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Research Article

ISOLATION, CHARACTERIZATION, SEROTYPING AND ANTIBIOGRAM STUDIES OF *E.coli* COLLECTED FROM DIARRHOEIC NEONATAL KIDS

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Abstract-Neonatal diarrhoea is a major threat responsible for high mortality in neonates particularly during the first week after birth. The present study was undertaken to find out the prevalence of *Escherichia coli* (*E. coli*) with neonatal kid diarrhoea. A total of 103 faecal samples were collected from diarrhoeic goat kids up to 4 weeks of age and processed for detection of *E. coli* by cultural and biochemical techniques. The enteropathogens detected *E. coli* 75 (72.81%) were recovered producing lactose fermenting pink colored colonies on MacConkey agar medium and colonies with greenish metallic sheen on EMB agar medium. Further, all the 75 isolates showed typical IMViC patterns of *E. coli viz.* Indole and Methyl- red positive, Voges Proskauer and Citrate negative. Out of 75 isolates, 35 isolates were sent to National *Salmonella* and *Escherichia* Center (NSEC), Central Research Institute, Kasauli. Of these 35 isolates, 22 were serotyped into nine different 'O' groups, while two isolates were untypable and six isolates were found rough. The serotypes obtained were O2, O84, O86, O87, O101, O118, O120, O128, O141 and O157. The most predominant serotypes were O120 (nine isolates), O141 (four isolates) and O157 (two isolates) while other serotypes were O2, O84, O86, O87, O101, O118 and O128 (one isolate each). Out of 75 isolates, very high resistance was showed against Cephalexin 72 isolates (96.00%), Kanamycin 70 isolates (93.33%). Moderate number of isolates were found to be resistant to Tetracycline 45 isolates (60.00%), while low prevalence of resistance showed against Ciprofloxacin 25 isolates (33.33%), Trimethoprim 16 isolates (21.33%), 15 isolates (20.00%) to Gatifloxacin and 12 isolate (16.00%) to Enrofloxacin.

Keywords- Kids diarrhoea, *E.coli,* Isolation, Characterization, Antibiogram.

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Introduction

In goat kids about 50% cases of neonatal diarrhoea were ascribed to *E.coli* [1]. Neonatal mortality is recorded to be as high as 60%, with enterotoxigenic strains of *E. coli* being principally involved [2]. In enterotoxiccoli bacillosis potent enterotoxin are produce by the pathogenic *E. coli* after adhering the mucosa and proliferation which encourage excessive secretion of fluid from intestinal mucosa [3]. Enterotoxigenic *E. coli* produce severe diarrhoea in goat kids mainly during the first two weeks of life with highest frequency of pathogenicity in kid younger than 3 days old [4].

E. coli is a genetically and phenotypically diverse species whose strains are identified on the basis of 'O' (Somatic), 'H' (Flagellar) and sometimes 'K'(Capsular) antigens, which collectively make up the serotype. Numerous classes of diarrhoea causing *E. coli* are now documented on the basis of production of virulence factors, such as Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterohaemorrhagic *E. coli* (EHEC) or Verocytotoxigenic *E. coli* (VTEC), Enteroaggregative *E. coli* (EAEC) and Diffusely adherent *E. coli* (DAEC) [5]. So, keeping in view of this background information, the present investigation was undertaken for Isolation as well as serotyping of *E.coli* isolates from diarrhoeic neonatal kids and Biochemical characterization and drug resistance pattern of *E.coli* isolates from diarrhoeic neonatal kid faeces.

Materials and Method

Collection of faecal samples

A total of 103 faecal samples were aseptically collected from Livestock Research Station, Navsari Agricultural University, Navsari and different villages of south Gujarat. The samples were collected directly from the rectum of diarrhoeic goat kids aging 1 day to 4 weeks showing the symptoms of diarrhoea. The samples were collected in sterilized screw caped plastic vials (Hi Media, Mumbai) and brought in laboratory on ice pack within 2-3 hours of collection. Sample was processed for isolation of *E. coli*. The Sex-wise and age-wise details of sample collected are shown in [Table-1].

Table-1 *Sex-wise and age-wise collection of faecal samples from diarrhoeic goat kids*

Age (weeks)	Male	Female	Total
0-1	11	23	34
1-2	12	15	27
2-3	14	9	23
3-4	9	10	19
Total	**46**	**57**	**103**

Isolation, identification, serotyping and biochemical characterization of *E. coli* isolates

Media and chemicals required for isolation and biochemical characterization of *Escherichia coli* were prepared as per Edwards and Ewing (1972) [6]. Primary isolation of *E. coli* was done using MacConkey agar medium. Lactose fermenting colony were selected and subcultured on Eosin Methylene Blue (EMB) agar

medium to detect the production of metallic sheen. Such colonies were selected and stored in nutrient agar slants for further studies. All the isolates were stained by Gram's staining method and observed under oil immersion objective for presence of gram negative rods.

Biochemical characterization of *E. coli* isolates

Biochemical tests like Indole, Methyl-red, Voges Proskauer, Citrate and Malonate utilization were employed for identification of *E. coli*, as per Edwards and Ewing (1972) [6].

Serotyping of *E. coli* isolates

Out of 75 *E.coli* isolates, 35 isolates were selected for serotyping and sent to National *Salmonella* and *Escherichia* Center (NSEC), Central Research Institute (CRI), Kasauli (Himachal Pradesh) on nutrient agar slant for serotyping.

Assay for antimicrobial drug resistance of the *E. coli* isolates

The bacterial isolates were subjected to *in vitro* antibiotic sensitivity test as per the method of Bauer *et al*. [7]. Isolates were grown in BHI broth overnight and plates of MH agar were seeded with about one ml of inoculum. The inoculum was allowed to dry. Antibiotic discs were placed on inoculated agar surface at about two cm apart. All plates were placed in refrigerator for 20 minutes. Diameter of the zones of inhibition was measured after the incubating the plates in upright position at 37°C for overnight. Zones were graded as sensitive and resistant after the comparing measurements with zone size interpretative chart furnished by the manufacturer.

Results and Discussion

Prevalence of enteropathogens

Neonatal mortality is recorded to be as high as 60%, with enterotoxigenic strains of *E. coli* being principally involved [2]. About 50% cases of neonatal diarrhoea were ascribed to *E.coli* [1]. In goat kids *E. coli*, Rotavirus, *Cryptosporidium parvum* and *Clostridium perfringens* types A, B and C are frequently involved as causative agents of neonatal kid diarrhoea [8]. All 103 faecal samples were tested for the presence of *E. coli* and 75 (72.81 %) samples were found positive for *E. coli.* In the present study in 0-1 week of age group 26 (76%) out of 34 samples were found positive for *E. coli* these findings corroborates the findings of Orden *et al.* [9] who found 68% positive samples of *E. coli*. In 1-2 week age group 19 (70%) out of 27 samples were found positive which is in contrast the findings of Cid *et al.* [10] who observed lesser percentage 36 per cent positive samples. However in present study in 3-4 week of age group moderate 12 (63%) samples found positive for *E. coli* out of 19 samples such findings were also reported by Munoz *et al.* [11] who found 33.3 per cent positive samples.

Isolation and identification of *E. coli* isolates

During the present study 103 faecal samples from diarrhoeic goat kids were processed for isolation of *E. coli.* Of these, 75 were found positive for *E. coli.* All the 75 isolates revealed characteristic features of *E. coli*, which were Gram negative bacilli, produced pink lactose fermenting colonies on MacConkey agar and characteristic greenish metallic sheen on EMB agar.

Biochemical characterization of *E. coli* isolates

In the present study all the isolates showed the typical biochemical reaction, characteristic of *E. coli*, as described by Edwards and Ewing (1972) [6]. All the isolates showed typical IMViC pattern of *E. coli viz.,* Indole and MR tests positive, VP and Citrate utilization tests negative. Malonate utilization was negative for all the isolates. These findings corroborate the findings of Arya [12]. All isolates produce yellow colour colony on Triple Sugar Iron slant (TSI) was also reported by Lehman [13]. Nitrate reduction test positive for all the isolates was also obtained by Tiso and Schechter [14]. Glucose fermentation test positive for all the isolates and all isolates found motile was also reported by Mittal *et al.* [15].

Serotypes of *E. coli* isolates

The serotypes obtained were O2, O84, O86, O87, O101, O118, O120, O128, O141 and O157. The most predominant serotypes were O120 (nine isolates), O141 (four isolates) and O157 (two isolates) while other serotypes were O2, O84, O86, O87, O101, O118 and O128 (one isolate each) and six isolates were found Rough.

The serogroups reported to be commonly prevalent in goat kid in India are – O2, O3, O5, O6, O8, O9, O14, O15, O17, O18, O21, O22, O23, O25, O26, O33, O35, O37, O38, O41, O44, O45, O49, O51, O52, O53, O55, O60, O65, O66, O69, O70, O73, O75, O76, O78, O80, O81, O82, O83, O87, O88, O91, O98, O99, O101, O102, O104, O112, O113, O114, O115, O116, O117, O120, O127, O131, O133, O136, O145, O147, O148, O152, O156, O157, O158, O160, O168 and O172 [16-22].

Serogroups O2 recorded in present study were also reported by Orden *et al.* [23] they observed onset and subsequent pattern of shedding of AEEC from the goat kids over a six months period and isolated O2 serogroup in three colonies. Serogroup O86 isolated in this study from diarrhoeic kid cases was also reported by Osman *et al.* [20] isolated O86 seogroup from 102 *E. coli* strains in diarrhoeic goats. Serogroup O87 recorded in present study were also reported by Horcajo *et al.* [19] found O87 serogroup from VTEC strain of *E. coli* in diarrhoeic goat kids. Serogroup O101 found in present study was also reported by Wani *et al.* [18] from nonmigratory goats. Serogroup O118 reported in present study were also reported by Salvadori *et al.* [24] from diarrhoeic calves they isolated 205 *E. coli* strains.

Serogroup O120 recorded in present study were also reported by Wani *et al.* [18] from 50 *E. coli* strains in migratory goats. Serogroup O128 found in present study was also reported by Horcajo *et al.* [19] from diarrhoeic goat kids. Serogroup O141 recorded in present study were also reported by Horcajo *et al.* [19] isolated O141 serogroup from 232 VTEC strains isolated from goats. Serogroup O157 was also reported by Wani *et al.* [18] isolated 50 *E. coli* strains from faecal samples of migratory goats and found O157 from two isolates.

In the present study two *E. coli* isolates were untypable. It is not uncommon to isolate strains of *E. coli*, which could not be typed with the available antisera as which is also reported by Ruchi *et al*. [22]. Also six *E. coli* isolates were found rough in present study. Similar findings is also reported by Wani *et al*. [18] they found 18 *E. coli* strains rough from the 170 *E. coli* strains from non-migratory goats.

Antimicrobial drug resistance pattern of the *E. coli* Isolates

Of the 75 isolates tested, two isolates (16.00%) were found resistant to enrofloxacin which correlates with the result, (12.00%) resistant isolates, obtained by Paul *et al.* [25]. Moderate per cent of isolates (33.33%) were found to be resistant to ciprofloxacin which is similar to the findings of Paul *et al.* [25] who observed lesser 30 per cent of *E. coli* isolates resistant to ciprofloxacin in their study. With cephalosporins group, 72 (96.00%) isolates showed resistance to cephalexin. Such findings were also reported by Mulik [26] who observed 89.74 % isolates resistant to cephalexin. In this study, 93.33 per cent isolates showed resistance to kanamycin, similar findings also have been reported by Cid *et al.* [27] who found 82 per cent kanamycin resistance. Further, a lesser per cent (21.33%) of isolates were found to be resistant to Trimethoprim. As against 46.00 per cent resistance was reported by Hariharan *et al*. [28].

Moderately high percentage (60.00%) of isolates was found to be resistant to antibiotic tetracycline in this study however Dulo *et al.* [21] reported 11 per cent of *E. coli* isolates from goats found resistant to tetracycline

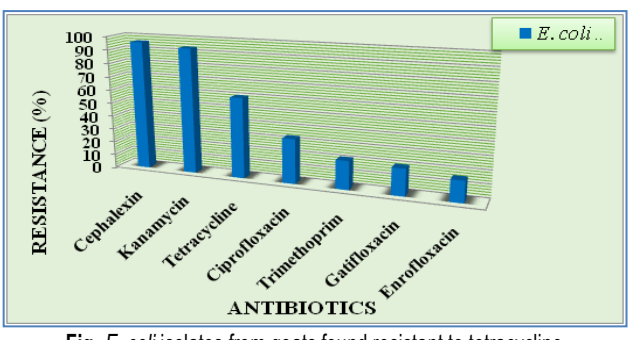

Fig- *E. coli* isolates from goats found resistant to tetracycline

Conclusions

E. coli detected in the present study was 75 (72.81%). All 75 isolates showed typical IMViC pattern of *E. coli viz*., Indole and M.R. positive, V.P. and Citrate negative. Out of 35 *E. coli* isolates, 10 different 'O' serogroups were recorded in different locations, while two isolates were found untypable and six isolates found rough.

The serotypes obtained were O2, O84, O86, O87, O101, O118, O120, O128, O141 and O157. The most predominant serotypes were O120 (nine isolates), O141 (four isolates) and O157 (two isolates) while other frequently occurring serotypes were O2, O84, O86, O87, O101, O118 and O128 (one isolate each). Antibiotic resistance patterns revealed 96 per cent resistance to Cephalexin, 93.33 per cent to Kanamycin, 60 per cent to Tetracycline, 33.33 per cent to Ciprofloxacin, 21.33 per cent to Trimethoprim, 20 per cent to Gatifloxacin and only 16 per cent of isolates were resistant to Enrofloxacin.

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Author Contributions: All authors are equally contributing in research design and planed a study. HP, IK, PS and KS collected the samples, carried out the laboratory work, analyzed the data. IK, AB and VD drafted and revised the manuscript. All authors have read and approved the final manuscript.

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