

# Isolation Identification and Characterization of Bacterial pathogens causing Calf Diarrhea with special reference to *Escherichia coli*.

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## Citation

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## Abstract

Though India is an agricultural country, livestock plays an important role. In bovine, calf mortality is due to many diseases, in that diarrhea or white scour plays a major role. Enterocolibacillosis is the disease caused by *Escherichia coli*. About 16 samples were collected and 12 samples were positive for *Escherichia coli*. Antibiotic susceptibility test were performed and finding out the sensitivity and resistance pattern. In this all the isolate were resistant to oxytetracyclin. DNA was isolated using conventional boiling and rapid cooling method. Polymerase chain reaction was carried out using tet (A) primer to detect the presence of tet (A) plasmid. Amplified product shows approximately 417 bp fragments in agarose gel electrophoresis viewed under UV illumination.

## INTRODUCTION

The livestock population of India is huge and animals as a whole play an importance role in the agricultural economy even though they often receive inadequate nourishment. Diarrhea in calves can be caused by a variety of pathogens including bacteria, viruses, protozoa and intestinal parasites. Among bacteria, enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* are known to be the most common and economically important agents (House, 1978), but other bacteria eg. *Campylobacter* spp. have also been identified as cause of enteric disease and diarrhea in calves. In acute neonatal diarrhea, an important disease of calves, 4 microorganism in particular, are of widespread occurrence and proven enteropathogenicity: rota virus, coronavirus, cryptosporidia and enterotoxigenic *Escherichia coli* (ETEC) (Acres et al., 1975). Overfeeding, overpopulation, cold temperature, bad hygiene, artificial feeding and colostrums deprivation are all predisposing factor which can be important in the complex etiology of the disease. Among these organism *Escherichia coli* is the main cause for the calf diarrhea as "white" scour.

The disease characteristically affects calves within the first 10-14 days of age, usually within the first week when there is sudden onset of profuse yellow or white diarrhea causing rapid and severe dehydration. The calf quickly becomes

recumbent. Accumulation of fluid in the abomasums and intestines gives the abdomen a bloated appearance. These enterotoxigenic *Escherichia coli* are shed into the environment by infected animals in the herd and are ingested by newborn calves soon after birth. There is some natural immunity to enterotoxigenic *Escherichia coli*; however, it often fails to protect calves born and raised under modern husbandry conditions.

## MATERIALS AND METHODS

### COLLECTION OF SAMPLE

Sterilized new polypropylene storage vial of 5ml capacity was used for collection of fecal sample from the diarrheic calves.

### MEDIUM

MacConkey agar, Eosin Methylene Blue agar, Brilliant Green agar, Xylose-Lysin Deoxycholate, Nutrient agar, Peptone water, MR-VP broth, Nitrate broth, Phenol Red Base broth, Simmon's citrate agar, Triple Sugar Iron agar, Christensen's urea agar base, Motility test medium and Muller Hinton agar. It was autoclaved at 121°C for 15 minutes.

### ANTIBIOTIC TEST

Antibiogram of the *Escherichia coli* isolates were carried

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out, as per the standard single disc diffusion technique according to Kirby- Bauer method ( Bauer et.al, 1965), modified by National Committee for clinical Laboratory Standard M2A4. The zone of inhibition was measured, recorded and interpreted according to the 3<sup>rd</sup> International supplement (1991).

### DNA ISOLATION

Isolation of DNA from the *Escherichia coli* was carried out by conventional boiling and rapid cooling method. A single colony of the test culture was inoculated in 10 ml nutrient broth and incubated at 37°C for 24 hours. The cells were harvested by centrifugation at 5000rpm for 10 minutes. The pellet was washed with phosphate buffer solution by centrifuging at 5000 rpm for 10 minutes. Supernatant was decanted and the pellet was washed with PBS again. Then the pellet is resuspended in 20µl nuclease free water and boiled for 5-10 minutes at 100°C and cooled rapidly on icebox. Then it was centrifuged at 1000 rpm for 5 minutes. Supernatant was collected and stored at 4°C as DNA template.

### RESULT AND DISCUSSION

Out of 16 fecal samples from the diarrheic calves 12 were positive for bacterial isolation on to media like MacConkey agar, EMB, BGA, XLD. All the 12 isolates produced positive reaction to lactose fermentation on MacConkey agar plate, metallic green sheen colonies on EMB plates, yellowish green colonies on BGA, yellow colonies on XLD. These findings were in correlation with the presentation of Cowan (1993) and John Barnes et al., (2003).

Gram staining were performed for all the isolates and revealed a Gram negative, non-acid fast, uniform staining non-spore forming bacilli. These findings were in correlation with the presentation of John Barnes et al., (2003).

All the 12 isolates evinced positive reaction for indole test by converting the amino acid tryptophane to indole with help of tryptophanase enzyme by the organism, Methyl Red test by utilization of glucose in the MRVP medium, triple sugar iron agar test, the isolates utilized all three sugars glucose, lactose and sucrose which results in acid slant or acid butt with gas production, carbohydrate fermentation test, utilized all three sugars by change of red colour (indicator phenol red) to yellow with gas production in Durham's tube in Phenol red base medium, nitrate reduction test by converting the nitrate to nitrite, catalase test by the development of air bubbles on adding 3% H<sub>2</sub>O<sub>2</sub> to the isolate in the glass slide.

All the 12 isolates were negative for Voges proskauer test where no acetone production was seen, citrate utilization test where no colour change in Simmon's citrate medium and also negative for oxidase test. These findings were in correlation with Bettelheim, (1994) and Cowan, (1993).

Figure 1

TABLE - 1  
Biochemical characters of *Escherichia coli* isolates

samples	Indole test	MR test	VP test	Citrate test	Nitrate test	TSI test	Urease test	Oxidase test	Carbohydrate Glu Lac Sac	Motility test	Species
E1	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E2	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E3	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E4	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E5	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E6	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E7	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E8	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E9	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E10	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E11	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>
E12	+	+	-	-	+	A/AG	-	-	+ + + +	+	<i>E.coli</i>

(+) = Positive reaction (-) = Negative reaction A/AG = Acid slant and acid butt with gas production.

Antibiotic susceptibility test were done for all the isolates. All the isolates were sensitive to ciprofloxacin(100%) followed by pefloxacin(100%), cephotaxime(100%), chloramphenicol(83.33%), co-trimoxazole(75%) and gentamicin(75%). In the present study the isolates were sensitive to gentamicin was similar to the findings of Jones et al., 1977. It was resistant to oxytetracyclin(100%), chlortetracycline(100%), ampicillin(66.66%) and amoxicillin(58.33%). Palmer et al, (1977) reported that the findings of amoxicillin was effective. Resistant to tetracycline resistant(100%) was similar to according to Galland et al.(2001). They found that 38.5 percent isolates were resistant to tetracycline. The variation in the antimicrobial pattern may be due to the use of antibiotic in that region during that period.

**Figure 2**

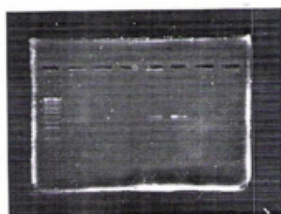
**TABLE-II**  
Antibiotic susceptibility test for *Escherichia coli*

Antibiotic disc	Isolates												Percentage	
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	Sensitive	Resistant
Am <sup>30</sup>	R	S	R	S	R	S	R	R	S	R	R	S	41.66	58.33
A <sup>10</sup>	R	R	S	S	R	R	R	S	S	R	R	R	33.33	66.66
Ce <sup>30</sup>	S	S	S	S	S	S	S	S	S	S	S	S	100	-
C <sup>30</sup>	S	S	S	R	S	S	R	S	S	S	S	S	83.33	16.66
C <sup>20</sup>	R	R	R	R	R	R	R	R	R	R	R	R	-	100
Ct <sup>30</sup>	S	S	S	S	S	S	S	S	S	S	S	S	100	-
Co <sup>30</sup>	S	S	S	R	S	R	S	S	R	S	S	S	75	25
E <sup>10</sup>	R	R	R	R	R	R	R	R	R	R	R	R	-	100
G <sup>30</sup>	S	S	S	R	S	R	S	S	R	S	S	S	75	25
O <sup>30</sup>	R	R	R	R	R	R	R	R	R	R	R	R	-	100
Pf <sup>5</sup>	S	S	S	S	S	S	S	S	S	S	S	S	100	-

Amoxycillin; A, Ampicillin; Ce, Cephotaxime; C, Chloroamphenicol; Ct, Chlortetracyclin; Cf, Ciprofloxacin; Co, Co-trimoxazole; E, Erythromycin; G, Gentamicin; O, Oxytetracyclin; Pf, Pefloxacin S, Sensitive; R, Resistant.

PCR amplification were done for two isolates. In the present study the PCR amplified fragments of approximately 417 bp was observed with use of tet(A) specific primers F- CGA GCC ATT CGC GAG AGC and R- GCC TCC TGC GCG ATC TGG in the 1.5% agarose with ethidium bromide stain electrophoresed and viewed under UV illumination. This findings were in agreement with that of Furushita et al., (2003).

**Figure 3**



Amplification of *Escherichia coli* isolates at 417 base pair  
M- Molecular weight marker (100 base pair)  
Lane 4 and 5 – positive sample

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