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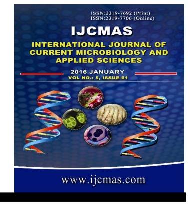

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Uropathogens and their antibiotic susceptibility pattern at a tertiary care teaching hospital in Coastal Karnataka, India

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A B S T R A C T

Urinary tract infections are common, easily diagnosed conditions with established laboratory methods of evaluation. Escherichia coli is by far the most common pathogen, accounting for about 80% of culture positive urinary tract infections. This study was undertaken to know the prevalence and antibiogram of uropathogens in our hospital. A total of 2191 clean catch midstream urine samples were processed in clinical microbiology laboratory during the study period. Uropathogens were identified by standard techniques and antibiotic sensitivity testing was carried out by Kirby Bauer Disk Diffusion method as per CLSI guidelines. Male to female ratio was 1:1.06. Escherichia coli was the predominant isolate accounting for 57.14% followed by *Klebsiella* species (17.62%), *Enterococcus* species (8.1%), *Pseudomonas aeruginosa* (6.98%), Staphylococcus species (3.97%). 64.44% of *E.coli* and 53.15% of *Klebsiella* produced ESBL. *E.coli* was highly sensitive to Amikacin (89.17%) and Nitrofurantoin (85.83%). Our study showed a high prevalence of UTI in females and in the elderly patients. Most of the gram negative bacilli showed increasing rates of resistance Cephalosporins, Fluoroquinolones and Trimethoprim-sulfamethoxazole. The resistance pattern is on the rise indicating the need to establish antibiotic policies and take stringent measures to ensure effectiveness of the same.

Introduction

Urinary tract infections (UTIs) are the most common bacterial infections in humans showing increasing trends in spite of availability and use of antibiotic agents, in all age groups and both genders (Orhue 2014, Shah *et al*., 2015). It is the most common site for nosocomial infection. 3.2% of catheterised patients have UTI, even with adequate aseptic precautions during instrumentation. 50%of the patients with indwelling catheters have UTI with multidrug resistant bacteria (Ananthanarayan and Paniker, 2009). UTI following instrumentation is a frequent cause of significant morbidity, sepsis and death (Kulkarni *et al*., 2014). A variety of organisms can cause UTI including bacteria, fungi and viruses; bacteria being responsible for 95% of the cases. Most common etiological agents are *Escherichia coli, Klebsiella species, Pseudomonas aeruginosa*, *Proteus mirabilis*, *Acinetobacter species, Enterococcus* species and *Staphylococcus* species (Orhue 2014). Treatment of UTI is often empiric and the extensive and inappropriate use of antibiotics has resulted in the emergence of multi drug resistant bacteria which is a major problem worldwide. Knowledge of the etiological agents of UTI and their antibiotic susceptibility pattern is necessary for ensuring appropriate treatment (Shah *et al.*, 2015).

This study was undertaken to know the prevalence of uropathogens in our hospital and their antibiogram.

Materials and Methods

The study was conducted in the Department of Microbiology, of a tertiary care teaching hospital. A retrospective analysis was made on urine culture and sensitivity between January 2013 and October 2013. A total of 2191 clean catch midstream urine samples collected in a wide mouthed sterile container were received in the clinical microbiology laboratory. They were further processed by standard loop method (a semi quantitative method) and inoculated onto Blood agar, MacConkey agar and Cysteine Lactose Electrolyte Deficient agar. The plates were incubated aerobically at 37˚C for 18-24 hours. A wet mount preparation was analyzed for the presence of pus cells. Positive urine culture was determined by significant bacteriuria i.e., isolation of one or two pathogens (but not more than two) with $>10^5$ colony forming units per ml and presence of pyuria (>6 pus cells per high power field). The organisms isolated were identified by their colony morphology, Gram Stain and relevant standard biochemical reactions (Collee JG *et al*.,). Antibiotic susceptibility testing was carried out by Kirby Bauer Disk Diffusion method. A lawn culture was made on Mueller Hinton Agar and following antibiotic disks were tested : CPM-Cefipime (30µg), CTX-Cefotaxime (30µg), CTR-Ceftriaxone (30µg), CAZ-Ceftazidime (30µg), PIT-Piperacillin- tazobactam (100/10 µg), PI-Piperacillin (100µg), AK-Amikacin (30µg), GEN-Gentamicin (10µg), TOB-Tobramycin (10µg), CIP-Ciprofloxacin (5µg), NX-Norfloxacin (10µg), COT-Cotrimoxazole (1.25/23.75µg), NIT-Nitrofurantoin (300µg), IMP-Imipenem (10µg), TE-Tetracycline (30µg), HLG-High level Gentamicin (120µg), P-Penicillin (10U), AMP-Ampicillin (10µg),CX-Cefoxitin (30µg), LZ-Linezolid (30µg), VA-Vancomycin (30µg). The plates were incubated aerobically at 37˚C for 18-24 hours. Zone sizes were measured and the drugs were interpreted to be sensitive or resistant according to CLSI guidelines (2013). Following drugs were tested for yeast isolates: Amphotericin B, Nystatin, Clotrimazole, Fluconazole, Ketoconazole. Antifungal susceptibility testing was carried out on Mueller Hinton Agar with Methylene blue.

Results and Discussion

A total of 2191 urine samples were received by the clinical microbiology laboratory during the period of study. Among these, 630 patients showed significant bacteriuria while 36 patients showed infection with yeast. DISCUSSION

Figure 1 shows gender distribution. There were 322 males and 344 females in our study accounting for 48.35% and 51.65% respectively. Male to female ratio was 1:1.06. Sharma *et al* (2011) have reported male to female ratio of 1:1.8 and Orhue (2014) reported a ratio of 1:1.6. Razak *et al*

(2012) and Shah *et al* (2015) have also observed female predominance in their studies. This finding of increased number of UTI in female sex can be attributed to the presence of short urethra in them.

Figure 2 and Table 1 show age distribution. UTI can occur at any age. Our study showed an increased rate of infection among patients of the age group of 41-70 years. Most of the cases belonged to the age group of 50-79 years (57.4%) in a study by Eshwarappa *et al* (2011) and 21-40 years (54.98%) in a study by Razak *et al* (2012).

Figure 3 & 4, Table 2 show distribution of the causative agents. In the present study we isolated 630 bacteria and 36 yeasts. Among the bacterial profile of UTI, Escherichia coli was the predominant isolate accounting for 57.14% followed by Klebsiella species (17.62%), *Enterococcus* species (8.1%), *Pseudomonas aeruginosa* (6.98%), Staphylococcus species (3.97%), *Acinetobacter* species (3.17%), Citrobacter species (1.11%), Enterobacter aerogenes (0.95%), Proteus mirabilis (0.79%) and Morganella morganii (0.001%). Among 25 Staphylococcal species isolated 19 were S.aureus. Out of the 7 Citrobacter species isolated, 4 were C.freundii and 3 were C.koseri.

Eshwarappa *et al* (2015), Shah *et al* (2015), Hussein NS (2014) and Razak *et al* (2012) have reported *E.coli* as the most commonly isolated bacteria in their study of UTIs, accounting for 66.9%, 61.02%, 39% and 37.95% respectively. Alemu *et al* (2012) in their study of UTI in pregnant women also found *E.coli* as the most common causative agent (47.5%). *E.coli* was the predominant isolate in various studies of UTIs in diabetic patients by Ampaire *et al* (2015), Janifer *et al* (2015) and Sibi *et al* (2011); the percentage of isolation being 50%, 43.36% and 39.4% respectively. UTI studies on paediatric patients by Sharma *et al* (2011) and Patel P *et al* (2014) revealed that *E.coli* caused majority of the infections (67.5% and 38.42% respectively).

Klebsiella species was the second most common organism isolated in our study (17.62%). Similar finding has been reported by Shah *et al* (2015), Eshwarappa *et al* (2015), Razak *et al* (2012) and Patel P *et al* (2014); percentage of isolation being 13.56%, 15.5%, 21.41% and 23.89% respectively.

Pseudomonas and Proteus species are mostly associated with hospital acquired UTIs rather than community acquired UTIs. In our study Pseudomonas has been isolated from 44 samples accounting for 6.98% of bacterial isolates. Similar results have been observed by Shah *et al* (2015) and Patel P *et al* (2014). However, isolation rates of 10.2% and 2% have been reported by Eshwarappa *et al* (2015) and Hussein NS (2014). Pseudomonas was the third common organism isolated in a study of catheter associated UTI by Kulkarni *et al* (2014) accounting for 14.1%.

A total of 36 yeasts were grown from the urine samples accounting for 5.41% of total isolates. 15 among them were Candida albicans (41.67%). Razak *et al* (2012) have reported 7.79% yeast isolates in their study.

Tables 3, 4, 5 and 6 show the antibiotic sensitivity pattern of bacterial isolates. *E.coli* was highly sensitive to Amikacin (89.17%) and Nitrofurantoin (85.83%) moderately sensitive to Piperacillin-tazobactam (78.33%) and Least sensitive was to Ceftazidime (37.78%), Ceftriaxone (35.56%), Cefotaxime (35.56%), Cefipime (39.44%), Ciprofloxacin (28.33%), Norfloxacin (27.5%) and Cotrimoxazole

(38.06%). Similar sensitivity pattern has been reported by Shah *et al* (2015), Razak *et al* (2012) and Hussein NS (2014). 64.44% of *E.coli* produced Extended Spectrum Beta Lactamase.

Klebsiella species was highly sensitive to Amikacin (76.58%) followed by Piperacillin-tazobactam (66.67%), Gentamicin (57.66%), Ciprofloxacin (54.05%), Cotrimoxazole (52.52%), Norfloxacin (51.35%), Cefipime (51.35%), Ceftazidime (50.45%), Ceftriaxone (46.85%), Cefotaxime (46.85%). Nitrofurantoin showed a susceptibility of 37.84%. Similar sensitivity pattern for Cephalosporins, Fluoroquinolones and Nitrofurantoin has been reported by Shah *et al* (2015), Razak *et al* (2012). 53.15% of *Klebsiella* produced Extended Spectrum Beta Lactamase.

Table.1 Age Distribution

AGE GROUP (in years)	Number of patients with UTI (n=666)	Percentage
0-10	18	2.70
11-20	21	3.15
21-30	84	22.95
31-40	72	19.67
41-50	116	31.69
51-60	117	31.97
61-70	143	39.07
71-80	94	25.68
81-90	14	3.83
91-100	02	0.55

Table.2 Bacterial Profile of UTI

Bacteria isolated	**Number**	**Percentage**
Escherichia coli	360	57.14
Klebsiella species	111	17.62
Enterococcus species	51	8.1
Pseudomonas aeruginosa	44	6.98
Staphylococcus species	25	3.97
Acinetobacter species	20	3.17
Citrobacter species	07	1.11
Enterobacter species	06	0.95
Proteus mirabilis	05	0.79
Morganella morganii	01	0.001

Table.3 Antibiotic Sensitivity Pattern of Enterobacteriaceae (in percentage)

	E.coli	*Klebsiella*	*Citrobacter*	*Enterobacter*	*Proteus*	*Morganella*
AK	89.17	76.58	85.71	33.33	100	100
GEN	60.83	57.66	71.43	33.33	80	100
CTR	35.56	46.85	57.14	16.67	80	100
CTX	35.56	46.85	57.14	16.67	80	100
CAZ	37.78	50.45	57.14	16.67	80	100
CPM	39.44	51.35	57.14	16.67	80	100
PIT	78.33	66.67	71.43	33.33	100	100
IMP	83.33	74.77	85.71	33.33	100	100
CIP	28.33	54.05	57.14	16.67	100	100
NX	27.5	51.35	57.14	16.67	80	100
COT	38.06	52.52	42.86	16.67	20	100
NIT	85.83	37.84	42.86	0	0	0

Table.4 Antibiotic Sensitivity Pattern of Nonfermenters (in percentage)

	Pseudomonas	*Acinetobacter*
AK	45.45	45
GEN	38.64	35
TOB	31.82	-
CTR	-	20
CTX	-	20
CAZ	31.82	25
CPM	34.09	30
PIT	-	35
PI	38.64	-
IMP	59.09	35
CIP	25	30
NX	13.64	30
COT	-	45

Table.5 Antibiotic Sensitivity Pattern of Gram Positive Bacteria (in percentage)

	Staphylococcus Species (n=25)	*Enterococcus* Species (n=51)
P	24	13.73
AMP	-	74.51
CX	76	-
CIP	17.33	25.49
NX	48	25.49
GEN	72	-
HLG	-	37.25
COT	56	-
NIT	80	82.35
TE	72	43.14
VA	100	100
LZ	100	100

Table.6 Overall Sensitivity of Commonly used Antibiotics for Gram Negative Bacteria

Antibiotics	Percentage of sensitivity
AK	71.9
PIT	65.4
NIT	53.61
COT	46.4
CTR	44.52
NX	34.29

Table.7 Antibiogram of Yeasts

Antifungal agent	Percentage of sensitivity
Amphotericin B	94.4
Nystatin	94.4
Clotrimazole	91.64
Fluconazole	88.89
Ketoconazole	80.56

Figure.1 Gender Distribution

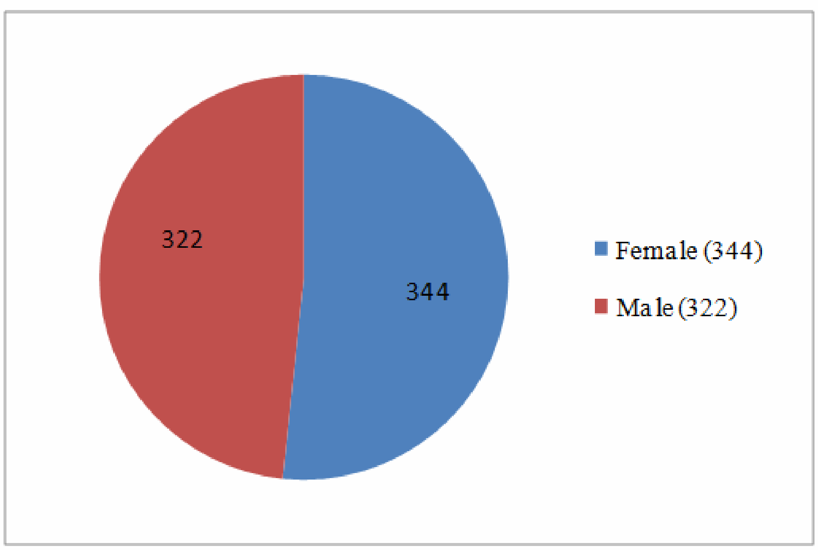


Figure.2 Age Distribution

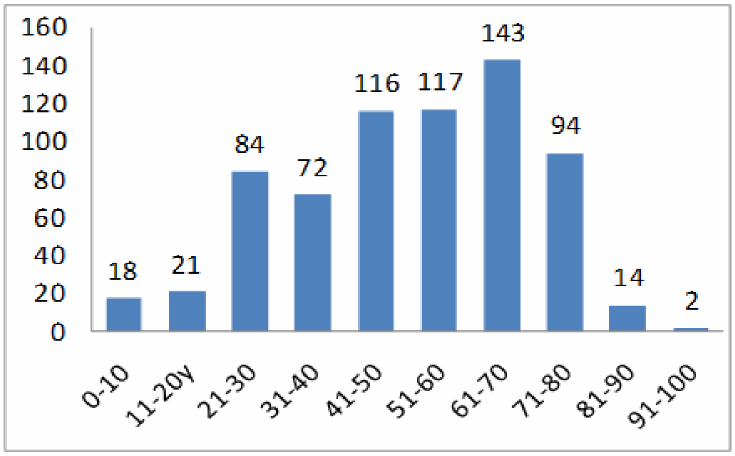


Figure.3 Distribution of Isolates

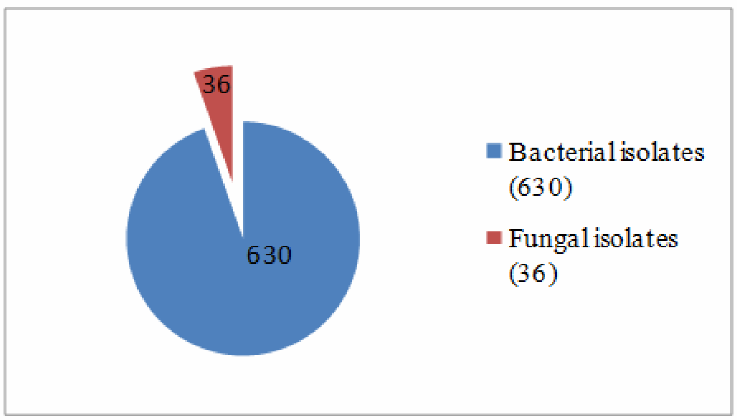
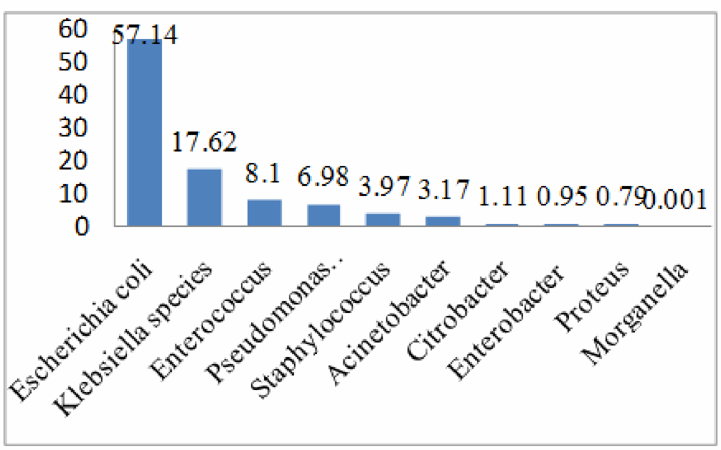


Figure.4 Bacterial Profile of UTI



The present observed that *Pseudomonas aeruginosa* isolated in our study was mostly resistant to many of the antibiotics. Amikacin showed maximum sensitivity (45.45%) followed by Gentamicin, Cefipime (34.09%), Ceftazidime (31.82%), Ciprofloxacin (25%), Norfloxacin (13.64%). Similar susceptibility pattern has been reported by Kulkarni *et al* (2014).

Over 80% of P.mirabilis were sensitive to all the drugs tested except for cotrimoxazole which showed susceptibility of only 20%. Only one Morganella morganii was isolated and it was susceptible to all the drugs tested except Nitrofurantoin. Staphylococcal species showed highest sensitivity to Vancomycin and Linezolid (100%) followed by Nitrofurantoin (80%), Gentamicin and Tetracycline (72%), Cotrimoxazole (56%), Norfloxacin (48%). Least sensitivity was seen against Ciprofloxacin (17.33%) and Penicillin (24%). Six among 19 S.aureus were Methicillin resistant (31.58%).

Candida showed maximum sensitivity to Amphotericin B and Nystatin (94.4%) followed by Clotrimazole (91.64%), Fluconazole (88.89%) and Ketoconazole (80.56%).

Our study showed a high prevalence of UTI in females and in the elderly patients. The increased prevalence in elderly could be attributed to co morbidities like diabetes mellitus. *E.coli* still remains the commonest causative agent for UTI with a high sensitivity rate to Nitrofurantoin. Most of the gram negative bacilli showed increasing rates of resistance to routinely tested and used antibiotics like Cephalosporins, Fluoroquinolones and Trimethoprim-sulfamethoxazole .Though the resistance patterns in our study are similar to other studies, it is on the rise due to the uncontrolled abuse of the available antibiotics. Hence it becomes very essential to analyse the causative agent for UTI, establish antibiotic policies and take stringent measures to ensure effectiveness of the same. Failing to do so might result in the rise of multi drug resistant bacteria due to irrational use of antibiotics.

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