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Research Article

Isolation and Antibiogram of Enterobacteria associated with Bovine calf Diarrhea

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ABSTRACT

Members of Family *Enterobacteriaceae* have been implicated in various pathogenic conditions of animals, birds and human beings. In recent past, the enterobacteria like *Salmonella* and *E. coli* have been found associated with severe food borne outbreaks. The study was conducted to find out association of enterobacteria in the cases of collibacillosis in bovine calves. A total of 125 faecal samples were collected from 3-6 months old bovine calves showing diarrhea and subjected to isolation and identification of enterobacteria. *E. coli* could be isolated from 110 samples, 53 samples yielded *Pseudomaonas*, while *Shigella* could be isolated from 23 samples followed by *Edwardsiella* from 13 samples, *Salmonella* from 9 samples, *Klebsiella* from 4 samples and *Proteus* from 3 samples. *E. coli* was found to be the major single bacterial agent associated with cases of collibacillosis either alone or in combination with other enterobacteria. *In vitro* sensitivity of the isolates was tested against seven antimicrobial agent's viz. Ampicillin, Chloramphenicol, Cloxacillin, Ciprofloxacin, Gentamicin, Streptomycin, and Tetracycline. Gentamicin was recorded to be the most effective antibiotic in case of calf diarrhea as highest number of isolates of *E. coli*, *Shigella*, *Edwardsiella*, *Salmonella*, *Klebsiella* as well as *Proteus* recovered from cases of calf diarrhea was found sensitive to Gentamicin.

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INTRODUCTION

Diarrheal diseases in calf constitute a major health problem by causing heavy morbidity and mortality particularly in the developing countries like India (Sojka, 1971, Malik et al., 2012). Neonatal calf diarrhea is one of the stumbling blocks for the dairy industry in our country, as it causes severe impact on productivity of calf at its maturity (Gow et al., 2005). About 64 percent of the cases of diarrhea in calves were ascribed to potentially infective pathogenic agents (Bareandeguy et al., 1987; Malik et al., 2013). Certain serotypes particularly O157:H7 of *E. coli* have emerged as an important food born pathogen for which the cattle are found to be a single major reservoir and major source of human infection (Chapman et al., 1993; Dhama et al., 2013). The present study was carried to find out the association of enterobacteria with the cases of neonatal calf diarrhea and to study their antibiogram.

MATERIALS AND METHODS

A total of 125 fecal samples were collected using sterile swabs from 3-6 months old bovine (cattle) calves from instructional livestock Farm Complex and cases presented to Teaching Veterinary Clinical complex of the College, showing diarrhea and processed for the isolation of enterobacteria as described by Cruickshank et al., (1957). The isolates were identified on the basis of cultural, morphological and biochemical characteristic including sugar fermentation test as described by Edward and Ewing (1972). Faecal sample were dispersed in peptone water and streaked on Mc Conkey's Lactose agar (MLA) plates. Following incubation at 37^0C, lactose fermenting colonies showing pink-red color and non-lactose fermenting colonies appearing colorless were picked up and taken into individual slants for further identification. For isolation of Salmonella, a loop full of sample was inoculated in tetrathionate broth and incubated at 37^OC for 24 hr. Following enrichment a loop-full of material from tetrathionate broth was streaked onto Brilliant green agar (BGA) plates and the plates were incubated at 37^OC for 24 hr. The pink red colonies appearing on BGA were taken into slants and processed for identification of Salmonella. The isolates thus obtained were subjected to morphology, biochemical tests and sugar fermentation as per Cruickshank et al., (1957) and identified as per Edward and Ewing (1972). In vitro sensitivity of the isolates were tested against seven antimicrobial agents viz. Ampicillin,

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Table 1: Antibiotic sensitivity pattern of enterobacterial isolates recovered from bovine calf diarrhea.

Organism	Isolates	Ampicillin	Chloram - phenicol	Cloxacillin	Ciprofloxacin	Gentamicin	Streptomycin	Tetracycline
E. coli	110	22 (20.00)	30 (27.27)	02 (1.81)	09 (8.18)	105 (95.45)	67 (60.90)	87 (79. 09)
Shigella species	23	07 (30.43)	09 (39.13)	02 (8.69)	12 (52.17)	19 (82.60)	12 (52.17)	07 (30.43)
Edwardsiella species	13	02 (15.38)	08 (61.54)	01 (7.69)	02 (15.38)	12 (92.31)	07 (53.85)	05 (38.46)
Salmonella species	09	- (-)	06 (66.67)	01 (11.11)	01 (11.11)	08 (88.89)	03 (33.33)	05 (55.56)
Klebsiella species	04	2 (50.00)	3 (75.00)	- (-)	01 (25.00)	04 (100.00)	04 (100.00)	04 (100.00)
Proteus species	03	- (-)	01 (33.33)	- (-)	- (-)	03 (100.00)	02 (66.66)	01 (33.33)

Chloramphenicol, Cloxacillin, Ciprofloxacin, Gentamicin Streptomycin, and Tetracycline using standard discs following the disc diffusion method described by Bauer et al. (1966).

RESULTS AND DISCUSSION

Out of 125 faecal samples processed, 110 (88.00%) samples yielded *E. coli*, 53 (42.40%) samples yielded *Pseudomonas*, 23 (18.40%) samples yielded *Shigella*, 13(10.40%) samples yielded *Edwardsiella*, 9 (7.20%) samples yielded *Salmonella*, 4 (3.20%) samples yielded *Klebsiella* and 3 (2.40%) samples yielded *Proteus* (Table 1). *E. coli* and *Pseudomonas* could be recovered as the single etiological agent in 29 (23.20 percent) and 6 (4.80%) cases, respectively, while involvement of *E. coli* with other enterobacteria was recorded in remaining 90 (64.80%) cases. In only 7.20 percent cases, the involvement of *Salmonella* and *Pseudomonas* was found. Over all, *E. coli* appeared to be an important causative agent either alone or in combination with other bacteria in the etiology of calf diarrhea. Hussain and Saikia (2000) also found *E. coli* to be the causative agent in majority of the cases (73.12 percent). Sojka (1971) observed that in spite of involvement of many bacteria in neonatal calf diarrhea, about 50 percent cases were ascribable to *E. coli* infection alone. *E. coli* has been reported to be the first organism, which rapidly colonize the alimentary tract of new born in great number, that's why its frequency of isolation is greater than other bacteria (Smith and Halls, 1967).

The antibiogram of enterobacterial isolates is presented in Table 1. On antibiogram studies, out of 110 *E. coli* isolates tested, 105 (95.45 percent) isolates showed sensitivity to Gentamicin followed by 87 (79.09 percent), 67 (60.90 percent), 30 (27.27 percent), 22 (20.00 percent) and 9 (8.18 percent) isolates showing sensitivity against Tetracycline, Streptomycin, Chloramphenicol, Ampicillin and Ciprofloxacin, respectively. Only two (1.81 percent) isolates showed sensitivity for Cloxacillin. Similar results were also recorded by Jones *et al*, (2000); Yadav and Sharda (2006) and Malik et al., (2013). Similar observations were also recorded with *Shigella* isolates where out of 23 isolates tested, 19 (82.60 percent) isolates showed sensitivity to Gentamicin followed by 12 (52.17 percent) isolates each for Streptomycin and Ciprofloxacin. Only 9 (39.13 percent) isolates were sensitive for Chloramphenicol and 7 (30.43 percent) each for Tetracycline and Ampicillin. The least sensitivity (2 isolates, 8.69 percent) was recorded for Cloxacillin. Shekhar et al., (2005) found 100 percent sensitivity of *Shigella* isolates against Gentamicin followed by 80 percent sensitivity against Cloxaxillin, Kanamicin, Chloramphenicol and Furazolidone. Among the *Edwardsiella* isolates (13), 12 (92.31 percent) isolates were found sensitive to Gentamicin whereas 8 (61.54 percent), 7 (53.85 percent) and 5 (38.46 percent) isolates were found sensitive to Chloramphenicol, Streptomycin and Tetracycline, respectively and 2 (15.38 percent of each) isolates were sensitive to Ampicillin and Ciprofloxacin. Only single (7.69 percent) isolate was found to be sensitive to Cloxacillin. Shome and Shome (1999) studied the antibiogram of *Edwardsiella* and reported highest sensitivity to Chloramphenicol followed by Penicillin, Erythromycin, Gentamicin, Kanamycin and Tetracycline whereas resistance against Ampicillin, Bacitracine, Co-trimoxazole, Neomycin, Polymyxin, Rifampicin, Streptomycin and Trimethoprim. All the isolates of *Salmonella* were found resistant against Ampicillin. Among 9 *Salmonella* isolates tested, 8 (88.88 percent) isolates were found sensitive to Gentamicin followed by 6 (66.67 percent) for Chloramphenicol, 5 (55.56 percent) for Tetracycline, 3 (33.33 percent) for Streptomycin and 1 (11.11 percent) for Cloxacillin and Ciprofloxacin each. Similarly, Pandey and Sharma (1994) reported 100 percent resistance of *Salmonella* isolates from cattle and chicken to Cloxacillin. However all the *Klebsiella* isolates showed highest degree of sensitivity for Gentamicin, Streptomycin and Tetracycline (4 each, 100 percent) followed by Chloramphenicol (3, 75 percent), Ampicillin (2, 50 percent) and Ciprofloxacin (1, 25 percent), while all the isolates were resistant against Cloxacillin (4, 100 percent). Similarly, all the isolates of *Proteus* were found sensitive to Gentamicin (3, 100 percent), followed by Streptomycin (2, 66.66 percent), Tetracycline (1, 33.33 percent) and Chloramphenicol (1, 33.33 percent) whereas all (3, 100 percent) were resistant to Cloxacillin, Ciprofloxacin and Ampicillin. The sensitivity pattern of the isolates indicates that the decreased use of Gentamicin for treatment in recent time

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has contributed significantly for its increase sensitivity whereas the antibiotics like Cloxacillin, Ciprofloxacin and Ampicillin are well resisted by the organisms due to their frequent use for treatment and preventive purposes in dairy industry. The results of present study indicate that Gentamicin is the most effective antibiotic in case of calf diarrhea as it is found to be highly sensitivite for *E. coli*, *Shigella*, *Edwardsiella*, *Salmonella*, *Klebsiella* as well as *Proteus* isolates recovered from cases of calf diarrhea.

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