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Research Article

Incidence and Drug Resistance Pattern of Collibacillosis in Cattle and Buffalo Calves in Western Utter Pradesh in India

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ABSTRACT

Diarrhea in farm animals, especially in neonatal calves is one of the most challenging clinical syndromes encountered by practicing large animal's veterinary practitioners throughout the world. Therefore looking into the intricacy of early age calf diarrhea primarily due to *E. coli*, the present study was designed to perform the isolation and identification of involved *E. coli* strains in calf diarrhea and also to establish their drug sensitivity pattern. During the study period total 109 faecal samples were collected from diarrheic cattle and buffalo calves less than three months of age from the university teaching veterinary clinics complex, DUVASU, Mathura, University dairy farm, nearby gaushalas located at Vrindavan and Mathura along with the Veterinary Hospitals of western Utter Pradesh located in district Muzaffarnagar, Baghpat, Meerut, Gautambudh Nagar, Ghaziabad and Saharanpur. After laboratory confirmation out of 109 processed samples, only 41 were positive for *E. coli*. Out of these 41 isolates only 13 revealed the toxin production, hence were pathogenic. On the basis of drug sensitivity test, Amikacin (87.80% sensitivity), Aztreonam (73.17%) and Gentamicin (51.21%) were found to be the effective drugs which could prohibit the growth of *E. coli* isolates obtained during sudy. However, the presence of 100% resistance against Ampicillin, Cefdinir, Co-trimoxazole, Cloxacillin, Erythyromycin, Lincomycin, Penicillin, Rifampin, Tetracyclin and Vancomycin is matter of concern particularly the resistance against Norfloxacin and Pefloxacin as fluroquinoles are very commonly used in cases of gastroenteritis.

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INTRODUCTION

Diarrhoea is one of the most common and multifactorial disease of neonates chiefly caused by *E. coli* and remains common devastating disease all over the world (Bhalerao et al., 2000; Kumar et al., 2013), particularly in calves less than three months of age (Malik et al., 2012 and 2013). The etiological agent E. coli is also considered as important food borne pathogen (Dhama et al., 2013a). Similarly, Sizov et al. (1984) also observed the presence of *E. coli* in 34 percent samples of calves in the age group of 3 to 10 days that died due to diarrhoea. Among the multiple predisposing factors of diarrhoea due to bacterial causes especially *E. coli*, risk is greater in calves in which there has been failure of passive transfer of maternal immunoglobulin (Gay 1983). Inspite of the complex etiology of calf diarrhea, the involvement of bacterial pathogens is still responsible for more than 50% cases of diarrhoea in neonatal calves (Kumar et al., 2012a,b) and *E. coli* is more or less consistently isolated during cultural examination of the intestinal contents of calves that succumb to diarrhoea complex during first three weeks of life (Boyd et al., 1974; Malik et al., 2012). Colibacillosis is one of the major causes of neonatal calf diarrhoea and it accounts for maximum neonatal calf mortality (Sherwood et al., 1983, Haggard, 1985, Mailk et al., 2012). Now *Escherichia coli* is seen as a pathogenic species with remarkable versatility in its ability to cause disease in both humans as well as animals. Depending upon the severity of condition and also availability of trend personnel antimicrobials are commonly used in therapy of diseased calves either orally or parentally. Some commonly used antimicrobials as sulbactam, ampicillin, neomycin, cephalosporin, tetracycline and sulphonamide- trimethiprim mixture all have all been used but day to day development of drug resistance problem envisages regular drug sensitivity results with the timely available antimicrobials in the market (Kumar et al., 2010). Similar to other part of world in India also microbiological work has been amply conducted for finding the association of different strains of *E. coli* with diarrhoea in calves by Sharma (1986), Chakraborty and Nag (1997) and Hussain and Saikia (2000). However, in last decade or so the development of drug resistance and the presence of multi drug resistant pathogenic bacteria are major problem in treating bacterial diseases (Kumar et al., 2012c; Anita et al., 2013; Dhama et al., 2013b). Use of antimicrobial in early stages of disease has been always improves survival rate and highly favored but it requires proper selection of drug in the given population (DuPont and Ericsson, 1993). Thus looking into the intricacy of calf diarrhoea due to *E. coli*, the present study was planned to assess incidence rate of *E. coli* in calf diarrhoea and antimicrobial drug sensitivity pattern using commonly available antibiotics in the treatment.

MATERIALS AND METHODS

The present study was undertaken on calves up to three months of age exhibiting symptoms of diarrhea at teaching veterinary clinical complex, Instructional Livestock Farm Complex (DUVASU, Mathura), Nearby Gaushalas located at Vrindavan, Mathura along with some Veterinary Hospitals located in district Muzaffarnagar, Baghpat, Meerut, Gautambudh Nagar, Ghaziabad and Saharanpur of Uttar Pradesh state, India (Table 1). During the period under study, a total of 109 faecal samples from diarrhoeic calves were collected from the calves showing diarrhea and brought to laboratory on ice.

The faecal samples of diarrheic calves of less than 3 months of age were collected directly from rectum. Approximately 20 gms of faecal content were collected from rectum of calves in sterilized containers (Edwards and Ewing, 1972). The faecal samples were processed for isolation of *Escherichia coli*, as per standard procedure described by Edwards and Ewing (1972) for the characterization of isolates on the basis of routine laboratory procedures which included cultural, morphological and biochemical parameters by growth on differential media MLA (MacConkey's lactose agar), selective media EMB (Eosin Methylene blue agar) of HiMedia (Mumbai), differential staining (Gram's staining) and confirmed with biochemical test as Indole production, Methyl red reduction, Vogous Proskaure test and citrate utilization (IMViC) pattern (Quin et al., 2002).

S. No.	Source	Number of Samples
1	Gaushalas, Vrindaban, Mathura	20
2	Govt. Veterinary Hospitals, Saharanpur	17
3	Teaching Veterinary Complex, DUVASU, Mathura	14
4	Govt. Veterinary Hospitals, Muzaffarnagar	12
5	Govt. Veterinary Hospitals, Baghpat	11
6	Govt. Veterinary Hospitals, Meerut	10
7	Govt. Veterinary Hospitals, Gautambudh Nagar	09
8	DDD farm, DUVASU, Mathura	08
9	Govt. Veterinary Hospitals, Ghaziabad	08
	TOTAL	109

Table 1: Place-wise distribution of samples (blood and faeces) from diarrheic calves

All the isolates were examined for their drug sensitivity pattern by disc diffusion method (Bauer et al., 1966). The present study included 20 commonly used antibiotic discs (Hi-Media, Mumbai) viz., amikacin (30 mcg), ampicillin (10 mcg), aztreonam (30 mcg), cefadroxil (30 mcg), Cefdinir (5 mcg), ciprofloxacin (30 mcg), co-trimoxazole (25 mcg), cloxacillin (5 mcg), erythyromycin (15 mcg), gentamicin (10 mcg), kanamycin (30 mcg), lincomycin (15 mcg), norfloxacin (10 mcg), nitrofurantoin (300 mcg), pefloxacin (5 mcg), penicillin (10 IU), rifampin (5 mcg), tetracyclin (30 mcg), tobramycin (10 mcg), vancomycin (30 mcg). For the preparation of bacterial lawn on plates, six hours young broth culture ($4.8x10^{10}$ c.f.u/ml) of each isolate was smeared over the nutrient agar medium by sterilized cotton swab. After the inoculation plates were allowed to dry at room temperature for 10-15 minutes. Then respective antibiotic discs were placed on the surface of inoculated medium by sterile forceps with uniform spacing between two discs and pressed gently to ensure full contact. For the final interpretation all the inoculated plates were incubated at 37^0C for overnight (Kumar et al., 2012c). After the incubation of 24 hrs, zone of inhibition was recorded in millimeters and compared with the chart provided by the manufacturer (HiMedia, Mumbai) for assessing the sensitivity of the antibiotics. The interpretation of results was performed as per the guidelines of NCCLS (2002).

Isolates of *E. coli* obtained in the study were tested for haemolysin production on 5 % sheep blood agar according to Beutin et al., 1989. For the confirmation of haemolysin production streaked blood agar plates with *E. coli* were incubated at 37^0C and examined at 4 hrs interval up to 24 hrs for the presence of zone of haemolysis.

RESULTS

Out of 109 faecal samples, 47 samples produced smooth, circular, convex, entire, pink colour colonies on MLA. Further isolated pink colonies from MLA were transferred on selective media EMB plates. After overnight incubation at 37^0C, out of 47 samples only 41 produced metallic sheen on EMB plates (Table 2). The presumptive *E. coli* colonies were subjected to Gram's staining and biochemical tests (Table 3). Thus, out of 109 faecal samples from diarrheic calves, *E. coli* could be confirmed from only 41 samples. Thus percent positivity of *E. coli* in diarrheic calves was 37.61% (Table 2).

All the isolates were examined for their drug sensitivity pattern by disc diffusion method (Bauer et al., 1966) with selected antimicrobial discs (Hi-Media, Mumbai) and results of antibiogram revealed the presence of drug resistance in *E. coli* isolates recovered from diarrheic calves against most of the commonly used antibiotics such as ampicillin, Cefdinir, co-

S. No.	Source	Number of Samples	Positive	Prevalence (%)
1	Gaushalas, Vrindaban, Mathura	20	08	40.00
2	Govt. Veterinary Hospitals, Saharanpur	17	06	35.29
3	Teaching Veterinary Clinical Complex, DUVASU, Mathura	14	05	35.71
4	Govt. Veterinary Hospitals, Muzaffarnagar	12	05	41.66
5	Govt. Veterinary Hospitals, Baghpat	11	04	36.66
6	Govt. Veterinary Hospitals, Meerut	10	03	30.00
7	Govt. Veterinary Hospitals, G.B. Nagar	09	03	33.33
8	DDD farm, DUVASU, Mathura	08	04	50.00
9	Govt. Veterinary Hospitals, Ghaziabad	08	03	37.50
	TOTAL	109	41	37.61

Table 2: Occurrence of *E.coli* in diarrheic calves

Table 3: Cultural, morphological and biochemical characteristics of isolates from diarrheic calves

Organisms	Cultural		Morphological	Biochemical
	MLA	EMB	Gram's staining	IMViC Pattern
E. Coli	Pink Colony	Metallic sheen	Gram Negative cocobacilli	++--

+ Positive test result, – Negative test result

Table 4: Results of antibiotic sensitivity test of *E. coli* isolated from diarrheic calves

Name of Antibiotic	Number of isolates (41)				
	Resistant	Intermediate	Sensitive	Sensitivity %	Resistant %
Amikacin	4	1	36	87.80	9.76
Ampicillin	41	-	-	0	100
Aztreonam	5	6	30	73.17	12.20
Cefadroxil	33	-	8	19.51	80.49
Cefdinir	41	-	-	0	100
Ciprofloxacin	32	4	5	15.63	78.05
Co-trimoxazole	41	-	-	0	100
Cloxacillin	41	-	-	0	100
Erythyromycin	41	-	-	0	100
Gentamicin	-	20	21	51.21	0
Kanamycin	10	15	16	39.02	24.39
Lincomycin	41	-	-	0	100
Norfloxacin	41	-	-	0	100
Nitrofurantoin	21	20	-	0	51.21
Pefloxacin	41	-	-	0	100
Penicillin	41	-	-	0	100
Rifampin	41	-	-	0	100
Tetracyclin	41	-	-	0	100
Tobramycin	29	12	-	0	70.73
Vancomycin	41	-	-	0	100

S- Sensitivity, I – Intermediate, R- Resistant

trimoxazole, cloxacillin, erythyromycin, lincomycin, norfloxacin, pefloxacin, penicillin, rifampin, tetracycline and vancomycin. Out of 20 drugs only three drugs mainly Amikacin (87.80% sensitivity), Aztreonam (73.17%) and Gentamicin (51.21%) were found to be the effective drugs which could prohibit the growth above 50% of isolates during the drug sensitivity test. The antibiotic discs of Kanamycin could showed only 39.0% sensitivity (Table 4).

In an effort to test the pathogenecity of isolates, all the 41 isolates of *E. coli* were tested for haemolysin production on sheep blood agar (SBA) as per Beutin et al. (1989). Haemolysin producing isolates of *E. coli* produced the typical hemolytic reaction after 4 hrs incubation on SBA whereas, after 24 hrs of incubation clear hemolysis was produced by 13 isolates. Isolates which produced no zone of haemolysis even after 24 hrs incubation on SBA were considered as non-hemolytic.

DISSCUSSION

In the present investigation a total of 109 faecal samples were collected from diarrheic calves (below three months of age), for isolation and confirmation of *E.coli*. Out of 109 faecal samples of diarrhoeic calves, 41 faecal samples were positive for *E. coli*. The prevalence of *E. coli* in diarrhoeic calves was 37.61%. Yadav and Gupta (1971) noticed 35% prevalence of *E. coli* in diarrhoeic calves. Srisuparbh (1978) reported 26.25% prevalence of *E. coli* in diarrhoeic calves. Shome and Shome (1996) reported 14 samples positive for *E. coli* out of 25 faecal samples of diarrhoeic calves. Navade et al. (2000) noticed 53.37% prevalence of *E. coli* in neonatal diarrhoeic calves. Gupta et al. (2006) observed only 23.72% prevalence rate of *E. coli* in diarrhoeic calves. The findings of these workers are more or less similar to the findings of present study. The prevalence rate of *E. coli* in diarrheic calves varies in the range of 30% to 50%. The incidence of *E. coli* showed the higher prevalence at university dairy farm, Gaushalas in and around Vrindavan and Mathura. The higher prevalence of *E. coli* in these areas may be due to poor managemental practices and predisposing factors like overcrowding and malnutrition, which are supposed to be primary cause of immunosupression. As *E. coli* is a commensal organism and is responsible for diarrhea in majority of weak, malnourished, debilitated and immunosuppressed calves particularly calves receiving less or no maternal antibodies through colostrums (Malik et al., 2012). Comparatively lower prevalence rate of *E. coli* in diarrheic cases of district Meerut and Gautambudh Nagar as compared to Muzaffarnagar and Ghaziabad may be due to better managemental practices, individual rearing and better awareness among livestock owners and trend of feeding calves for colostrums. Major reason behind the practice in western Uttar Pradesh is that still individual livestock owners hesitate to sell the milk and main purpose of rearing these animals is production of milk for their own household consumption and also the breeding of animals for agriculture requirement.

The resistance of all the *E. coli* isolates procured during the study period against commonly used antibiotics such as ampicillin, Cefdinir, co-trimoxazole, cloxacillin, erythyromycin, lincomycin, norfloxacin, pefloxacin, penicillin, rifampin, tetracycline and vancomycin is a matter of serious concern as Norfloxacin, pefloxacin and tetracycline are vary commonly used and prescribed antibiotics in veterinary and human practices even today. This resistance might be due to indiscriminate use of these antibiotics irrespective of etiological agents (Verma et al., 2007; Kumar et al., 2010) as well different drug interaction due to concurrent

administration is performed irrespective of type of drug and their interaction (Rahal et al., 2007 and 2013). The findings of study are in contrast to the findings of Tripathi and Soni (1982), who observed 69.69% *E. coli* sensitive for ampicillin and 69.70% to Chloramphenicol. Bali et al, (2000) similar to our findings reported pefloxacin as an effective drug in enteric colibacillosis in calves and Chowdhury and Das (2003) found a high degree sensitivity of *E. coli* isolates to Ciprofloxacin, however, in present study the recovered isolates from the cases of diarrheic calves revealed 100% resistance against fluroquinolones group of antibiotics as norfloxacin and pefloxacin. The resistance against the antibiotics used for the treatment of disease conditions caused by gram positive bacteria as penicillin and tetracycline is supported by the findings of Tripathi and Soni (1982), who observed that 45% of *E. coli* showed resistance to Streptomycin, (75.75%) to oxytetracycline. Whereas Boyd et al, (1974) obtained 100% resistance against Penicillin G. In an another study similar to the findings of present study, Fairbrother et al., (1978) also observed 100% resistance with erythromycin, penicillin and resistance up to 95% against tetracycline. Similarly Awasthi and Rao (1980) recorded 90-95% resistance against penicillin and oxytetracyclin. The transfer of resistance factor from resistant microorganisms through R or RTF might be the cause of this high rate of resistance in *E. coli* isolates (Yeoman, 1980) and the other probable reason might be due to under dosing and irrational therapy particularly for duration of treatment (Kumar et al., 2012a, b,c). In the present study, isolates of *E. coli* isolated from the cases of diarrhea in calves showed that 87.80% were sensitive to amikacin, 73.17% to aztreonam and 51.21% to gentamicin. Chowdhary and Das, 2003 and Sato et al., 2005 have already reported high rate of sensitivity against gentamicin.

The hemolytic property of *E. coli* strain associated with diarrhea were recognized long back as early as by Dudgeon et al. (1921). This hemolytic activity is due to presence of toxin haemolysin and play important role in virulence of *E. coli* (Smith and Longgod, 1972). Hemolytic activities have been shown to be toxic to a wide range of mammidium cells including sheep erythrocytes (O'Hanley et al., 1991). ETEC and VTEC strains of E. coli produce alpha hemolysin and entrohemolysin, respectively, which are responsible for pathogenecity. Enterohemolysin is produced by VTEC but rarely by non-VTEC *E. coli* strains, which possess alpha hemolysins show typical clear zone of hemolytic reaction on sheep blood agar after 24 hr of incubation. Strains having entrohemolysin did not exhibit hemolytic reaction. During the study to assess the presence of haemolytic activity, thirteen isolates which revealed clear zone of hemolysis on SBA after 24hr of incubation were alpha hemolysin producing VTEC strains. The prevalence rate of these is 31.70% in the occurrence of *E. coli.* Thus the present study revealed 11.92% overall prevalence rate of alpha hemolytic VTEC strains.

The above findings also suggest that there is a constant need of screening of faecal samples of diarrheic calves this is especially important to formulate a suitable broad spectrum and effective treatment against the *E. coli* and to reduce or prevent the losses.

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