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Bacteriological Profile of Uropathogens and their Antimicrobial Susceptibility Pattern in Isolates from a Tertiary Care Hospital

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A B S T R A C T

Urinary Tract Infection (UTI) is one of the most common infections observed in clinical practice among the community and hospitalized patients. Since the pattern of susceptibility is constantly changing, monitoring the changing trends has become more important. It provides information of the pathogenic organisms isolated from patients as well as assists in choosing the appropriate antimicrobial therapy. This retrospective study aims to analyze various uropathogens and their antimicrobial susceptibility pattern which would assist in selecting the most appropriate antibiotic therapy and for treatment of UTI in a tertiary care hospital. 700 urine isolates were studied retrospectively from November 2016 to January2017 which were cultured on to Blood agar and MacConkey agar plate. The plates that showed colonies $>10^5$ were considered significant and were identified by standard biochemical tests & sensitivity of the organisms was performed by Kirby – Bauer method on Mueller Hinton agar. Out of the 700 samples processed,48.6% (340) gave positive urine culture, of which 73 (61.86%) were *Escherichia coli* 69% (107), *Klebsiella spp.*,11.6%(18), *Proteus spp.*, 9.7%(15), *pseudomonas spp*.,8.4% (13), *Acinetobacter spp.*,1.3%(2) and *Coagulase Negative Staphylococcus*(CONS) 67% (130), *Candida spp*.,24.7%(48), *Enterococci spp.*, 8.3%(16) respectively. Susceptibility patterns of each isolates have been determined. Resistance pattern observed was ESBL was about 87%, MBL 8% and AmpC7% among the Gram negative organisms. This study discourages the indiscriminate use of antibiotics which in turn would prevent further development of bacterial drug resistance. For this, a proper knowledge of susceptibility pattern of uropathogens is necessary before prescribing empirical antibiotic therapy.

Keywords

Urinary tract infection, Antimicrobial susceptibility, Extended-spectrum β-lactamases, Amp C, Metallo Beta Lactamases (MBL).

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Introduction

Urinary Tract Infection (UTI) is one of the most common infections observed in clinical practice among the community& hospitalized patients (Khan *et al*., 2001). Despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in human population. Since the antibiotic susceptibility pattern is constantly changing, monitoring the antimicrobial susceptibility has become mandatory (Charania *et al*., 1980; Gupta *et al*., 2002). It provides information on the pathogenic organisms isolated from patients as well as assists in choosing the most appropriate antimicrobial therapy (Deshpande *et al*., 2011). The uses of antibiotics have an influence in the spread of antimicrobial resistance among bacteria. Antibiotic resistant microorganisms have been a source of ever-increasing therapeutic problem. Continued mismanaged selective pressure has contributed towards the emergence of multiple drug resistant (MDR) bacteria (Cohen *et al*., 1992). Treatment of UTI cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. In spite of the availability and use of the antimicrobial drugs, UTIs caused by bacteria have been showing increasing trends in recent years (Razak *et al*., 2012). The emergence of antibiotic resistance in the management of UTIs is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation. The current knowledge of susceptibility pattern is mandatory for the proper management of UTI.

This retrospective study aims to analyze various uropathogens and their antimicrobial susceptibility pattern in a tertiary care hospital, which assist in selecting the most appropriate antibiotic therapy in treatment of Urinary Tract Infection.

Materials and Methods

A retrospective analysis of 700 consecutive urine samples received at the microbiology laboratory in a tertiary care hospital over a period of 3 months from November 2016 to January 2017. Samples were mid – stream urine specimens obtained by clean catch method received from various outpatient departments and inpatient wards were transported to the diagnostic laboratory in sterile leak proof container were processed immediately.

All the specimens were inoculated onto Blood agar and MacConkey agar plate and incubated overnight at 37^{o}C. Samples that showed a colony count of $>10^{5}$ were considered significant. Bacterial isolates were identified based on the colony morphology, Grams staining and biochemical reactions. Antimicrobial susceptibility testing was done using Muller Hinton agar by modified Kirby-Bauer disc diffusion method and their resistance pattern was analyzed according to CLSI guidelines 2016. The data was recorded and analyzed.

Antimicrobial Agents used: Ampicillin (10µg), Amikacin (30µg), Gentamycin (10µg), Ciprofloxacin (5µg), Cefotaxime (30µg), Ceftriaxone (30µg), cefepime (30µg), Cotrimoxazole (1.25/23. 75 µg), Norfloxacin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Nitrofurantoin (300µg), Imipenem (10µg), Meropenem (10µg), Piperacillin-tazobactum, (100/10µg), Vancomycin (30µg), Linezolid (30µg).

Results and Discussion

A total of 700 urine culture reports were analyzed in the present study between November 2016 and January 2017. Among the total of 700 samples received, 48.6% (340) showed positivity for microbial growth and 2.7 %(9) were polymicrobial (Table 1). The predominant growth of single bacteria was seen in 97.3% (331) samples out of which 52.9% (180) were females and 47.1 % (160) were males (Table2), 54 % (183) from outpatient and 46 % (157) from inpatient department. Among the organisms isolated Gram positive was 56%(194) and Gram negative was 44%(155).The most common organisms isolated were *Escherichia coli* 69% (107), *Klebsiella spp.*,11.6% (18), *Proteus spp.,* 9.7%(15), *Pseudomonas spp*.,8.4%(13), *Acinetobacter spp.*,1.3%(2) and *Coagulase Negative Staphylococcus (CONS)* 67%(130), *Candida spp*., 24.7%(48), *Enterococci spp.*, 8.3%(16) respectively (Table 3). *Enterococci spp.,* showed 100% susceptibility to vancomycin and Linezolid, 68.8% sensitivity to Ampicillin and 56.3% sensitivity to Nitrofurantoin (Table 4). *E. coli* showed 96.3% sensitivity to Amikacin, Imipenem and Meropenem, 94.4% sensitivity to Piperacillin-tazobactum. 89.7% sensitivity to Nitrofurantoin. *Klebsiella* showed 94.4% sensitivity to Imipenem and Meropenem and 72.2% to pip-taz and Amikacin. *Proteus* showed 100% sensitivity to Imipenem, Meropenem and pip-taz., 86.7% sensitivity to Amikacin and 60% sensitivity to Ciprofloxacin and Ofloxacin. *Pseudomonas spp.,* showed 76.9% sensitivity to pip-taz, Imipenem and Meropenem, 69.2% sensitivity to Cefipime and 61.5% sensitivity to Ciprofloxacin, Ofloxacin and Amikacin. *Acinetobacter spp*., showed 100% sensitivity to Amikacin, all the cephalosporins, pip-taz and carbapenems (Table 5). Regarding the drug resistance pattern, *E. coli* showed 65.4%(70) of ESBL, AmpC 2.8% (3) and MBL 3.7%(4), *Klebsiella spp.,* showed ESBL 44.4%(8), 22.2%(4) AmpC and MBL5.6% (1). In *Proteus spp.*, there were 60% (9) ESBL producers and in *Pseudomonas spp*., there were 23.1 % (3) MBL producers (Table 6).

Urine culture is very much important for the treatment of UTI in both males and females. It is also essential to isolate and identify the bacteria which cause urinary tract infection. In addition to that the susceptibility pattern of these bacteria is very important to avoid the development of drug resistance. A total of 700 urine culture reports were analyzed in the present study between November 2016 and January 2017. In the present study, isolation and identification of uropathogens were performed and 48.6% (340) showed significant growth of bacteria. So, remaining majority 51.4% (360) of the cases showed either insignificant bacteriuria or no growth with urine from the suspected cases of UTI. The reason of low growth rate may be due to irrational use of antibiotic which is available in the local market in this country and these are given without prior culture and antibiotic sensitivity pattern. In addition to that, incomplete dose is another factor. Prior antibiotic therapy before sending urine samples for culture and sensitivity and other clinical conditions like non-gonococcal urethritis could be the factors responsible for insignificant bacteriuria or no growth. Among the total of 700 samples received, 2.7%(9) were polymicrobial, the predominant growth of single bacteria was seen in 97.3% (331) samples out of which 52.9% (180) were females and 47.1%(160) were males. The male to female ratio was 1:1.125 and 54% (183) from outpatient and 46 % (157) from inpatient department. The age and sex distribution of the patients diagnosed with UTI among the hospitalized patients and those attending the outpatient department followed the natural epidemiological pattern of UTI. There were a higher number of young adult female patients diagnosed as UTI cases. Yusuf *et al.*, showed the ratio is more than two times more frequent in female than male (ratio male: female=1:2.2).

It is well established that female are more commonly infected with UTI than male due to anatomical position of urethra, influence of hormone and pregnancy. The international studies have shown that UTIs in women are very common; therefore, one in five adult women experience UTI in her life and it is extremely common, clinically apparent, worldwide patient problem (Abdullah *et al*., 2015). Among the organisms isolated Gram positive was 56% (194) and Gram negative was 44% (155). The most common organisms isolated from this study were *Escherichia coli* 69%(107), *Klebsiella spp.,*11.6%(18), *Proteus spp*., 9.7%(15), *Pseudomonas spp*., 8.4%(13), *Acinetobacter spp.,* 1.3%(2), *Coagulase Negative Staphylococcus*

67%(130), *Candida spp*., 24.7%(48), which correlates with the study conducted by Mathivathana, Usha *et al*., (2013) which showed isolation of (61.86%) were *Escherichia coli*, (18.64%) were *Klebsiella spp.,* (12.71%) were *Pseudomonas spp.*, *Proteus spp*. (0.08%) and *Acinetobacter spp.* (0.08%). Polymicrobial infection mounted to 12 (10.16%). 8 isolates of *Candida* were obtained. Gram‑positive organisms have received more attention recently as a cause for bacteriuria and UTI. *Coagulase negative Staphylococcus, S. aureus, streptococci*, and *Enterococci* have been reported in small numbers by various authors, but they are recognized as important causes of UTI. *Enterococci spp*., 8.3% (16) were isolated. *Enterococci spp*., showed 100% susceptibility to vancomycin and Linezolid, 68.8% sensitivity to Ampicillin and 56.3% sensitivity to Nitrofurantoin. We found similar occurrence rate as 13.5%, and 5.8% for *Enterococci*, and *Coagulase negative Staphylococcus*, respectively and 23 cases of candiduria. In our study, *E.coli* showed 96.3% sensitivity to Amikacin, Imipenem and Meropenem, 94.4% sensitivity to Pip-taz. 89.7% sensitivity to Nitrofurantoin. *Klebsiella* showed 94.4% sensitivity to Imipenem and Meropenem and 72.2% to pip-taz and Amikacin. *Proteus* showed 100% sensitivity to Imipenem, Meropenem and pip-taz.86.7% sensitivity to Amikacin and 60% sensitivity to Ciprofloxacin and Ofloxacin. *Pseudomonas spp.,* showed 76.9% sensitivity to pip-taz, Imipenem and Meropenem69.2% sensitivity to Cefipime and 61.5% sensitivity to Ciprofloxacin, Ofloxacin and Amikacin. *Acinetobacter spp*., showed 100% sensitivity to Amikacin, all the cephalosporins, pip-taz and carbapenems. Similar study by Mathivathana *et al*., showed overall Sensitivity to Imipenem was 100%, Nitrofurantoin was 90.57%, Amikacin was 83.02%, fourth generation cephalosporin was 43.4%, Fluoroquinolones was 32.1% and Third Generation Cephalosporin was 30.8%.

Regarding the drug resistance pattern, in the present study, *E.coli* showed 65.4%(70) of ESBL, AmpC 2.8% (3) and MBL 3.7%(4), *Klebsiella spp.*, showed ESBL 44.4%(8), 22.2%(4) AmpC and MBL5.6% (1). In *Proteus spp*., there were 60% (9) ESBL producers. Another study showed the percentage of ESBL producers was 69.2%. Maximum ESBL producers were found among *E. coli* isolates i.e. 80.9% followed by *Klebsiella spp.*, (75%). A study done by Mathur *et al*., (2011) and Umadevi *et al*., (2002) showed 68% and 75% prevalence of ESBL producers respectively. Additionally, Extended-spectrum β-lactamase (ESBL)-producing *E. coli* tended to be isolated more often in these studies**.** In another recent study 29.5% of *E. coli* were suspected to produce Extended-spectrum beta-lactamase (ESBL) and amikacin and nitrofurantoin were the drugs to which >90% of *E. coli* were susceptible**.** *E. coli* was found to be sensitive to imipenem (97.9%) followed by nitrofurantoin (91.5%), amikacin (76.6%) and piperacillin-tazobactam (68%). Babypadmini *et al*., showed the susceptibility of ESBL producers to imipenem, nitrofurantoin and amikacin to be 100%, 89% and 86% respectively. In the present study, Amp C production was 25% of which 22.2% (4) from *Klebsiella spp*., and 2.8% (2) from *E.coli*. Study conducted by Mitesh patel *et al*., (2010) showed (45.61%) were positive for AmpC β-lactamase enzyme production. In the present study, MBL production was observed in 32.4%. In *Pseudomonas spp.*, there were 23.1%(3) MBL producers. Sowmya *et al*., (2015) showed 15.3% Imipenem resistance among *Pseudomonas* strains, however a higher resistance rate have been reported by Varaiya *et al*., (2015) (25%).

Table.1 Growth of Urine culture among the study population (n=700)

Growth	Number	Percentage(%)
Positive	340	48.6
Polymicrobial	9	2.7
Monomicrobial	331	97.3
No growth	360	51.4

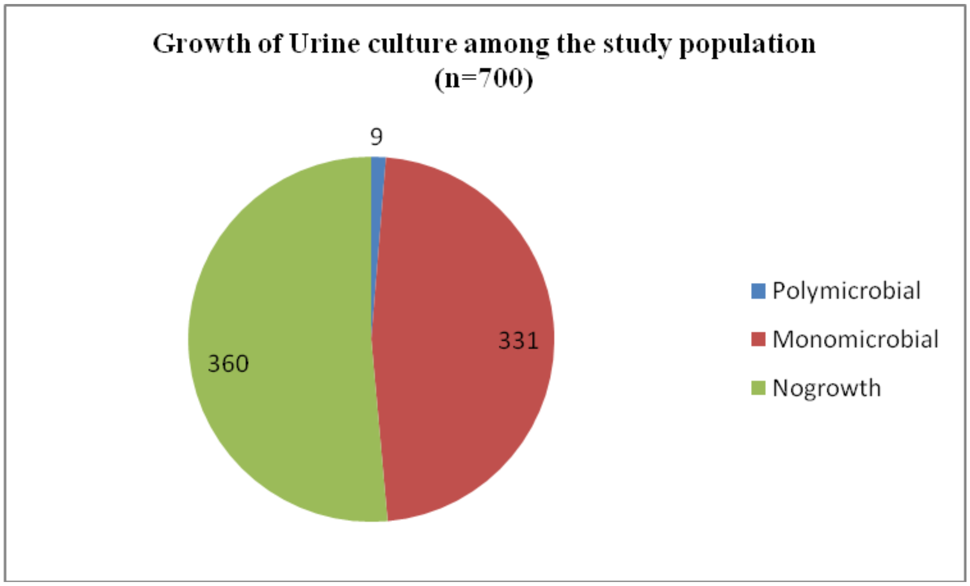

Table.2 Gender distribution of culture positive cases(n=340)

Gender	Number	Percentage(%)
Female	180	52.9
Male	160	47.1

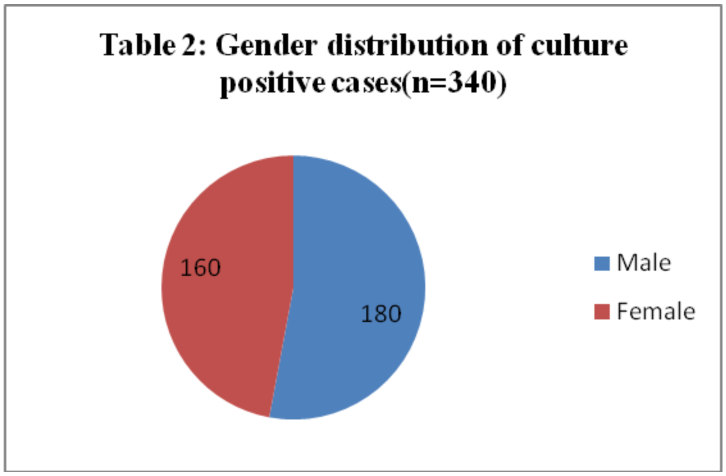

Table.3 Bacteriological profile of Culture positive organisms (n=340)

Bacteria	Number	Percentage(%)
Escherichia coli	107	69
Klebsiella spp.,	18	11.6
Proteus spp.,	15	9.7
Pseudomonas spp.,	13	8.4
Acinetobacter spp.,	2	1.3
CONS	130	67
Candida spp.,	48	24.7
Enterococci spp.,	16	8.3

Table.4 Antimicrobial susceptibility pattern of *Enterococci* spp., (n=16)

Antibiotics	S	%	R	%
Ampicillin (10µg)	11	68.8	5	31
Amikacin (10µg)	6	37.5	10	63
High level Gentamycin (120µg	6	37.5	10	63
Norfloxacin 10µg	1	6.25	15	94
Ciprofloxacin 5µg	1	6.25	15	94
Nitrofurantoin 300µg	9	56.3	7	44
Vancomycin 30µg	16	100	0	0
Linezolid 30µg	16	100	0	0

Table.6 Distribution of antimicrobial resistance pattern among the isolates

Organism	ESBL (No/%)	AMP C (No/%)	MBL (No/%)
E.coli(n=107)	70(65.4)	3(2.8)	4(3.7)
Klebsiella spp.,(n=18)	8(44.4)	4(22.2)	1(5.6)
Proteus spp.,(n=15*)*	9(60)	-	-
Pseudomonas spp.,(n=13)	-	-	3(23.1)

Table.5 Antimicrobial susceptibility pattern of Gram negative organism (n=155)

Antibiotics	E.coli (n=107) (No/%)	Klebsiella spp.,(n=18) (No/%)	Proteus spp.,(n=15) (No/%)	Pseudomonas spp., (n=13) (No/%)	Acinetobacter spp.,(n=2) (No/%)
Ampicillin (10µg)	9 (8.4)	0(0)	1(6.7)	-	-
Amikacin (30µg)	103(96.3)	13(72.2)	13(86.7)	8(61.5)	2(100)
Gentamycin (10µg)	55(51.4)	8(44.4)	10(66.7)	5(38.5)	1(50)
Norfloxacin (10µg)	30(28)	7(38.9)	8(53.3)	5(38.5)	1(50)
Ciprofloxacin (5µg)	30(28)	7(38.9)	9(60)	8(61.5)	1(50)
Ofloxacin (5µg)	31(29)	7(38.9)	9(60)	8(61.5)	1(50)
Ceftriaxone (30µg)	29(27.1)	5(27.8)	6(40)	0	2(100)
Cefotaxime (30µg)	27(25.2)	5(27.8)	6(40)	-	2(100)
Cefipime (30µg)	37(34.6)	6(33.3)	7(46.7)	9(69.2)	2(100)
Cotrimoxazole(1.25/23.75µg)	35(32.7)	4(22.2)	3(20)	-	1(50)
Nitrofurantoin (300µg)	96(89.7)	2(11.1)	3(20)	-	-
Piperacillin – tazobactum(100/10µg)	101(94.4)	13(72.2)	15(100)	10(76.9)	2(100)
Imipenem (10µg)	103(96.3)	17(94.4)	15(100)	10(76.9)	2(100)
Meropenem (10µg)	103(96.3)	17(94.4)	15(100)	10(76.9)	2(100)

In conclusion, the results of the present study showed that higher rate of resistance is prevalent in a tertiary care hospital, which is the result of the irrational use of antibiotics and implementation of appropriate infection control measures to control the spread of these strains in the hospital.

Moreover, our study concludes that *E. coli* and other isolates were more sensitive to imipenem, nitrofuranotin and piperacillin-tazobactam compared to the other antibiotics tested and therefore these may be the drugs of choice for treatment of infections that are caused by ESBLs. With the increasing use of carbapenems for treating infections with ESBL producing organisms, the problem of MBL production is also increasing. This study discourages the indiscriminate use of antibiotics which helps to curb further development of bacterial drug resistance. For this, a proper knowledge of susceptibility pattern of uropathogens in the given locality is necessary before prescribing empirical antibiotic therapy.

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