DETERMINATION OF ANTIBIOGRAM PATTERN IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM CATTLE FAECES

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ABSTRACT

Escherichia coli is the one of the major challenge in regards to antibiotic resistance phenomena. It has not only significantly associated with many animal diseases but also important for high level of antibiotic resistance. The present study has designed to determine antibiogram pattern of *E. coli* strains obtained from cattle diarrhoeic faeces. Total 31 genotypically confirmed *E. coli* isolates were obtained from 40 samples and screened for 16 antibiotics. It was found that 90.32% isolates were sensitive against cefepime, chloramphenicol and ceftrioxane followed by 87.09% sensitive to levofloxacin, 80.64% to polymyxin-B, 77.41% gentamicin, 67.74% ampicillin/sulbactam, 54.83% ticarcillin and tetracycline, 51.61% streptomycin, 48.38% ampicillin, 41.93% isolates were sensitive to cephotaxime. The all isolates were 100% resistant to penicillin and rifampicin. Further, this study suggested molecular characterization of isolates in context of genetic mechanisms involve in antibiotic resistance.

Key words**:** *Escherichia coli,* antibiogram, cattle, faeces

Introduction

Escherichia coli is the type species of the genus Escherichia, which contains commonly motile gram negative bacilli within the family Enterobacteriaceae. The bacteria are necessary commensal part of the intestinal flora for the proper digestion of food. *E. coli* may also associate with several intestinal and extra-intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicaemia and gram-negative pneumonia (Carter, 1985). Antibiotic resistance is a fast growing problem to hampered animal diseases management and socioeconomic status of society. Probably it may cause by indiscriminate use of antibiotics in humans and animals, also including use in food products of animals as growth promoter (Sharma *et al.,* 2013).

Laboratory analysis has important role in management of various infections especially antibiotic sensitivity test is significant for proper prescription of antibiotics. In recent years, the multidrug resistance *E. coli* has emerges which make complicated the situation by the emergence of resistance to most of first-line antimicrobial agents (Jensen *et al.,* 2006). Antibiotic drug- resistance is spreads worldwide. *Escherichia coli* has important connecting link in resistance transmission because of versatile nature of disease pattern including as major, opportunistic and commensal pathogen. This study has designed to find out antibiogram pattern of *E. coli* to find proper efficient antibiotics and to reduce risk of antibiotic resistance by decreasing indiscriminate use.

Materials and Methods

Sample collection

Fourty faecal samples from cattle from Bikaner city were collected aseptically with the help of sterile cotton swabs soaked in nutrient broth and placed in sterile test tubes, taking all precautions to avoid contamination. The samples were transported to the laboratory as soon as possible for further processing. The samples were inoculated on nutrient agar and Mac-conkey agar plates and then processed for isolation and identification of *Escherichia coli* (Quinn *et al.,* 1994). All the samples were proceeded for phenotypic and biochemical tests.

Genotypic confirmation (23S rRNA based Ribotyping)

All the 31 phenotypically confirmed isolates were confirmed to be *E. coli* by 23S rRNA ribotyping described by Khaled *et al.* (2010) with some minor modification. The Primer-1F - 5' GCT TGA CAC TGA ACA TTG AG 3' and Primer-2R - 5' GCA CTT ATC TCT TCC GCA TT 3' was used. (Fig. 1).

Antibiotic sensitivity testing

Antibiotic susceptibility testing was done as per the disc diffusion method (Bauer *et al.,* 1966) against 16 antibiotics of different classes. In brief, the isolates were inoculated in sterile 5 ml nutrient broth, incubated for 18 hr at 37oC and then the opacity was adjusted to 0.5 McFarland opacity standards with normal saline solution (Quinn *et al.,* 1994). Each inoculum was then spread over the surface of Mueller-Hinton agar with the help of sterile swab. Plates were allowed to dry for 10 min at 37°C and then antibiotic discs were carefully placed on the surface with enough space around each disc for diffusion of the antibiotic. Plates were incubated for 24 hr at 37^0C and the zone of inhibition of growth of the organism around each disc was measured in millimeters. After inhibition zone measurement, interpretation of resistant, sensitive and intermediates drown as breakpoints defined by the Clinical and Laboratory Standards Institute (CLSI, 2007).

Results and Discussion

A total of 31 *Escherichia coli* isolates were obtained from 40 diarrhoeic faecal samples of cattle with 77.50% frequency. All isolates were confirmed phenotypic as well as genotypic by 23S rRNA based Ribotyping with 662bp amplicon size (Fig. 1). Antibiogram pattern of *E. coli* isolates rvealed that 100%

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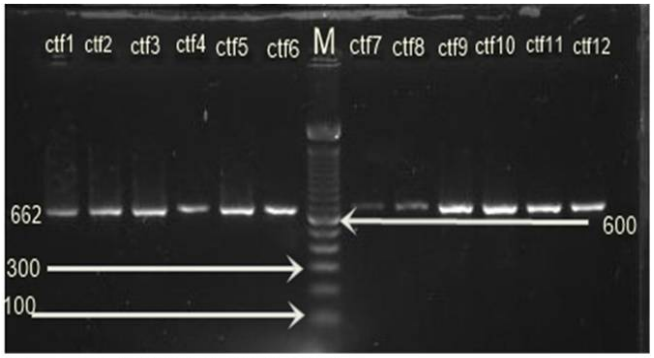
isolated were resistant to two antibiotics named as penicillin and rifampicin. Out of total 16 antibiotics, cefepime, chloramphenicol and ceftrioxane were most effective with 90.32% efficacy, followed by levofloxacin 87.09% efficient, polymyxin-B 80.64%, gentamicin 77.41%, ampicillin/sulbactam 67.74%, ticarcillin and tetracycline 54.83%, streptomycin 51.61%, ampicillin (48.38%) and cephotaxime was 41.93% efficient against the studied isolates. Remaining other antibiotics were variable effective against *E. coli* as mentioned in Table 1. In the present investigation, 70.96% and 51.61% *E. coli* isolates were intermediate against erythromycin and cephotaxime, respectively.

In agreement to present study, higher isolation frequency was reported by Sharma *et al.* (2013) who detected 18 *E. coli* isolates from 21 faecal samples of enteritis in pet dogs in the same study area. It may indicated more prevalence of *E. coli* isolates among pets associated with interaction of human society with pets in compare to domestic animal such as cattle. The observed high efficacy of cefepime, chloramphenicol and ceftrioxane suggested that they could be used for management of diarrhoeic infections of cattle which associated with *E. coli*. The results of present study are comparable with Pachaury



M = 100bp Molecular marker
Ctf1-12 = Cattle diarrheic fecal sample

Fig.1: Genotypic confirmation *of E. coli* isolates obtained from faeces of cattle

and Kataria (2012) who has been also studied resistance pattern of *E. coli* obtained from cattle faecal samples.

Similar to present study Sharma *et al.* (2013) and Singh *et al.* (2016) detected antibiogram pattern of *E. coli* isolates obtained from dog and equines suffered by gastrointestinal disorders, respectively. They have also observed that cephalosporins, quinolones and aminoglycoside are more effective while penicillin and macrolides are less effective in enteric infections associated with *E. coli*. The observed resistance of isolates may correlate with use of antibiotics in particular area. The earlier studies from same area have been also reported that it could be inferred that antibiotics with low cost, easily available and widely used by veterinary practitioners in the particular area are least effective in that zone (Pachaury and Kataria, 2012; Sharma *et al.,* 2013). The resistance may contribute by inherent property of organism as well as interaction with other resistant organisms (Pandey *et al.,* 2016). Intermediate property of some isolates may indicate conversion stage, which suggested that the isolates are changing themselves from sensitive to resistant phenotypes by various meaning of mechanisms of antibiotic resistance (Goncuoglu

Table1: Antibiogram pattern of *E. coli* isolates obtained from faeces of cattle

S. No.	Antibiogram disc	Per cent		
		Sensitive	Intermediate	Resistant
1	Ampicillin (AMP)	48.38	25.80	25.80
2	Ampicillin/Sulbactam (A/S	67.74	22.58	9.67
3	Cefepime(CPM)	90.32	6.45	3.22
4	Cephotaxime(CTX)	41.93	51.61	6.45
5	Ceftriaxone(CTR)	90.32	3.22	6.45
6	Chloramphenicol (C)	90.32	3.22	6.45
7	Erythromycin(E)	-	70.96	29.03
8	Gentamicin(GEN)	77.41	16.12	6.45
9	Levofloxacin(LE)	87.09	3.22	9.67
10	Penicillin G (P)	-	-	100
11	Polymyxin-B (Pb)	-	19.35	80.64
12	Rifampicin(RIF)	-	-	100
13	Streptomycin(S)	51.61	38.70	9.67
14	Tetracycline(TE)	54.83	29.03	16.12
15	Ticarcillin (TI)	54.83	35.48	9.67
16	Trimethoprim(TR)	22.58	29.03	48.38

et al., 2010).

In conclusion, results of present study provides pointer of the basic information of antibiotic resistance pattern of *E. coli* isolated from fecal samples of diarrhoeic cattle. It emphasizes the need of proper monitoring, surveillance and regulated use of antibiotic to reduce the pressure of this global problem. It further suggested exploring the genetic mechanisms, which govern antimicrobial resistance of *E. coli* isolates.

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