SEROTYPING AND ENTEROTOXIGENICITY OF *ESCHERICHIA COLI* ISOLATED FROM URINARY TRACT INFECTIONS

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ABSTRACT

One hundred and forty patients with symptomatic urinary tract infection were investigated for serotyping and enterotoxin production. *Escherichia coli* constituted 63 per cent of the 119 culture positive cases followed by *Klebsiella spp* and *Proteus spp*. *E. coli* serotype O5 was the most prevalent (29.3%) of the typable strains followed by O17 and O25. Out of the 75 *E. coli* strains, 30 (40%) were enterotoxin producers. Four per cent produced both heat-stable enterotoxin and heat-labile enterotoxin, 21.3 per cent produced only heat stable, whereas 13.3 per cent produced only heat labile toxin. *E. coli* serotype O5 was the commonest enterotoxin producer (45.5%), followed by rough strains (52.9%) and untypable strains (30.7%). Other ET producing strains were O1, O25, O55, O57 and O60. The enterotoxin production of the most prevalent serotype O5 possibly contributes as a virulence marker in UTI cases.

MJAFI 1997; 53 : 83-86

KEYWORDS : Enterotoxins; *Escherichia coli*; Serotyping; Urinary tract infections.

Introduction

E*scherichia coli*, the most common organism in the faecal flora is a well known pathogen outside its ecologic niche. In urinary tract infections (UTI), it is the most common isolate both in general and hospital practice [1]. Initially gut *E. coli* colonize the periurethral area, ascend in the urinary tract, adhere by the release of exopolysaccharide polymers and pili, survive with the help of osmoprotective glycine betaine in urine and then lodge and multiply forming a bacterial biofilm [2]. Currently *E. coli* causing diarrhoea are divided into 6 groups, depending upon virulent factors and lesions produced by them. These groups are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli*, enteroaggregative *E. coli*, enterohaemorrhagic *E. coli*, enteroinvasive *E. coli*, and diffusely adherent *E. coli*. ETEC produce two types of enterotoxin (ET) namely, heat labile ET (LT) and heat stable ET (ST), by an ent plasmid [4]. Though ETEC are recognized as important causes of childhood diarrhoea very little work has been done to evaluate their ability to cause UTI. The present work has been carried out to find the

prevalent serotypes and their ability to produce enterotoxins as possible virulence markers in UTI.

Material and Methods

A total of 140 patients with symptoms of UTI admitted to Command Hospital (Southern Command) Pune were investigated. Early morning midstream urine was collected in wide mouthed 100 mL sterile containers with due precautions. After routine examination for pus cells and protein the samples were inoculated on cysteine lactose electrolyte deficient (CLED) medium by standard wire-loop method and incubated for 24 hours at 37°C. *E. coli* were identified by their colony characters and biochemical reactions [5].

For serotyping the isolates were sent to the National *Salmonella-EC* Centre, CRI, Kasauli where serotyping was carried out by type-specific antisera [5].

Tests for ST : The classical infant mouse model, as proposed by Dean [6], was used. In short, 1-4 day old albino mice were selected and separated from their mothers just before the test. The oro-oesophageal route as proposed by Lallier [7] was

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Fig. 1 : Normal Verocell monolayer.

used. Four mice were used for each test strain. The culture filtrate in trypticase soy broth was heated to 100°C for 30 minutes to destroy the LT. Two per cent Evans-blue was added to the test material and 0.1 mL was administered orally to each mouse. They were kept at room temperature for 3-4 hours and then sacrificed. The intestines were observed for swelling and then dissected. The intestines of all 4 mice were weighed together and compared with the weight of the rest of the body. The ratio of the weight of the intestines to that of the body was interpreted as under:-

Ratio < 0.07 Negative

Ratio 0.07-0.09 Borderline positive

Ratio > 0.09 Strongly positive

A known positive enterotoxigenic strain H10407 and a known negative strain ECK12 were used for comparison.

Test for LT: The Vero cell method of Spiers et al was used [8]. Monolayer of Vero cells were grown in tissue culture tubes. Two tubes were inoculated with 0.5 ml each of culture filtrate and incubated at 37°C for 18 hours. Morphological changes in the form of ballooning, cell wall thickening, and formation of filamentous tendrils or dendrites in at least 50 per cent of cells indicated the presence of LT (Figs 1,2).

Results

Out of 140 samples 119 were culture positive. *E. coli* accounted for 75 (63%) of these, followed by *Klebsiella* (15.9%). The distribution of different

Fig. 2 : Verocells showing morphological changes due to LT.

pathogens is shown in Table 1. Amongst the *E. coli*, serotype O5 was the most common serotype causing UTI (29.3%) followed by O17 and O25 (5.3% each). Rough strains were 22.6 per cent and non-typable strains were 17.3 per cent (Table 2). Forty seven (61.3%) out of 75 strains of *E. coli* were nontoxigenic. Out of the remaining 29 toxin producers, 3 (4%) produced both ST and LT, 16 (21.3%) produced only ST and 10 (13.3%) only LT. The other serotypes which produced ET were O1, O25, O55, O57 and O60 (Table 3).

TABLE 1

Distribution of different pathogens isolated from 119 cases of UTI

Species	No (%)
<i>Escherichia coli</i>	75 (63%)
<i>Klebsiella spp</i>	19 (15.9%)
<i>Proteus spp</i>	6 (5.1%)
<i>Pseudomonas spp</i>	6 (5.1%)
<i>Staphylococcus aureus</i>	6 (5.1%)
<i>Streptococcus faecalis</i>	5 (4.2%)
<i>Serratia spp</i>	1 (0.8%)
<i>Providentia spp</i>	1 (0.8%)
Total	119 (100%)

Discussion

Occurrence of *E. coli* and *Klebsiella spp* as most prevalent in the present series is similar to findings reported in literature earlier. In general *E.*

TABLE 2
Serotypes of 75 *Escherichia coli* isolated

Serotype	No of sampleS (%)
O5	22 (29.3)
O17	4 (5.3)
O25	4 (5.3)
O60	3 (4.0)
O1	3 (4.0)
O57	2 (2.6)
O2	2 (2.6)
O15	1 (1.3)
O20	1 (1.3)
O22	1 (1.3)
O35	1 (1.3)
O55	1 (1.3)
Rough	17 (22.6)
Non typable	13 (17.3)
Total	75 (100%)

TABLE 3
Correlation between serotype and enterotoxin production

Serotype	No. of isolates	LT & ST	Only LT	Only ST	Percent
O5	22	1	5	4	45.5
O17	4	—	—	—	Nil
O25	4	—	1	—	25.0
O60	3	1	—	—	33.0
O55	1	—	1	—	100.0
O1	3	—	—	2	66.0
O57	2	—	—	1	50.0
O2	1	—	—	—	Nil
O15	1	—	—	—	Nil
O20	1	—	—	—	Nil
O22	1	—	—	—	Nil
O35	1	—	—	—	Nil
Rough	17	—	2	7	52.9
Untypable	13	1	1	2	30.7
Total	75	3	10	16	

LT = Heat-labile enterotoxin; ST = Heat-stable enterotoxin

coli is commoner in domiciliary UTI cases (70-90%) than in the nosocomial UTI (50-55%) [1,5].

In one of the earlier Indian studies [4], out of 392 strains, 264 were typeable, 28 untypeable and 6 were rough. O20 was the commonest of the typeable strains followed by O78. Studies carried out

by workers abroad [9] did not show any isolate of serotype O5, whereas this has been consistently found by Indian workers [10-11] including the present study. This reflects the prevalence of this serotype in the Indian population. In the study by Prabhu et al [11] serotype O5 was isolated in 1.7 per cent of UTI cases. The other O serotypes that are found commonly in UTI viz. O1, O2, O17, O22 and O25 were also isolated. O1 occurred in 4 per cent cases in this study, which is consistent with other Indian studies [10,11].

Klipstein et al [12] in their study of enterotoxigenicity of *E. coli* isolated from urine cultures found that 46.2 per cent were enterotoxigenic. The toxins were of intermediate potency. This is corroborated by the present study where the result of ST showed that 96 per cent were weakly positive (ratio in the range of 0.07-0.09). Though ST is poorly antigenic, recently Ram et al [13] detected ST by ELISA, when ST had been made antigenic either by combining with bovine IgG/albumin or by polymerization with toluene. By this method they detected ST in 392 (70.4%) out of 552 cases of diarrhoea. In their study ELISA was marginally more sensitive than the classical suckling mouse model suitable for screening and quantitation, but the problem is lack of availability and considerably higher cost. Result of test for LT showed that, only 15 per cent of the strains were strongly positive. Since O5 is most prevalent and 45.5 per cent strains are enterotoxigenic, they retain the virulent potential UTI.

Studies on correlation between serotypes and ET production of *E. coli* for UTI are limited. Therefore, most of the correlations are done from *E. coli* isolates from diarrhoea cases. In these studies the common ET serotypes were O6, O8, O15, O25, O27, O63, O78, O115, O148, O153, O159 and O167 [5,13]. In this study O5 was a major ET producer (45.5%), followed by serotypes O17 and O25, which agrees with the study by Basu [11]. More recently, highly sensitive DNA probes 850 bp for LT and synthetic sta II probe for ST, have been used for detection of ET [3], where 1.5 per cent of 262 strains were producers of ET. Shovarams study [13], using co-agglutination test for LT and ELISA for ST, showed that maximum number of O78 strains i.e. 30.3 per cent out of 30 isolates, produced ET followed by O114 (20%).

As ETEC are more prevalent in developing countries they retain the potency to produce ET while causing UTI and can spread in a close environment producing new cases of diarrhoea. In India O5 serotype is the most common strain both in UTI and ETEC related diarrhoeas. Since LT production is ent plasmid mediated simultaneous carriage of other plasmid mediated properties, like aerobactin mediated iron uptake and colonization factor antigen which contributes greatly to virulence may also be present [5]. However more studies are required to be carried out in this direction.

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