

Original Research Article

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Isolation, Serotyping and *In-vitro* and *In-vivo* Antibacterial Sensitivity of *Escherichia coli* Strains Isolated from Diarrheic Lambs in Kashmir

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ABSTRACT

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This study was aimed to isolate the *Escherichia coli* strains and to compare the *in-vitro* and *in-vivo* antibacterial sensitivity of *Escherichia coli* strains isolated from diarrheic lambs. Different strains of *Escherichia coli* were isolated from the clinically affected lambs. Principal serotypes of *E. coli* recovered were O20 (10.09 %), O11 (8.25%), O123, O84 and O22 (7.33% each), O107 (5.50%), O89 and O92 (3.66% each), O69 and O5 (2.75% each), O157, O15, O36, O159 and O60 (1.83% each). Serogroups O26, O23, O17, O157, O153 and O5 with established zoonotic importance were among those recovered from diarrhoeic lambs. The antimicrobial susceptibility pattern of the isolates showed isolates were sensitive to four antibacterials, viz. gentamicin (61.33%), ciprofloxacin (60%), neomycin (54.6%) and co-trimoxazole (50.6%). while as, the isolates were resistant to amoxicillin and oxacillin. The therapeutic efficacy (*in-vivo*) of four highly effective antibacterials were evaluated and treatment regimen used in four different groups of clinical cases indicated, gentamicin and ciprofloxacin most effective drugs followed by neomycin and co-trimoxazole which was comparable with their *in-vitro* efficacy.

Introduction

Epidemiological studies on diarrhea in lambs, have shown that colibacillosis is one of the most important causes of neonatal lamb diarrhea that is followed by mortality and is responsible for 14-30 per cent of lamb deaths (Ahmed, 2009). The disease is caused by pathogenic serotypes of *Escherichia coli* (Gyles, 1992). *Escherichia coli* (*E. coli*), a member of family *Enterobacteriaceae* is a short Gram negative, non-spore forming motile bacillus. According to modified Kauffman scheme, the organism is serotyped on the basis of its somatic (O), flagellar (H)

and capsular (K) surface antigens (Lior, 1996). The distribution of different serotypes of *E. coli* varies with the geographical regions and their prevalence in man and animals in a particular area. Over 700 antigenic types or serotypes of *E. coli* have been recognized based on these three antigens (Nataro and Kaper, 1998). The detection and accurate identification of *E. coli* is enormously important as the distribution of different serotypes and the prevalence in animals varies with the geographical regions (Hassan and Sheikh, 2013). Although antibiotics are

frequently used for both treatment and prevention of *E. coli* scours in lambs and calves to minimize losses (Novotna *et al.*, 2005) but in recent years, the emergence of drug resistant strains following extensive and indiscriminate use of antimicrobial agents for therapy and prophylaxis has led to loss of their efficacy (Schoenian, 2006). Resistance to almost all front line antimicrobials is demonstrated by *E. coli* isolates from lambs (Ahmed, 2010). Thus the information on drug resistance or sensitivity trends in a geographic area is helpful to veterinarians in selection of appropriate drugs for empirical therapy.

Materials and Methods

Three hundred rectal swabs were collected from diarrheic lambs below the age group of 8-weeks irrespective of their sex and breed, for the isolation of *Escherichia coli*. Rectal swabs were collected aseptically and processed within 2 to 4 hours for the isolation and identification of *Escherichia coli* (Barrow and Felham, 1992).

For isolation of *E. coli*, MacConkey agar (MCA) and Eosin methylene blue agar (EMB) were used as differential and selective plating media. Afore mentioned faecal samples were initially screened on MCA. Pink colored colonies on MCA plates (Plate-2) were considered to be lactose fermenting isolates. Two such pink colonies from each MCA plate were picked up and transferred to EMB agar plate. Colonies with greenish metallic sheen (Plate-3) on EMB agar plates were considered to be of *E. coli*. In all 256 *E. coli* isolates obtained from the faecal samples were used for further study.

Serogrouping: Confirmed *E. coli* isolates were sent to National Salmonella and Escherichia Centre, Central Research Institute, Kasouli (Himachal Pradesh) for serotyping.

***In-vitro* sensitivity:** The antimicrobial susceptibility test was performed using Kirby-Bauer disk diffusion method on Muller Hinton agar (HiMedia-India). Isolates were tested for the following antibiotics; ciprofloxacin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), ampicillin (10 µg), oxacillin (10 µg), enrofloxacin (30 µg), neomycin (10 µg), co-trimoxazole (Thrimethoprim- sulfmethoxazole) (25 µg), trimethoprim (30 µg), gentamycin (10 µg), amoxicillin (10 µg) and (30 µg) cefmetazole (HiMedia- India). According to the width of the inhibitory zone, the pattern of drug sensitivity was determined as susceptible and resistant.

***In-vivo* clinical study:** The clinical trials were designed on thirty lambs exhibiting the symptoms of diarrhea and dehydration that were divided into five groups (six animals each). Only those antibacterials were given which showed moderate to high sensitivity *in-vitro* pattern against *E. coli* isolates, which were readily available and economically feasible.

Results and Discussion

The cultural examination of faecal samples collected from diarrhoeic lambs, revealed presence of *Escherichia coli* in 256 fecal samples out of total 300 samples. All the *E. coli* isolates were sent to National Salmonella and Escherichia Centre Kasouli (Himachal Pradesh) for serotyping. Isolates of *E. coli* were typed for 'O' antigen. Out of 109 *E. coli* isolates, 93 belonged to 34 different 'O' serogroups while, twelve were untypable, and four were rough isolates. The prevalence of serogroups O20 was highest (10.09 %) followed by O11 (8.25%), O123, O84 and O22 (7.33% each), O107 (5.50%), O89 and O92 (3.66% each), O69 and O5 (2.75% each), O157, O15, O36, O159 and O60 (1.83% each) while as, serogroups of O1, O17, O19, O12,

O26, O153, O23, O132, O88, O4, O43, O9, O56, O2, O154, O69, O68, O102 and O103 were the least (0.91% each). Serogroups O12, O84, O2, O19, O60, O26, O15, O103 and O22 reported in the present study were also reported by Butaev (1978) from lambs and Rathore *et al.*, (2003) in animals and humans. Serogroups O43, O153, O157, O20, O107, O26, O88, O26, O15 and O132 were reported by Wani *et al.*, (2003-04) from diarrhoeic lambs and calves in Kashmir. Serogroups viz. O11, O22, O23, O26, O4, O132 and O103 have been reported by Blanco *et al.*, (1996) from diarrhoeic lamb. Cid *et al.*, (2001) reported the association of O20, O22 and O4 serogroups with lamb diarrhea (Morris and Sojka, 1985).

The *E. coli* isolates were tested against eleven commonly used antibiotics. Of the major isolates, 61.33 per cent of the isolates were sensitive to gentamicin followed by ciprofloxacin (60%), neomycin (54.6%), whereas co-trimoxazole (50.6%), cefmetazole (53.4%), tetracycline (48%) and enrofloxacin (46.7%) were moderately sensitive. Penicillin and trimethoprim showed resistant against 88 per cent and 34.6 per cent isolates respectively; while as maximum numbers of isolates were resistant to oxacillin (93.3%) and amoxicillin (84%).

Here highest percentage of isolates were sensitive to gentamicin and ciprofloxacin, similar findings have been previously reported (Wani *et al.*, 2003; Gulsun *et al.*, 2005; Gazelle *et al.*, 2012) and in partial agreement with Cid *et al.*, (1996). Aminoglycosides, such as gentamicin are highly effective as they have a selective, rapid bactericidal action especially, on Gram negative bacilli. Two members of quinolone group (ciprofloxacin and enrofloxacin) were tested for their efficacy against isolated strains, with average sensitivity of 80-90 per cent, however ciprofloxacin was highly

sensitive against most of the isolates. This might be due to fact that this antibiotic had not been extensively used in lambs against the disease from this place. The high sensitivity against neomycin was also reported in lambs (Blanco *et al.*, 1996). High sensitivity of *E. coli* isolates may be due to the uncommon use of this antibiotic. Co-trimoxazole showed good sensitivity against *E. coli* isolates from diarrhoeic lambs and this finding is in agreement with earlier reports of (Edrington *et al.*, 2009 and Purkayastha *et al.*, 2010). Cefmetazole, a second generation cephalosporin moderately sensitive was placed third in order of sensitivity (Orden *et al.*, 1999) and (Hariharan *et al.*, 2004). In the present study higher per cent of isolates were resistant to amoxicillin; similar findings have been reported (Saravanbava 1990). The resistance recorded against penicillin derivatives (amoxicillin and oxacillin) in the present study may be due to their extensive use. *In-vitro* drug sensitivity pattern indicated that most identified serotypes were highly sensitive to four antibacterials, viz. gentamicin, ciprofloxacin, neomycin and co-trimoxazole.

The treatment (*in-vivo* efficacy) of the clinical cases of colibacillosis in lambs was studied on four trail groups of six lambs each (Table 2). Therapeutic efficacy of four different antibacterials viz. ciprofloxacin, gentamicin, neomycin and co-trimoxazole was evaluated (which showed higher *in-vitro* sensitivity against *E. coli* isolates) against clinical colibacillosis in lambs. The efficacy of the drugs was assessed as per the rate and degree of recovery of clinical symptoms. Gentamicin and ciprofloxacin, at dose rate of 4mg/kg body weight daily for five days, proved to be most effective drugs wherein, drugs helped in restoration of the disturbed haemato biochemical changes and abating clinical symptoms.

Table.1 *In-vitro* drug sensitivity pattern of *E. coli* isolates

S. No.	Antimicrobials	Symbol	Disc content	Highly sensitive		Moderate sensitive		Low sensitive		Resistant	
				No.	%	No	%	No	%	No	%
1.	Amoxicillin	Ac	300mg	-	-	-	-	12	16	63	84
2.	Ciprofloxacin	Cf	10mcg	45	60	27	36	2	2.7	1	1.3
3.	Gentamicin	G	10mcg	46	61.3	25	33.6	2	2.7	2	2.7
4.	Tetracycline	T	30mcg	26	34.6	36	48	11	14.6	2	2.7
5.	Penicillin	P	10 units	-	-	-	-	66	88	9	12
6.	Neomycin	N	30mcg	41	54.6	29	38.7	3	4	2	2.7
7.	Enrofloxacin	En	10mcg	30	40	35	46.7	7	9.3	3	4
8.	Trimethoprim	Tr	5mcg	14	18.6	26	34.6	18	24	17	22.7
9.	Oxacillin	Ox	1mcg	-	-	3	4	2	2.7	70	93.3
10.	Cefmetazole	Cf	30mcg	12	16	40	53.4	20	26.7	3	4
11.	Co-trimoxazole (trimethoprim/sulphamethoxazole)	Co	1.25/23.75 mcg	38	50.6	32	42.7	3	4	2	2.7

Table.2 Therapeutic trials (*In-vivo*) in clinical cases of colibacillosis in lambs

Groups	Name of the antibiotic	Dose	No. of cases treated	Day I	Day II	Day IV	Day VI	No. of cases Cured	%age	No.of cases Delayed	%age
I	Control Group	-	six	-	-	-	-	six	100.00	-	-
II	Control Group	-	six	-	-	-	-	-	-	six	100
III	Ciprofloxacin (ciprobid)	4mg/kg (250 mg tab-1/4 th twice daily)	six	+	+	+++	++++	six	100.00	-	-
IV	Co-trimoxazole (Biotrim)	15-30 mg/kg BW (Bolus 1/10 th orally)	six	+	++	++	+++	four	66.68	two	33.34
V	Gentamicin (Gentamicin Sulphate)	4mg/kg 0.3 ml iv/ im BID daily	six	+	++	+++	++++	six	100.00	-	-
IV	Neomycin	10mg/kg BW (Bolus 1/12 th orally)	six	+	+	+++	+++	five	83.35	one	16.66

+ = Slightly improved; ++ = Improved; +++ = Recovery; ++++ = Complete recovery

Another group of lambs was treated with another conventional gut acting antibacterial neomycin, dosage of 10mg/kg body weight for five days that confirmed 83.35 per cent efficacy in this study. The infected group of lambs treated with co-trimoxazole at the dose rate of 15-30 mg/kg body weight, registered 66.8 per cent efficacy in this study. Similar efficacy of antibacterials against *E. coli* diarrhea was reported by Sakia and Harashma (1985). Treatment regimen (*in-vivo* efficacy) used in four different groups of clinical cases

of colibacillosis indicated gentamicin and ciprofloxacin most effective drugs followed by neomycin and co-trimoxazole corresponding with *in-vitro* study.

In conclusion, one hundred nine isolates of *E. coli* were identified, characterized and got serotyped. Ninety three serotypes belonged to 34 different 'O' serogroups. Prevalence of serogroups O20 and O11 was highest with per cent prevalence of 10.09 and 8.25 respectively. Isolation of *E. coli* serogroups

especially O26, O23, O17, O157, O153, O2 and O5 was alarming because of their zoonotic importance. *In-vitro* drug sensitivity pattern indicated that most identified serotypes were highly sensitive to four antibacterials, viz. gentamicin, ciprofloxacin, neomycin and co-trimoxazole. The therapeutic efficacy of four highly sensitive *in-vitro* drugs was evaluated, viz. gentamicin, ciprofloxacin, neomycin and co-trimoxazole. Gentamicin at 4mg/kg body weight and Ciprofloxacin at 4mg/kg body weight were found 100 per cent effective at 144 hour post treatment. Neomycin at 10mg/kg body weight was 83.35 per cent effective and co-trimoxazole at 15-30 mg/kg body weight was 66.8 per cent effective at 144 hour post treatment.

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