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Hemolysin Production and Antibiogram Pattern of Uropathogenic *E. Coli* Isolates from South India.

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ABSTRACT

Escherichia coli is the most common urinary pathogen accounting for 50% - 90% of all uncomplicated urinary tract infections (UTIs). Most haemolytic *E. coli* secretes alpha haemolysin. A descriptive study was conducted at a tertiary care hospital in South India from June to December 2011 to determine hemolysin production and antibiogram pattern of Uropathogenic *E. coli*. A total of 105 *E. coli* isolates from urine samples were studied. *E. coli* isolates were identified using standard microbiological techniques. They were screened for haemolysin production, determining a zone of lysis around each colony on 5% sheep blood agar plates after overnight incubation. Antimicrobial susceptibility pattern was determined by Kirby-Bauer disc diffusion method. Descriptive statistics like percentages were analyzed. Among 105 isolates tested, 67 were female and the predominant age group affected was between 20- 30 years. Only 10.5% (11 isolates) were found to be hemolytic. 80.9% were found to be sensitive to nitrofurantoin and 75.2% to amikacin, while 80% and 83.8% were found to be resistant to norfloxacin and co-trimoxazole respectively. Haemolysin producing *E. coli* contributed to a small percentage of organisms causing UTI. Nitrofurantoin and amikacin were the most sensitive drugs, which can be recommended as empirical therapy for treatment of UTI.

Keywords: Uropathogenic *Escherichia coli*; urinary tract infections; virulence; haemolysin

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INTRODUCTION

Escherichia coli a common inhabitant of the gastrointestinal tract of humans and is the most common cause of Urinary tract infections (UTI) [1-5]. Certain strains of *E. coli* acquire specific virulence factors which increase their ability to cause a broad spectrum of diseases. The most common infections in humans are urinary tract infections (UTIs) and are caused by bacteria originating often from the faecal and perineal flora [6].

The Extra-intestinal pathotype *E. coli* (ExPEC) strains have the capacity to exist in the gut normally, however, they can disseminate and colonize the blood, the central nervous system, and the urinary tract, thereby producing disease. ExPEC strains includes two pathotypes namely, neonatal meningitis *E. coli* (NMEC) and uropathogenic *E. coli* (UPEC) [1].

E. coli express several important virulence factors which have implications in pathogenesis of urinary infection [1,7-9]. Hemolysin production, which is one of the virulence factors, is associated with pathogenic strains of *E. coli* in humans, especially those causing more clinically severe forms of UTI [1,2]. Most hemolytic *E. coli* strains secrete a cytolytic protein toxin called alpha hemolysin. Approximately a half of UPEC strains causing upper UTIs, and a third causing lower UTIs, produce a hemolysin (HlyA) belonging to the repeat toxins (RTX) family [1,2,7,8]. More than 80% of all UTIs, including both asymptomatic bacteriuria (ABU) and symptomatic UTIs are caused by UPEC.

This study was undertaken to determine the hemolysin production in Uropathogenic *E. coli* and to assess the utility of Fluroquinolones as empirical antibiotic therapy, by determining the susceptibility pattern of these *E. coli* isolates to various antibiotics.

MATERIALS AND METHODS

Study design

Urine samples were collected either as midstream urine samples (MSU) or as catheterized samples, from inpatients as well as the outdoor patients of a tertiary care hospital in south India over a period of 6 months (June 2011 to December 2011). All samples were inoculated into 5% sheep blood agar and cysteine lactose electrolyte deficient media (CLED) (HiMedia, Mumbai, India), using a sterile calibrated loop delivering 0.001 ml of urine. All cultured plates were incubated overnight at 37°C and colony count was performed.

Diagnostic criteria

A total of 105 *E. coli* strains were isolated from these urine samples during the study period. Identification of *E. coli* was done by standard microbiological procedures. Culture results were interpreted as significant if there were more than 10^5 colonies per ml of urine. All *E. coli* isolates were subjected to microscopic and appropriate biochemical tests for proper identification in a systematic way according to standard microbiological techniques.

All isolates were screened for production of haemolysin, a virulence marker of UPEC isolates. This was done by determining the presence of a clear zone of lysis around each colony on 5% sheep blood agar plates after overnight incubation.

Antimicrobial susceptibility profile was determined by Kirby-Bauer disc diffusion method on Mueller Hinton agar (HiMedia) using the following antibiotic discs: Trimethoprim-sulfamethoxazole (1.25/23.75mg), gentamicin (10mg), amikacin (10mg), ceftriaxone (30mg), imipenem (10mg), nalidixic acid (30µg), norfloxacin (30µg) and piperacillin- tazobactum (110 µg). All the plates were incubated overnight at 37°C and zone of inhibition were interpreted according to the standard CLSI guidelines [10]. *E. coli* ATCC 25922 was used as the reference strain for quality control purposes.

Statistical analysis

All the data were compiled and analyzed by MS Excel software. Descriptive statistics like percentages were analyzed.

RESULTS

Among 105 UPEC isolates identified, the highest isolation rates were found to be among females (n=67, 63.8%). The age group of 21- 30 years was predominant among female patients (n=27, 25.7%), followed by the age group of 51-60 years. The lowest isolation rates were found in the age group of 0-10 years (n= 1, 0.95%). In males, the predominant age group affected was between 51-60 years (n=9, 8.6%), followed by the age group of 61-80 years. While the age group of 11-20 years had the lowest isolation rates (n=1, 0.95%). UTI was found to affect females (63.8%) more commonly than males (36.2%). The age distribution is shown in Table-1.

Table 1.Comparison of uropathogenic *E. coli* isolates in males and females in various age groups

Age Interval (years)	Males (n=38)	Females (n=67)
0-10	4	1
11-20	1	11
21- 30	3	27
31-40	2	10
41-50	5	5
51-60	9	7
61-70	7	4
71-80	7	2

Most of the isolates were from the department of Obstetrics and Gynaecology (n = 46, 43.8%), followed by Urology (n= 25, 23.8%). Least number of isolates were from Orthopaedics (n=2, 1.9%), and Pediatrics (n=4, 3.8%). The distribution of isolates from various departments is detailed in Table-2.

Table 2. Distribution of uropathogenic *E. coli* in various departments

Departments	Number of isolates (%)
Obstetrics & Gynaecology	46 (43.8%)
General Surgery	8 (7.6 %)
General Medicine	20 (19 %)
Urology	25 (23.8 %)
Paediatrics	4 (3.8 %)
Orthopaedics	2 (1.9 %)

Out of the 105 isolates, only 10.5% (11 isolates) were found to produce haemolysin. Most of the hemolytic strains were isolated from females (n=8, 72.3%).

Analysis of the antimicrobial susceptibility testing of 105 *E. coli* strains isolated from both inpatients and outpatients with UTI showed that 80.9% were found to be sensitive to nitrofurantoin and 75.2% to amikacin, while 91.4% and 83.8% were found to be resistant to nalidixic acid and co-trimoxazole respectively. Table 3 depicts the antibiotic pattern of *E. coli* strains for various drugs.

Table 3.Antibiotic susceptibility pattern of uropathogenic *E. coli*

Antibiotics	Sensitive	Intermediate	Resistant
Nitrofurantoin	85(81%)	6(5.7%)	14(13.3%)
Amikacin	79 (75.2%)	14 (13.3%)	12(11.43%)
Gentamicin	32(30.5%)	7(6.67%)	66(62.9%)
Ceftriaxone	29(27.62%)	1(0.95%)	75(71.43%)
Norfloxacin	21(20%)	0	84 (80%)
Co- trimoxazole	17(16.2%)	0	88(83.8%)
Nalidixic acid	9(8.6%)	0	96(91.43%)

DISCUSSION

Urinary tract infections (UTI) are one of the most common bacterial infections. The spectrum of UTI varies from asymptomatic bacteriuria to symptomatic cystitis, pyelonephritis and blood stream infections [2]. The most frequent pathogen causing UTI among Gram-negative bacteria is *E. coli*, and is associated with acute renal failure and various urological complications.

Various virulence factors which are involved in the pathogenesis of UTI by UPEC strains can be categorised into two categories, viz. surface virulence factors and secreted virulence factors. Type 1 fimbriae, S fimbriae and F1C fimbriae, capsular material and the endotoxic LPS molecules comprise surface virulence factors [1,7]. Whereas, secreted virulence factors are alpha–haemolysin (α-haemolysin), Cytotoxic necrotising factor 1 (CNF1), Secreted autotransporter toxin (SAT), cytolethal distending toxin (CDT), and low molecular weight siderophores like aerobactin, enterobactin and yersiniabactin [1,7].

Alpha – haemolysin is a pore-forming toxin of the 'repeat toxin' (RTX) family with a target cell spectrum including erythrocytes, leukocytes, endothelial-and renal epithelial cells.[1,8] Similar to streptolysin-O, they induce the synthesis of specific antibodies during infection and of high titres of anti- α haemolytic antibodies could be seen in patients suffering from infection caused by them. These hemolysin molecules insert into lipid-containing membranes, producing cation-selective channels which increase the erythrocyte membranes permeability to Ca2+, K+, mannitol, and sucrose [1,8].

In our study of 105 UPEC isolates, we found that UTI was more frequent in females than males. Among females the, predominant age group affected was between 21- 30 years, followed by the age group of 51-60 years. The reasons for UTI in young females may be due to various factors like, short urethra, sexual intercourse, lack of post coital voiding and usage of diaphragms and spermicides. In postmenopausal women, estrogen deficiency, leads to alteration of the vaginal flora, leading to recurrent UTI's [2,11,12].

We documented the pattern of antimicrobial resistance among our 105 UPEC isolates during the study period of six months. We observed that isolates from both inpatients and outpatients with UTI showed increased sensitivity to nitrofurantoin (80.9%) and amikacin (75.2%). Similar findings were observed in various studies also [9,13,14]. The reason for high sensitivity to nitrofuratoin, may be due to their narrow spectrum of activity, decreased prescription of these drugs and their narrow tissue distribution [15]. Though the drug has side effects it is still an important first – line drug against UTI before culture and sensitivity as, it achieves 85% higher cure rates and serum concentrations than either co-trimoxazole or quinolones [3,4]. These findings suggest there is merit in antibiotic cycling.

Amikacin is a drug administered parenterally in the hospital settings to treat severe and complicated UTIs. Hence they are less frequently used and this may contribute to its high sensitivity. In the present study, amikacin showed the second highest sensitivity rates, namely 75.2% [12,16].

We observed that resistance rates were higher among antimicrobials that have been used for long as empirical choice like co-trimoxazole. Similar findings were documented by various studies [5,16,17]. This may be due to increased consumption of these antibiotics, self-medication, transfer of resistant isolates and non-compliance with medications [4,9,11,15]. During the past decade the prevalence of *E. coli*'s resistance to co-trimoxazole has increased, posing a significant problem, as this agent is frequently prescribed for uncomplicated UTI in many developed and developing countries. Now The Infectious Diseases Society of America (IDSA) recommends, co-trimoxazole as a first line of therapy of uncomplicated UTI's in regions where resistance is below 20%, otherwise an alternative therapy with a Fluoroquinolone, especially ciprofloxacin and nitrofurantoin, or fosfomycin is recommended [16]. Fluoroquinolones are considered to be the first-line treatment in patients with pyelonephritis or complicated UTIs. In our study, resistance to nalidixic acid was found very high (91.43%) and norfloxacin showed a resistant rate of 80%.This was in accordance with the results of various other studies [13,15-17]. Thus, Fluroquinolones are not useful as empirical therapy for UTIs. Instead, Nitrofurantoin could be considered as an antibiotic choice for empirical therapy.

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CONCLUSIONS

Haemolysin producing *E. coli* contributed only to a small percentage of organisms causing UTI. Fluroquinolones can no longer be used as empirical choice for therapy, instead Nitrofurantoin and Amikacin can be recommended.

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