Evaluation of Antimicrobial Efficacy Against *E. coli* Isolates of Different Serogroups Obtained from Diarrhoeic Neonates of Calves, Kids, and Lambs

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ABSTRACT

Fourteen antimicrobials were evaluated their efficacy against 115 *Escherichia coli* isolates recovered from faecal samples collected from neonatal diarrhoeic calves (66), kids (25) and lambs (24). Of these isolates 37 were nontypeable, four were of rough type and 74 isolates were typeable for 'O' antigen belonging to 19 different serogroups (O2, O3, O5, O17, O21, O22, O25, O41, O45, O55, O60, O65, O70, O76, O114, O116, O147, O152, and O158). Evaluation of antimicrobial efficacy by disc diffusion method revealed that the most effective antibiotics were ciprofloxacin, sparfloxacin and triple sulpha followed by co-trimoxazole, chloramphenicol, nitrofurantoin, gentamicin, nalidixic acid, polymixin-B and amoxyclav in decreasing order of their efficacy while ampicillin and cephotaxime were found totally ineffective against these isolates. All the isolates showed multiple drug resistance to various antibiotics ranging from two to ten antibiotics. The resistance patterns were independent of serogroups of isolates and the animal species from which isolates were obtained. However, there was a variation in resistance pattern for same antibiotic *vis-a vis* locations of their isolation.

Key words: antibiogram, E. coli, diarrhoeic faeces, animal neonates, efficacy

ABSTRAK

Empat belas antimikroba dalam penelitian ini dievaluasi efikasinya terhadap 115 *Escherichia coli* yang diisolasi dari sampel feses pedet (66), anak kambing (25), dan anak domba (24) neonatal yang mengalami diare. Berdasarkan isolat yang dihasilkan, 37 merupakan isolat tak bertipe, 4 isolat tipe *rough*, dan 74 isolat tipe *antigen* 'O' yang memiliki 19 serogrup yang berbeda (O2, O3, O5, O17, O21, O22, O25, O41, O45, O55, O60, O65, O70, O76, O114, O116, O147, O152, and O158). Evaluasi efikasi antimikroba yang dilakukan dengan metode difusi cakram menunjukkan bahwa antibiotik yang paling efektif adalah *ciprofloxacin, sparfloxacin,* dan *triple sulpha* yang diikuti secara berturut-turut oleh *co-trimoxazole, chloramphenicol, nitrofurantoin, gentamicin, nalidixic acid, polymixin*-B dan *amoxyclav*, sedangkan *ampicillin* dan *cephotaxime* tidak efektif terhadap isolat-isolat tersebut. Seluruh isolat menunjukkan multiresisten terhadap dua hingga 10 antibiotik. Pola resistensi tergantung kepada serogrup dan spesies ternak asal isolat. Namun demikian terdapat suatu pola resistensi untuk antibiotik yang sama dengan lokasi isolasinya.

Kata kunci: antibilogram, E. coli, feses diare, ternak baru lahir, efikasi

INTRODUCTION

Neonatal diarrhoea is one of the major causes of mortality in young calves, lambs and kids resulting in economic loss to dairy industry. Out of many diverse causes, *Escherichia coli* is recorded as the major etiological agent of diarrhoea in young ruminants. The indiscriminate use of antibmicrobials in treating such infections has led to emergence of multi-drug resistant strains which are difficult to be controlled in future outbreaks. This situation needs either new therapeutic agent with novel mechanisms of action to circumvent these infections or the most effective antibiotics among the available lot be used after testing against the organisms. There is increased public and scientific interest regarding the administration of antimicrobials to animals due primarily to zoonotic bacterial pathogens (White & McDermott, 2001). The emergence of antibacterial resis-

tance among pathogens that affect animal health is of growing concern in veterinary medicine as these resistant pathogens in animals may lead to potential health risk for humans (Medina *et al.,* 2011). This is why the monitoring of antimicrobial susceptibility in pathogenic bacteria and in commensals in animals is also recommended by OIE (Acar & Rostel, 2001). Serotyping is a common method used for the characterization of clinical isolates of *E. coli* and has a broad use in epidemiology and also in medical diagnosis. The existing association between serotype and pathotype makes this method a valuable tool for typing *E. coli* (Ramteke & Tewari, 2007).

The present paper puts on record *in vitro* testing of 14 antibiotics against *E. coli* isolates obtained from cattle calves, goat kids and lambs in order to record the antibiogram and to see any relationship between antibiogram and serogroup they belong to.

MATERIALS AND METHODS

One hundred and fifteen *E. coli* isolates obtained from diarrhoeic faecal samples from calves (66), kids (25) and lambs (24) belonging to organised and unorganised dairy farm at seven different locations in and around Bikaner city located in the Thar desert of west Rajasthan state were identified as per procedures described by Cowan & Steel (1975) and Quinn *et al.* (1994).

The antibiogram for the isolates was determined by disc diffusion method as per technique of Bauer *et al.* (1966). In brief, biochemically confirmed *E. coli* isolates were grown in Muller-Hinton broth for 6 h at 37 °C. Turbidity was adjusted to 0.5 McFarland standard and Muller-Hinton agar plates were seeded with the cultures. Different antibiotic discs (Hi-Media Laboratories, Mumbai, India) were placed on the inoculated medium. Antibiotic sensitivity plates were incubated at 37 °C for 24 h and zone of inhibition was recorded and compared as per manufacturers instructions for each antibiotic.

Serogrouping for "O" antigen of the isolates was obtained from National Salmonella and *E. coli* centre, Kasauli (H.P), India by agglutination reaction using standard 'O' antisera.

RESULTS AND DISCUSSION

Diameter of zone of inhibition was measured and results were interpreted as sensitive, intermediate and resistant respectively as per the manufacturer's instructions (Table 1, Figure 1). In the present study the most of the antibiotics were effective in inhibiting the growth of *E. coli* and the resistance shown by these isolates towards antibiotics was comparatively less. Among the used antibiotics ciprofloxacin, sparfloxacin and triple-sulpha were recorded effective against all the isolates whereas ampicillin and cephotaxime were totally ineffective. The rest of the antibiotics showed a variable efficacy against these *E. coli* isolates.

The high sensitivity of *E. coli* for ciprofloxacin, sparfloxacin, triple sulpha, co-trimoxazole, chloramphenicol, gentamicin, nalidixic acid and nitrofurantoin indicated that they could be used to circumvent the *E. coli* infection in this area. This might be attributed to

the fact that these antibiotics have not been used with greater frequency in the treatment of enteric infection in Bikaner region. Similar to our findings, no resistance for ciprofloxacin and low resistance for gentamicin were reported by Read *et al.* (2004) against *E. coli* isolates.

Our observations towards ampicillin resistance were also similar to those of many other workers from various parts of the world *viz.* Cid *et al.* (1996) from Spain; Bradford *et al.* (1999) and Donalson *et al.* (2006) from USA; Khan *et al.*(2002) from India and Karczmarczyk *et al.* (2011) from Ireland. The resistance recorded to penicillin (ampicillin) in the present study might be due to β-lactamase production by the isolates. In addition, the indiscriminate use of antibiotics exerts a selection pressure which leads to development of drug resistance in the isolates.

When the antibiogram data were analysed *vis-a-vis* animal species we could not find any relationship between the isolates from a particular animal species and their susceptibility or resistance pattern towards a particular antibiotic. This finding indicated that the antibiotics in the area were used irrespective of the animal species and/ or there would have been cross-infection of the animal species by the same isolates as they are generally housed in close association. In addition to it, the human population also lives in the close proximity of animal population with very high frequency of human-animal interactions especially in a village set up in India. Hence the antibiogram pattern obtained may be because of use of antibiotics used in animal as well as human population in that particular area.

When the antibiogram data were analysed *vis-à-vis* location from where the samples were collected for isolation of *E. coli* it was recorded that there was a variation in resistance pattern of same antibiotic at different locations which might be due to frequency of

Table 1. Antibiogram pattern of *E. coli* isolated from neonatal diarrhoeic animals

| Antibiogram disc	Concontra- tion (mcg/disc)	Percent		
		Sensi- tive	Interme- diate	Resis- tant
Ampicillin (A)	10	-	-	100
Amoxyclav (Ac)	30	36.52	63.47	-
Azithromycin (At)	15	46.95	1.73	51.3
Cephotaxime (Ce)	30	-	-	100
Chloramphenicol (C)	30	83.47	5.21	11.3
Chlortetracycline (Ct)	30	20	68.69	11.3
Ciprofloxacin (Cf)	5	100	-	-
Co-trimxazole (Co)	25	97.39	-	2.6
Gentamicin (G)	10	74.78	10.43	14.78
Nalidixic acid (Na)	30	73.91	-	26.08
Nitrofurantoin (Nf)	300	66.95	26.08	6.95
Polymixin B (Pb)	300	20.86	-	79.13
Sparfloxacin (Sc)	5	100	-	-
Triple-sulpha (S3)	300	100	-	-
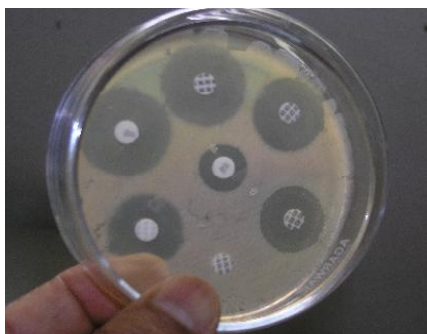
Figure 1. Antibiogram plate showing zone of inhibition of respective antibiotic discs for *Escherichia coli* isolates of diarrhoeic neonates

antibiotic used at a particular location for treatment of enteric infections.

All the isolates showed multiple drug resistance to various antibiotics ranging from two to ten antibiotics. Karczmarczyk *et al.* (2011) demonstrated mobility of resistance genes using configuration assays with a representative selection of *E. coli* isolates and they found high molecular weight plasmids responsible for resistance to multiple antimicrobial compounds.

Seventy nine isolates were resistant to four or more antibiotics. Predominant resistance pattern was observed for ampicillin azithromycin, cephotaxime and polymixin-B. Out of 49 lamb and kid isolates, resistance to ampicillin, azithromycin, cephotaxime and polymixin-B (AAtCePb) was observed for 32 (65.30%) isolates. Resistance to ampicillin, azithromycin, cephotaxime and nalidixic acid (AAtCeNa) was observed for 28 (57.14%) while resistance to ampicillin, azithromycin, cephotaxime and gentamicin (AAtCeG) was observed for 15 (30.61%) isolates. Out of 66 calf isolates, resistance to ampicillin, cephotaxime and polymixin-B (ACePb) was observed for 59 (89.39%) isolates. Resistance for ampicillin, cephotaxime and gentamicin (ACeG) was observed for 14 (21.21%) isolates, resistance for ampicillin, cephotaxime and chloramphenicol (ACeC) was observed for 13(19.69%) isolates and resistance for ampicillin, cephotaxime and azithromycin (AAtCe) was shown by 10(15.15%) isolates.

Multiple drug resistance patterns obtained in present study could be compared with the findings of Bradford *et al*. (1999) and Khan *et al*. (2002). These workers revealed various combination of multiple antimicrobial drug resistance for the *E.coli* isolates. Studies on antimicrobial resistance of *E.coli* from different animal species showed an increase in the incidence of resistance over the years as a result of the wide spread use of antimicrobial drugs in animals (Cid *et al*., 1996), the problem further aggravated by the transfer of *E. coli* from livestock to poultry to human (Kapoor & Kulshreshtha, 1994).

In the present study of the total 115 *E. coli* isolates typed for 'O'antigen 37 (32.17%) were non-typeable, four (0.03%) were of rough type and 74 (64.43) were typeable into 19 different serogroups of which O22 was the most common with prevalence of 12.17%. Two se-

rogroups (O5 and O147) were common to all the three animal species, lambs and kids harboured only one common serogroup (O55) and lambs and calves shared three common serogroups (O22, O25, O116) while four serogroups (O17, O60, O76 and O152) were common to calves and kids. The antibiogram was analysed against the serofroups of the isolates but no relationship was recorded between them.

CONCLUSION

The highest rate of resistance has been detected against the antimicrobial drugs most commonly used either as feed additives or as curative agents in farm animals.

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