A comparative study on antimicrobial susceptibility of *campylobacter* spp. Isolates from fecal samples of domestic animals and poultry in Tonekabon and Shiraz, Iran

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ABSTRACT:

During the past decade *Campylobacter* has been shown to be responsible for enteritis in human and animal. The natural habitats of most *Campylobacter* species are the intestines of birds and other warm-blooded animals. These organisms may enter the environment, including drinking water, through the feces of animals, birds or infected humans. Fecal samples of Domestic Animals and Poultry were subjected to survey frequency of occurrence of pathogenic *Campylobacter* spp. in Tonekabon and Shiraz. Antimicrobial susceptibility of the isolates was assessed to evaluate the rate of antibiotic resistant campylobacter's in both cities. The method for isolation of pathogenic *Campylobacter* spp. was Kapandis Baseri (prêt-KB) and for antimicrobial susceptibility of the isolates was disk diffusion and E-test. A total of 28 and 37 *Campylobacter* spp. were isolated in Tonekabon and Shiraz, respectively. All pathogenic *Campylobacter* spp. isolates were sensitive to Ciprofloxacin, however, varied responses to other antibiotics have been observed among the isolates. In addition, lowest MIC values were found for Ciprofloxacin and Gentamicin and highest MIC values were found for Erythromycin, Chloramphenicol, Gentamicin and Tetracycline. Overall, based on our observations, domestic animals and poultry should be considered as reservoirs of *Campylobacter* spp. in both cities. Although, frequency of existence of antibiotic resistance *Campylobacter* in Tonekabon was relatively high, Ciprofloxacin resistant *Campylobacter* were isolated neither from Tonekabon nor Shiraz. The Result obtained from data statistical analyses showed significant correlation ($P<0.05$) between the isolation rate of susceptible strains of *Campylobacter* to Cefalexin, Cefalotin and Ampicillin in Tonekabon and Shiraz.

Keywords: *Campylobacter*; Domestic Animals; Poultry; Antibiotics

INTRODUCTION

Members of the *Campylobacter* genus are gram negative, curved, S-shaped, non-spore forming and microaerophilic bacteria commonly found in animal feces. *Campylobacter* is the most common cause of bacterial acute gastroenteritis in human beings [1]. 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 farces of animals, birds, or infected humans [2]. These organisms are unable to grow but may survive in the environment for several weeks at temperatures around 4°C [3]. The genus *Campylobacter* comprises 14 species, out of which, *C. jejuni*, *C. coli* and *C. lari* are responsible for cases of gastroenteritis. Infective dose of this bacterium is very small; it has been estimated that only 500 cells of *C.jejuni* can cause human illness [4].

In 1999, the center for disease control and prevention estimated that more than two million *Campylobacter* infections occurred annually in the US, which accounted these bacteria as the most common cause of food borne illnesses [5]. Extensive reports in developed countries pointed out that the consumption of contaminated poultry meat is major source

of *Campylobacter* infection [6&7]. On the other hand, European food safety authority's report in 2005 stated that during the last 30 years human campylobacteriosis has dramatically increased in industrialized countries [8]. The epidemiologic survey in developing countries illustrated different levels of isolation of *C.jejuni* from the samples in Bangkok, Thailand, Nairobi, Kenya and India [9]. Moreover, similar to the developed countries, poultry was reported as major source of infection in those countries [9].

However, antimicrobial chemotherapy in case of patients with acute *Campylobacter* enteritis involves treatment with Erythromycin, Tetracycline and Fluoroquinolones [10,11] but the resistant strains of *Campylobacter* to Erythromycin, Tetracycline's and Fluoroquinolones from developed [12&13] and developing countries [14] were isolated. For instance, due to increasing fluoroquinolone-resistant campylobacter's in Thailand, from 0-84% during 1990-1995 and Austria still questions on use of Fluoroquinolones for treatment of patients suffering from *Campylobacter* enteritis remained [15-17]. Therefore, based on foregoing evidence and because, investigations on bacteriological, pathological, clinical and epidemiological aspects of campylobacter's in Iran, the present study was undertaken to determine antimicrobial susceptibility of pathogenic campylobacter's isolates from environment in both area as a comparative study.

MATERIALS AND METHODS

Isolation of *Campylobacter* from environmental samples: In all, 260 faecal samples were collected from healthy domestic animals and poultry at different farms of Tonekabon and Shiraz. From these, 140 samples were collected from cow, sheep, horse and poultry in Tonekabon and 120 samples were collected from cow, horse and poultry in Shiraz. 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 *Campylobacter* detection in this study was pre-treatment-Kapandis Baseri (prêt- KB) method and medium was blood and antibiotic free Kapandis Baseri (KB) medium [18].

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 and 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 [19]. 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 campylobacter's 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 [20] and E-test [21]. For disc diffusion test, the antibiotic discs were Chloramphenicol 30 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 were in accordance with National Committee for Clinical Laboratory Standards [22]. The antibiotic strips for E-test were Tetracycline, Erythromycin, Gentamicin, Ciprofloxacin 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 ($1.5x10^8$ 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, five 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. The number of sample calculated by this formula:

$$n = \frac{z^2 \times p\,(1-p)}{d^2}$$

Statistical analyses of the data were carried out using SPSS computer software (SPSS 16) and Chi Square test.

RESULTS:

Isolation and identification of *Campylobacter* spp.: twenty eight and thirty seven *Campylobacter* spp. were isolated from faecal samples of domestic animal and poultry in Tonekabon and Shiraz respectively. Out of twenty eight *Campylobacter* isolates in Tonekabon 12 were belonged to *C. jejuni*, 8 to *C. coli* and 8 to *C. lari* and out of thirty seven isolates in Shiraz, 15 had 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 area. In addition, present finding showed that frequency of existence of antibiotic sensitive strains of *Campylobacter* in Shiraz was relatively high. For instance, all *Campylobacter* strains isolates in Tonekabon were resistant to Ampicillin whereas, the sensitive strains of *Campylobacter* to this antibiotic were found among the isolates in Shiraz. Furthermore, the rate of existence of Cephalothin and Cephalexin resistant strains of C*ampylobacter* in Tonekabon was relatively high (Table 1).

Minimal Inhibitory Concentration (MIC) of antibiotics against *Campylobacter* isolates: Minimal inhibitory concentrations of five 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 Tables 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 and Gentamicin (2 μg mL^{-1}) and highest MIC values were found for Chloramphenicol, Erythromycin, Gentamicin and Teracycle (64 μg mL^{-1}). Furthermore, the range of MIC values for Ciprofloxacin was narrow while, for the other antibiotics tested it 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. The Result obtain from Statistical analyses of data showed significant correlation ($P<0.05$) between the isolation rate of susceptible strains of *Campylobacter* to Cefalexin, Cefalotin and Ampicillin in Tonekabon and Shiraz. However, no significant correlation was found between the isolation rates of susceptible strains to the rest of Antibiotics in Both cities.

Table 1: susceptibility of environmental campylobacter's isolates from domestic animals and poultry in Tonekabon and Shiraz by disc diffusion method.
Percentage of *campylobacter* isolates sensitive to:

	No.of Isolated	C	CN	Cf	Am	CP	T	E	GM
† *C.jejuni*	12	83	17	25	0	100	83	75	67
† *C.coli*	8	75	0	13	0	100	88	75	88
† *C.lari*	8	75	13	0	0	100	88	100	75
‡ *C.jejuni*	15	73	47	53	87	100	93	93	87
‡ *C.coli*	10	80	60	40	90	100	90	90	90
‡ *C.lari*	12	83	58	33	75	100	92	92	92

† *Campylobacter* isolates from domestic animals and poultry in Tonekabon, ‡ *Campylobacter* isolates from domestic animals and poultry in Shiraz, C, Chloramphenicol,. CN, Cephalexin,. Cf, Cefotaxim,. Am, Ampicillin,. CP, Ciprofloxacin,. T, Tetracycline,. E, Erythromycin,. GM, Gentamicin,.

Table 2: Minimal inhibitory concentrations of antibiotics against environmental campylobacter's isolates from domestic animals and poultry in Tonekabon
MICs (µg mL^{-1}) against isolates of

	*C.jejuni**			*C.coli*†			*C.lari*‡		
Antibiotics	Range	MIC 50	MIC 90	Range	MIC 50	MIC 90	Range	MIC 50	MIC 90
Erythromycin	8-64	16	64	8-32	16	32	8-32	16	32
Gentamicin	8-64	8	32	2-32	4	32	2-32	8	32
Ciprofloxacin	2-4	2	4	2-4	2	4	2-4	2	4
Chloramphenicol	16-64	16	32	16-64	32	64	8-64	16	64
Tetracycline	8-32	8	32	4-32	4	16	8-32	16	32

*12 isolates, † 8 isolates, ‡ 8 isolates were tested. Cumulative 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 campylobacters isolates from domestic animals and poultry in Shiraz
MICs (µg mL^{-1}) against isolates of

	*C.jejuni**			*C.coli*†			*C.lari*‡		
Antibiotics	Range	MIC 50	MIC 90	Range	MIC 50	MIC 90	Range	MIC 50	MIC 90
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
Ciprofloxacin	2-4	2	4	2-4	2	4	2-4	2	4
Chloramphenicol	16-64	16	64	16-64	32	64	16-64	32	64
Tetracycline	8-64	8	32	4-32	4	16	4-32	8	64

*15 isolates, † 10 isolates, ‡ 12 isolates were tested. Cumulative 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 campylobacter's. Present findings 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 harbor 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 [23&24]. A number of potential risk factors related to campylobacteriosis is untreated water, poor food hygiene and handling practices [25]. In order to find out the likely sources of *Campylobacter* it is necessary to characterize strains, which are commonly isolated from food chain and environment and to identify these strains in the human infections.

On the other hand, present data showed that pathogenic *Campylobacter* isolates from domestic animals and poultry in both areas 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 C*ampylobacter* isolates from domestic animals and poultry in south of Iran was relatively high. Although, parallel to present data isolation rate of

antibiotic sensitive *Campylobacter* in developing countries was high [26- 28] the rate of antibiotic resistant *Campylobacter* is increasing in developed countries [17,29]. In general, due to high frequency of occurrence of Ampicillin resistant *Campylobacter* spp. in these countries, 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 [18]. In addition, the existence of antibiotic sensitive *Campylobacter* in Iran with high frequency increased possibility to select effective antibiotics for treatment of *Campylobacter* enteritis.

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