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RESEARCH ARTICLE

SEROTYPES, HEMOLYSIN PRODUCTION AND DRUG RESISTANCE AMONG UROPATHOGENIC *ESCHERICHIA COLI* ISOLATED AT A TERTIARY CARE HOSPITAL IN MUMBAI

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ABSTRACT

Background & Objectives: Uropathogenic *Escherichia coli* (UPEC) harbors virulence properties which are usually absent in non-pathogenic *E. coli.* The study was undertaken to characterize the properties like hemolysin production, serotypes and multi drug resistance (MDR) in routinely administered antimicrobial drugs in the UPEC isolates.

Design & Methods: Laboratory confirmed 105UPEC isolates, obtained by semi quantitative culture method from clinically symptomatic cases of urinary tract infections (UTIs) along with fecal isolates of *E. coli* from 50 healthy individuals were included as controls. All isolates were subjected to serotyping and haemolysin production on sheep blood agar. Antibiorgram was done using modified Kirby-Bauer's disc diffusion method by the standard Clinical & Laboratory Standard Institute (CLSI) guidelines.

Results: All 105 serotyped strains showed high prevalence to O25 followed by O120 and others. Total 25 different serotypes were observed. Hemolysin production observed in 14 (13.33%) of uroisolates and 3 (6%) of control strains. MDR was found in 47 (44.76%) isolates with preponderance to 3 classes: Ampicillin, Co-trimoxazole and Gentamycin. No correlation between serotypes, haemolysin production and MDR was found.

Interpretation & Conclusion: Antimicrobial susceptibility profiles are necessary to be evaluated in India where a severe misuse of antibiotics at all levels can be seen. Stringent policies of antimicrobial use and infection control in all hospitals is a need of the hour. Detection of hemolysin production, serotyping and MDR in the isolates is reasonably easy and screening them in clinical microbiology laboratory of a tertiary care hospital is a worth consideration.

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INTRODUCTION

'Urinary tract infections (UTIs) are defined as the presence and active multiplication of microorganisms within the urinary tract. This is one of the commonest presentation ofbacterial infections seeking treatment in clinical practice' (Banerjee, 2009 and Sharma, 1997). *Escherichia co*li is the predominant bacteria usually originate in the fecal flora (Shrikhande *et al*., 1999 and Raksha *et al*., 2003)."Certain serotypes of *E. coli* are consistently associated with uropathogenicity and are designated as uropathogenic *E.coli* (UPEC)" (Raksha *et al*., 2003; Kausar *et al*., 2009; Vagrali, 2009 and Fule *et al*., 1990).

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Such UPEC harbors virulence properties which are usually absent in non-pathogenic *E. coli.* The appearance of different serotypes varies from place to place and time to time. Though the etiology of UTI is not clearly understood, it has been stated that the various virulence –associated characteristics might be contributing to infectivity independently or in together (Fule *et al*., 1990).Spectrum of manifestations can be seen as: pyelonephritis, blood stream infection, asymptomatic bacteriuria and symptomatic cystitis (Naveen and Mathai, 2005). Apart from geographical variation, there is an established correlation reported in various virulence factors and prevalent serotypes (Fule *et al*., 1990). 'The UPEC strains are responsible for the majority of UTIs that occur in 70-90% of the seven million cases of acute cystitis and 2, 50,000 cases

of pyelonephritis reported annually in the United States' (Foxman and Brown, 2003 and Griebling, 2007).

Both community and public health resources have been drastically affected by these organisms socially as well as economically (Harding and Ronald, 1994).It is routinely practiced in almost all UTI cases, to start an empirical antimicrobial treatment before the availability of laboratory results of urine culture (Molina-Lopez *et al*., 2011). However, before initializing and optimizing the use of UTIs empirical antibiotic therapy,it is necessary that clinicians should have the knowledge of susceptibility patterns of UPEC strains in that specific geographical locations or populations (Bours *et al*., 2010). The increased incidence of multi drug resistant (MDR) *E.coli* strains to commonly used antimicrobial agents do complicate the clinical management of UTIs (van de Sande-Bruinsma *et al*., 2008). The occurrence of *E.coli* strains that are resistant to fluoroquinolones, trimethoprim and cephalosporins have been increased in recent years. Such strains have special clinical importance due to the availability of limited therapeutic options (Paterson and Bonomo, 2005).

This study was undertaken with an aims and objective to study the prevalence and possible relationship between UPEC characteristics such as haemolysin production and antibiotic drug resistance in the strains isolated in our tertiary care hospital. Also to know the distribution of different O serotypes of *E. coli* in and around Mumbai.

MATERIALS AND METHODS

A total of 105 *E. coli* isolates recovered fromclinically symptomatic UTI cases of all age groups attending outpatient department of Sir J.J. Hospitals, Mumbai were studied over a period of two years from December 2009 to December 2011for the various serotypes and hemolysin production in the Department of Microbiology, Grant Government Medical College. Fecal isolates of *E.coli* from 50 healthy individuals were included as controls. Urine samples were processed as per the standard protocol. All urine samples having growth of single morphotype of colonyon MacConkey's agar with counts 105 colonies/ml were considered significant (Campus *et al*., 1994).A count 104-105 colonies/ml was considered "probably significant" and a count of <104 colonies/ml as insignificant growth of perineal or urethral flora (Campus *et al*., 1994).The additional features noticed in terms of colony morphology were, whether they were mucoid/nonmucoid and biochemical reactions, whether showed hemolysis on sheep blood agar after overnight incubation, whether they were typical and atypical isolates. An isolate was considered as typical if it was a lactose fermenter and aerogenic and atypical if it was non-lactose fermenter and anaerogenic. Atypical isolates were further tested for sorbitol, cellobiose and adonitol fermentation. Thus, lactose fermenting *E.coli* strains by standard biochemical tests (Forbes *et al*., 2002) obtained from cases of significant or probably significant counts were screened for virulence markers. The isolates were maintained by inoculating into nutrient agar deeps and preserved at 4^0C.The cytolytic protein toxin secreted by most hemolytic *E.coli* isolates is known as alpha haemolysin (Cavalieri *et al*., 1984). The isolates were sub cultured on sheep blood agar plates and incubated overnight at 37^0C. 'Hemolysis was detected by determining a clear hemolyticzone (β- hemolysis) around or beneath each colony (Raksha *et al*., 2003; Naveen and Mathai, 2005 and Fatima *et al*., 2012).Isolates stored on nutrient agar were sent to National *Salmonella* and *Escherichia* Center, Central Research Institute, Kasauli (HP) for 'O' serotyping. In-vitro antibiotic susceptibility testing was done with 10 antimicrobial agents using modified Kirby-Bauer's disc diffusion method on Muller Hinton Agar plates. The plates were incubated at 37^0C for 18-24 hours. Isolates were characterized as sensitive, intermediate and resistant based on diameter (size measured in millimeters) of the Zones of Inhibition (ZOI);in accordance with the Clinical and Laboratory Standard Institute (CLSI) Guidelines (CLSI, 2010). The commercial antibiotic discs (Hi Media Laboratories, Mumbai) used in the present study were Ampicillin (10µg/ml),Co-trimoxazole(25µg/ml), Gentamycin (10µg/ml), Norfloxacin (10µg/ml), Nitrofurantoin (300µg/ml), Ciprofloxacin (5µg/ml), Amikacin (30µg/ml), Cefazolin (30µg/ml), Meropenem (10µg/ml).*Escherichia coli* ATCC 25922, *Pseudomonas aerug* inosa ATCC 27853 were used as controls (Raksha *et al*., 2003 and Molina-Lopez *et al*., 2011).
MDR was defined as resistance to three or more different classes of antibiotics. Chi square test was used to analyze the data statistically. EPI Info version 5 was used for this analysis (Molina-Lopez *et al*., 2011).

OBSERVATIONS AND RESULTS

A total of 105 *E.coli* isolates were selected from symptomatic cases of UTIs. These were investigated for their serotype, haemolysin production and MDR among the strains. Fecal isolates of *E. coli* from 50 healthy individuals were included as controls. Haemolysin production was observed in 14 (13.33%) of urinary isolates and 3 (6%) of control strains. The difference between cases and controls for haemolysin production was highly significant ($p<0.001$). Serotyping of 105 strains revealed that they belong to 25 diverse O-antigen type able groups (Table1).

Table 1. Shows distribution of *E.coli* serogroups

Serogroups	Uropathogenic (N=105)	Fecal (N=50)
O25	29	Nil
O120	14	Nil
UT	12	5
Rough	9	Nil
O60	7	3
O130	6	3
O5	3	Nil
O20	3	5
O100	3	5
O3	2	3
O56	2	Nil
O163	2	Nil
O76,O86	1 each	6,6
O159	1 each	4
O8,O131	1 each	3,3
O6	1 each	2
O88	1 each	2
O9,O44,O55,O63,O78,O166	1 each	Nil

UT: - Untypeable

Among the 25 identified serotypes, the following were the most frequent: O25 with 29 isolates (27.61%), O120 with 14 isolates (13.33%) and UT with 12 isolates (11.42%). The O86

and O166 were two unusual serotypes detected in the present study similar to the study reported by Molina-Lopez *et al*. All 105 studied UPEC strains expressed highest resistance rates to the following: Ampicillin, 98 isolates (93.33%); Co-trimaxazole, 88 (83.80%); Norfloxacin, 84 (80%); Gentamycin, 49(46.66%); Nitrofurantoin, 38 (36.19%); Ciprofloxacin, 19 (18.09%); Cefazolin, 17 (16.19%); Amikacin, 13 (12.38%); Netilmycin, 12 (11.42%) and Meropenem, 3 (2.85%) (Table2).

Table 2. Prevalence of antibiotic drug resistance among 105 UPEC isolates

Antibiotics	Resistance	
	No.	%
Ampicillin	98	93.33
Co-trimaxazole	88	83.80
Norfloxacin	84	80
Gentamycin	49	46.66
Nitrofurantoin	38	36.19
Ciprofloxacin	19	18.09
Cefazolin	17	16.19
Amikacin	13	12.98
Netilmycin	12	11.42
Meropenem	3	2.85

Forty seven (44.76%) isolates were resistant to the three classes of antibiotics: penicillin's (Ampicillin), trimethoprim / sulfamethoxazole (TMP/SMX) (Co-trimaxazole) and aminoglycosides (Gentamycin) of the total tested antimicrobial agents of class fluoroquinolones and cephalosporin's; hence were designated as MDR.

DISCUSSION

UTIs are the most common infectious diseases worldwide affecting all age group peoples including men, women and children. The increased prevalence of MDR *E.coli* strains in the community worsen the prognosis of UTIs. Serious problems do occur in the treatment of MDR strains especially in the poor- resource settings where there is no alternate effective antibiotics is available (Shanthi and Kayathri, 2012). In our hospital set up and probably in every hospital, UTIs are one of the most commonly diagnosed infections, althoughthe causative agents do vary in their antimicrobial susceptibility pattern from time to times and from place to place (Banerjee, 2009). UPEC is receiving an increasing attention due to the high degree of morbidity and mortality of UTIs. "Cell morphology and molecular biology studies have revealed that UPEC express several surface structures and secrete protein molecules; some of them cytotoxic, peculiar to the strains of *E.coli* causing UTI (Raksha *et al*., 2003).''

As a group, strains of *E.coli* causing UTIs can be differentiated from fecal strains by their expression of specific O-polysaccharide antigens. Urinary isolates are more commonly type able with batteries of common O antisera than fecal strains (Molina-Lopez *et al*., 2011). The presence of virulence factors in UPEC strains supports the concept of association of UPEC with urinary pathogenicity (Vagrali, 2009). Hence it is important to identify UPEC from non UPEC isolates in the urinary samples. However, in patients with urological abnormality, *E.coli* with lower virulence can cause infections. In non-pathogenic *E.coli*, virulence properties are absent while they are present in UPEC (Zahera *et al*., 2011). The certain serogroups of UPEC possesses specific virulence properties which increases the uropathogenicity (Fatima *et al*., 2012). Majority of the studies reported that *E.coli* isolates from UTI belonged to a limited number of 'Uropathogenic' serotypes, mainly O1, O2, O4, O6, O7, O8, O18, O25 and O75 (Shrikhande *et al*., 1999; Fule *et al*., 1990; Fatima *et al*., 2012 and Johnson, 1991). However, in many studies, the serotypes O4 and O6 have been found to be the most frequently isolated from urine (Fule *et al*., 1990 and Bhalla and Aggarwal, 1989).In the present study the most frequent serotype was O25 (27.61%), which is well established as associated with UTIs, similar to the findings by Jose Molina-Lopez *et al*. (2011) and Nazish Fatima *et al*. (2012). In the UPEC strains, serogroup O25 has traditionally not been recognized as prevalent worldwide (Molina-Lopez *et al*., 2011). The α-hemolysis is a cytolytic protein toxin usually secreted by the most hemolytic *E.coli* strains can be seen as a zone of lysis on blood agar plates (Smith, 1963). Such hemolytic *E.coli* strains are associated with greater frequency in the serotypes O2, O4, O6, O20 and O25 (Fatima *et al*., 2012).

α-haemolysin is recognized as one of the most important virulence factor, due to its strong pro-inflammatory action which leads to the secretion of chemotaxins and IL-6;who triggers the pathogenesis of renal disease (Fatima *et al*., 2012). For the establishment of acute pyelonephritis, α-haemolysin is not a mandatory parameter, but it facilitates tissue injury and survival of the pathogen in the renal parenchyma. Such injury initiates bacterial entry into the blood stream. UPEC usually has this haemolysin (Naveen and Mathai, 2005), as was observed in our study in 14 (13.33%) of uroisolates and 3 (6%) of control strains. However, the difference between cases and haemolysin producing controls was highly significant ($p<0.001$) similar to Johnson *et al*.(1991)who reported haemolysin production in 38% urinary and 12 % of fecal isolates respectively. In another study conducted by Kausar *et al*. (2009) reported haemolysin production in 42 (21%) of uroisolates and 8 (16%) of control strains. However, in the present study, though the nature of haemolysin was not further characterized it could have a cytotoxic necrotizing factor. Antibiotics have potential to interfere with the attachment of *E. coli* to uroepithelialcells by changing their bacterial surface. The antibiotic resistant strains maintain their capacity of adherence leading to effective colonization, finally leading to an established infection of urinary tract (Fule *et al*., 1990).

In this study, the MDR was found in 47 (44.76%) isolates with preponderance to three classes: penicillin's (Ampicillin), TMP/SMX (Co-trimaxazole) and aminoglycosides (Gentamycin) of the total tested antimicrobial agents of class fluoroquinolones and cephalosporin's. Fluoroquinolones, TMP/SMX or nitrofurantoin are usually recommended for the empirical treatment of uncomplicated UTIs (Molina-Lopez *et al*., 2011). However, nitrofurantoin is considered the most effective drug among all, can be administered orally, represents an alternative for empirical oral therapy of uncomplicated cystitis and hence been recommended by the Infectious Disease Society of America (IDSA) (Warren *et al*., 1999). Here, we found a high level resistance to TMP/SMX

(83.80%) and low level resistance to nitrofurantoin and fluoroquinolones (36.19 % and 18.09 %, respectively) exactly opposite to the study reported by Molina-Lopez *et al*. (2011) who reported high level resistance to fluoroquinolone and TMP/SMX (55.5-60.6 % and 56.2 %, respectively). Here, Ampicillin, Co-trimaxazole, Norfloxacin, Gentamycin used, showed high level resistance. Similar findings were reported by many workers around the world (Banerjee, 2009). The obtained resistance to these antibiotics, might be due to these antibiotics which are in use for a long period and must been abused; as a result the organism might have discovered the mechanisms of shuffling their mode of action (Banerjee, 2009). No correlation between serotypes, haemolysin production and MDR was observed in this study. In the context of the emerging MDR UPEC strains, therapy should be administered only after culture and sensitivity testing. Such practice would help in the proper treatment of individual patient and also reduce the indiscriminate use of the antibiotics and can stop further drug resistance (Raksha *et al*., 2003).These things has to be kept in mind while developing guidelines for UTIs treatment who shows high prevalence of drug resistance among uropathogens (Zahera *et al*., 2011).Our study had shown that susceptibility pattern is necessary to obtain sensitivity reports before starting the antibiotic treatment in suspected UTIs. The laboratory reports should be available to the clinicians before initiating the therapy as the knowledge of drug sensitivity pattern in that particular area provides the guidance regarding the empirical treatment of UTI (Banerjee, 2009).

Conclusion

UPEC strains plays an important role in the aetio-pathogenesis of UTI. Periodic review and Renewal of antibiotic policy along with the periodic review is required for the control of acquiring drug resistant strain. In-depth studies at molecular level are necessary for the better understanding of different virulence factors; as most urovirulent strain express multiple virulence factors simultaneously. The laboratories should be well equipped for better management of these isolates; and should encourage to evaluate an accurate bacteriological record and their antibiogram. We believe that the methods of detection of the virulence markers are reasonably easy and screening them in a clinical microbiology laboratories is a worthwhile exercise.

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