

Characterization of *Campylobacter Jejuni* from Mastitis

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ABSTRACT : Mastitis is the potential threat in the dairy industry and caused by different types of bacteria. *Campylobacter jejuni* is significant food borne pathogen and causing clinical mastitis in bovines. *Campylobacter* is also associated with abortion, enteritis, colitis and in human severe urinary tract infection. There is less evidence of report on the mastitis caused by *Campylobacter* and their antibiogram studies. Hence this study is planned to determine the occurrence of *Campylobacter* mastitis in bovines and document their antibiotic sensitivity for effective treatment control of mastitis.

Keywords: Mastitis, antibiogram, public health.

INTRODUCTION

Bovine mastitis remains a major challenge to the world wide dairy industry and is the most prevalent production disease in dairy herd worldwide (Seegers *et al.*, 2003). *Campylobacter jejuni* continue to be the Major food borne pathogen and it causes human bacterial gastritis (Skirrow, 1994). In 1886, Escherich observed organisms resembling *Campylobacter* in stool samples of children with diarrhoea. In 1913, Mcfaydean and Stockman identified *Campylobacter* in fetal tissues of aborted sheep. In 1972, the Belgium microbiologist, first isolated campylobacter from stool samples of patients with diarrhea (Kist, 1985). Apart from causing bovine mastitis campylobacter jejuni organisms are excreted directly from milk and cause food borne infections in humans. Among various human food borne infections *Campylobacter jejuni* (Robinson and Jones 1981) is the leading cause of bacterial food borne diseases of humans throughout the world for which milk forms a potential source. The present study was conducted to know the prevalence of *Campylobacter* from clinical mastitis milk samples of Buffaloes.

MATERIAL AND METHODS

Total of 26 clinical mastitis milk samples were collected from buffaloes suffered with mastitis, from VCC, VCRI, Namakkal. Milk samples were collected by taking utmost sterile, aseptic precautions to avoid external contaminations.

Isolation of *Campylobacter* Spp. was carried out as per the method described by Backer *et al* (1987). Milk samples were streaked with the help of a sterile swab on the surface of the Preston *Campylobacter* selective agar contain 7% laked horse blood (SR0048c, Oxoid, Basingstoke, UK) and Preston *Campylobacter* selective

supplement (SR117E, Oxoid) and incubated at 42°C for 48 hours in an anaerobic jar with Anaero Higas pack (Himedia Pvt. Ltd. Mumbai) to provide microaerophilic environment (Açik and Çetinkaya 2006). The isolates were presumptively identified based on colony appearance, microscopic morphology, motility, oxidase test, hippurate hydrolysis test, inhibition by nalidixic acid, growth in the presence of 1% glycine and 1.55 Sodium chloride as per Collee *et al.* (1989).

Biotyping pattern of the *Campylobacter* isolates was performed based on Hippurate hydrolysis, rapid production of H₂S and DNA hydrolysis tests as per Lior (1984).

Antibiotic sensitivity pattern of the isolates were performed by disc diffusion method as per Bauer *et al.* (1966) on 5% sheep blood agar using different commercially available antibiotics viz., Ciprofloxacin, Enrofloxacin, Chloramphenicol, Gentamicin, Streptomycin. The antibiotic discs were procured from Hi-media Pvt. Ltd. Mumbai.

RESULTS AND DISCUSSION

Out of 26 clinical mastitis samples from buffaloes Case No., 1368, 1337, 1425, 1443, 1532, 1913 (6 samples, 23%) were found to be positive for *Campylobacter jejuni* based on colony appearance, microscopic morphology, motility, oxidase test, hippurate hydrolysis test, inhibition by nalidixic acid, growth in the presence of 1% glycine and 1.55 Sodium chloride as per Barrow and Feltham (1992).

The colonies were usually flat, non-haemolytic, watery, gray (sometimes with a pink tinge), spreading and often large. At times they may appear like drops of water, spreading along the streak marks. *Campylobacter jejuni*

appeared as slender, curved to spiral Gram negative rods. Coccoid transformation could be observed with old cultures. Corkscrew type motility was observed in hanging drop method. All the isolates gave positive Oxidase test were sensitive to nalidixic acid, able to grow on medium containing 1% glycine and 1.5% NaCl inhibited the growth of the isolates.

Biotyping of the *Campylobacter* isolates revealed that all the isolates hydrolysed hippurate and negative to produce H₂S. Only two (Case. No 1368, 1337) isolates were showed positive reaction to DNA hydrolysis. This finding well accorded with reports of Suresh Varma *et al.* (2005).

Table 1: Characteristics of the isolates.

Character	<i>Campylobacter jejuni</i>
Motility	+
Growth under anaerobic conditions	+
Oxidase	+
Growth on medium containing 1.5% Na Cl	-
Growth on medium containing 1% Glycine	+
Hippurate hydrolysis	+
Rapid H ₂ S test	-
DNA hydrolysis	-
Sensitivity to Nalidixic acid	+
Sensitivity to Cephalothin	-

Note: + a positive reaction; - a negative reaction

Antibiotic sensitivity of the isolates were showed that all the 6 isolate were sensitive to Ciprofloxacin and Enrofloxacin (100%). This finding is correlated well with the findings of Porter and Duguid (1989). All the isolates were 100% resistant to penicillin. Only 4 isolates were sensitive to Chlarampenicol (66%) and

Gentamicin (66%). The isolates (2) showed sensitive to Streptomycin (33%). This wide variation to various antibiotics may be due to the indiscriminate uses of antibiotics in mastitis therapy Suresh Varma *et al.* (Loc. cited).

Table 2: Antibiotic sensitivity Pattern of *Campylobacter jejuni* isolates.

Antibiotics	Isolate No.	Percentage of Sensitivity
Ciprofloxacin	1368, 1337, 1425, 1443, 1532, 1913	100%
Enrofloxacin	1368, 1337, 1425, 1443, 1532, 1913	100%
Chloramphenicol	1368, 1425, 1443, 1913	66%
Gentamicin	1368, 1425, 1443, 1913	66%
Streptomycin	1337, 1425	33%
Penicillin	1368, 1337, 1425, 1443, 1532, 1913	Resistant

The present finding clearly indicated that the *Campylobacter jejuni* is one of the causative factor in clinical mastitis of buffaloes. The present study revealed that the presence of *C. jejuni* may major threat as a food borne pathogen. Khanna *et al.* (1996) reported that contaminated food products are the principle sources of infection to man. Chattopadhyay *et al.* (2001) recorded many outbreaks of *Campylobacter* in human beings. Unpasteurised milk, raw milk contaminated with *campylobacter* is the principle factor in the out breaks of human campylobacteriosis.

SUMMARY

The present was conducted to identify the prevalence of *C.jejuni* in clinical mastitis of buffaloes. 6 isolate could be isolated from 26 milk samples. Biotyping and antibiotic sensitivity pattern of the isolates were performed and their zoonotic and public health importance were discussed.

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