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Isolation, Identification and Antibiotic Sensitivity Pattern of *Escherichia coli* Isolated from Diarrheic and Non diarrheic Calves

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ABSTRACT

The present study was conducted to isolate, identified *Escherichia coli* from healthy and diarrheic cattle and buffalo calves and determine their antibiotic sensitivity pattern to commonly used antibiotics. A total 100 *E. coli* isolated from 104 rectal swabs sample from healthy and diarrheic cattle and buffalo calves were subjected to antibiotic sensitivity pattern against 08 different antibiotics by Kirby-Bauer disk diffusion method. The antibiotic sensitivity pattern showed that the *E. coli* isolated from healthy and diarrheic cattle calves showed higher resistance against ampicillin (88.88%), tetracycline (44.44%) and ampicillin (62.50%), co-trimoxazole (54.16%), respectively. While, healthy cattle calves showed higher resistance against ampicillin (46.15%) and cotrimoxazole (23.07%) whereas from diarrheic buffalo ampicillin (52.17%) and cotrimoxazole (39.13%) were recorded.

Introduction

The emergence and spread of antimicrobial resistant bacteria is an increasing problem and a threat to global public health (WHO 2017). Various studies have been conducted worldwide to isolate pathogenic bacteria that may be a cause of concern for human or animal health. Neonatal calf diarrhea is multifactorial symptom which despite of continuous research is major cause of poor growth in young calves (Lorenz *et al.*, 2011). The economic aspect of diarrheal diseases in calves and their mortality and morbidity is a matter of great concern to the livestock owners. Gram negative bacteria are a major therapeutic challenge in both livestock and human beings. More than 85% of the diarrhoea is due to the members belonging to family Enterobacteriaceae, particularly *E. coli*. *E. coli* is a Gram negative, short rod shaped, flagellated, motile, oxidase negative, facultative anaerobic bacterium (Markey *et al.*, 2013). *E. coli* is a commensal microbe, which is the major part of normal aerobic microbial population of the intestine of humans and warm blooded animals and plays an important role in host metabolism, immunology and nutrition (Tenaillon *et al.,* 2010).

Materials and Methods

Rectal swabs sample of healthy and diarrheic cattle and buffalo calves were collected from organised and unorganised dairy farms in and around Jabalpur (Madhya Pradesh) in peptone water and incubated at 37°C for 18 hours. A total of 104 samples were processed for isolation of *E. coli* using standard microbiological techniques and their sensitivity profile for antibiotics as per the standard microbiological protocols. The swabs were inoculated on Mac Conkey's agar and Eosin methylene blue agar plates at 37°C for 24 hours. The primary identification of the bacterial colonies from the positive cultures performed with the help of colony appearance. Furthermore, identification and characterization of the *E. coli* isolates was conducted on the basis of colony morphological characteristics, Gram's staining and biochemical tests such as indole, methyl red, Voges Proskauer, citrate utilization and triple sugar iron agar (Markey *et al.,* 2013). Antibiotic susceptibility pattern of the isolates was determined by using Kirby-Bauer disc diffusion test recommended by Clinical and Laboratory Standards Institute (CLSI, 2013) against 08 antibiotics belonging to 06 different class of antibiotics. Disc used were Aminoglycosides (gentamicin 10μg), Polymyxin (colistin 10μg), Fluoroquinolone (levofloxacin 10μg, ciprofloxacin 5μg), Penicillins (ampicillin 10 μg, choramphenicol 30 μg), Tetracyclines (tetracyclines 30 μg), Sulphonamides (cotrimoxazole 25 μg). An inoculum was prepared for each bacterial isolate by adjusting the turbidity to 0.5 McFarland standards, which was then spread on Muller-Hinton agar plates. The antibiotic discs were then set on the agar plates and incubated overnight at 37°C for 24 hours. The zones of inhibition for the antibiotics were measured in mm and were further classified according to CLSI guidelines.

Results and Discussion

Out of 104 fecal samples, 100 samples were positive for *E. coli* (Table 1). The small bright pink colour colony on MacConkey agar indicating the lactose fermenter (fig. 1) when further streaked on EMB showed characteristics metallic green sheen (fig.2) which was further characterized by biochemical test (fig. 3). *E. coli* was Indole and MR positive and VP and Citrate negative and yellow color butt and slant in TSI agar slant (fig.4). On the basis of morphological and biochemical characteristics 96.15% samples were positive for *E. coli.* Antibiotic susceptibility pattern of the 100 *E. coli* isolates were determined by Kirby Bauer disc diffusion method against 08 different antibiotics (fig.5). *E. coli* isolated from healthy and diarrheic cattle calves showed higher resistance against ampicillin (88.88%), tetracycline (44.44%) and ampicillin (62.50%), co-trimoxazole (54.16%), respectively. Healthy and diarrheic buffalo calves were showed higher resistance against ampicillin (46.15%), cotrimoxazole (23.07%) and ampicillin (52.17%), cotrimoxazole (39.13%), respectively.

In the present study, 100 fecal samples (healthy and diarrheic cattle and buffalo calves) were positive for *E. coli*. A similar finding was reported by the earlier worker Masud *et al.,* (2012). The present study shows much higher isolation rate than Paul *et al.,* (2010) (46 %), Masud *et al.,* (2012) (30.71%) and Gebregiorgis and Tessema (2016) (36.8 %). The reason why the result of the current study varies from the other reports might be due to variations in farm management conditions. As documented in Radostits *et al.,* (2007), gaps in management specifically calf handling practices, inadequate nutrition, exposure to severe environment, insufficient attention to the newborn calf or a combination of these.

Resistance to antimicrobial drugs among bacterial pathogens is an emerging problem. Clinically important antimicrobials are extensively used in food animal for disease prevention, treatment and growth promotion. It is suggested that two-third of antimicrobials produced globally are consumed in the livestock sector (CDDEP, 2015). Several studies shows that widespread use of agricultural antimicrobials contributes to increased clinical resistance to antimicrobials (Chang *et al.,* 2015 and Marshall and Levy, 2011).

Table.1 Isolation of *Escherichia coli* by conventional method

S.No	Particulars	Non diarrheic cattle calves	Diarrheic cattle calves	Non diarrheic buffalo calves	Diarrheic buffalo calves	Total
1	Sample size	27	25	26	26	104
2	Total *E. coli* isolated	27	24	26	23	100
3	Percentage positive	100%	96.00%	100%	88.46%	96.15%

Table.2 Antimicrobial resistance profile of *E. coli* in non diarrheic cattle calves

S.No.	Antibacterial Agent	Concentration mcg/disc	Non diarrheic cattle calves(n=27)			
			Susceptible	Intermediate	Resistant	Total
1	Ampicillin	10	03 (11.11%)	0 (0%)	24 (88.88%)	27
2	Chloramphenicol	30	12 (44.44%)	10 (30.03%)	05 (18.51%)	27
3	Ciprofloxacin	05	25 (92.59%)	01 (3.70%)	01 (3.70%)	27
4	Co-Trimoxazole	25	12 (44.44%)	07 (25.92%)	08 (29.62%)	27
5	Colistin	10	27 (100%)	0 (0%)	0 (0%)	27
6	Gentamicin	10	26 (96.29%)	0 (0%)	01 (3.70%)	27
7	Levofloxacin	05	26 (96.29%)	01 (3.70%)	0 (0%)	27
8	Tetracyclin	30	03 (11.11%)	07 (25.92%)	17 (62.96%)	27

Table.3 Antimicrobial resistance profile of *E. coli* in diarrheic cattle calves

S.No.	Antibacterial Agent	Concentration mcg/disc	Diarrheic cattle calves(n=24)			
			Susceptible	Intermediate	Resistant	Total
1	Ampicillin	10	09 (37.50%)	0 (0%)	15 (62.50%)	24
2	Chloramphenicol	30	15 (62.50%)	07 (29.16%)	02 (8.30%)	24
3	Ciprofloxacin	5	16 (66.66%)	01 (4.16%)	07 (29.16%)	24
4	Co-Trimoxazole	25	10 (41.66%)	03 (12.50%)	13 (54.16%)	24
5	Colistin	10	20 (83.33%)	0 (0%)	04 (16.66%)	24
6	Gentamicin	10	22 (91.66%)	0 (0%)	02 (8.30%)	24
7	Levofloxacin	05	17 (70.83%)	01 (4.16%)	06 (25.0%)	24
8	Tetracyclin	30	07 (29.16%)	07 (29.16%)	10 (41.66%)	24

Table.4 Antimicrobial resistance profile of *E. coli* in diarrheic buffalo calves

S.No.	Antibacterial Agent	Concentration mcg/disc	Diarrheic buffalo calves(n=23)			
			Susceptible	Intermediate	Resistant	Total
1	Ampicillin	10	11 (47.82%)	0 (0%)	12 (52.17%)	23
2	Chloramphenicol	30	17 (73.91%)	04 (17.39%)	02 (8.69%)	23
3	Ciprofloxacin	5	14 (60.86%)	04 (17.39%)	06 (26.08%)	23
4	Co-Trimoxazole	25	14 (60.86%)	0 (0%)	09 (39.13%)	23
5	Colistin	10	22 (95.65%)	0 (0%)	01(4.34%)	23
6	Gentamicin	10	22 (95.65%)	01 (4.34%)	0 (0%)	23
7	Levofloxacin	05	18 (78.26%)	0 (0%)	05 (21.73%)	23
8	Tetracyclin	30	09 (39.13%)	0 (0%)	14 (60.86%)	23

Table.5 Antimicrobial resistance profile of *E. coli* in Non diarrheic buffalo calves

S.No.	Antibacterial Agent	Concentration mcg/disc	Non diarrheic buffalo calves(n=26)			
			Susceptible	Intermediate	Resistant	Total
1	Ampicillin	10	15 (57.69%)	0(0%)	11 (42.30%)	26
2	Chloramphenicol	30	24 (92.30%)	02 (7.69%)	0 (0%)	26
3	Ciprofloxacin	5	20 (84.61%)	01 (3.84%)	05 (19.23%)	26
4	Co-Trimoxazole	25	19 (73.07%)	01 (3.84%)	06 (23.07%)	26
5	Colistin	10	26 (100%)	0 (0%)	0 (0%)	26
6	Gentamicin	10	26 (100%)	0 (0%)	0 (0%)	26
7	Levofloxacin	05	19 (73.07%)	03 (11.53%)	04 (15.38%)	26
8	Tetracyclin	30	15 (57.69%)	01 (3.84%)	10 (38.46%)	26

Fig.1 Gram's Staining of *Escherichia coli* (100X)

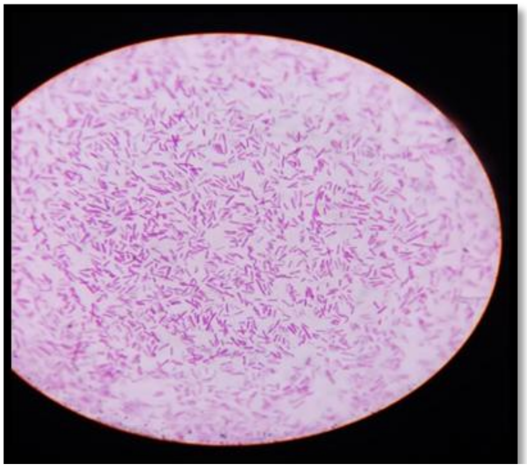


Fig.2 Growth of *E. coli* on MacConkey agar **Fig.3** Growth of *E. coli* on EMB Agar

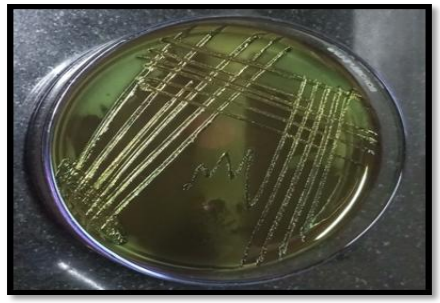
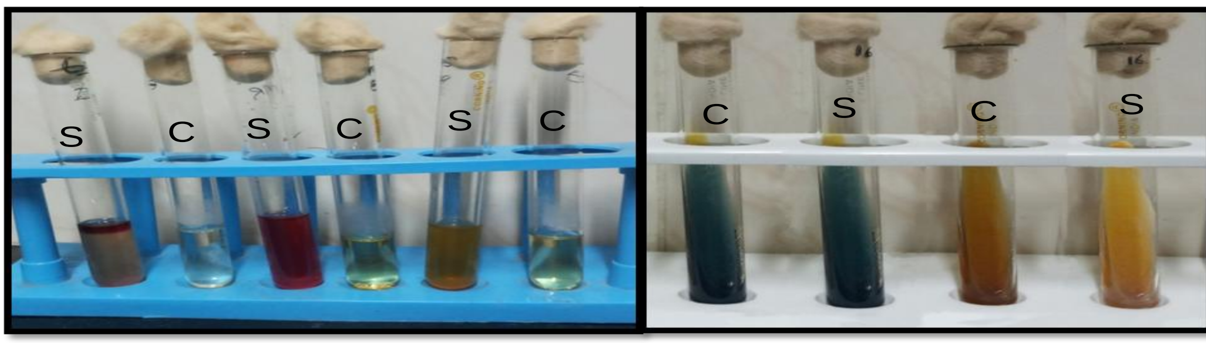
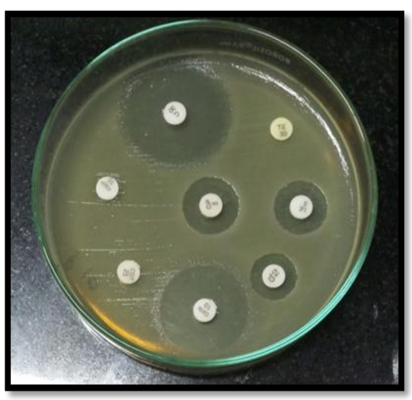


Fig.4 IMViC test and TSI Agar test for *Escherichia coli*



S – Sample C - Control

Fig.5 Multidrug resistance profile of *Escherichia coli*



In present study, Antibiotic sensitivity pattern was recorded against 08 different antibiotics revealed the multidrug resistance profile in *E. coli* isolates from diarrheic and healthy cattle calves were 66.66% and 48.14% and for buffalo calves it was 47.82% and 38.46%, respectively. *E. coli* isolates from healthy and diarrheic calves were resistant to at least one of the antimicrobials tested.

The isolates from healthy cattle calves showed higher resistance to ampicillin

(88.88%) followed by 44.44% for tetracycline while the antibiotics colistin, levofloxacin, gentamicin and ciprofloxacin were sensitive (Table 2). Highest rate of antibiotic resistance was observed in *E. coli* isolates from diarrheic cattle calves against ampicillin (62.50%), co-trimoxazole (54.16%) and tetracycline (37.50%) while chloramphenicol and gentamicin were effective (Table 3). Similar to the present findings, Kmet and Bujnakova (2018) reported highest antimicrobial resistance to ampicillin followed by tetracycline for the *E. coli* isolated from calves. Malik *et al.,* (2013) observed highest antimicrobial resistance to the ampicillin and tetracycline and highest susceptibility to gentamicin for the *E. coli* isolated from diarrheic calves in UP, India. The variation in antibiotic resistance can be due to indiscriminate use of antibiotics in particular area.

Highest rate of antibiotic resistance was observed in *E. coli* isolates from diarrheic buffalo calves against tetracycline and ampicillin (52.17%) followed by co-trimoxazole (39.13%) while colistin followed by gentamicin, levofloxacin and chloramphenicol were effective (Table 4). In healthy buffalo calves, highest antimicrobial resistance was observed to ampicillin (46.15%) followed by tetracycline (38.46%), while colistin, gentamicin and chloramphenicol antibiotics were sensitive (Table 5). Similar to the present results, Srivani *et al.,* (2017) reported higher antimicrobial resistance against tetracycline (63.21%) followed by ampicillin (48.11%), whereas highest susceptibility to chloramphenicol and gentamicin for the *E. coli* isolated from diarrheic buffalo calves in Andhra Pradesh and Telangana states. Majueeb *et al.,* (2013) observed higher susceptibility to chloramphenicol for the *E. coli* isolated from buffalo calves in Jammu, India. The *E. coli* isolated from diarrheic buffalo calves (Nizza *et al.,* 2010) in Italy showed higher sensitivity to colistin as in the present findings.

It can be concluded from the present study that both diarrheic and healthy calves harvested multi drug resistance *E. coli*.

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