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Isolation and identification and antibiogram of *E. coli* from faecal samples of calves

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Abstract
Aim: The goal of the current study was to understand how *E. coli* affected calf diarrhea in cattle and buffalo calves as well as to Known more about their metabolic profiles (biochemical profiles) and antibiotic susceptibility test.
Material and methods: 100 samples were collected from the ILFC (Instructional Livestock Farm Complex Veterinary College) in and around Bidar district (Navbad and Kamthana). According to Paul *et al.* (2010), *E. coli* isolates were isolated and identified. *E. coli* isolates that had been identified culturally were also tested for metabolic profile (biochemical) and antibiotic sensitivity test.
Result: 85 (85%) of the 100 samples that were collected were positive for *E. coli* species. Each of the 100 samples was inoculated into nutrient broth and left to incubate for 24 hours at 37 °C. A loop of nutrient broth was selectively plated into MacConkey agar after 24 hours, and looked for the appearance of small, pinpoint pink colonies. The pinpoint pink coloured colonies were then streaked onto eosin methylene blue agar, where the appearance of colonies with black centres and metallic sheen was watched for. The prevalence *E. coli* was reported to be present 85% (85/100). *E. coli* isolates showed sensitivity for antibiotics like Ciprofloxacin, Co-trimoxazole, and Ceftriaxone-tazobactum. Further, *E. coli* isolates were resistant to penicillin, streptomycin, erythromycin, kanamycin, amoxyclav, and amoxicillin were the most common.

Keywords: *E. coli*, MacConkey, ABST

Introduction
Calf diarrhoea is a substantial cause of morbidity and mortality in calves during the first three weeks of life, resulting in enormous economic loss. *Rotavirus*, *coronavirus*, enterotoxigenic *Escherichia coli*, *Salmonella* spp., and *Cryptosporidium parvum* are the agents responsible for this mixed illness (Veena *et al.*, 2023) [19]. In addition to the animal's non-infectious characteristics (such as nutritional and immunological health), the environment or management also have a significant impact on the development of disease in calves. According to Izzo *et al.* (2011) [9], neonatal calf diarrhoea is a complicated condition (NCD). Due to its complexity, NCD is difficult to effectively regulate (Cho and Yoon, 2014) [5]. According to Achá *et al.* (2004) [1], there are often higher cases of diarrhoea in October and November than during March to October. Despite this, the molecular identification of virulence factors for *E. coli* aids in classification and can shed light on the range of potential advantages of employing vaccines, some of which are not now available against all kinds. To treat sick calves, antibiotics are usually given parenterally or orally. Some examples of frequently used antimicrobials that have been used include sulbactam, ampicillin, neomycin, cephalosporin, tetracycline, and a sulphonamide-trimethoprim combination (Izzo *et al.*, 2011) [9]. The growth of drug resistance and the presence of dangerous bacteria that are multidrug resistant, however, have become important barriers to treating bacterial infections over the past ten or so years (Anita *et al.*, 2013) [2].

Materials and Methods
The current study was conducted in the Department of Veterinary Public Health and Epidemiology at the Veterinary College, Bidar, using a total of 100 field samples, consisting of rectal swabs from diarrheal buffalo and cattle calves (1 week to 1 year of age), which were aseptically collected into nutrient broth (NB) from different areas in the ILFC (Instructional livestock complex) and in the surrounding Bidar district (Navbar and Kamthana).

Isolation and identification of *E. coli* from fecal samples

A loopful of culture from nutrient broth was also streaked onto MacConkey agar following the standard approach, and it was then incubated at 37 °C for 24 hours. The presence of *E. coli* was observed from the luxuriant development and pinpoint-sized pink colonies that appeared on the petri plates. Additionally, one positive colony from MacConkey agar was streaked simultaneously on eosin methylene blue agar, and those isolates that displayed colonies with a metallic sheen with black centres were recognised as positive isolates and put through biochemical testing (Metabloic profile).

All of the isolates were stained with Gram's stain to analyse the cell morphology. The smear was made on an ungreasy, clean microscope glass slide by choosing pink colonies that had grown on MacConkey agar plates. The smear was heat fixed, stained using Gram's method, and viewed under a microscope to assess the morphological traits and staining reaction. The isolates were also put through a battery of (Metabollic profile) biochemical tests, including the inode, methyl red, voges proskauer, and citrate tests. Additionally, one colony from MacConkey agar was inoculated into MacConkey Broth.

Antibiotic sensitivity test (ABST)

The conventional disc diffusion method was used to conduct the antibiotic sensitivity test. 2 ml of nutrient broth and a part of the inoculum from the pure culture on the nutritional agar were combined well. 24 hours were spent keeping this at 37 °C. The bacterial inoculum was adjusted to 0.5 on the McFarland scale and then applied to the Mueller Hinton agar plates using a sterile swab. According to the usual approach for the disc diffusion method as published by Bauer *et al.* (1966) [4], the antibiotic discs were put aseptically to the agar surface with a distance between centres of at least 30mm apart and inspected after incubation for 12-24 hours at 37°C. Then, using an antibiotic zone scale, the zone of inhibition was represented in millimetres. By comparing the values of the zone of inhibition obtained for each antibiotic disc against the reference chart provided with the discs, sensitivity/resistance was evaluated. The interpretation was carried out in compliance with the Clinical Laboratory Standard Institute's (CLSI, 2020) [6] performance standards for antimicrobial susceptibility testing. Antibiotic discs utilised in the investigation and findings are shown in Table 1.

Results and Discussion

***E. coli* isolation and identification in diarrhoeic calf samples**

In the current study, data from ILFC and the area surrounding the bidar district (Navbad and Kamthana) gathered from diarroheic calves revealed an *E. coli* prevalence of 85/100 (85%).

El-Seedy *et al*. (2015) [7] reported 119 (93.7%) bacterial isolates were found in 127 collected faecal samples from diarrheal calves, which included 96 (75.6%) *E. coli* strains and 23 (18.1%) *Salmonella* serovars which is slightly higher than the present study. Tweleve farms in the southwest Nigerian states of Oyo and Ogun were the subject of a study by Sunday *et al*. (2016) [16], 120 (14.5%) of the 825 calves that he gathered that were younger than 6 months old showed signs of diarrhoea. They also found 120 pieces of excrement from diarrheal calves among this. They were isolated, identified in accordance with standard practise, and tested for the presence of bacterial pathogens. The two farms in the states of Oyo and Ogun had the greatest prevalence rates of diarrheal calves, with rates of 50% (25 out of 50 calves) and 23% (12 out of 52 calves), respectively. 19 out of the 120, or 15.8%. *E. coli* was analysed by Ashraf *et al.* (2017) from 47 faecal samples with an infection rate of (47%) followed by *Pseudomonas aeruginosa* 4(4%) and *Salmonella* Typhimurium. According to Veena *et al.* (2023) [19], 105 out of 110 diarrhoeic faecal sample isolates had a prevalence of 95.45% for *E. coli.* When 120 faecal samples from diarrheal calves under three months old were collected in dry and wet seasons by Mona *et al*. (2020) [11], they revealed that 71 (59.5%) and 36 (30%) of the samples tested positive for E. coli and Salmonella species, respectively, while 26 (21.66%) had mixed E. coli and *Salmonella* species infection, which is somewhat similar to the current study.

Isolation and identification of *E. coli* from diarrhoeic calves

All 85 samples from the current investigation had pinpoint pink colonies on MacConkey agar, followed by metallic-shaded colonies with black centres on eosin methylene blue agar (Table 2, Plates 1 and 2). Additionally, the isolated were oxidase negative and catalase positive. When inoculated into MacConkey broth, every isolate formed a yellow hue. They additionally tested positive for indole and methyl red and negative for citrate and voges proskauer biochemically. Table 2, Plate 3, and Table 4.

Henben *et al.* (2022) [8] found the similar result similar to present study where he cultured the suspicious on nutritional media and incubated aerobically for 24-48 hours at 37 °C. Then, pure colonies were subcultured on MacConkey agar for 24 to 48 hrs. at 37 ºC followed by plating on Eosin methylene blue agar Further, the isolates were subjected to by morphological evaluation like grams staining. Lactose fermenter and non-lactose fermenter groups of putatively isolated bacteria were further divided using MacConkey agar colonial characteristics. In a different investigation, Walid *et al.* (2020) [18] isolated *E. coli* from faeces and intestinal samples were put into Trypticase soya broth and cultured for 24 hours at 37 °C. The enriched samples were then selectively plated on Oxoid's MacConkey and Eosin Methylene Blue (EMB) agar plates and incubated at 37 °C for 24 hours. Biochemically, metallic sheen colonies on EMB agar with lactose fermenting capabilities on MacConkey agar were found using Simmon's citrate and triple sugar iron (TSI) agar medium (Oxoid, UK). After being treated with nutritious broth, the faeces samples collected by Mohammad *et al.* (2022) [10] were incubated at 37 °C for an overnight duration. The enhanced cultures from each sample were then streaked onto EMB and SS agar media, respectively, to isolate *E. coli* and *Salmonella* spp. A single colony that was supposed to exist was streaked across the same media in order to establish pure cultures.

Neonatal diarrhoea still affects newborn calves and is a critical health problem, even though the cattle industry has made tremendous strides. It is also associated with high death and morbidity rates as well as large economic expenses. Losses. *E. coli* is the bacteria that is most common. calf diarrheal illness as a normal gut resident, it was expected that a decent number of the isolates would contain non-pathogenic *E. coli*. The animals with diarrhoea may have been exposed to other infectious agents in addition to these isolates, which may not be harmful.

Antimicrobial susceptibility test of *E. coli* isolates

The antibiogram patterns of the *E. coli* isolates in our study investigation showed varying sensitivity when tested against 15 commonly used antimicrobial medicines. The most sensitive drugs were found to be ceftriaxone+tazobactum (68%), Co-trimoxazole (62%), ceftriaxone (61.5%), ciprofloxacin (60.89%), levofloxacin (59%), and gentamicin (50.87%). The isolates additionally shown 100% resistance to the antibiotics pencillin (100%), streptomycin (100%) and erythromycin (100%) followed by kanamycin (61.10%), cefixime (50%), tetracycline (50%) and ampicillin (40%) followed by chloramphenicol (39%), bacitracin (39%) and amoxyclav (35%).

Veena *et al.* (2023) [19] found findings that were equivalent to those of the current study. The medications with the highest levels of sensitivity were co-trimoxazole (67%), ceftriaxone+tazobactum (64.09%), ciprofloxacin (63.89%), ceftriaxone (61.45%), levofloxacin (59.88%), cefaperazone (55.88%), and gentamicin (47.87%). The results also showed that all 105 isolates were completely resistant to metronidazole, nitrofurantoin, penicillin, and streptomycin. Polymyxin B was next, with 61.11 percent resistance, followed by kanamycin, cefixime, doxycycline, tetracycline, cephalexin, amoxycillin, and chloramphenicol (40.67%). Sruthy *et al.* (2019) [15] reported that all isolated *E.coli* also revealed that all 105 isolates had 100% resistance to metronidazole, nitrofurantoin, penicillin, and streptomycin, followed by polymyxin B (61.11%), kanamycin (61.04%), cefixime (53.98%), doxycycline (52.89%), tetracycline (50.85%), cephalexin (47.46%), amoxycillin (40.7%), and chloramphenicol (40.67%).Our results, were consistent with those of Sruthy (2019) [15], found that all 41 isolates had 100% resistance to metronidazole, followed by 82.93% for penicillin, 80.49% for ceftazidime, 80.49% for amoxicillin plus clavulanic acid, 75.61% for furazolidone, 70.74% for ceftriaxone, 68.30% for amoxycillin, and 56.10% for amoxicillin plus s+sulbactam (56.10%). tetracycline (79.5%), streptomycin (67%), ampicillin (54%), and trimethoprim-sulfamethoxazole (43%) each exhibited a percent resistance of, according to Nader *et al.* (2019) [13]. Ceftazidime (14.8%), amoxicillin-clavulanic acid (13.6%), and aztreonam (11.3%) had the lowest drug resistance rates, whereas none of the isolates had imipenem resistance. *E. coli* isolates were resistance to penicillin, erythromycin, cotrimoxazole, and nitrofurantoin was reported to occur most frequently, according to Mubita *et al.* (2008) [12]. According to El-Seedy *et al.* (2019) [7], *E. coli* isolates only shown great sensitivity to marbofloxacin, spectinomycin, and neomycin.

The use of human pharmaceuticals to treat infected animals, the transmission of human pathogens to animal hosts where resistance has already developed, and the excretion of these chemicals are the main causes of the rise in antibiotic resistance to *E. coli* infection. Antibiotics and their metabolites can be found in human sewage and sludge, and when applied to animals via irrigation or sludge fertiliser made with sewage water, they may acquire resistance.

Table 1: List of antimicrobials agents, codes, concentrations and spectrum of activity

Sl. No.	Antibiotic	Code	Disc content	Spectrum of activity
1.	Ampicillin	AMP	2mcg	Narrow
2.	Amoxyclav	AMy	30mcg	Narrow
3.	Bacitracin	B	10 Units	Narrow
4.	Chloramphenicol	C	30mcg	Broad
5.	Ciprofloxacin	CIP	5mcg	Broad
6.	Ceftriaxone	CTR	30mcg	Broad
7.	Cefixime	CF	30mcg	Broad
8.	Co-trimoxazole	COT	30mcg	Broad
9.	Erythromycin	E	15mcg	Broad
10.	Gentamicin	GEN	50 mcg	Broad
11.	Kanamycin	K	30mcg	Broad
12.	Levofloxacin	LF	5mcg	Broad
13.	Pencillin	P	10Units	Narrow
14.	Tetracycline	TE	30mcg	Broad
15.	Streptomycin	S	10mcg	Narrow

Table 2: Colony morphology, staining, biochemical tests of *E. coli* species from calf diarrhea

Sl. No.	Isolates /Species	Prevalence of *E. coli* (%)	Pin point pink colored colonies on MacConkey agae G	Black centered colonies with metallic sheen	Grams reaction	Motility at 37 ºC	Yellow colour on MacConkey broth	Biochemical tests						
								Indole	Methyl red	Voges-Proskauer	Citrate	Nitrate	Catalase	Oxidase
1.	*E.coli* (isolates)	85	+	+	-	+	+	+	+	-	-	+	+	-

Table 3: Antimicrobial susceptibility pattern of *E. coli* isolates

Bacterial species isolated	S/R	Amoxicillin	Amoxyclav	Cefaperazone	Cefixime	Ceftriaxone	CIT#	Chloramphenicol	Ciprofloxacin	Co-trimoxazole	Gentamicin	Kanamycin	Levofloxacin	Pencillin	Streptomycin	Tetracycline
E.coli		R	R	S	R		S	S		S	S	R	S	R	R	R

S: Sensitivity, R: Resistant, CIT: Ceftriaxone+tazobactum

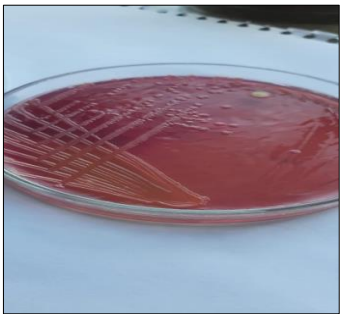

Plate 1: of MacConkey plate showing Pink lactose fermenting colonies

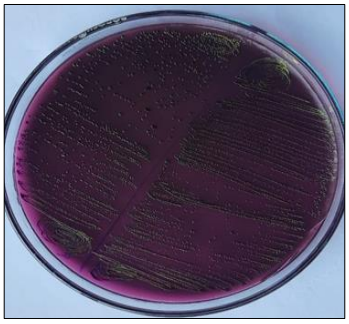

Plate 2: of *E. coli* Showing Metallic sheen appearance on EMB plate

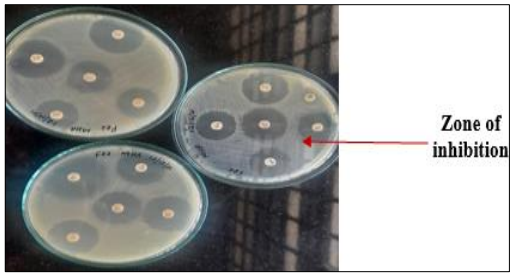

Plate 3: of Antibiotic sensitivity test

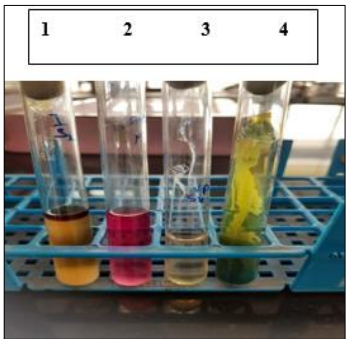

Plate 3: 1. Indole: + 3. Voges-Proskauer:- Methyl red: + 4.Citrate

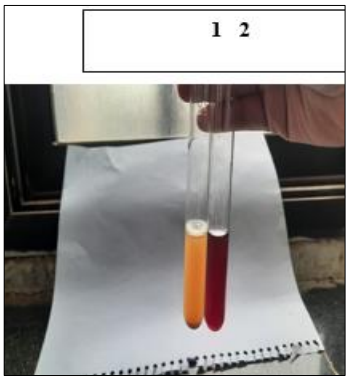

Plate 4: MacConkey broth: 1.Yellow colour: + 2. Red

Conclusion

100 samples altogether were gathered from the ILFC and the bidar district. *E. coli* was determined to be 85% prevalent. Additionally, every isolate tested positive for pinpoint colonies on MacConkey agar and a metallic sheen with a black center on EMB agar. The *E. coli* isolates tested demonstrated ciprofloxacin, co-trimazole, and ceftriaxone-tazobactum sensitivity. Further, pencillin, streptomycin, erythromycin, kanamycin, amoxyclav, and amoxicillin were the most frequently used antibiotics for treating *E. coli* isolates.

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