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Original Article

Etiology and Antimicrobial resistance pattern of Uropathogens in a hospital from suburban Mumbai

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ABSTRACT

Background: Urinary tract infection (UTI) remains one of the common infections in OPD as well as hospitalized patients with significant morbidity. Area specific studies are intended to acquire knowledge of pathogens and their antimicrobial susceptibility pattern which is essential for appropriate empiric therapy. **Aim:** To evaluate the distribution of bacterial pathogens responsible for UTI & their resistance to antimicrobials in our setting. **Methods:** Early morning freshly voided mid-stream urine samples from patients with suspected UTI were tested for significant bacteriuria using calibrated loop, Blood agar & MacConkey agar. Isolates were identified by ATB instrument using API identification strips and antimicrobial susceptibility test was done by ATB instrument using ATB strips according to CLSI guidelines. **Results:** Out of 658 urine samples tested, 155 (23.56%) yielded significant pathogens. Majority i.e. 108 (69.68%) of the isolates were from females. E. coli 67 (43.22%) was observed to be the most predominant organism followed by Klebsiella pneumoniae 25 (16.13%) and Coagulase negative Staphylococci 22 (14.19%). It was observed that Enteric gram negative bacilli (GNB) were highly resistance to commonly prescribed antimicrobials like Ampicillin 89 (90.82%), Amoxicillin - Clavuli nic acid 67(68.37%), Norfloxacin 61 (62.24%) and Cotrimoxazole 54 (55.10%). Imipenem was found to be the most effective drug against Enteric GNB with zero resistance followed by Piperacillin-Tazobactam 12 (12.24% resistance) and Nitrofurantoin 41 (41.84% resistance). Gram positive cocci (GPC) were highly resistant to the routinely used drugs like Penicillin 36 (90%), Erythromycin 31(77.5%) and Amoxicillin-Clavuli nic acid 26 (65%). Linezolid was found to be the most effective drug against GPC with zero resistance followed by Vancomycin 1 (2.5% resistance) and Nitrofurantoin 2 (5% resistance). **Conclusion:** E coli was the most common uropathogen, Multidrug resistance in uropathogens denotes the importance of judicious use of antimicrobials. Imipenem and Piperacillin-Tazobactam were the most promising drugs against Gram negative bacilli. Linezolid and Vancomycin were highly effective drugs against GPC.

1.Introduction:

Urinary tract infection (UTI) remains the most common bacterial infection in human beings in spite of the widespread availability of antimicrobials.[1]. Estimated annual global incidence of UTI is 250 million, costing the global economy more than 6 billion US $[2]. Manifestations of UTI vary from mild symptomatic cystitis to pyelonephritis & septicemia. In almost all patients with suspected UTI, antimicrobial treatment initiates before laboratory urine culture reports are available, [3] thus antimicrobial resistance may escalate in uropathogens due to frequent use of antimicrobials. Inadequately treated UTIs and failure of empirical therapy may lead to significant morbidity & even mortality[4]. Distribution of uropathogens and their susceptibility to antimicrobials is variable regionally and geographically[5]. However a large proportion of uncontrolled use of antimicrobials has invariably resulted in development of antimicrobial resistance which in recent years has become a major problem worldwide[6].

As uropathogens and their antimicrobial resistance pattern is changing constantly, identifying the uropathogens and monitoring their antimicrobial susceptibility is pivotal. It provides the information about the organism associated with UTI and reports about antimicrobial susceptibility pattern, thus helps in most

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appropriate empirical antimicrobial therapy and curtails the spread of antimicrobial resistance. Very few studies on uropathogens in recent decades are available from this area; therefore the present study was conducted to know he organisms causing UTI (uropathogens) and their antimicrobial resistance profile in our setup. This is the first report of such kind from this institute.

2.Material & Methods:

Study design:

A retrospective cohort study carried out on the patients attending ESIC hospital, Andheri, during January 2011 to December 2011.

Setting:

Department of Microbiology, ESI-PGIMSR & ESIC model hospital, Andheri, Mumbai

Sample collection & analysis:

Patients with suspected UTI attending Outpatient Department and admitted patients of ESIC Hospital, Andheri were enrolled in this study. Early morning freshly voided mid-stream urine samples were collected in a sterile container and were immediately transported to Bacteriology Laboratory, Dept of Microbiology for further processing. A modified semi quantitative technique using standard calibrated bacteriological loop was performed to transfer the 0.01 ml of urine sample on Blood agar & MacConkey agar (Himedia Ltd, Mumbai). The plates were incubated at 37^0C for 18-24 hours. The colony count for significant bacteriuria i.e. 10^5 bacteria per milliliter was done using semi quantitative method[7]. A single positive culture per patient was included in this study.

Pure isolates were identified by ATB instrument using API identification strips (Biomerieux Ltd., France) and antimicrobial susceptibility test was done by ATB instrument using ATB strips (Biomerieux Ltd., France) according to CLSI guidelines[8]. The antimicrobials used for detecting antimicrobial susceptibility of uropathogens were Ampicillin, Cotrimoxazole, Norfloxacin, and Amoxicillin-Clavulanic acid, Amikacin, Ceftriaxone, Nitrofurantoin, Ceftazidime, Piperacillin, Ticarcillin, Piperacillin-Tazobactam, Imipenem, Penicillin, Erythromycin, Gentamicin, Clindamycin, Linezolid and Vancomycin.

3.Results:

Out of 658 urine samples collected, 155 (23.56%) samples yielded significant pathogens either bacteria or fungus. (Table I) Majority i.e. 108 (69.68%) of the isolates were from females and 47 (30.32%) from males. Isolates from adult patients were 126 (81.29%) & remaining 29 (18.71%) were from pediatric age group. (Table I) Uropathogens isolated from hospitalized patients were 98 (63.23%), however 57 (36.77%) were from outdoor patients.

Majority of uropathogens were Enteric gram negative bacilli (GNB) 98 (63.22%) followed by gram positive cocci (GPCs) 40 (25.8%). Candida species were isolated from 13 (8.39%) cases and remaining 4 (2.58%) were nonfermentative gram negative bacilli (NFGNB).

In all uropathogens, E. coli 67 (43.22%) was observed to be the most predominant organism followed by Klebsiella pneumoniae 25 (16.13%). (Table I) Other important uropathogens isolated were Coagulase negative Staphylococci (CONS) 22 (14.19%), Enterococci 14 (9.03%) and Candida 13 (8.39%). (Table I)

E. coli was found to be highly resistant to the routinely used drugs like Ampicillin 60 (89.55%), Amoxicillin-Clavulanic acid 47(70.15%), Norfloxacin 41 (61.19%) and Amikacin 38 (56.72%). Imipenem was found to be the most effective drug against E. coli with zero resistance followed by Piperacillin-Tazobactam 11 (16.42% resistance) and Nitrofurantoin 22 (32.84% resistance). (Table II)

Klebsiella pneumoniae was also found to be highly resistant to the routinely used drugs like Ampicillin 23 (92%), Nitrofurantoin 17 (68%), Amoxicillin-Clavulanic acid 16 (64%) and Ceftriaxone 16 (64%). Imipenem was found to be the most effective drug against Klebsiella with zero resistance followed by Piperacillin-Tazobactam 1 (4% resistance) and Amikacin 10 (40% resistance). (Table II)

It was observed that enteric GNB were highly resistance to commonly prescribed antimicrobials like Ampicillin 89 (90.82%), Amoxicillin - Clavulanic acid 67(68.37%), Norfloxacin 61 (62.24%) and Cotrimoxazole 54 (55.10%). Imipenem was found to be the most effective drug against Enteric GNB with zero resistance followed by Piperacillin-Tazobactam 12 (12.24% resistance) and Nitrofurantoin 41 (41.84% resistance). (Table II)

Among NFGNB, the most effective drugs were Imipenem and Piperacillin Tazobactam, However total resistance was observed against Ampicillin, Cotrimoxazole and Amoxicillin-Clavulanic acid followed by Norfloxacin (75%). (Table III)

CONS were found to be highly resistant to the routinely used drugs like Penicillin 20 (90.91%), Amoxicillin - Clavulanic acid 16 (72.73%) and Erythromycin 15 (68.18%). Linezolid and Vancomycin were found to be the most effective drug against CONS with zero resistance followed by Nitrofurantoin 1 (4.55% resistance). (Table IV)

Enterococci were found to be highly resistant to the commonly used drugs like Erythromycin 13 (92.86%) and Penicillin 12 (85.71%). Linezolid was found to be the most effective drug against Enterococci with zero resistance followed by Vancomycin and Nitrofurantoin 1 (7.14% resistance) each. (Table IV)

Table IV shows that GPC were highly resistant to the routinely used drugs like Penicillin 36 (90%), Erythromycin 31(77.5%) and Amoxicillin-Clavulanic acid 26 (65%). Linezolid was found to be the most effective drug against GPC with zero resistance followed by Vancomycin 1 (2.5% resistance) and Nitrofurantoin 2 (5% resistance).

Table - 1. Age & gender wise distribution of uropathogens

Sr.NO	Organisms isolated	Male	Female	Adult	Pediatric	Total (%)
1.	E. coli	25	42	57	10	67 (43.22)
2.	K. pneumoniae	6	19	22	3	25 (16.13)
3.	Enterobacter	1	2	1	2	3 (1.94)
4.	P. mirabilis	1	0	0	1	1 (0.65)
5.	Serratia	0	1	1	0	1 (0.65)
6.	Citrobacter	1	0	0	1	1 (0.65)
7.	P. aeruginosa	1	1	1	1	2 (1.29)
8.	Acinetobacter	1	1	2	0	2 (1.29)
9.	CONS	1	21	20	2	22 (14.19)
10.	Enterococci	5	9	9	5	14 (9.03)
11.	S. aureus	2	2	4	0	4 (2.58)
12.	Candida	3	10	9	4	13 (8.39)
Total		47 (30.32%)	108 (69.68%)	126 (81.29%)	29 (18.71%)	155

a) Sr. No. 1 to 6= Enteric GNB 98(63.22%), Sr. No. 7& 8= NFGNB 4 (2.58%) and

Sr. No. 9 to 11 =GPC 40 (25.80%)

b) **Abbreviations:** E. coli = Escherichia coli, K. pneumoniae = Klebsiella pneumoniae, P. mirabilis = Proteus mirabilis, P. aeruginosa = Pseudomonas aeruginosa, CONS=Coagulase negative Staphylococci, S. aureus = Staphylococcus aureus.

Table 2. Antimicrobial resistance among enteric Gram negative bacilli

	E. coli 67 (%)	K. pneumoniae 25 (%)	Entero-bacter 3 (%)	P. mirabilis 1	Serratia 1	Citro-bacter 1	Total 98(%)
Ampicllin	60(89.55)	23(92.00)	3(100)	1	1	1	89(90.82)
Cotrimoxazole	34(50.75)	14(56.00)	3(100)	1	1	1	54(55.10)
Norfloxacin	41(61.19)	17 (68.00)	2(66.66)	1	1	1	61(62.24)
Amoxicillin-Clavulanic acid	47(70.15)	16 (64.00)	2(66.66)	0	1	1	67(68.37)
Amikacin	38(56.72)	10 (40.00)	2(66.66)	0	1	0	51(52.04)
Ceftriaxone	23(34.31)	16 (64.00)	1(33.33)	0	1	1	42(42.86)
Nitrofurantoin	41(41.84)	17(68.00)	1(33.33)	1	0	0	41 (41.84)
Piperacillin-Tazobactam	12(12.24)	1 (4.00)	0	0	0	0	12(12.24)
Imipenem	0	0	0	0	0	0	0

Table 3. Antimicrobial resistance among NFGNB

Antibiotic	P. aeruginosa n=2	Acinetobacter n=2	TOTAL n=4
Ampicillin	2	2	4
Cotrimoxazole	2	2	4
Norfloxacin	1	2	3
Amoxicillin-Clavulanic acid	-	2	4
Amikacin	1	0	1
Ticarcillin	1	1	2
Piperacillin	1	1	2
Ceftazidime	1	0	1
Nitrofurantoin	1	0	1
Piperacillin-Tazobactam	0	0	0
Imipenem	0	0	0

Table 4: Antibiotic resistance among Gram positive cocci

	S. aureus 4 (%)	CONS 22 (%)	Enterococci 14 (%)	Total (%)
Penicillin	4(100)	20(90.91)	12(85.72)	36(90)
Erythromycin	3(75)	15(68.18)	13(92.86)	31(77.5)
Amoxicillin-Clavulanic acid	3(75)	16(72.73)	7(50)	24(60)
Oxacillin	2(50)	8(36.36)	-	-
Clindamycin	1(25)	10(45.45)	8(57.14)	19(47.5)
Linezolid	0	0	0	0
Vancomycin	0	0	1(7.14)	1(2.5)
Cotrimoxazole	2(50)	7(31.82)	-	-
Gentamicin	2(50)	7(31.82)	8(57.14)	17(42.5)
Norfloxacin	2(50)	10(45.45)	7(50)	19(47.5)
Nitrofurantoin	0	1(4.55)	1(7.14)	2(5)

4.Discussion:

UTI accounts for a large proportion of antibacterial drug usage & have large socioeconomic impact. Majority of the treatment begins or done totally empirically. Hence knowledge of common uropathogens & their regional susceptibility pattern is crucial to optimize the appropriate therapeutic strategy & to avoid the emergence of bacterial resistance which is responsible for increasing number of therapeutic failure.

In the present study, 23.56% samples yielded significant pathogens from suspected UTI cases. In India prevalence of uropathogens ranges from 10.86% to 45.32% [9-11] and in abroad from 8.7% to 17.9 %. [12-15]. This indicates that urine culture is essential for a definitive diagnosis of UTI.

Like majority of the studies, [16-19] females were affected more than males principally owing to anatomical & physiological factors like shorter urethra. In our study, 81.29% isolates were from adult patients & 18.71% were from pediatric age group. It is because of the fact that majority of the adult patients were from reproductive age group. Although 63.23% isolates were from hospitalized patients but there was remarkable isolates 36.77% from outdoor patients which reflects the problem of UTI in hospitals as well as in the community at large.

Majority of uropathogens were enteric GNB 63.22% followed by GPC 25.8%. This is in accordance with the other studies. [9-10]. Amin M et al [14] reported 94% GNB and 5.6% GPC. Enteric GNB colonize the urogenital mucosa with adhesion, pili, fimbriae and P1 –blood group phenotype receptor[20]. In this study, Candida was isolated from 8.39% cases and remaining 2.58% were NFGNB like other study[13]. While Khan et al [21] reported Candida as 2nd commonest uropathogen isolated. Similarities and differences in type of distribution of uropathogens may result from different environmental conditions and host factors, healthcare practices, socioeconomic status, hygienic practices in each country.

E. coli (43.22%) was observed to be the predominant uropathogen. E. coli is single most common species isolated in UTI patients all over the world. [11,14,16,19,21,22]. Klebsiella pneumoniae was the next most common uropathogen similar to most other studies [16-19]. Al Benwan et al [22] reported Streptococcus agalactiae as 2nd most common pathogen, some authors [12-24] reported Staphylococci as the second common isolate. This is because of the regional and geographic variation. Other important uropathogens isolated in this study were CONS and Enterococci.

Antimicrobial resistance is emerging as a big problem for public health which threatens the lives of hospitalized individuals as well as those with chronic conditions and adds considerably to healthcare cost by increasing the hospital stay.

The present study revealed that E coli were highly resistant to multidrugs (3 or more) like Ampicillin, Amoxicillin-Clavulanic acid and Norfloxacin it is because most of the empirical therapy for UTI starts with these drugs. Our reports are consistent with other studies. [19,21,23,24] Imipenem and Pipeacillin- Tazobactam were highly effective against E coli. Similar trends were reported by other authors. [13],[18],23] Unlike our studies Behadin J et al [19] reported Amoxicillin - Clavulanic acid as the highly effective drugs against E coli; this may be because of local variation of drug susceptibility in different hospitals.

Like other studies, [13-18] Klebsiella pneumoniae was found to be highly resistant (multi resistant) to the routinely used drugs like Ampicillin, Nitrofurantoin, Amoxicillin - Clavulanic acid, and Norfloxacin. Imipenem was found to be the most effective drug against Klebsiella pneumoniae with zero resistance. Akram et al [18] reported 88% sensitivity of Imipenem against Klebsiella pneumoniae. Piperacillin-Tazobactam was highly effective drug after Imipenem followed by Amikacin. Unlike E coli Nitrofurantoin was not effective against Klebsiella. (Table II)

In this study like other studies [9-24] Enteric GNB were multidrug resistant. This is because of the fact that earlier exposure of these isolates to the commonly used drugs like Amoxicillin - Clavulanic - Clavulinic acid, Norfloxacin and Cotrimoxazole might have increased the resistance development. Although, Norfloxacin was considered as one of the drug of choice for the treatment of UTI, the increasing resistance rate necessitates that widespread empirical

use of Norfloxacin should be discouraged because of potential promotion of resistance. It suggests that these drugs cannot be used as empirical therapy for UTI particularly in our set up. Like other studies, [13-20] Imipenem was the most promising drug against enteric GNB with zero resistance followed by Pipeacillin-Tazobactam. It is because these drugs are not easily accessible and relatively expensive compared to other. Imipenem has the widest coverage against gram negative isolates. This study suggests that Imipenem should be kept as the reserve drug for complicated UTI caused by these organisms.

NFGNB isolated in this study were associated with hospital acquired infections. Against NFGNB, the most effective drugs were Imipenem and Piperacillin-Tazobactam, like Sood et al,[9] however total resistance was observed against Ampicillin, Cotrimoxazole and Amoxicillin-Clavulanic acid followed by Norfloxacin like study by DAS et al. [20] An earlier study reported in 2005 by Ukey et al [25] from this state shows that Tobramycin and Gentamicin were highly effective drugs against NFGNB isolated from UTI cases.

CONS have emerged as a pathogen in UTI. CONS were found to be multidrug resistant to the routinely used drugs like Penicillin, Amoxicillin - Clavulanic acid and Erythromycin like other study. [26] Linezolid and Vancomycin [26] were found to be the most effective drug against CONS with zero resistance followed by Nitrofurantoin (4.55% resistance).

Enterococci often pose a problem in complicated UTI, in patients with indwelling catheters or in patients receiving broad spectrum antimicrobials for another infection. In this study, Enterococci were found to be highly resistant to the drugs like Erythromycin and Penicillin. [9-11] Linezolid was found to be the most effective drug against Enterococci with zero resistance followed by Vancomycin and Nitrofurantoin. Although Vancomycin was effective against Enterococci but 7% resistance similar to study by Sood et al [9] may suggest that this may be the beginning of Vancomycin resistant Enterococci (VRE) in our set up. Earlier study [11] from this area reported no VRE in 2006.

GPC were found to be highly resistant to the two or more drugs like Penicillin, Erythromycin and Amoxicillin - Clavulanic acid. Linezolid was found to be the most effective drug against GPC with zero resistance. Vancomycin was highly effective drug after Linezolid followed by Nitrofurantoin. [9-18] This suggests that Linezolid and Vancomycin can be used as reserve drugs. Low resistant to Nitrofurantoin possibly is because of its multiple mechanism of action despite being used for many years in UTI. [15] Its use should be encouraged to counter increasing bacterial resistance.

The rise in antimicrobial resistance in this study emphasizes the importance of sound hospital infection control policies, rational antimicrobial prescribing practices. Antimicrobial resistance survey from various hospitals can be useful for comparison between resistance rates at national levels. The study should be periodically repeated to know any significant change in the antimicrobial susceptibility of uropathogens over time.

We recommend that every hospital should have its own antimicrobial policy based on microbiological data to combat rise in emergence of antimicrobial resistance.

5.Conclusions

E coli was the most predominant uropathogen, adult females were more affected. Multidrug resistance to commonly used antimicrobials in uropathogens has caused considerable alarm which suggests the importance of judicious use antimicrobials. Imipenem and Piperacillin-Tazobactam were the most promising drugs against Gram negative bacilli. Linezolid and Vancomycin were highly effective drugs against GPC. Nitrofurantoin can be considered as the alternative option in the empirical treatment of UTI.

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