

Original Research Article

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Microbiological Profile of Urinary Tract Infections with special Reference to Antibiotic Susceptibility Pattern of *Escherichia coli* Isolates

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ABSTRACT

Urinary tract infections, caused by multidrug resistant uropathogenic *Escherichia coli* (UPEC) are alarmingly escalating. Widespread utilization of empirical antibiotics without performing anti-microbial susceptibility testing led to expanding resistance to different classes of antimicrobial agents generally prescribed among UPEC isolates in our country. Therefore, this study aims to detect the prevalence and the antibiotic susceptibility pattern of UPEC isolates, in Tanta University Hospitals, Egypt. A total of 200 urine samples were collected from July 2015 to August 2016. Urine count and culture were done. Antibiotic susceptibility testing was performed according to the clinical and laboratory standards institute guidelines (CLSI 2016). *E. coli* was the predominant isolate (53.85%) followed by *Klebsiella* spp. (17.58%), *Candida* spp (10.99%), *Proteus* spp. (6.04%) *Coagulase negative Staphylococcus* (3.85%) *Enterococcus* spp. (2.75%), *Pseudomonas* spp. (2.19%), *Acinetobacter baumannii* (1.65%), *Enterobacter* spp. (0.55%) and *Serratia marcescens* (0.55%). The highest percentage of UPEC isolates were non susceptible to Ampicillin, Cefazolin (100%) for each, followed by Nalidixic acid (91.84%), Trimethoprim/sulfamethoxazole (87.9%), Norfloxacin (82.65%), beta-lactam inhibitors (81.3%), Ceftriaxone (79.60%), Cefotaxime (74.49%), Ceftazidime (64.3%), Gentamicin (26.53%), Amikacin (22.45%), Nitrofurantoin (21.4%) and Imipenem (20.41%). Therefore, physicians must follow antibiotic stewardship and change their treatment pattern based on performing antibiotic susceptibility testing.

Keywords

Urinary tract infections
Uropathogenic *E. coli*,
Antimicrobial resistance, Antibiotic susceptibility,
Antibiotic stewardship

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Introduction

Urinary tract infections (UTIs) are a standout amongst the most widely recognized bacterial infections in hospital and community based setting around the world (Maleki *et al.*, 2017). This infection is applied to a variety of clinical conditions ranging from asymptomatic

bacteriuria, to symptomatic UTI, to severe kidney infection with consequential sepsis, requiring hospitalization (Kumar *et al.*, 2016a).

This type of infection is distressingly rising because of the development of drug resistance of causative pathogens (Chander, 2016).

Escherichia coli is the most widely recognized Gram-negative extra intestinal pathogen isolated from urine culture in patients with UTIs, whether complicated or uncomplicated, accounting for 70–80 % of community-acquired infections and 40– 60 % of healthcare-associated infections (Hisano *et al.*, 2015). Uropathogenic *Escherichia coli* (UPEC) utilize a variety of virulence factors such as surface structural components, like lipopolysaccharide, capsule, flagella, pili, outer membrane proteins, as well as secreted toxins and siderophore. Consequently, these mechanisms are double edged weapon as they play an important role in UTI pathogenesis furthermore; they are attractive candidates for the development of new drugs and vaccines (Werneburg *et al.*, 2015 & O'Brien *et al.*, 2016).

Worldwide, Antimicrobial resistance among UPEC is a major health concern because of its expanding resistance to different classes of antimicrobial agents generally prescribed (Polse *et al.*, 2016).

The increasing resistance to several broad-spectrum antibiotics, utilized in the treatment of UTIs, for example (ampicillin, trimethoprim/sulfamethoxazole, fluoro - quinolons, and cephalosporin), among clinical UPEC strains complicates treatment, increases costs, and decreases the effectiveness of the available antibiotics against this infection (Biedenbach *et al.*, 2016).

Hospitalized patients in urology department have a tendency to acquire UPEC infections with higher antimicrobial resistance (Lavigne *et al.*, 2016).

The proper selection of empirical antimicrobials and the effectiveness of antibiotic treatment necessitate a good understanding of the typical bacteriology involved in UTIs as well as the local

resistance patterns, and the specific patient's antimicrobial and microbiologic history (Bartoletti *et al.*, 2016).

Studies show that the physicians prescribe antibiotics such as trimethoprim/sulfamethoxazole, cephalosporins and fluoroquinolones in spite of the expanding bacterial resistance to these drugs (Schmiemann *et al.*, 2012).

There is limited information about the antibiotic resistance patterns among UPEC isolates in our hospital. Therefore, this study aims to detect the prevalence and antibiotic susceptibility patterns of UPEC strains isolated from patients admitted to the urology department in Tanta university hospitals.

Materials and Methods

This study was carried out on 200 patients (100 inpatients and 100 outpatients) showing clinical pictures of urinary tract infections admitted to the Urology Department and outpatient urology clinics in Tanta University Hospitals, during the period of research from July 2015 till August 2016.

The study incorporated patients who admitted to the hospital and were supposed to have UTI and who gave their informed consent.

Ethical approval for this study was provided by ethics and research committee, Faculty of Medicine, Tanta University.

Collection of samples

Clean-catch midstream urine was collected into a wide mouth sterile container. Proper instructions were given to the patients regarding the method of collection. Later samples were labeled with patient's age and sex on container. The samples were transported to the laboratory of microbiology

and processed within 30 minutes for isolation and identification of the causative pathogens.

Urine count

Semi quantitative culture of urine samples on Cysteine lactose electrolyte deficient (CLED) agar (Oxoid, UK) was used for diagnosis of significant bacteruria or candiduria. The semi quantitative culture was done by inoculating a loopful 1µl of well mixed uncentrifuged urine using a sterile calibrated bacteriological loop on CLED agar by surface streak method and colonies were counted after overnight incubation at 37°C aerobically. The number of colonies obtained was multiplied by 1000 to obtain the CFU/mL.

The significant colony count is a pure growth of $\geq 10^5$ CFU/mL in case of bacteriuria and $\geq 10^4$ CFU/mL in case of candiduria (Collee *et al.*, 2006).

Isolation and identification of uropathogens

Cultures of urine samples were done for cases with significant urine count; these cultures were done on blood, MacConkey's agars and sabouraud dextrose (Oxoid, UK). The plates were inoculated using a sterile bacteriological loop then all plates were incubated aerobically at 37 °C for 24 hrs (Cheesbrough, 2006).

The produced colonies were identified by the traditional microbiological methods including microscopic examination, culture characteristics and biochemical reactions for detection of the causative organisms UPEC isolates were confirmed by using integral system enterobacteri (Liofilchem Italy), which is a 24-well system containing desiccated biochemical and antibiotic substrates for the biochemical identification and susceptibility testing of enterobacteria. Briefly the system was inoculated with the bacterial suspension of the micro-organism under examination and

incubated at 36 °C \pm 1°C for 18-24 hours. The tests for identification and for the susceptibility testing are interpreted by assessing the change in color of the various wells additionally antimicrobial susceptibility profile was confirmed by disk diffusion method.

Antimicrobial susceptibility testing for *E. coli* isolates

Kirby-Bauer disc diffusion method

Antibiotic susceptibility testing of the UPEC isolates were done by Kirby-Bauer disc diffusion method using Clinical Laboratory Standards Institute guidelines (CLSI, 2016) criteria on Muller-Hinton agar (Oxoid, UK).

In this study 7 groups of antibiotics were used which were quinolones, cephalosporins, aminoglycosides, aminopenicillin, carbapenems, sulfonamides and nitrofurantoin.

The used antibiotics discs (Oxoid, UK) were Amikacin (30µg), Gentamicin (10µg), Trimethoprim / Sulfamethoxazole (1.25/23.75 µg), Cefazolin (30µg), Cefotaxime (30µg), Imipenem (10µg), Ampicillin (10µg), Cefoxitin (30 µg), Ceftriaxone (30µg), Ceftazidime (30µg) Cefpime (30 µg), Nitrofurantoin (300 µg), Amoxicillin / Clavulanic acid (20/10µg), Nalidixic acid (30µg) and Norfloxacin (10µg).

American Type Culture Collection (ATCC) strain *E. coli*. ATCC 25922 was used as quality control strain in antimicrobial susceptibility testing.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-

Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

Results and Discussion

Characteristics of study participants

Table 1 gives demographic data and clinical characteristics of the study population. The patient ages ranged between 24 and 65 years with mean \pm SD of age 47.49 ± 9.26 . Of 200 cases, 73 (36.5%) were male and 127(63.5%) were female.

As regard to the risk factors for UTIs, urinary stones (60.0%), diabetes (36.3%) and prostatic hyperplasia (11.1%) were the important risk factors for developing UTI other risk factors were nephropathy (8.1%), cancer bladder (5.9%), cancer prostate (4.4%), renal failure (4.4%) and hydronephrosis (3.0%). (21.5%) of patients were suffered from hypertension, ascites, cholangiocarcinoma, hepatic encephalopathy and cancer colon.

Prevalence and distribution of uropathogens

The urine count of 200 urine samples on CLED agar yielded a total of 172 (86.0%) significant bacterial growths including 162 (94.19%) (Single organism) and 10 (5.81%) mixed infection (Two organisms).

The 172 culture positive samples of urine yielded 182 bacterial isolates including both single growth (162) and mixed growths (10) of two organisms each. Aerobic Gram-negative bacteria were the dominant pathogens (n=150; 82.42%) Enterobacteriaceae are the predominant pathogens (n=143; 78.57%), while 7 (3.85 %)

strains belonged to the non-fermenting rods (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*). Gram-positive cocci (*Enterococcus* spp and *Coagulase negative Staphylococcus*) were isolated from 12 patients and accounted for 6.6 % of all the isolates. Regarding to fungal infection, *Candida* was isolated from 20 patients accounting (10.99 %) as shown in table 2.

E. coli was the predominant isolate (53.85%) followed by *Klebsiella* spp. (17.58%), *Candida* spp (10.99%), *Proteus* spp. (6.04%) *Coagulase negative Staphylococcus* (3.85%) *Enterococcus* spp. (2.75%), *Pseudomonas* spp. (2.19%), *Acinetobacter baumannii* (1.65%), *Enterobacter* spp. (0.55%) and *Serratia marcescens* (0.55%). Mixed infection showed combination of *E. coli* and *Klebsiella* spp in 3 cases. *E. coli* and *Candida* in 1 case *Klebsiella* and *Candida* in 2 cases. *Candida* and *Enterococcus* spp in 2 cases. *E. coli* and *Acinetobacter baumannii* in 2 cases respectively.

Antibiotic susceptibility pattern of UPEC isolates

According to the antibiogram of the isolated UPEC by disc diffusion method, The highest percentage of UPEC isolates were resistant to Ampicillin, Cefazolin (100%) for each, followed by Nalidixic acid (91.84%), Trimethoprim/sulfamethoxazole (87.9%), Norfloxacin (82.65%) and beta-lactam inhibitors (81.3%), Ceftriaxone (79.60%), Cefotaxime (74.49%), Ceftazidime (64.3%), Gentamicin (26.53%), Amikacin (22.45%), Nitrofurantoin (21.4%) and Imipenem (20.41%) as shown in table 3.

In the current study, regarding age distribution, a higher frequency of UTIs was in the age 24 to 65 years. The mean \pm SD was 47.49 ± 9.26 years.

Our result is inconsistent with the study of (Lavigne *et al.*, 2016) who found that the mean \pm SD of age of UTI cases was 65.8 \pm 18.2 years this difference may be attributed to the difference in selection of patients.

Female are more liable to develop UTI than males. This result is in accordance with other studies showed that females have a higher frequency of UTI compared to males (Salunke and Gidamudi, 2017; Mangal *et al.*, 2017) reported similar finding.

Females are more susceptible to UTI because of anatomical reasons or other host factors. The anatomical site of the urogenital tract in females is in charge of bacterial contaminations caused by both internal and external flora (Shanthi and Kayathri, 2012). Diagnosis of UTIs is a good example of the need for close collaboration between the clinician and the microbiologist.

In the present study, the prevalence of UTI was 86.0%. This result is in agreement with the study of (Moue *et al.*, 2015) who reported that prevalence rate of isolation of UTI was 79.5 %.

Our result also more or less correlates with the study of (Haque *et al.*, 2015) who found that, the prevalence of UTIs was 59.67 % (including 94.41 % unimicrobial and 5.59 % mixed infection by two organisms).

In comparison to our result; (Pratap *et al.*, 2016) found that prevalence rate of UTI was 32.5%.

This difference may be attributed to the difference in the transport of samples, in the number of selected cases, the media selection and local prevalence rate. Furthermore, general condition of the patients, even the standard personal hygiene and education status may be responsible.

The current study observed aerobic Gram-negative bacteria were the dominant pathogens with Enterobacteriaceae the predominant pathogens

This finding was in agreement with previous studies study done by (Stefaniuk *et al.*, 2016; Kumar *et al.*, 2016 b) in which similar results were reported.

E. coli was the predominant isolate followed by *Klebsiella* spp., *Candida* spp., *Proteus* spp., *Coagulase negative Staphylococcus*, *Enterococcus* spp., *Pseudomonas* spp., *Acinetobacter baumannii*, *Enterobacter* spp., and *Serratia marcescens*

This result is in agreement with the previous studies of (Salunke and Gidamudi, 2017; Sanjee *et al.*, 2017; Vranic *et al.*, 2017; Wong *et al.*, 2017) in which similar organisms were isolated, with *E.coli* was the most prevalent isolate

The highest rate of isolation of *E. coli* causing UTI may be attributed to most of the bacterial organisms causing UTI originate from the faecal flora and among these facultative anaerobes, *E. coli* constitutes the major portion superimposed by various virulence factors that facilitate the ascent of bacteria from faecal flora, up the urethra into the bladder They have several factors responsible for their attachment to uroepithelium such as adhesins, pili and fimbriae (Das *et al.*, 2006). According to the antibiogram of the isolated UPEC The highest percentage of UPEC isolates were resistant to ampicillin, and cefazolin (100%) for each, followed by trimethoprim/sulfamethoxazole (87.9%), beta-lactam inhibitors (81.3%), ceftriaxone (79.60%), cefotaxime (74.49%), ceftazidime (64.3%). The higher rate of sensitivity among UPEC isolate were towards amikacin (77.55%), imipenem (76.53%), nitrofurantoin (75.5%) and gentamicin (71.43%).

Table.1 Demographic data and clinical characteristics of studied group of suspected UTIs (n=200)

Demographic data	Outpatients (100)	Inpatients (100)	Total (n=200)	P Value
Sex				
Male	20 (20%)	53(53%)	73(36.5%)	X ² p <0.001*
Female	80(80%)	47(47%)	127(63.5%)	
Age (y)				
Min. –Max.	24.0 -56.0	40.0 -65.0	24.0 – 65.0	t _p <0.001*
Mean± SD	42.03 ± 7.96	52.96±6.97	47.49 ± 9.26	
Antibiotic intake				
Yes	58(58%)	100(100%)	158(79%)	X ² p <0.001*
No	42(42%)	0(0%)	42(21%)	
Underlying risk factors				
Yes	35(35%)	100(100%)	135(67.5)	X ² p <0.001*
No	65(65%)	0(0%)	65(32.5)	
Culture result				X ² p =0.103
Significant growth	90(90.0%)	82(82.0%)	172(86.0%)	
Non-significant growth	10(10.0%)	18(18.0%)	28(14.0%)	

X²p: p values for Chi square test, t_p: p values for Student t-test *: Statistically significant at p ≤ 0.05 and highly significant at p ≤ 0.001*

Table.2 Distribution of uropathogens isolated from positive urine culture of both group

Organism in UTIs		No. of isolates (n= 182)%	Outpatients (n=90) N%	Inpatients (n=92)N%	x ² p value
Gram –ve (n=150) (82.4%)	<i>Escherichia coli</i>	98 (53.85%)	56(62.22%)	42(45.65%)	0.299
	<i>Klebsiella spp</i>	32(17.58%)	14(15.56%)	18(19.56%)	
	<i>Proteus spp</i>	11(6.04%)	3(3.33%)	8(8.7%)	
	<i>Pseudomonas aeruginosa</i>	4(2.19%)	1(1.11%)	3(3.26%)	
	<i>Acinetobacter baumannii</i>	3(1.65%)	1(1.11%)	2(2.17%)	
	<i>Enterobacter spp</i>	1(0.55%)	0(0%)	1(1.1%)	
	<i>Serratia marcescens</i>	1(0.55%)	0(0%)	1(1.1%)	
Gram +ve (n= 12)(6.6%)	<i>Coagulase negative Staphylococcus</i>	7(3.85%)	5(5.56%)	2(2.17%)	
	<i>Enterococcus</i>	5(2.75%)	2(2.22%)	3(3.26%)	
Fungi (n= 20) (10.99%)	<i>Candida spp.</i>	20(10.99%)	8(8.89%)	12(13%)	
Total		182(100)	90(100%)	92(100%)	

Table.3 Antibiotic susceptibility of isolated UPEC strains disc diffusion method (n=98)

Antibiotics (µg)	Symbol	Sensitive(mm) (N)%	Intermediate (mm) (N)%	Resistant(mm) (N)%
Amikacin(30)	AK	76 (77.55)	-	22 (22.45)
Gentamicin (10)	CN	70 (71.43)	2 (2.04)	26 (26.53)
Trimethoprim/ sulfamethoxazole (25)	SXT	9 (9.9)	2 (2.02)	87 (87.9)
Nitrofurantoin (300mcg)	F	74 (75.5)	3 (3.1)	21(21.4)
Ampicillin (10µg)	AMP	-	-	98 (100.0)
Cefazolin (30µg)	CZ	-	-	98 (100.0)
Cefotaxime (30µg)	CTX	25(25.51)	-	73 (74.49)
Ceftazidime (30 µg)	CAZ	30 (30.6)	5 (5.1)	63 (64.3)
Ceftriaxone(30µg)	CRO	20 (20.40)	-	78 (79.60)
Cefoxitin (30g)	FOX	35 (35.71)		63 (64.29)
Cefepime	FEP	37(37.76)		61(62.24)
Amoxicillin / clavulanic acid (20/10µg).	AMC	24 (18.7)	-	74 (81.3)
Imipenem (10µg)	IPM	75 (76.53)	3(3.06)	20(20.41)
Nalidixic acid	NAL	8(8.16%)	0	90(91.84%)
Norfloxacin	NOR	17(17.35%)	0	81(82.65%)

The study of (Maleki *et al.*, 2017) found that the UPEC isolates were most resistant to ampicillin (82.9%), cefalotin (78.1%), and tetracycline (61.1%). Antibiotic resistance to cefazolin (49.5%), ceftriaxone (38.2%), cefotaxime (30.2%), ceftazidime (26.1%), ciprofloxacin (42.2%), and cotrimoxazole (60.1%). The isolates were most sensitive to nitrofurantoin (95.9%), gentamicin (77.2%), and amikacin (71.5%)

This finding is in agreement with the study of (Thangavel *et al.*, 2017) who reported that the higher rate of sensitivity among UPEC isolate were towards Amikacin (96.3 %), Imipenem (96.3 %), and Nitrofurantoin (89.7%). On the other hand, the study of (Chander, 2016) high resistance levels were detected against gentamicin (91.11%) while Nitrofurantoin recorded the least resistance levels (13.33%) among the *E. coli* isolates.

In comparison to our result (Munkhdelger *et al.*, 2017) found that antibiotic resistance was cephalotin (85.1%), ampicillin (78.4%), and trimethoprim/sulfamethoxazole (70.9%) in

the UPEC strains. Sensitivity values above 50% were found to gentamicin (57.4%), ciprofloxacin (58.1%), ceftazidime (66.2%), cefuroxime (76.4%), cefoxitin (93.9%), nitrofurantoin (94.6%), and imipenem (98%).

(Munkhdelger *et al.*, 2017) explained the higher sensitivity rate towards nitrofurantoin due to the lower frequency of the use of this drug. Regarding to resistance to nalidixic acid our result also is in agreement with (Haque *et al.*, 2015) who reported similar result

The susceptibility patterns of *E.coli* against different antibiotics vary in different geographical regions, eventually leading to empirical therapy which is based on the local susceptibility profiles. Bacterial biofilm are often associated with long-term persistence of organism in various environments. Bacteria in biofilm display dramatically increased resistance to antibiotics (Tajbakhsh *et al.*, 2016).

The highest rate of resistance among UPEC is mainly due to unwise use of antibiotics. The

increased accessibility of antibiotics probably an important factor in antibiotic resistance, Discontinuation of treatment due to negligence of patient or unavailability of drugs also develops antibiotic resistance (Chander, 2016).

The practice of prescribing antibiotics to treat UTI without bacterial characterization led to increased resistance among uropathogens and to decrease infectiveness of oral therapies. Despite clinical symptoms of UTIs have been ameliorated by numerous antibiotics, UPEC persistence and resistance to antibiotics represent a serious problem (Blango and Mulvey, 2010). Therefore important measures are mandatory to ensure rational and well-informed use of antibiotics; Antibiotic stewardship should be introduced in clinical practice to avoid the risk of infection episodes in which the urologist cannot risk systemic inflammatory response syndrome or sepsis (Bartoletti *et al.*, 2016).

In conclusion, the high incidence rate of UTIs reported in this study should be of great concern, as not only do UTIs pose a threat to health, but they also impose an economic and social burden. Increasing rate of resistance to commonly used antibiotics is an alarming sign for future of healthcare sector. Injudicious use of antibiotics could be the most important reason for such high rate of increasing resistance. Therefore, empirical treatment should be carefully selected based upon the susceptibility data of isolates as there is variation in antibiotic susceptibility pattern of UPEC. Antimicrobial stewardship has become a highly important measure in the struggle to preserve the effectiveness of available antimicrobials

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