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Study of *Enterobacteria* responsible to cause diarrhea in large animal and its drug resistance pattern

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Abstract

Study was conducted to know the association of enterobacteria which are responsible to cause diarrhea in large animals. A total 100 fecal samples were collected in different age groups, which are come in different areas of eastern region of utter-Pradesh. Overall 132 isolates were recovered only out of which, 82 isolates belonged to *E. coli*, 29 isolates belong to *Pseudomonas spp.*, 13 to *Shigella species* and only 08 to *Edwardsiella species*. Most prominent are *E. coli*. A considerable variation pattern was observed in the susceptibility patterns of different organisms isolated from diarrheic large animals against various chemotherapeutic agents tested. Antibiotics viz. Gentamicin was showing highest sensitivity drug followed by Tetracycline, Streptomycin, Chloramphenicol, Ampicillin, Ciprofloxacin and Cloxacillin was showing resistant in all microorganism associated with diarrhea.

Keywords: Antibiotics, Diarrhoea, *E. coli*

1. Introduction

Large animals (cattle and buffalo) population is the backbone of our animal husbandry and contribute more than 5000 crores daily to Indian GDP. Infectious diarrhea, which is an important to cause mortality especially young animals resulting huge economic losses to our dairy industry. There are numerous factor involvements which causes diarrhea, they all can be categorized into three groups one is the age, involvement of pathogenic strain of bacteria and environmental factor. Some of the risk factor is colostrums deprivation, overcrowding, adverse climate condition, inferior milk replacer which may also precipitate the disease in the calves. The importance of enteric pathogens affecting neonatal calves of domestic animal was reviewed by Tzipori (1985) [15]. He reported that *Enterotoxigenic E. coli* (ETEC), Rotavirus, Cryptosporidium species and Coronavirus were responsible for 80-95 percent infection in neonatal calf worldwide. Cryptosporidium was found in 30- 50 percent cases on worldwide basis. Hussain and Saikia (2000) [7] has reported single infection with the *E. coli* infection 73 percent cases and mixed infection with two or more bacteria in 27 percent cases of diarrhea. Some of the enteric organisms are associated with diarrhea but In all, *E. coli* is one of them which are most pathogenic, other occur as normal flora of alimentary tract (Hussain and Saikia, 2000) [7]. Wide spectrum use of antibiotic for disease control make or develop the resistant strain of bacteria.

2. Materials and Methods

A total 100 fecal samples were collected in different age groups, which are come in different areas of eastern region of utter-Pradesh and processing was done in the laboratory of the Department of Veterinary Microbiology.

2.1. Isolation of *E. coli*

A loop full of the sediment was inoculated into 2 ml Mac Conkey lactose broth for enrichment. After 18 hr of incubation at 37 °C, a loop full of culture was streaked on Mac Conkey Lactose Agar (MLA) plates and the plates were incubated at 37 °C. After 24 hr of incubation, lactose fermenter (rose-pink) colonies were picked up and streaked onto Eosin Methylene Blue (EMB) agar plates and incubated at 37 °C for 24 hr.

The samples producing a greenish metallic sheen on EMB plates were transferred on to Nutrient Agar slants and further processed for identification of the culture.

2.2. Identification of *E. coli*

E. coli cultures were identified using the procedures described by Cruickshank *et al.* (1975) [5] as follows:

2.2.1. Gram's staining

For Gram's staining, the isolates were grown overnight at 37 °C and a smear was prepared on a microscopic glass slide with a loop full of culture. The smear was air-dried and heat-fixed before pouring Crystal violet stain (0.3% w/v). After one min, excess stain was washed off with water. Gram's iodine (0.4% w/v) was then added and allowed to react for 30 sec. before being rinsed off with water. The smear was then de-stained with ethanol (95% v/v) and again stained with the secondary stain, safranin (0.4% v/v) for one minute. The smear was then washed with water for 5 sec. and observed under oil immersion objective. The cultures showing pink coloured rods (Gram negative), arranged singly or in pairs, were suspected as *E. coli*.

2.2.3. Motility test

The motility of the test cultures was observed by hanging drop technique as described by Cowan and Steel (1975) [4]. Hanging drop slide was prepared from overnight broth culture using cavity slides and examined under high power (45 X) objective.

2.2.4. Catalase test

The method described by Quinn *et al.* (1994) [11] was followed. A loop-full of overnight culture of bacterial isolate was mixed with a drop of 3% hydrogen peroxide over a clean glass slide. The production of gas bubbles or effervescence within a few seconds was considered as catalase positive.

2.2.5. Oxidase test

The procedure of Faller and Schleifer (1981) [6] was used. Filter paper was impregnated with 1 percent (w/v) aqueous solution of tetramethyl-p-phenylene-diamine dihydrochloride. Bacterial cultures were smeared across the filter paper with a glass rod. Appearance of dark purple colour within 5-10 seconds indicated positive oxidase test.

2.2.6. Indole test

The procedure of Quinn *et al.* (1994) [11] was followed for this test. The isolates were inoculated in 2ml of peptone water and incubated at 37 °C for 4 hr. Few drops of xylene were added to the culture and mixed thoroughly to dissolve the indole. About 0.2 ml of Kovac's reagent was then added. A pink layer of xylene was considered as positive for indole production.

2.2.7. Methyl red test

The isolates were inoculated in glucose phosphate peptone water (GPPW) and incubated at 37 °C for 24 hr. One drop of methyl red solution was then added and the tubes showing bright red colour were considered positive.

2.2.8. Voges Proskauer test

The test cultures were inoculated in 2.0 ml of glucose phosphate peptone water (GPPW) and incubated at 37 °C for 24 hr. One ml of KOH (40% w/v) and 3 ml of α -naphthol solutions were then added and cultures were shaken

thoroughly. Appearance of bright cherry red colour was considered to be positive.

2.2.9 Citrate test

The test culture was inoculated upon Simmon's citrate agar slants and incubated at 37 °C for 48 to 72 hr. The original, green colour of the medium turned to blue in positive cases. The isolates showing negative result were suspected to be *E. coli*.

2.2.10. Triple Sugar Iron test

The isolates were inoculated upon the TSI slants by stab inoculation method into the butt and the slants were incubated aerobically at 37 °C for 24 hr. The slants were then observed for the following changes:

2.2.11. Sugar fermentation reaction

The sugars used for fermentation reaction were glucose, lactose, sucrose, fructose, maltose and mannitol. Solutions A & B were prepared separately, where solution A comprised of a 10% sugar solution, in distilled water and solution B, peptone water, to which, Andrade's indicator was added in 1% strength and mixed thoroughly. Solution B was dispensed in 2.5 ml quantity in sugar-fermentation tubes with Durham's tubes. The tubes were autoclaved at 15 lbs pressure for 15 min. Solution A, for all the sugars except glucose, were also sterilized by autoclaving, while glucose was sterilized by steaming. Using a sterile pipette, 0.25 ml of solution A was added to the tubes containing solution B, to make 1% sugar medium. The test culture was then inoculated and tubes were incubated at 37 °C for 24 hr. before the observations based on colour change and gas production were made.

2.2.12. Antibiotic resistance pattern

Antimicrobial drug sensitivity of the isolates was tested for different antibiotics (Table- 2) using the modified disc diffusion method of Bauer *et al.* (1966) [2]. The test organism was inoculated in 2 ml nutrient broth and incubated at 37 °C for 24 hr. Thereafter, a uniform lawn of culture was prepared on Muller Hinton agar plates, with the help of sterile swab soaked in the culture and plates were allowed to stand for 5 min. Standard discs of antibiotics (Oxoid) were placed on the surface of agar, keeping a disc to disc distance of about 12 mm and the discs were slightly pressed with the help of forceps to make complete contact with the agar surface. The plates were then incubated at 37 °C for 24 hr. Sensitivity of *E. coli* strains to an antibiotic was determined by appearance of zone of growth inhibition around the disc. Diameter of the clear zone of inhibition was measured with zone scale and compared with standard inhibition zone chart, supplied by the disc manufacturer to interpret the results.

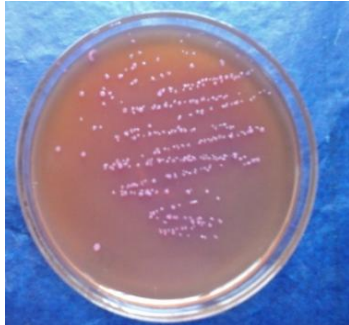
3. Results

A total number of 100 fecal samples from different age groups were examined in the laboratory of Department of Veterinary Microbiology. Out of one hundred, 132 isolates were recovered. Among these 132 bacterial isolates, 82 (62.12 percent) isolates were of *E. coli*, 29 (21.96 percent) isolates of *Pseudomonas Species*, 13 (9.84 percent) isolates of *Shigella* species and 08 (06.06 percent) isolates of *Edwardsiella* species. (Table 1)

Tentatively identify the *Enterobacteria* on the basis of biochemical test, IMViC test and sugar fermentation test.

The antibiotic sensitivity test was carried out with all the enterobacteria isolated against seven antibiotic viz.

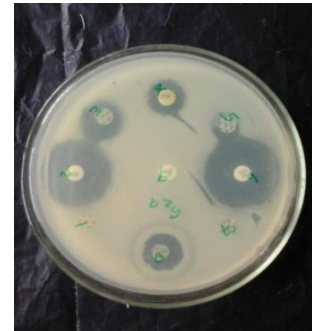
Ampicillin, Chloramphenicol, Cloxacillin, Ciprofloxacin, Gentamicin, Streptomycin and Tetracycline. In this study, out of 82 isolates of *E. coli*, 78 (95.12 percent) were found to be highly sensitive for Gentamicin followed by Tetracycline (71, 86.58 percent), Streptomycin (67, 81.70), Chloramphenicol (30, 36.58), Ampicillin (22, 26.82), Ciprofloxacin (09, 10.97 percent) and Cloxacillin (02, 2.43 percent). Table 2



Enterobacteriaceae on MLA plate



E. coli on EMV plate



Antibiogram study

Among *Shigella* species, 12 (92.30) out of 13 isolates tested were positive for Gentamicin followed by 11 (84.61 percent) as well as Streptomycin 09 (69.23 percent), 08 (61.53 percent) for Chloramphenicol, 06 (46.15 percent) for Tetracycline and 05 (38.46 percent) for Ampicillin. Table 2

Out of 08 isolates of *Edwardsiella*, the highest 07 (87.50 percent) isolates were sensitive to Gentamicin followed by 05 (62.50 percent) for Chloramphenicol against Streptomycin 04 (50.00 percent) against Tetracycline 03 (37.50 percent), against Ampicillin 02 (25.00 percent) and Least sensitive/ resistant against Cloxacillin 01 (12.50 percent). Table 2

Table 1: Bacteria isolated from diarrhea

Sl. No.	Types of Isolates	No. of isolates recovered	Percentage (%)
1	<i>Escherichia coli</i>	82	62.12
2	<i>Pseudomonas species</i>	29	21.96
3	<i>Shigella species</i>	13	09.84
4	<i>Edwardsiella</i>	08	06.06
	Total	132	

Table 2: Antibiotic sensitivity pattern of Enterobacterial isolates recovered from diarrhea in large animals

Name of Micro-organism	No. of isolates	Ampicillin	Chloramphenicol	Cloxacillin	Ciprofloxacin	Gentamicin	Streptomycin	Tetracycline
<i>E. coli</i>	82	22	30	02	09	78	67	71
<i>Shigella species</i>	13	05	08	03	12	11	09	06
<i>Edwardsiella Species</i>	08	02	05	01	01	07	04	03

4. Discussion

Diarrheal diseases in animals specially young constitute a major health problem by causing heavy morbidity and mortality particularly in the developing countries like India (Sojka, 1971) [14]. Bareandeguy *et al.*, (1987) [1] reported that 94 percent of the cases of diarrhea in calves were due to potentially infective pathogenic agents. A total 100 fecal samples were collected in different age groups, which are come in different areas of eastern region of Uttar-Pradesh. Overall 132 isolates were recovered only out of which, 82 isolates belonged to *E. coli*, 29 isolates belong to *Pseudomonas spp.*, 13 to *Shigella species* and only 08 to *Edwardsiella species*. Isolation of these bacteria from calf diarrhea was also reported by Joon and Kaura (1993) [8]. Hussain and Saikia (2000) [7] recovered *E. coli* (93), *Citrobacter* (8), % *Proteus* (6), *Klebsiella* (4) and *Enterococci* (4) from 93 fecal samples collected from cases of calf diarrhea. Wani *et al.*, (2004) [16] also reported presence of *E. coli* and other enterobacteria responsible for causing diarrhea from an organized dairy farm in Kashmir Valley and from Srinagar (India). Borah and Das (1996) [3] and Kumar *et al.*, (2005) [9] reported highest sensitivity of *E. coli* isolates to Gentamicin. Similarly results were reported by Madhu Babu

et al., (1998) [10]. Shekhar *et al.*, (2005) [12] found 100 percent sensitive to Gentamicin and highest resistance show against Penicillin G. Shome and Shome (1999) [13] studied the antibiogram of *Edwardsiella* and reported highest sensitivity to Chloramphenicol and resistant against Ampicillin.

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