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Isolation and biotyping of *Salmonella* and *Escherichia coli* associated with neonatal buffalo calves

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ABSTRACT

Diarrhoea is a predominant cause of mortality in neonatal buffalo calves leading to significant economic losses. Faecal samples from 149 neonatal buffalo calves were bacteriologically screened for *Salmonella* spp and *Escherichia coli*. Six isolates of *Salmonella* spp. could be recovered, out of which 4 belonged to *Salmonella* Infantis (6,7: r:1, 5), while one each belonged to *Salmonella* Gallinarum (1,9,12:-) and *Salmonella* Bareilly (6,7:y:1,5). Out of the 61 identified isolates of *E. coli*, 7 were rough (R), 1 was untypable (UT) and the rest 53 belonged to 35 different 'O' serogroups. Faecal recovery of *E. coli* belonging to O157 serogroup was not successful. *In vitro* drug sensitivity testing indicated gentamicin, norfloxacin and ciprofloxacin with respective sensitivities of 89.18%, 78.37% and 72.97% to be effective against *E. coli*. The isolates were, however, refractory to ampicillin, tetracycline and furazolidone. Isolation of multi-drug resistant *E. coli* warrants a careful selection of antibiotics for animal use from a public health perspective.

Key words: Antibiogram, Buffalo, *E. coli*, Neonatal calf diarrhoea, *Salmonella*

Calf-hood morbidity can affect the animal's rate of weight gain, days of first calving, dairy herd survivorship and presumably the future productivity. Diarrhoea is a leading cause for the mortality of buffalo neonates (Nizza *et al.*2010). Based on a retrospective epidemiological analysis of data on necropsy examination of buffalo calves over a span of 14 years, pneumoenteritis, pneumonia and enteritis were found to be the principal infectious causes, which together constituted for 80% of the total mortality in buffalo calves with maximum mortality being recorded in neonatal calves < 1 month of age (Rana *et al.*2010).

As regarding infectious diarrhoea of bacterial etiology in buffalo calves, *Escherichia coli* and *Salmonella* spp. are incriminated to be the major causative agents. World-widely a lot of work on serotyping and antibiogram of *E. coli* isolates from animals has been done, but recent literature lacks reports on the involvement of *Salmonella* spp. and *E. coli* serotypes in buffaloes from this area. Kaura (1988) in a two-year surveillance on the incidence of *Salmonella* serotypes in diarrhoeic calves at some of the organized farms in Haryana, Punjab and Uttar Pradesh reported salmonellosis to the tune of 5.96% in buffalo calves at Central Institute for Research on Buffaloes, Hisar. The present communication reports the spectra of *E. coli* and *Salmonella* serotypes isolated from buffalo neonates at organized herd of this institute. Such information would be useful with a long-term objective of developing relevant disease control programme, thus improving the overall production performance of the animals.

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MATERIALS AND METHODS

Sample collection and isolation of E. coli and Salmonella

Faecal samples from 149 (131 diarrhoeic and 18 apparently healthy) neonatal buffalo calves of either sex were collected and brought to the laboratory under aseptic conditions and processed immediately for bacterial isolation. For isolation of *Salmonella*, selective enrichment was done in Rappaport-Vassiliadis broth followed by incubation at 42°C for 24 h. The samples were streaked onto MacConkey's Lactose Agar (MLA) and incubated at 37°C for 24 h. Following incubation, lactose fermenting colonies were streaked on eosin-methylene blue (EMB) agar plates. Lactose negative colonies were streaked on brilliant green agar (BGA) plates. Typical *E. coli* colonies on EMB agar displaying green metallic sheen and pink coloured *Salmonella* spp. colonies on BGA were isolated as pure cultures on non-selective media and subjected to biotyping. Screening for *E. coli* O157:H7 serotype from buffalo calf faeces was also done using CT-SMAC medium.

Biochemical characterization of isolates

The purified cultures were identified on the basis of their morphological, cultural and biochemical characteristics (Barrow and Feltham 2003, Quinn *et al.* 1994) using conventional biochemical tests.

Serotyping of the isolates

Purified presumptive cultures of *E. coli* and *Salmonella* spp. were confirmed and serotyped at National Salmonella and Escherichia Centre, the referral typing laboratory, Central Research Institute, Kasauli, Himachal Pradesh.

In-vitro drug sensitivity testing of isolates

Antibiogram spectrum of 37 *E. coli* isolates was determined by modified Bauer-Kirby disc-diffusion technique (Quinn *et al.*1994) using 15 different antibiotic discs on Mueller-Hinton agar. The antibiotic discs tested were ampicillin (10 µg), ciprofloxacin (10 µg), colistin (10 µg), co-trimoxazole (25 µg), gentamicin (10 µg), nitrofurantoin (300 µg), streptomycin (10 µg), tetracycline (30 µg), cefotaxime (30 µg), cefalexin (30 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), furazolidone (50 µg), norfloxacin (10 µg) and oxytetracycline (30 µg). The results were recorded by measuring the diameter of clear zone of inhibition around each antibiotic disc as per manufacturer's instructions.

RESULTS AND DISCUSSION

Isolation and biotyping of E. coli *and* Salmonella *spp.*

Serotyping of *E. coli* occupies a central place in the history of this pathogen (Lior 1996). *E. coli* of specific serogroup can be associated with reproducibility of certain clinical syndrome. The distribution of different serotypes of *E. coli* varies with geographical regions and their prevalence in man and animals in a particular area. A shift in 'O' serogroups along with their virulence factors has also been observed (Soderlind *et al.*1988). Bacteriological screening of faecal samples led to the isolation of 61 isolates of *E. coli*, of which 7 were rough (R), 1 was untypable (UT) and the rest 53 belonged to 35 different 'O' serogroups, viz. O5(3), 119(2), 91, 60(4), 129, 8(2), 172, 186, 86(2), 81, 87, 117, 139, 37, 9, 106, 143, 83, 15, 25, 52, 21(2), 164, 1, 12(4), 26, 75, 4(3), 159(3), 76, 3(2), 140, 138, 112, 114(2). Faecal recovery of *E. coli* belonging to O157 serogroup, however, was not successful.

Faecal presence of serogroups O4, O9, O1, O25, O60 and O87 has previously been documented in buffalo calves from India (Singh *et al.* 2007). Our results are in accordance with Vagh and Jani (2010) who reported the isolation of *E. coli* O5, O25, O3, O8, O4 and rough isolates from diarrhoeic buffalo calves in Gujarat state of India. Kaura *et al.*(1991) reported serogroup O4 as verotoxigenic along with other O-serogroups O8, O9, O26 and O119 from buffalo calf diarrhoea The isolation of EPEC serotype O86 from two apparently healthy buffalo calves is a cause of concern as Holmes and Gross (1990) reported that this serotype is normally predominant in infantile enteritis. Mohammad *et al.* (1986) reported verotoxin producing serogroups O3, O5, O75, O76, O86, O117 and O159 in diarrhoeic buffalo calves and interestingly 11 of the 41 O-groupable strains in their study belonged to well-recognized human enteropathogenic *E. coli* (EPEC) O-groups. Kanwar *et al.* (2007) reported O9 and O76 as ETEC isolates on the basis of rabbit ligated ileal loop assay. Some of the *E. coli* isolates (O4, O15, O26 and O86) have also been reported to be associated with neonatal meningitis, septicemia, urinary tract and intestinal infection in human (Orskov *et al.*1977).

Khan *et al.*(2009) reported a higher prevalence of *Salmonella* (16.3%) than *E. coli* (15.3%) in the faecal samples of buffalo calves in Pakistan. Out of the 6 identified serovars of *Salmonella* in the present study, 4 belonged to *S.* Infantis (6,7: r:1, 5), while one each belonged to *S.* Gallinarum (1,9,12:-) and *S.* Bareilly (6,7:y:1,5). Our findings, however, differ with those of Kaura (1988) who documented the presence of *S.* Richmond (6,7:y:1,5), *S.* Typhimurium (1,4,5,12:1:1,2), *S.* Weltevreden (3,10:r:z6) and *Salmonella* rough in the faeces of buffalo calves.

Antibiogram analysis of the isolates

Frequent and indiscriminate use of antimicrobial agents for treatment exerts a selection pressure which leads to the development of multiple drug resistance (MDR) among different strains of bacteria (Hussain and Saikia 2000) and therefore, periodical screening for the drug susceptibility pattern of bacterial isolates becomes essential not only for successful treatment but also for preventing emergence of drug-resistant strains. Since buffalo calves may harbour multidrug-resistant *E. coli*, a continued surveillance for emerging antimicrobial resistance along with a careful selection of appropriate antibiotics is recommended (Nizza *et al.* 2010) for its control.

Drug sensitivity profile of *E. coli* isolates has been presented in Table 1. A high degree of resistance of *E. coli* isolates to ampicillin (penicillin), cefalexin and cefotaxime (first and third generation cepholosporins, respectively) observed in this study might suggest the development of ESBL (extended spectrum β lactamases) in *E. coli*.

In vitro drug sensitivity testing of *E. coli* against a battery of 15 antibiotics indicated a marked heterogeneity in the sensitivity pattern. Gentamicin followed by norfloxacin and ciprofloxacin with respective sensitivities of 89.18%, 78.37% and 72.97% were found to be effective against *E. coli*. High sensitivity of *E. coli* isolates to gentamicin and norfloxacin has earlier been documented (Hussain and Saikia 2000 and Ribeiro *et al.* 2000). Colistin, streptomycin and co-trimoxazole had moderate efficacy against *E. coli* isolates. A perusal of the results indicates that absolute resistance against ampicillin, furazolidone and tetracycline was demonstrable in the assay with MDR being a common feature amongst the isolates. MDR has been reported among *E. coli*

Table 1. Drug sensitivity profile of *E. coli* isolates

Antibiotic	Number of isolates			Percentage Sensitivity
	Sensitive	Intermediate	Resistant	
Ampicillin (10µg)	-	4	33	Nil
Ciprofloxacin (10 µg)	27	-	10	72.97
Colistin(10 µg)	24	2	11	64.86
Co-trimoxazole (25 µg)	23	-	14	62.16
Gentamicin (10 µg)	33	1	3	89.18
Nitrofurantoin (300 µg)	15	19	3	40.54
Streptomycin (10 µg)	24	10	3	64.86
Tetracycline (30 µg)	-	-	37	Nil
Cefotaxime (30 µg)	2	12	23	5.40
Cefalexin (30 µg)	1	9	27	2.70
Chloramphenicol (30 µg)	4	9	24	10.81
Nalidixic acid (30 µg)	13	10	14	35.13
Furazolidone (50 µg)	-	2	35	Nil
Norfloxacin (10 µg)	29	-	8	78.37
Oxytetracycline (30 µg)	9	7	21	24.32

isolates from diarrhoeic calves (Joon and Kaura 1993). Extensive use of antibiotics for healthcare and treatment of animals might explain for this phenomenon. Keeping in view the MDR of *E. coli* isolates and their possible human transmission, careful and judicious use of antibiotics is advisable for routine healthcare practices of animals.

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