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Prevalence and Antibiogram of *Campylobacter* Infections in Dogs of Mathura, India

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ABSTRACT

With the increasing trends of pet ownership the chances of campylobacteriosis are also increasing as these pets are kept in close visicinity of owners. The prevalence and antimicrobial sensitivity profiles of *Campylobacter* isolates from faeces of dogs attended in veterinary practice at Teaching Veterinary Clinical Complex, Mathura, India. During the period of investigation (October 2009 to April 2010), 100 rectal swabs from dogs were collected and transported to the laboratory for further investigations. Bacteriological examination revealed 51.00% prevalence rate of *Campylobacter* isolates in dogs faecal samples. The disc-diffusion method was used to know the susceptibility of all the 51 *Campylobacter* isolates against 10 commonly used antimicrobials in pet animal practice. High rates of resistance were observed to erythromycin (90.20%), tetracycline (88.23%), ampi-cloxacillin (88.23%), ciprofloxacin (80.39%), enrofloxacin (68.63%) and aymoxycillin-clavulanic acid (19.61%). All the *Campylobacter* isolates were susceptible to amikacin, levofloxacin and streptomycin. Erythromycin and ciprofloxacin are drugs for treatment of human campylobacteriosis. The high resistance rate to these drugs among *Campylobacter* isolates from dog faeces is of public health significance as dogs are supposed to be the main source of infection in human beings.

Key words: Prevalence, *Campylobacter*, dogs, drug resistance

INTRODUCTION

Enteropathogenic bacteria have long been recognized as a significant problem owing to their pathogenicity potential to animals and their zoonotic risk to humans. Among them, *Campylobacters* have been considered to be important pathogens causing human gastroenteritis, arthritis, meningitis globally (Goldberg and Rubin, 1988; Peterson, 1994; Baserisalehi *et al*., 2006; Humphrey *et al*., 2007; Frederick and Huda, 2011) and leading to serious impact on public health (Ethelberg *et al*., 2004). Dogs are contaminated by oral-fecal contact and by manifesting gastroenteritis or acting as healthy carriers, serve as a potential source of infection to humans (Bruce and Fleming, 1983; Goossen *et al*., 1991; Burnens *et al*., 1992; Ene *et al*., 1992; Moreno *et al*., 1993; Torre and Tello, 1993; Fernandez *et al*., 1994; Robinson and Pugh, 2002; Workman *et al*., 2005; Sabry, 2009). Dogs in developing countries like India often live

in close proximity to humans (with the possibility of direct transmission of pathogens) and have not been examined thoroughly for *Campylobacter* sp. carriage. There is a dearth of information and research on the prevalence and antimicrobial resistance studies of *Campylobacters* in dogs in India in spite of the reports in Europe and other parts of the world on dogs as a potential sources of infections for humans.

This study therefore, ascertained the prevalence and drug resistance profiles of *Campylobacter* spp. isolated from dogs in Mathura, India in order to provide updated information and the suspected role of dogs in its zoonotic significance.

MATERIALS AND METHODS

Rectal swabs were collected aseptically from 100 dogs presented to veterinary practice at Teaching Veterinary Clinical complex (University Veterinary Hospital), DUVASU, located in Mathura, India and transported at 4°C to the laboratory on the day of collection for the isolation of *Campylobacter* spp.

Isolation and identification of *Campylobacter* spp.: All the samples were processed in *Campylobacter* Enrichment Hi Veg TM Broth Base (HiMedia, Mumbai) with addition of polymixin B sulphate, rifampicin, trimethoprim and cycloheximide (*Campylobacter* selective IV, HiMedia, Mumbai) and incubated at 42-43°C under micro aerophilic conditions for 24 h. After incubation, the inoculums was streaked onto selective media (*Campylobacter* selective agar, HiMedia, Mumbai) supplemented with 10% defibrinated lysed sheep blood and reconstituted contents of *Campylobacter* selective-I (HiMedia, Mumbai) containing polymixin B, vancomycin, trimethoprim and cephalothin and incubated for 48 h at 42-43°C under micro aerophilic conditions. Characteristic *Campylobacter* colonies were picked up and subjected to presumptive identification like Gram's staining, motility, oxidase and catalase test and further subjected to biochemical test for confirmation (Skirrow and Benzamin, 1980; Gracia *et al*., 1985).

Antimicrobial sensitivity assay: All the *Campylobacter* isolates were assessed for their antimicrobial susceptibility testing by the disc-diffusion method following the NCCLS guidelines (NCCLS, 2002). The following antimicrobial agents were used at the indicated concentrations (μg $disc^{-1}$ except where specified): using 10 commonly used antibiotic discs (Hi-Media, Mumbai) viz., amikacin (30 g), amoxycillin-clavulanic acid (20/10 μg), ampicloxacillin (10 μg), ciprofloxacin (30 μg), chloramphenicol (30 μg), enrofolxacin (10 μg), erythyromycin (15 μg), levofloxacin (5 μg), streptomycin (10 μg) and tetracyclin (30 μg).

RESULTS

Campylobacter spp. was isolated from 51 dogs of the total 100 dogs (percent positivity 51.00%). The results of antibiotic sensitivity test for the ten antimicrobial agents for *Campylobacter* spp. is shown in Table 1. Using the disc diffusion method, 46 out of 51 isolates of *Campylobacter* (90.20%) were resistant to erythromycin, 45 to tetracycline (88.23%), 45 to ampi-cloxacillin (88.23%), 41 to ciprofloxacin (80.39%), 35 to enrofloxacin (68.63%) and 10 to amoxycillin-clavulanic acid (19.61%). All the *Campylobacter* isolates were susceptible to amikacin, choramphenicol, levofloxacin and streptomycin.

Table 1: Antibiotic sensitivity test of *Campylobacter* spp. isolates of dogs

	No. of isolates (51)				
Antimicrobial agents	Resistant	Intermediate	Sensitive	Sensitivity (%)	Resistant (%)
Amikacin	0	0	51	100.00	0.00
Amoxycillin-clavulanic acid	10	10	31	60.79	19.61
Ampi-cloxacillin	45	0	06	11.76	88.23
Ciprofloxacin	41	0	9	17.64	80.39
Chloramphenicol	0	5	46	90.19	0.00
Enrofolxacin	35	0	16	31.37	68.63
Erythyromycin	46	0	5	9.80	90.20
Levofloxacin	0	5	46	90.19	00.00
Streptomycin	0	1	50	98.04	00.00
Tetracyclin	45	0	6	11.76	88.23

S: Sensitivity, I: Intermediate, R: Resistant

DISCUSSION

Elucidating the shedding patterns and prevalence of *Campylobacters* in the faeces of dogs is a prerequisite for effective healthcare strategy against zoonotic infections. The species distribution of *Campylobacter* isolates from dogs and other animals differs considerably between publications and years (Hald and Madsen, 1997; Lopez *et al*., 2002; Sandberg *et al*., 2002; Hald *et al*., 2004; Workman *et al*., 2005; Mohammad and Mohagheghi, 2006; Baserisalehi *et al*., 2007b; Huat *et al*., 2010). The introduction of antimicrobial agents in human and animal therapy has had a great impact on population. The first agents were introduced during the 1930s and resistance to these drugs gradually emerged with their worldwide use. *Campylobacter* spp. is classified as an emerging human pathogen and recently, concern regarding the prevalence of campylobacteriosis has increased because of the frequent isolation of antimicrobial-resistant strains from humans and animals. After exposure to ten antibiotics, several isolates showed multiple resistances to most of the antibiotics used. Studies related to the sensitivity to antibiotics of *Campylobacter* spp. in different countries show different degrees of resistance to the same drug (Guevremont *et al*., 2006; Han *et al*., 2007; Little *et al*., 2008; Moran *et al*., 2009). In this study, majority of *Campylobacter* spp. isolates showed resistance to at least 5 of the antibiotics tested, indicating multi-drug resistance. In the present work, all isolates were sensitive to amikacin, chloramphenicol, levofloxacin and streptomycin. Sensitivity to chloramphenicol by all *Campylobacter* isolates was also observed in pigs (Saenz *et al*., 2000; Guevremont *et al*., 2006), whereas, similar resistance patterns for *Campylobacter* spp. was obtained from humans (Bardon *et al*., 2009) and chickens (Miflin *et al*., 2007).

Majority of the isolates were resistant to erythromycin, tetracycline, ampi-cloxacin, ciprofloxacin and enrofloxacin. Resistance to tetracycline by *Campylobacter* isolates from humans, dogs and other animals may range from 15 to 94% (Modolo *et al*., 1991; Gaudreau and Gilbert, 1998; Saenz *et al*., 2000; De Vega *et al*., 2005). The great variability in this antibiotic's efficacy is probably due to its worldwide use in cattle, both at therapeutic or low doses; this would increase selective pressure on bacteria. Clinical assays, however, have shown the therapeutic efficacy of tetracycline in treating dogs with Campylobacteriosis and a decrease in re-excretion rate (Abrahans *et al*., 1990; Burnens *et al*., 1992). High resistance to ampi-cloxacillin for *Campylobacter* spp. i.e., 57.3% (Little *et al*., 2008) and 65.7% (Saenz *et al*., 2000) was also recorded in samples from

pigs and 43.1% (Han *et al*., 2007) and 40.8% (Miflin *et al*., 2007) in samples from chicken. Similar results were obtained in this study, where 88.23% of the strains showed resistance to ampi-cloxacillin.

Campylobacter was frequently sensitive to quinolones; however, an increased resistance to these drugs is seen, probably due to genetic mutations interfering with bacterial DNA girase (Greene and Watson, 2003). Selective pressure caused by the indiscriminate use of these drugs in aviculture is a contributory factor. Previous studies (Saenz *et al*., 2000; Norma *et al*., 2007; Biasi *et al*., 2011) reported the greatest resistance of Campylobacter isolates to quinolones among various antibiotics similar to results obtained in the current study, in which 80.39% of the isolates were resistant to ciprofloxacin. Contrary to our findings, sensitivity to ciprofloxacin for all the 152 strains of *Campylobacter* spp. isolated from chicken in Australia (Miflin *et al*., 2007); 70 isolates from domestic animals and poultry from India (Baserisalehi *et al*., 2007a); isolates from environmental samples (Baserisalehi and Bahador, 2008) was also reported earlier, whereas ciprofloxacin resistance in only 0.3% of isolates was confirmed from cattle in Canada (Inglis *et al*., 2005). The resistance patterns displayed by *Campylobacter* isolates from dogs to fluoroquinolone (ciprofloxacin) and macrolides (erythromycin) classified as second line and first line antimicrobials are of particular importance, since patients suffering from Campylobacteriosis are usually treated with these antimicrobials agents (Uaboi-Egbenni *et al*., 2011).

Recent scientific studies has shown that *Campylobacter* antimicrobial resistance can be related to some specific genes and the dissemination of these genes of microorganisms to their progeny and across to other unrelated bacteria species through extrachromosomal DNA fragment called the plasmid from one animal species to another and to humans is possible (Baserisalehi and Bahador, 2008; Apata, 2009). Antimicrobial resistance observed in the present work might be due to the indiscriminate and irrational use of antimicrobials (Tambekar *et al*., 2007) in animals for preventive or therapeutic purposes irrespective of etiological agents. Given the relevance of the genus *Campylobacter* in human gastroenteritis, its occurrence in companion animals such as dog and the fact that majority of *Campylobacters* showed multi-drug resistance, a continuous surveillance and monitoring of the prevalence and the antimicrobial resistance of *Campylobacter* spp. in dogs and other pet animals is essential to the implementation of effective policies for controlling and preventing contamination and infection by this pathogen. The use of antibiotics as threauptic and prophylaxis for animals should be carefully evaluated and monitored because acquisition of antibiotic resistant strains of *Campylobacters* by man has serious health implications.

CONCLUSION

In view of the heterogeneity in the results reported in the literature in comparison to data in this study, we recommend that *Campylobacter* antimicrobial susceptibility tests be performed for therapeutic purposes with the strict hygienic measures to prevent transmission from pets to owner. Our results indicate amikacin, chloramphenicol, streptomycin and levofloxacin as drugs suitable for the treatment of canine Campylobacteriosis. This also opens up therapeutic possibilities for these drugs in human Campylobacteriosis.

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