International Journal of Molecular and Clinical Microbiology 1 (2011) 21-24

International Journal of
Molecular and Clinical Microbiology

Antimicrobial Susceptibility of Thermophilic *Campylobacter* spp. Isolated from Environmental Samples in Tonekabon

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ARTICLE INFO

Article history:
Received 9 September 2010
Accepted 24 February 2011
Available online 11 April 2011

Keywords:
Thermophilic *Campylobacter*
Antimicrobial Sensitivity
Environment Samples

ABSTRACT

The major purpose of this study was isolation, identification and antimicrobial susceptibility of thermophilic *Campylobacter* spp. from different sources including domestic animals (cow, sheep, horses), poultry, river water and sewage in Tonekabon. *Campylobacter* spp. was isolated using prêt-KB method and identified by phenotyping tests. Antimicrobial susceptibility of the isolates against different antibiotics and Minimal Inhibitory Concentration (MIC) values were determined by disc diffusion and double dilution methods respectively. In general, 32 strains of thermophilic *Campylobacter* were isolated from all of the sources. The results obtained indicated that frequency of occurrence of *Campylobacter* in poultry was high and in sewage was low. In addition, thermophilic *Campylobacter* isolates were sensitive to Amikacin and Ciprofloxacin and resistant to Ampicillin, Amoxicillin, Penicillin, Amoxiclave and Vancomycin. The lowest values of MIC were found for Ciprofloxacin, while the highest value was found for Streptomycin. Overall, our observations, illustrated that pathogenic *Campylobacter* were existed in all of the sources in north of Iran. Furthermore, they were sensitive to Amikacin and Ciprofloxacin and resistant to β lactam antibiotics.

1. Introduction

Members of the *Campylobacter* genus are gram negative, curved and S-shaped microaerophilic bacteria (Baserisalehi and Bahador, 2008). *Campylobacter* is one of the most important causes of bacterial acute gastroenteritis in human being. The natural habitat of these bacteria is intestine of bird and warm blooded animals. Pathogenic (thermophilic) *Campylobacter* can enter to the water and foods through the feces of warm blooded animals, birds and infected humans (Scotter et al., 1993). Entrance of Pathogenic *Campylobacter* into the human body causes enteritis with abdominal pain and watery diarrhea symptoms. *Campylobacter* genus consist 14 species in which *C. jejuni*, *C. coli* and *C. lari* are responsible for gastroenteritis (Alfredson et al., 2003; Luber et al., 2003). Although, *Campylobacter* enteritis is self limited, but antibiotic therapy must be considered in severe cases. However, Erythromycin, Tetracycline and Fluoroquinolones have been used for treatment of acute *Campylobacter* gastroenteritis, but recently some resistant strains have isolated in developed

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and developing countries (Taylor and Courvalin, 1998; Isenbarger et al., 1999). These resistances in *Campylobacter* spp. are related to the antibiotic usage in veterinary medicine and prophylaxis. In such cases, we will face to bacteria with increased levels of antibiotic resistances, by which the treatment process will be more difficult (Pidock et al., 2000). Therefore, based on forgoing evidence, the present study was undertaken to isolate pathogenic *Campylobacter* from environmental samples in order to achieve maximum information concerning their antimicrobial susceptibility.

2. Material and methods

2.1. Isolation of Campylobacter spp. from environmental samples

A total of 160 samples were collected from domestic animal (cow, horse, sheep), poultry feces, sewage, and river water from different areas in Tonekabon area. The fecal sample was collected from each animal using sterile stick and polyethylene bag and transferred to the laboratory within one hour of sampling. The river water and sewage samples were collected in 500 ml sterile bottles and transported to the laboratory at ambient temperature and stored at 4˚C before they were analyzed within 2h. All samples were subjected to detection of *Campylobacter* immediately upon arrival in the laboratory.

The pret-KB method was used for isolation of campylobacters (Baserisalehi et al., 2004). One gram of the collected fecal samples was emulsified in sterile phosphate buffered saline (pH 7.0, 0.1 M) at 10% (w/v) concentration. The suspension was centrifuged at 8500 rpm for 10 min, and then kept at room temperature. After 10-15 min, a loopful of supernatant was withdrawn and spread onto the Mueller-Hinton agar. The plates were incubated at 37^0C for 48 h under microaerophilic conditions and examined daily for 5 days.

Campylobacter identification was performed by subjecting all suspected colonies to microscopic examination of wet mount under dark field microscopy, gram staining, glucose fermentation, oxidase and catalase tests. The isolates exhibiting characteristic motility of *Campylobacter* were characterized by using standard *Campylobacter* phenotypic identification tests recommended by 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 different temperatures (25, 37 and 42°C), hippurate hydrolysis, urease production, resistance to Nalidixic acid (30 µg) and Cephalothin (30 µg).

2.2. Antibiotic susceptibility by disc diffusion method

In this study, the antimicrobial susceptibility of *Campylobacter* spp. isolates was determined by disc diffusion test (Bauer et al., 1966). For disc diffusion test, Ampicillin (10 µg), Amikacin (10 µg), Amoxicillin (25 µg), Cephalexin (30 µg), Ceftriaxone (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Amoxi Clave (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Tetracycline (30 µg), Penicillin (10 µg), Streptomycin (10 µg), Tobramycin (10 µg) and Vancomycin (30 µg) (PADTAN TEB - Iran) were used. The disc strengths and the zone size interpretation were done in accordance with National Committee for Clinical Laboratory Standards.

To perform the disc diffusion test, each culture was grown in 5 mL of Muller-Hinton broth until the turbidity corresponded to 0.5 McFarland standard tubes (1.5×10^8 cells mL^{-1}). This microbial suspension was spread out on the surface of Muller-Hinton agar by sterile swap and various antibiotic discs were placed on it. After incubating the plates at 37^oC under microaerophilic conditions for 48h, the inhibition zone were recorded.

3. Results

3.1. Isolation and identification of Campylobacter spp.

Thirty two *Campylobacter* spp., Comprising 16 *Campylobacter jejuni*, 9 *Campylobacter coli* and 7 *Campylobacter lari*, were isolated from fecal samples of domestic animals, poultry, river water and sewage in investigated area.

3.2. Antibiotic susceptibility of Campylobacter isolates

The results obtained for antibiotic susceptibility of *Campylobacter* isolates from environmental samples by disc diffusion method indicated that all strains were sensitive to Amikacin and Ciprofloxacin, while they showed different levels of susceptibility to other antibiotics including 98% to Gentamicin, 92% to Erythromycin, 90% to Tetracycline and Streptomycin, 82% to Tobramycin, 81% to Chloramphenicol, 4% to Cephalexin and 2% to Ceftriaxone. All *Campylobacter* spp. isolates were resistant to Ampicillin, Amoxicillin, Amoxi Clave, Penicillin and Vancomycin (table 1).

3.3. MIC of antibiotics against environmental isolates of Campylobacter spp.

Minimal inhibitory concentrations of eight important antibiotics against *Campylobacter* spp. isolates from environmental samples were determined. As shown in table 2, varied ranges of MIC values were observed for different antibiotics due to varied responses of *Campylobacter* isolates. The lowest MIC values were found for Ciprofloxacin (2 μg mL^{-1}), while Streptomycin (128 μg mL^{-1}) showed the highest value. Furthermore, the range of MIC values for Ciprofloxacin was narrower than other antibiotics tested.

4. Discussion

According to our data, domestic animals and poultry are of the major reservoirs of *Campylobacter* in investigated areas. Thus, direct contacts with infected animals and consumption of animal food products can cause enteritis in human beings (Aydin et al., 2007). Generally, antimicrobial chemotherapy in case of patients with acute *Campylobacter* enteritis involves treatment with erythromycin, Tetracyclines and Fluoroquinolones (Alfredson et al., 2003; Luber et al., 2003) but resistant strains of *Campylobacter* to erythromycin, Tetracyclines and Fluoroquinolones have been isolated in both developed (Taylor and Courvalin, 1998) and developing countries (Feierl et al., 1999). For instance, campylobacter strains resistant to fluoroquinolone increased from zero to 85% in Thailand and Austria during years 1990-1995 (Feierl et al., 1999).

The results of this study regarding antimicrobial susceptibility of the isolates illustrated that all pathogenic *Campylobacter* isolates were sensitive to Amikacin and Ciprofloxacin and resistant to Ampicillin, Amoxicillin, Penicillin, Amoxiclave and Vancomycin. These data were parallel to some reports obtained from developed and developing counties (Taylor and Courvalin, 1998; Isenbarger et al., 2002) although the rate of antibiotic resistances has been reported to be relatively higher in developed countries (Ge et al., 2003).

In conclusion, due to high occurrence frequency of Ampicillin, Amoxicillin, Amoxi Clave, penicillin, and Vancomycin resistant *Campylobacter* spp., it is suggested that these antibiotics do not be considered as drugs of choice for treatment of campylobacteriosis. In contrast, Ciprofloxacin could be used as drugs of choice, and Tetracycline, Gentamicin and Erythromycin as alternatives for treatment of campylobacteriosis in this geographical area.

Table 1. Antibiotic susceptibility of environmental campylobacters isolated from environmental samples in north of Iran by disc diffusion method

		Percentage of *campylobacter* isolates sensitive to:														
	No. of Isolated	C	CN	CRO	Am	AMX	CP	T	E	GM	AN	CoA	P	S	TOB	V
C.jejuni	16	94	13	7	0	0	100	94	88	94	100	0	0	94	75	0
C.coli	9	78	0	0	0	0	100	89	89	100	100	0	0	89	100	0
C.lari	7	71	0	0	0	0	100	86	100	100	100	0	0	86	71	0

C, Chloramphenicol,. CN, Cephalexin,. CRO, Ceftriaxone,. AM, Ampicillin,. AMX, Amoxicillin,. CP, Ciprofloxacin,. T, Tetracycline,. E, Erythromycin,. GM, Gentamicin,. AN, Amikacin,. CoA, Amoxi-Clave,. P, Penicillin,. S, Streptomycin,. TOB, Tobramycin,. V, Vancomycin.

Table 2. Minimal inhibitory concentrations of antibiotics against campylobacters isolated from environmental samples in north of Iran

	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
Amikacin	16-32	16	32	8-16	8	16	8-16	16	16
Erythromycin	8-64	32	64	8-16	8	16	8-32	16	32
Gentamicin	8-64	8	32	8-32	8	32	8-64	8	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
Streptomycin	16-32	16	32	8-128	16	32	8-64	16	64
Tetracycline	8-64	8	32	8-32	4	16	4-32	8	64
Tobramycin	16-32	16	32	4-8	4	8	4-32	4	32

Acknowledgments

I would like to thank Dr. Ghane for the critical reading of the manuscript and his useful comments.

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