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Prevalence and Antibiogram of Bacterial Uropathogens in a Tertiary care Teaching Hospital

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A B S T R A C T

Urinary Tract Infections (UTIs) are very common in clinical settings and many organisms are ground to be multi-drug resistant. Therefore, analyzing antibiotic susceptibility patterns will not only help in therapeutic difficulties but also decrease the indiscriminate use of antibiotics which are causing the development of MDR. Bacterial Isolates with a colony count of more than 10^5/ml were included in the study. The uropathogens were isolated using Urochrome UTI agar and MacConkey agar. Identification was done by standard biochemical reactions and then subjected to antibiotic susceptibility testing against 18 antibiotics of different classes using Kirby-Bauer's disc diffusion method. Out of a total of n=4385 urine samples processed 974 (22.2%) were found to have significant bacteriuria and n=3413(77.8%) were found to be negative. Gram-negative bacteria were found to be 78.7% while gram-positive cocci accounted for the remaining 21.3 % of the total pathogens, *E. coli* (54%), *Enterococcus spp* (18%), *K. pneumoniae* (15%). Regular monitoring and surveillance is the need of the hour given the constantly rising drug resistance. It is necessary to make a local antibiogram about the hospital environment in discussion with the physicians to provide an updated and effective empirical treatment of UTIs.

Introduction

Urinary tract infection (UTIs) is defined as the invasion of pathogens to the urinary tract tissues extending from the renal cortex to the urethra which includes the prostate, urinary bladder, kidney (Najar *et al.*, 2009).UTI is one of the important causes of morbidity in the general population. It is also the common cause of nosocomial infection among hospitalized patients (Ronald *et al.*, 1991). It is estimated that there are about 150 million urinary tract infections annually worldwide (Stamm *et al.*, 2001). Factors which are associated with UTIs and accelerate the chance of increasing the infection are catheterization, pregnancy, sex, age, kidney tumors, neurological disorders, urethral structures, immune-suppression, enlargement of the prostate, congenital/acquired anomalies of the bladder, poor personal hygiene, obstruction of the urinary tract, spermicidal contraception, sexual contraception, diabetes mellitus, etc. As the main causative agent of

UTIs are bacteria, the best choice for its treatment is the use of antibiotics (Sanjeel *et al.,* 2017). The Infectious Disease Society of America (IDSA) identified uropathogens as ―ESKAPE pathogens which are *Enterococcus faecium, S. aureus, Klebsiella spp., Acinetobacter spp., Pseudomonas spp*., and *Enterobacter spp* for new effective therapies (Prakash *et al.,* 2013). The emergence of resistant microorganisms to one or several antimicrobial agents is due to their indiscriminate use (Jaya Sankarankutty *et al.,* 2014). Monitoring of the antimicrobial susceptibilities become more important as the pattern of sensitivity is constantly changing. Multidrug resistance (MDR) among uropathogens is a global public health problem (Mathai *et al.,* 2001). In UTI cases there is a need to start treatment before the final microbiological results are available. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTIs and their resistance patterns may help the clinician to choose the right empirical treatment (Stamm *et al.,* 2001).

Materials and Methods

This Retrospective study was done in the Department of Microbiology, ESIC Medical College and Hospital, Sanathnagar, Hyderabad from the period of January 2018 to December 2019. Institutional Ethical committee permission was obtained for the study as per the protocol.

Inclusion criteria

Bacterial Isolates with a colony count of more than 10^5/ml were included in the study.

Exclusion criteria

1) Bacterial Isolates with a colony count of less than10^5/ml organisms.

2) When contaminants are grown

3) When Gram-positive budding yeast was isolated

A total of n=4385 urine samples were received based on the inclusion and exclusion criteria. The uropathogens were isolated using Urochrome UTI agar and MacConkey agar. Identification was done by standard biochemical reactions and then subjected to antibiotic susceptibility testing against 18 antibiotics of different classes using Kirby-Bauer's disc diffusion method as per Clinical and Laboratory Standard Institute (CLSI) guidelines.

Results and Discussion

Out of a total of n=4385 urine samples processed 974 (22.2%) were found to have significant bacteriuria and n=3413(77.8%) were found to be negative. Gram-negative bacteria were found to be 78.7% while gram-positive cocci accounted for the remaining 21.3 % of the total pathogens. The distribution of various microorganisms has been summarized in Table 1. *E. coli* (54%), *Enterococcus spp* (18%), *K. pneumoniae* (15%), *Proteus spp* (3.1%), *P. aeruginosa* (2.3%), CoNS (2.2%), *Citrobacter spp* (1.3%) were the most prevalent microorganisms in UTI patients. The most common isolate was *E. coli* (54%) followed by *Enterococcus* (18%) and *Klebsiella spp* (15%).

Out of the n=974 culture-positive samples, n=767(78.74%) were Gram-Negative bacteria and n=207(21.25%) were Gram-Positive bacteria. Amongst the Gram-negative bacteria, the most common were *E. coli, Klebsiella spp, Proteus spp*, *Pseudomonas spp* followed by less common ones like *Citrobacter*, *Enterobacter spp*, and *Acinetobacter baumannii* complex. The distribution of various gram positives and negative organisms in urine cultures was as depicted in Table 2.

Table.1 Distribution of various bacterial isolates in urine

Sl. No	Bacterial isolate	Percentage (N = 974)
1	*E. coli*	54%
2	*Enterococcus*	18%
3	*Klebsiella spp*	15%
4	*Proteus spp*	3.1%
5	*Pseudomonas aeruginosa*	2.3%
6	CoNS	2.2%
7	*Citrobacter spp*	1.3%
8	*Staphylococcus aureus*	0.6%
9	*Enterobacter spp*	1.02%
10	Non-Fermenter Gram-Negative Bacilli	0.6%
11	*Acinetobacter spp*	0.7%

Table.2 Frequency and distribution of Gram-positive & Gram-negative bacterial isolates in UTI cases

Gram-Negative bacteria	Percentage (N = 767)	Gram-Positive bacteria	Percentage (N = 207)
E. coli	68.18%	*Enterococcus*	84.5%
Klebsiella spp	18.5%	CONS	10.6%
Proteus spp	4.2%	*Staphylococcus aureus*	3.38%
Pseudomonas aeruginosa	2.9%		
Citrobacter spp	1.3%		
Enterobacter spp	0.9%		
NF GNB	1.92%		
***Acinetobacter baumannii* complex**	2.1%		

Table.3 The antimicrobial susceptibility pattern of various Gram-negative organisms

	E.coli	***Klebsiella***	***Pseudomonas spp***	***Proteus spp***	***Citrobacter spp***	***Enterobacter spp***
AMC	32	--	--	5.8	--	--
PIT	56	64	--	53	35	45
CFM	21	--	--	20	23	--
CTX	24	27	--	20	30	40
CAZ	--	--	53	22	29	40
CPM	25	19	65	22	29	50
IPM	80	68	85	--	71	100
MRP	78	63	80	52	71	100
GEN	92	78	45	62	43	58
AK	94	79	68	70	71	60
NET	92	79	46	51	72	80
NIT	96	60	34	88	58	40
CIP	35	39	45	35	29	45
NX	30	47	26	25	43	40
COT	48	55	--	29	29	80
TE	56	65	--	--	15	40

Table.4 Antimicrobial susceptibility of various Gram-Positive Bacteria

	Enterococcus	*S. aureus*	CoNS
P	35.4	20	--
AMP	67.9	--	--
GEN	--	60	40
HLG	-	--	--
NIT	69	40	46.6
CIP	32.9	40	14
NX	21	--	20
CD	11.8	20	47
E	21.3	20	40
TE	29	--	53
COT	--	40	40
TEI	97.6	20	34
LZ	97.5	60	55
VAZ	98.8	88	--

Fig.1 Susceptibility pattern of *E.coli* to various Antimicrobials

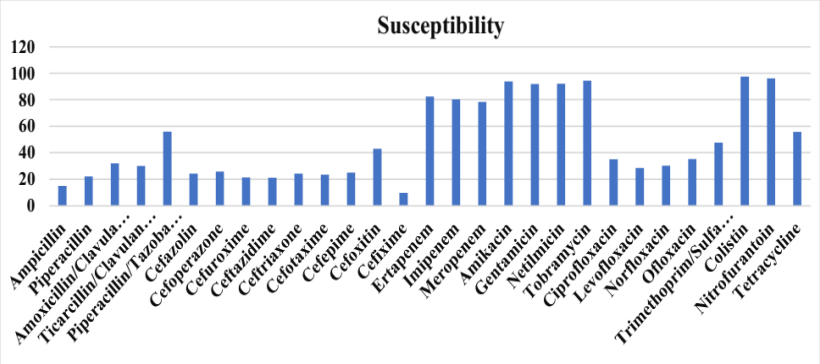


Fig.2 Susceptibility pattern of *Enterococcus spp* to various Antimicrobials

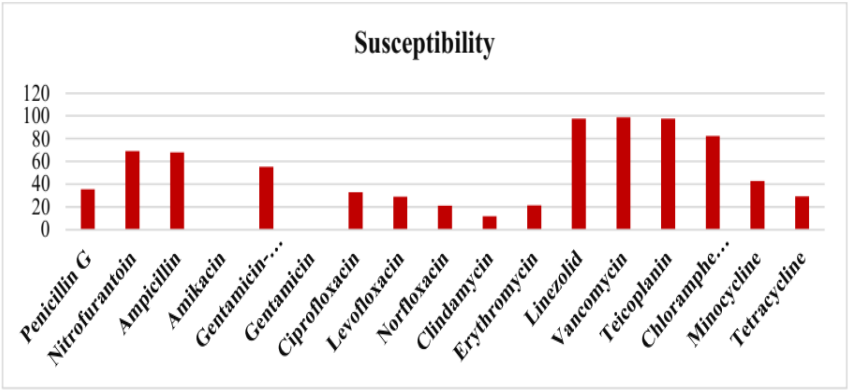
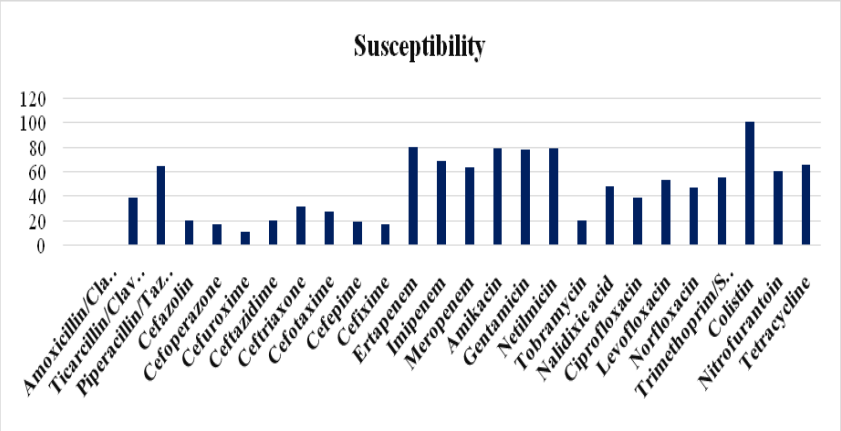


Fig.3 Susceptibility pattern of *Klebsiella spp* to various Antimicrobials



Amongst the various antimicrobials, it was found that the various gram-negative bacteria were less susceptible to the various cephalosporins (ESBL producers). Reduced susceptibility was seen to the first line agents prescribed for uncomplicated UTI i.e Cotrimoxazole & fluoroquinolones. CFM-Cefixime (10 µg) CAZ-Ceftazidime (30µg), CTX-Cefotaxime (30 µg), CPM-Cefipime (30 µg),CIP-Ciprofloxacin (5 µg), NX-Norfloxacin (10 µg),TE -Tetracycline (10µg), MI-(30 µg), IPM- Imipenem (10 µg), PIT-Piperacillin/ Tazobactam (100/ 10µg), AMC-Amoxycyllin/Clavulanate (20/10 µg), COT-Co-Trimoxazole 25 µg (23.75/ 1.25 µg), GEN- Gentamicin (10 µg), AK-Amikacin (30µg), AMP-Ampicillin (10µg), NET-Netilimicin (30 µg), NIT- Nitrofurantoin (300 µg). The distribution of susceptibility pattern is as depicted in Table 3.

Amongst the Gram-positive bacteria, the most susceptible antimicrobial agents were Vancomycin, linezolid. Lowered susceptibility to first-line agents like cotrimoxazole and quinolones was noted. Nitrofurantoin showed susceptibility between 40- 69% based on the organism. E-Erythromycin (15 µg), CD-Clindamycin (2µg) TE -Tetracycline (10 µg), VA-Vancomycin (E-strip used), LZ-Linezolid (30 µg) COT- Co-Trimoxazole 25 µg (23.75/ 1.25 µg), P- Penicillin G (10 Units), AMP-Ampicillin (10µg), NX- Norfloxacin (10 µg), CIP-Ciprofloxacin (5 µg), GEN- Gentamicin (10 µg), HLG – High-level Gentamycin (120 µg), NIT- Nitrofurantoin (300 µg), TEI-Teicoplanin (30 µg). The antimicrobial susceptibility pattern of the various organs is as depicted in Table 4.

The antimicrobial susceptibility pattern of the 3 most predominant isolates i.e. *E. coli, Enterococcus spp, and Klebsiella spp* are shown in Fig. 1, Fig. 2, and Fig. 3, respectively.

In the present study, Culture Positivity was 22.2%. The most common isolates were *E. coli* (54%) followed by *Enterococcus spp* (18%) and *Klebsiella spp* (15%). It was observed that the prevalence of Gram-negative bacteria (78.76%) was much higher than the Gram-positive bacteria (21.23%). In our study, among Gram-positive cocci, *Enterococcus* (84.5%), CoNS (10.6%), *S. aureus* (3.38%). Shakya *et al.,* (2017) in their study in Nepal found 80.9% *E. coli* isolated from their samples followed by 3.8% *Klebsiella pneumoniae* and 0.7% *Klebsiella oxytoca*. Daoud *et al.,* (2011) in Beruit found *E.coli* as the common pathogen in 60.64% of samples followed by *Klebsiella pneumoniae* and *Proteus spp., Pseudomonas aeruginosa,*

Enterococcus spp., and *Streptococcus agalactiae*. *E. coli* occurred more frequently in women (69.8%) than in men (61.4%). In a similar study done by Setu *et al.,* (2016) in Bangladesh found the most common organisms in UTI were *Escherichia coli* (63.93%) followed by *Klebsiella pneumoniae* (17.09%), other bacterial species, named *Pseudomonas spp, Enterobacter, Acinetobacter spp*. *Citrobacter spp, Proteus spp,* and *Morganella*. Sankarankutty *et al.,* (2016) in Tumkur, Karnataka, India found Gram-positive cocci in 20.6% cases. *Staphylococcus aureus* was isolated in 13.5% and *CoNS* in 2.5% of the cases. In another study by Theodore (2006) *Staphylococcus aureus* was seen in 14.5% and *CoNS* in 5% of the isolates. *E. coli* can colonize the urogenital mucosa with adhesins, pili, fimbriae, and P1 blood group phenotype receptor this probably explains its higher isolation from UTI (Das *et al.,* 2006). In our study, 80% *E. coli* was sensitive to imipenem, in contrast, to a study by Sanjee *et al.,* (2017) found 8.57% of the *E. coli* was sensitive to Imipenem. In our study sensitivity of *E. coli* to Ciprofloxacin (35.1%), ceftriaxone (24.3%), Nitrofurantoin (96.1%), Clotrimazole (47.5%) and Amoxyclav (6.6%). Sanjee *et al.,* (2017) showed Ciprofloxacin (45.71%), ceftriaxone (37.14%), Nitrofurantoin (62.86%), Clotrimazole (45.7%) and Amoxyclav (11.41%). The most effective antibiotic for the *E. coli* isolates observed was Nitrofurantoin (86.95%). A study by Shaifali *et al.,* (2012) which is similar to our study. The antimicrobial susceptibility pattern of *E. coli* varies widely by region as seen in various studies Shaifali *et al.,* (2012) Kothari *et al.,* (2008). The *Klebsiella* isolates in our study were found to be 100% sensitive to Colistin, followed by Ertapenem (79.3%), followed by Amikacin (79%), whereas susceptibility to Nitrofurantoin Cotrimoxazole and Ciprofloxacin was 60%, 55.2% and 38.5% respectively. The observation was similar to the study done by Akram *et al.,* (2017) where *Klebsiella* isolates showed higher susceptibility against Imipenem (88%) and Amikacin (59%) & (57%) isolates were susceptible to Cotrimoxazole. Whereas, High efficacy of Nitrofurantoin (90.90%) followed by Cotrimoxazole and Tetracycline (81.81%) both were observed against the *Klebsiella* isolates in the study done by Shaifali *et al.,* (2012). In our study *Enterococcus* spp was found most susceptible to Vancomycin (98%), Linezolid (97.5%). Susceptibility to nitrofurantoin was found to be (69%) & Ciprofloxacin (32.9%).There is a paucity of literature characterizing the appropriate choice of antibiotics for enterococcal UTI. Data suggest that enterococcal UTIs are associated with low complication rates independent of the agent chosen for therapy, making the case for use of narrow-spectrum agents when feasible (Eugene Lin *et al.,* 2012) Amoxicillin, Nitrofurantoin, or Fosfomycin. The experience with linezolid or fluoroquinolones is limited. The findings of our study show that most of the microorganisms show a trend of resistance towards cephalosporins, and commonly used fluoroquinolones, and reduced susceptibility to cotrimoxazole which are considered one of the 1st line agents for uncomplicated UTI's.

In conclusion the antimicrobial resistance pattern amongst various isolates tends to vary from one geographic region to the other. Regular monitoring & surveillance is the need of the hour given the constantly rising drug resistance. It is necessary to make a local antibiogram about the hospital environment in discussion with the physicians to provide an updated and effective empirical treatment of UTIs. A continuous check & constant reassessment of the antimicrobial susceptibility pattern of urinary pathogens would help in preventing the furthermost of the resistance. Our study shows

Nitrofurantoin as a promising empirical therapy in place of quinolones & Cotrimoxazole for uncomplicated UTI's.

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