

BACTERIAL PROFILING FROM THE BOVINE CALVES'S DIARRHEA AND ITS ANTIBIOTIC SENSITIVITY PATTERN AROUND CHANDRAPUR DISTRICT IN MAHARASHTRA

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Abstract: The present study was aimed to study the bacterial investigations from the bovine calves's diarrhea and antibiotic sensitivity test. The thirty (30) fecal samples were collected aseptically through rectal swabs and inoculated into nutrient broth and ABST Performed for enteric bacteria. The fecal samples were collected primarily tested for lactose fermenting and non fermenting enteric bacteria on MacConkey Agar (MCA), *Escherichia coli* on EMB Agar and *Salmonella spp* on Salmonella Shigella (SS) agar. The bacterial identification was done by gram staining and colony characters on MCA, EMB Agar and SS Agar. *Escherichia coli* 15(50%) was found most frequently enteric bacteria followed by *Salmonella spp* 11 (36.66%) and symbiotic association of *Escherichia coli* and *Salmonella spp* 2 (6.66 %). The antibiotic sensitivity pattern for enteric bacteria are sensitive to the Nitofurantoin (93.33%) Cefixime/Clavulanic Acid (76.66%), Cefalexin (76.66%), Amoxyclav (40%), Ampicillin (60%), Amoxicillin (40%), Penicillin-G(23.33%), Metronidazole (16.66%).

Keywords: Calves diarrhea, enteric bacteria, *Escherichia coli*, *Salmonella spp*, ABST.

INTRODUCTION

Livestock is an integral part of the agricultural production system in any country and plays an important role in socio-economic development of millions of rural household people in national economy. In New born calves there is higher mortality than their adult and it is one of major effect on economy in livestock industry. Diarrhea is a leading cause of economic losses to the dairy industry and major cause of calf mortality and morbidity during first few weeks of life [1]. Diarrhea in farm animals, especially in neonatal calves is one of the most challenging clinical signs encountered by large animal veterinary practitioner. It involves significant economic loss for labor and capital, calf mortality, loss in calf value and veterinary costs. The pre-weaning mortality of dairy calves was estimated at 10.8% level with diarrhea responsible for more than half of those deaths [2, 3, 4]. A number of infectious (bacteria, viruses, parasites) and non-infectious factors cause diarrhea in neonatal calves. The

most important infectious agents are Rota virus, Corona virus, E. coli. Salmonella species. Calf diarrhea caused by bacterial infection and viral infection has a bad effect on the dairy industry. Diarrhea caused by a various bacteria has been recognized as one of the most public clinical problems for calves worldwide. Among these bacteria Eschirechia coli (E. coli) as “white scour”, Salmonella typhimurium (S. typhimurium), Clostridium perfringens (C.perfringens) and Staphylococcus aureus (St. aureus) are to be the major microbial causes of diarrhea in calves.

The incidence of diarrhea in calves under 30 days of age varies between 10 % and 20 %. Calf diarrhea has an adverse effect on the calves’s health status, longevity in the herd and productivity performance. In order to increase the productivity per livestock unit without increasing livestock numbers and to devise preventive measures as well as to reduce losses during the initial months of life. It is important to identify the etiological agents involved in calf diarrhea.

Materials and method

Collection of Samples:

The fecal rectal swabs were collected in the month of September 2018 from organized and unorganized dairy farms from Chandrapur district in Maharashtra. The thirty (30) fecal rectal swabs collected from diarrheic calves having age group of first week to eight week by using sterile cotton swabs. The fecal swabs samples were kept on ice gel packs carried to the Department of Veterinary Microbiology and Animal Biotechnology, Nagpur Veterinary College under aseptic condition for immediate processing for bacterial culture, microscopic examination and antibiotic sensitivity test.

Detection of bacterial fecal pathogens

Bacteriological culturing and Examination:

All fecal samples were inoculated into freshly prepared nutrient broth test tube and incubated aerobically at 37⁰c overnight in the incubator. The subsequently streaking was done onto nutrient agar (NA) plates and MacConkey agar (MAC) plates for 24-48 hrs 37⁰c in the incubator. The morphological examination of colony growth on agar NA plate and MAC plate. The presence of growth on MAC plates was used as primary criteria to proceed for isolation and identification of E. coli. Furthermore the colony characteristics observed on MAC plate was used to classify suspected bacteria isolated into two groups: lactose fermenter (LF) and non lactose fermenter (NLF). Suspected E. coli colonies were presumptively identified their lactose fermenting character (pink colonies), suspected E. coli further sub

cultured Eosin methyl blue (EMB) agar medium to identify selectively *E. coli*. The characteristic colonies on EMB were identified based on green metallic sheen or blue-black to brown color shown in figure 1.

Isolation and identification of salmonella

All suspected colony grown on MAC Plate were subculturing done onto salmonella Shigella agar (SSA) Plate and kept aerobically at 37⁰c for 18-24 hrs in the incubator. Furthermore colony characters observed on SSA plate. The colony of salmonella spp visible colourless with black centre as shown in figure.2

Microscopic Study by Staining Method:

The bacterial colony was isolated from suspected fecal samples on nutrient agar identified by using gram staining method [5].

Antibiotic Sensitivity Tests

Kirby-Bauer disc diffusion method

After confirmation of bacterial growth on Nutrient agar (NA) and MacConkey Agar (MCA), faecal samples were inoculated in to nutrient broth and incubated at 37⁰c for overnight. The 20 ul to 30 ul inoculums poured on nutrient agar plate and by using sterile plastic spreader the inoculums was speared uniformly. Antibiotic discs were applied aseptically to the surface of the nutrient agar plate at an appropriate distance with the help of sterile forceps and incubated at 37⁰C for 24 hours, aerobically. The enteric bacteria were tested by the antibiotic discs which are procured from Himedia Pvt. Ltd. As namely Amoxyclav (AMC), Ampicillin (AMP), Amoxicillin(AMX), Cefixime/Clavulanic acid (CMC), Cefalexin (CN), Nitrofurantoin (NIT), Metronidazole (MT), Penicilline-G(P). The results were recorded and interpreted based on the diameter of the zone of inhibition, denoted as resistant (R), Intermediate (I) or Sensitive (S).

Results

Identification and characterization of enteric bacteria from faecal samples

The thirty (30) faecal samples were collected from age group of first week to 3 month of calves from diarrheic and non diarrheic calves. These faecal samples were processed for the enrichment of bacteria in nutrient broth at 37⁰C in the incubator for overnight. The streaking of culture was done on nutrient agar and MacConkey agar. The growth of bacteria on both plate obtained. On gram staining the morphology of bacteria shown the gram negative, short rods. The Colony characters on MacConkey agar shown the lactose fermented (LF) Pink colony and non lactose fermented (NLF) colorless colony. The fifteen (50%) samples were

positive for *Escherichia coli* on EMB agar which has appearance of metallic sheen appearance and eleven (36.66%) samples were positive for *salmonella spp* on SS Agar the bacterial colony appeared like colorless with black centre.

Identification of colony characters of *Escherichia coli* and *salmonella spp* on Culture media

Culture media	Colony Morphology	
	<i>E.coli</i>	<i>Salmonella spp.</i>
Nutrient agar (NA)	rounded, smooth, whitish to grayish colony	Circular, small, smooth colony
Mac Conkey agar (MCA)	Pink lactose fermented	Colorless, pale translucent colony
Eosin methylene blue (EMB)Agar	Moist circular colonies with dark centers, yellow green metallic sheen	Light purple to colorless
Salmonella Shigella agar(SS Agar)	Pink color colony	smooth colony with black center

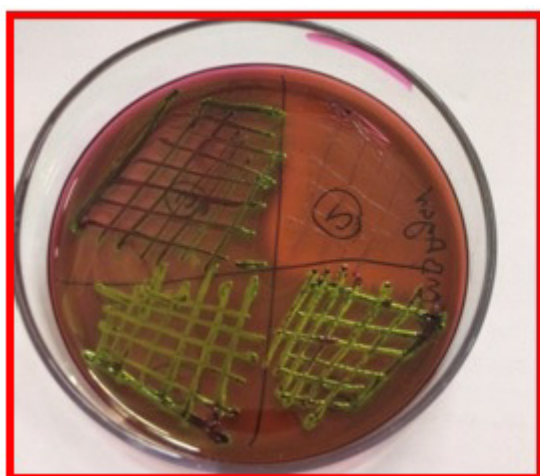


Fig.no.1 Metallic Sheen on EMB



Fig.no.2 Smooth colony with Black center on SSA

Antibiotic sensitivity test:

The antibiotic sensitivity test was done for eight antibiotics in this study revealed that the antibiotic Nitrofurantoin are sensitive for 93.33%, Cefalexin are sensitive 76.66%, Clavulanic acid/ Cefixime are sensitive 76.66% are sensitive and Ampicillin are sensitive 60% for enteric bacteria. The antibiotics sensitivity for all eight antibiotics mentioned in table as sensitive, intermediate and resistant against the enteric bacterial infection are as mentioned below.

The antibiotic sensitivity test for enteric bacteria

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Nitrofurantoin	93.33	6.66	0
Cefalexin	76.66	13.33	10
Clavulanic acid/ Cefixime	76.66	6.66	16.66
Ampicillin	60	33.33	6.66
Amoxyclav	40	43.33	16.66
Amoxicillin	40	36.66	23.33
Penicillin-G	23.33	23.33	53.33
Metronidazole	16.66	33.33	50

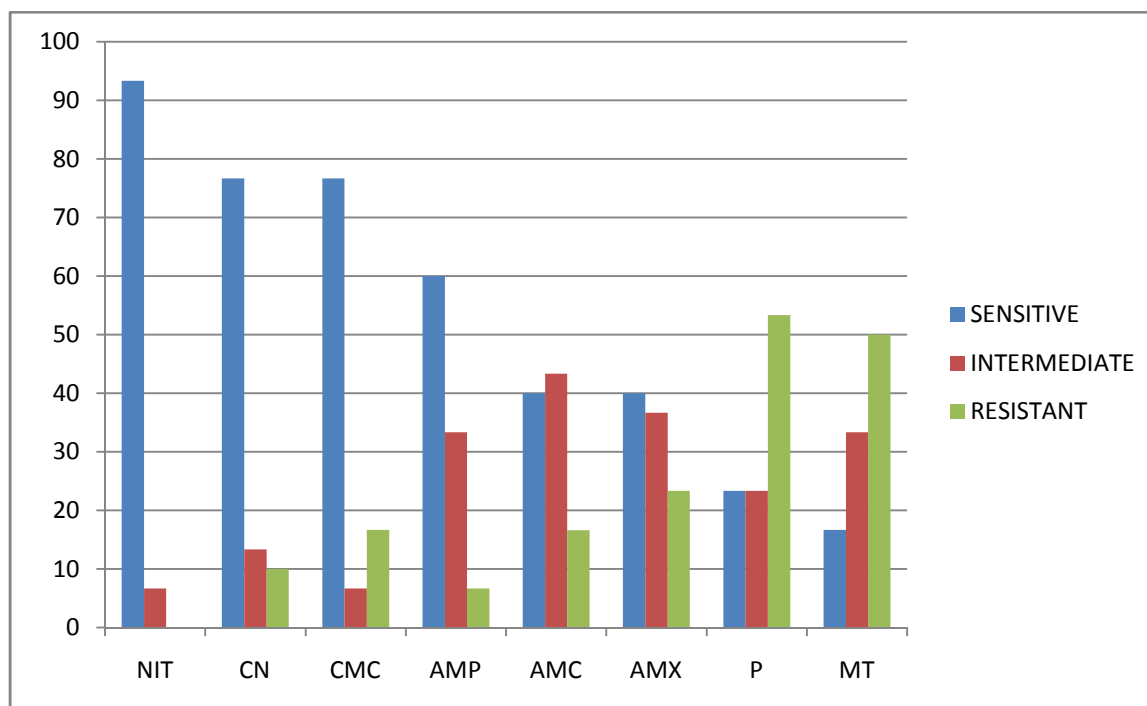


Chart: Antibiotic vs. Percentage

Discussion

Identifications of bacterial pathogen from the faecal samples

In the present investigations, the *E. Coli* and *salmonella spp* enteric bacteria were isolated from calves diarrhea, to determine the association of different risk factors and also determine the antibiotic sensitivity test in organized and non organized dairy farms around the Chandrapur district of Maharashtra. The present study focused on isolation of *E.coli* and *salmonella spp*.

The detection of *E. coli* in this study 15 (50%) out of thirty (30) diarrheic samples which is less than 53 (70.7%) were studied by Yeshiwas T and Fentahun W Molla [6] out of 75 diarrheic samples which is higher than the reports of Masud et al. [7] who out of 50 samples, 22 (44%), Dereje [8] who out of 58 fecal samples, 25 (43.1%) and less than Paul et al. [9] who out of 100 fecal samples, 76 (76%). Several authors reported prevalence of *E. coli* associated diarrhea in calves which varied from 25.0 % to 49.8 % published from 2002 to 2014 (Malik et al., 2013; Ansari et al., 2014) [10,11]. This high and low prevalence may be due to the difference in climatic conditions, sample size, feeding managements, personal hygiene, and the type of age where sample collected as well as farm size.

In the present finding diarrhea is highly associated with the age of calves. Accordingly below two weeks of calves were most high risk being affected with diarrhea. This is due to efficiency of colostrums absorption in gut and farm Managmental practices.

Conclusion

E.coli and *salmonella* infections are one of the major bacterial pathogens affecting dairy industry in terms of mortality and loss of production. It affected on public health. Thus hygiene Managmental practices should be strictly implemented to control *E.coli* and salmonella infections and food borne illness in humans.

References

- [1] Radostits O M, Blood D C, Gay CC, Blood D C and Hinchkliff K W 2000 Veterinary Medicine . 9th Edn, ELBS-Bailliere Tindal, London pp: 563-618.
- [2] Pereira RV, Santos TM, Bicalho ML, Caixeta LS, Machado VS (2011) Antimicrobial resistance and prevalence of virulence factor genes in fecal *Escherichia coli* of Holstein calves fed milk with and without antimicrobials. J Dairy Sci 94: 4556-4565.
- [3] Wu G, Mafura M, Carter B, Lynch K, Anjum MF (2010) Genes associated with *Escherichia coli* isolates from calves with diarrhea and/or septicaemia. Vet Rec 166: 691-692.
- [4] De Verdier K, Nyman A, Greko C, Bengtsson B (2012) Antimicrobial resistance and virulence factors in *Escherichia coli* from Swedish dairy calves. Acta Vet Scand 54: 2.
- [5] Merchant IA, Packer RA (1967) Veterinary Bacteriology and Virology. (7th Edn) Ames, Iowa, Iowa State University Press 752.
- [6] Yeshiwas T, Fentahun W M. The Prevalence of *E. coli* From Diarrheic Calves and Their Antibiotic Sensitivity Test in Selected Dairy Farms of Debre Zeit, Ethiopia. Adv Biotech & Micro.2017; 6(1): 555680.

- [7] Masud M, Fakhruzzaman M, Nazir H (2012) Isolation of *E. coli* from apparently healthy and diarrheic calves in Bangladesh and their antibiogram. *J Bang Soc Agric Sci Technol* 9: 45-48.
- [8] Dereje W (2012) Isolation and identification of Enterobacteria species from diarrheic calves in and around Addis Ababa, Ethiopia.
- [9] Paul K, Khan M, Mahmud S (2010) Isolation and characterization of *E. coli* from buffalo calves in some selected areas of Bangladesh. *Bangl J Vet Med* 8: 23-26
- [10] Mailk S, Kumar A, Verma AK, Gupta MK, Sharma SD, Sharma AK and Anu Rahal (2013). Incidence and Drug Resistance Pattern of Colibacillosis in Cattle and Buffalo Calves in Western Uttar Pradesh in India. *Journal of Animal Health and Production* 1: 15–19.
- [11] Ansari RAIH, Rahman MM, Islam MJ, Das BC, Habib A, Belal SMSH and Islam K (2014). Prevalence and antimicrobial resistance profile of *Escherichia Coli* and *Salmonella* isolated from diarrheic calves. *Journal of Animal Health and Production* 2: 12–15.