values were found for ampicillin and chloramphenicol with 256 μg mL^{-1} in case of the isolates in India and 64 μg mL^{-1} in case of the isolates in Iran. Furthermore, the range of MIC values for ciprofloxacin was narrow while, for the other antibiotics tested was wide. Besides, good correlation was found between sensitivity data of *Campylobacter* isolates by disc diffusion method and lowest MIC value obtained for ciprofloxacin in E-test.

Table 1: Susceptibility of environmental campylobacters isolates from domestic animals and poultry in India and Iran by disc diffusion method

Campylobacter spp.	No. of isolates	Percentage of *Campylobacter* isolates sensitive to								
		Ch*	Ce*	Co*	Cf*	Am*	Ci*	Te*	Er*	Ge*
†*Camp. jejuni*	27	33	0	18.5	0	0	100	33	59.2	74.0
†*Camp. coli*	18	50	0	50.0	0	0	100	100	33.4	38.9
†*Camp. lari*	25	36	0	0.0	0	0	100	76	44.0	32.0
‡*Camp. jejuni*	15	73	48	88.5	54	87	100	93	93.0	87.0
‡*Camp. coli*	10	80	62	90.0	43	85	100	87	87.0	87.0
‡*Camp. lari*	12	86	56	87.0	34	73	100	93	93.0	93.0

† *Campylobacter* isolates from domestic animals and poultry in India, ‡ *Campylobacter* isolates from domestic animals and poultry in Iran, *Ch, Chloramphenicol,. Ce, Cephalexin, Co, Co-trimoxazole,. Cf, Cefotaxime,. Am, Ampicillin,. Ci, Ciprofloxacin, Te, Tetracycline,. Er, Erythromycin, Ge, Gentamicin

Table 2: Minimal inhibitory concentrations of antibiotics against environmental *Campylobacter* isolates from domestic animals and poultry in India by E-test

Antibiotics	MICs (μg mL^{-1}) against isolates of								
	*Camp. Jejuni**			*Camp. coli*†			*Camp. lari*‡		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Ampicillin	128-256	128	256	128-256	128	256	128-256	128	256
Ciprofloxacin	2-4	2	4	2-4	2	4	2-4	2	4
Erythromycin	8-32	8	32	8-32	16	32	8-32	16	32
Gentamicin	2-64	8	32	8-64	16	32	8-64	16	32
Tetracycline	8-64	32	64	4-32	4	16	4-64	16	64
Chloramphenico	16-256	64	256	16-256	32	256	16-256	64	256

*27 isolates, † 18 isolates, ‡ 25 isolates were tested. Cnmulative percentage of the MIC concentration at which 50% (MIC_{50}) and 90% (MIC_{90}) of the bacterial isolates were inhibited from growth

Table 3: Minimal inhibitory concentrations of antibiotics against environmental *Campylobacter* isolates from domestic animals and poultry in Iran by E-test

Antibiotics	MICs (μg mL^{-1}) against isolates of								
	*Camp. Jejuni**			*Camp. coli*†			*Camp. lari*‡		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Ampicillin	8-64	16	64	16-64	32	64	32-64	32	64
Ciprofloxacin	2-4	2	4	2-4	2	4	2-4	2	4
Erythromycin	8-32	8	32	8-32	8	32	8-32	16	32
Gentamicin	2-32	4	32	8-64	8	32	8-32	16	32
Tetracycline	8-64	8	32	4-32	4	16	4-32	8	64
Chloramphenicol	16-64	16	64	16-64	32	64	16-64	32	64

*15 isolates, † 10 isolates, ‡ 12 isolates were tested. Cnmulative percentage of the MIC concentration at which 50% (MIC_{50}) and 90% (MIC_{90}) of the bacterial isolates were inhibited from growth

DISCUSSION

The present study clearly demonstrated the significance of domestic animals and poultry as extensive reservoirs of campylobacters. Present finding illustrated that frequency of occurrence of *Campylobacter* was high in the both areas of investigation. In addition, presence of different species of *Campylobacter* suggested that the domestic animals and poultry harbour a variety of the pathogenic *Campylobacter* spp. Therefore, close contact of the people with infected animals and consumption of contaminated animal food products can be a cause of *Campylobacter* enteritis.

Although, pathogenic *Campylobacter* spp. were detected in both of the regions, frequency of occurrence of them in India was relatively high. Similar to present data Baserisalehi *et al.* (2007) expressed, high faecal carriage of campylobacters for domestic animals and poultry in India increased the risk of infections among the people who living and working in the farms of this area. They also opined that existence of campylobacters in the environment depended on weather status of the countries as well as diet of the animals. Hence, suitable environmental conditions and favourable diet might be considered as reasons for existence of campylobacters in India with high frequency compared to Iran.

On the other hand, present data showed that pathogenic *Campylobacter* isolates from domestic animals and poultry in both of the countries were sensitive to ciprofloxacin while, varied responses to the other antibiotics were found among the isolates. Furthermore, the results obtained from susceptibility of the isolates to the antimicrobial agents elucidated that frequency of occurrence of antibiotic sensitive *Campylobacter* isolates from domestic animals and poultry in Iran was relatively high. Although, parallel to

present data isolation rate of antibiotic sensitive *Campylobacter* in developing countries was high (Isenbarger *et al*., 2002; Taylor and Courvalin, 1988), the rate of antibiotic resistant *Campylobacter* is increasing in developed countries (Ge *et al*., 2003). In general, due to high frequency of occurrence of ampicillin resistant *Campylobacter* spp. in India, the ampicillin could not be a drug of choice for treatment of campylobacteriosis. Tetracycline and gentamicin are recommended as alternative treatment, while ciprofloxacin would be a drug of choice for treatment of campylobacteriosis in this geographical area. Similar to India, ciprofloxacin should be considered as a drug of choice for treatment of campylobacteriosis in Iran. In addition, the existence of antibiotic sensitive *Campylobacter* in Iran with high frequency increased possibility to select effective antibiotics for treatment of *Campylobacter* enteritis. Nevertheless based on foregoing evidence ciprofloxacin resistance is not yet a problem in these regions as it is in Styria, Austria (Feierl *et al*., 1999).

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