PATHOGENICITY AND ANTIBIOGRAM OF *ESCHERICHIA COLI* ISOLATED FROM DIARRHOEIC COW CALVES

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ABSTRACT

Thirty two isolates of *Escherichia coli* recovered from diarrhoeic samples in cow calves were tested for virulence factors by a battery of *in vivo* (pathogenicity and enterotoxigenicity) and *in vitro* methods. Four isolates (12.50%) belonging to O88, O97, O98 and O159 serogroups proved fatal to Swiss albino mice on intraperitoneal inoculation of whole bacterial culture. Similarly the cell free filtrate of 4 (12.50%) isolates, belonging to O14, O88, O110 and O138 serogroups caused death of mice within 6 to 20 hours post intravenous inoculation (hpi) of intravenous inoculation. None of the isolates produced STa or LT enterotoxins in suckling mouse and mouse paw oedema test, respectively. Five (15.63%) isolates were found resistant towards the lytic action of cow calf serum, whereas 10 (31.25%) were sensitive and 17 (53.12%) intermediate sensitive to serum. The *in vitro* sensitivity of the isolates to antimicrobial drugs was found 100% to ciprofloxacin and sulphadiazine, followed by ceftriaxone and ceftriaxone/tazobactum (96.88%), cefotaxime (71.90%), amoxyclav (68.75%) and amikacin (46.9%).

KEY WORDS: *E.coli*, cow calves, diarrhoea, antibiogram, enterotoxins, pathogenicity.

INTRODUCTION

Escherichia coli is one of the leading cause of neonatal calf diarrhea which frequently results in substantial economic loss in the dairy industry (Bradford et al., 1999). Several *E. coli* serotypes, causing morbidity and mortality, have been isolated from diarrhoeic calves (Wani et al., 2004). Since *E. coli* is a normal inhabitant of the gastrointestinal tract, it is difficult to establish its role in diarrhea by mere isolation and identification. Therefore, it is imperative to characterize the isolates in terms of specific virulence factors. Moreover, repeated sub lethal exposure to antibacterial agents has not only promoted adaptive resistance, but also conferred decreased sensitivity to antibiotics in *E. coli* strains (Udaykar and Sharda, 2009). The present study report the phenotypic assessment of some of these virulence factors of *E. coli* strains isolated from cow calves suffering with diarrhoea.

MATERIAL AND METHODS

Bacterial isolates: Thirty two isolates of E. coli recovered from diarrhoeic cow calves maintained in the Department of Veterinary Microbiology, College of Veterinary Sciences & A.H., Mhow (M.P.) were used in the present study. Serotyping was done at CRI, Kasauli.

Animal pathogenicity test: The pathogenicity of whole bacterial culture of each *E. coli* isolate was studied in healthy adult Swiss albino mice weighing 20 to 22 g (Boro et al., 1983). The test was conducted by inoculating 0.1 ml of 18 h incubated brain heart infusion (BHI) broth containing approximately 3×108 viable bacteria per ml via intra peritoneal route into mouse. Two mice per isolate were used after due permission from the college level animal ethics committee. The control mice received an equal volume of sterile broth. The experimental animals were housed in separate cages and observed up to 96 hours post inoculation (hpi).

Pathogenicity of cell free filtrate: The cell free filtrate (CFF) of all the *E. coli* strains was prepared as per the method described by Giannella (1976). The filtrate was checked for sterility on nutrient

agar slant and subsequently stored at –20^{0}C till use. The pathogenicity of CFF was studied in healthy Swiss albino mice weighing 20 to 22 g by inoculating 0.1 ml CFF via intra venous route (Yadav et al., 2006). Two mice per isolate were used after due permission from the college level animal ethics committee. The control mice received an equal volume of sterile broth.

Enterotoxigenecity: Suckling mouse assay was conducted as per the method described by Dean et al. (1972) and modified by Giannella (1976) to demonstrate the production of heat stable enterotoxin (STa) by E.coli isolates. Similarly, the production of heat labile enterotoxin (LT1) was assessed by mouse paw oedema test (Harne et al., 1990).

Serum bactericidal assay: Overnight grown cultures of *E. coli* on Muellor Hinton agar (MHA) plates were harvested and cells were suspended in Hanks's Balanced Salt Solution (HBSS). Bacterial suspension (0.05 ml) was incubated with an equal volume of bubaline calf serum in sterile 96 well microtitre plates (Laxbro) and incubated at 37^{0}C in an orbital shaker for 3 h; control wells contained 0.05 ml HBSS instead of serum. Ten µl of each sample from control well was spread on MHA plates at 0 min to determine viable colony count. Similarly, 10µl of inoculum from each well containing serum was spread on MHA plates after 3 h of incubation. The strains were termed as serum sensitive if viable colony count dropped to 5% of initial value, and serum resistant if > 90% organisms survived after 3 h of incubation. Strains that have values in between were termed as intermediate sensitive (Seigfried et al., 1994).

In vitro antibiotic sensitivity: The *in vitro* antibiotic sensitivity test of the *E. coli* isolates against seven antibiotics (Hi Media Ltd.) was conducted by disc diffusion method of Bauer et al (1966) with minor modifications. Diameters of the clear zone of inhibition were measured and the interpretation of the results was made in accordance with the instructions supplied by the manufacturer.

RESULTS AND DISCUSSION

In vivo pathogenicity and toxigenicity: 4 (12.50%) out of 32 *E. coli* isolates, caused death of Swiss albino mice within 96 hpi . The positive isolates belonged to the serogroup O88, O97, O98 and O159. When whole bacterial culture was inoculated via intraperitoneal route. Amongst these pathogenic isolates, one belonging to O159 serogroup was scored as highly pathogenic (+++) showing mortality within 7 to 18 hpi. Moderate pathogenicity (++) was exhibited by 2 isolates belonging to O88 and 098, which caused death of mice within 19-30 hpi, whereas one strain of O97 resulted in mortality within 31-96 hpi and hence categorized as low pathogenic (+). Twenty eight isolates did not prove fatal, but resulted in signs of illness, and can thus be categorized as non-pathogenic. Blanco et al. (1993) and Yadav et al. (2006) reported O159 and O88 serogroups of *E. coli* to be pathogenic to mice via intraperitoneal route, respectively. Various degrees of pathogenicity recorded are in accordance with reports of Singh and Gupta (1996), whereas Blanco et al. (1993) reported a much higher percentage of pathogenicity. The pathogenecity observed in present study can be attributed to colonization and growth of bacteria and/or production of toxins in lower intestine. Growth of shigatotoxigenic *E Coli.* (STEC) in the intestine leads to fatal infections (Shimizu et al, 2003).

The CFF of 4 (12.5%) isolates of *E. coli* resulted in death of mice within 6 to 20 hpi. These isolates belonged to the serogroup O14, O88, O110 and O138. Yadav et al. (2006) also recorded mortality in adult Swiss albino mice due to CFF derived from strains of 8 different serogroups including O88. It is conferring that on colonization by bacteria, synthesis and /or release of toxins may be enhanced by factors such as bile, trypsin and antimicrobial agents present in intestine (Sears and Kaper, 1996).

Among 32 isolates none of them produced either STa or LT enterotoxin. These results are in accordance with low prevalence and percentage of ST producing strains from diarrheic cow calves reported by Salvadori et al. (2003) and Hussain et al. (2008).

Serum bactericidal assay: Five (15.63%) of isolates, belonging to serogroup O12, O14, O28, O98 and O138, were resistant towards the lytic action of calf serum as more than 90% organisms survived after incubation with serum. The findings are supported by report of Kausar et al. (2009), but in contrast to that of Seigfried et al. (1994). On the contrary, 10 (31.25%) isolates were sensitive to serum, out of which one each belonged to O20, O69, O88, O97, O105, O106, O108, O110, O166 and one was untypable. Intermediate sensitivity pattern was exhibited by 17 (53.12%) isolates.

In vitro antibiotic sensitivity test: The highest sensitivity was attributed to ciprofloxacin and sulfadiazine (100%), followed by ceftriaxone alone and in combination with tazobactum (96.88%), cefotaxime (71.90%), amoxyclav (68.75%) and amikacin (46.9%). Twelve (37.5%) isolates showed resistance to only one antibiotic, while 5 (15.6%), 4 (12.5%) and 1 (3.12%) exhibited simultaneous resistance to 2, 3 and 5 antibiotics respectively.

The fluoroquinolones have been shown to have excellent *in vitro* activities against *E. coli* isolates of human and animal origin, including STEC strains. Pan and Bhatia (2010) also reported high sensitivity towards ciprofloxacin. Wani et al. (2007) concluded that 3rd generation cephalosporins are highly effective against pathogenic *E. coli* strains, which supports high sensitivity of ceftriaxone revealed in this study. Results of Mitra et al. (2009) are also in agreement with finding of present work as they mentioned ceftriaxone as 2nd drug of choice. However, an intermediate activity (71.9%) towards cefotaxime was observed, which is contrary to report of Aksoy et al. (2007) and comparable to that of Alhaj et al. (2007). Cefotaxime and ceftriaxone are third generation cephalosporins, which have enhanced activity against the Enterobacteriacae. Cefotaxime was the first of the third generation cephalosporins to become available in veterinary medicine and hence, used more frequently than ceftriaxone, which could be the possible reason for an increased resistance towards the former in our study. Bovine *E. coli* strains that produce some potential virulence factors are more sensitive to quinolones than those that do not express these factors (Orden et al., 1999).

In present work, none of the *E. coli* was found resistant to sulphonamides. Our findings are in partial concurrence with those of Aksoy et al. (2007), whereas reports of Patil et al. (1999) and Regobelo et al. (2006) are contradictory. Seventeen (53.10%) isolates were found resistant to amikacin. Pan and Bhatia (2010) also reported about 50% *E. coli* isolates resistant to amikacin. Similarly, the resistance (31.25%) exhibited towards amoxicillin/clavulanic acid is in partial concurrence to reports of Regobelo et al. (2006) and Pan and Bhatia (2010). However, Aksoy et al. (2007) reported a high sensitivity (94.8%) of *E. coli* strains to amoxyclav. The drug sensitivity of coliform organisms varies with place, time, species, and even disease to disease in the same animal (Radostits et al., 2000)

Multiple drug resistant isolates of *E. coli* strains from animals have been reported worldwide (Orden et al., 2000; Wani et al., 2007; Arya et al., 2008). Use of antimicrobials in livestock production is suspected to significantly contribute to multiple drug resistance in species of bacteria, which are common to humans and animals (Acar and Rostel, 2001).

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