Aetiological Profile and Antibiogram of Urinary Isolates Causing UTI in Patients Attending a Tertiary Care Hospital of Western Odisha

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ABSTRACT

BACKGROUND
Although UTI is a female disease, males are also susceptible during the neonatal period and old age. Asymptomatic bacteriuria, cystitis and acute urethral syndrome are the most common clinical types. Etiological profile is variable in different geographical areas, but E. coli is the most common agent worldwide. Klebsiella, Proteus, Pseudomonas are important causes of hospital acquired UTI. Culture sensitivity of early morning mid-stream urine collected by clean catch technique is the gold standard method of diagnosis of UTI. Sensitivity to 3rd generation cephalosporins and cotrimoxazole is variable in different areas but aminoglycoside, nitrofurantoin and carbapenem are almost sensitive worldwide. Resistance to nitrofurantoin and carbapenem has been reported in many areas of the world. Before starting empirical therapy, physician should know the local etiological profile and antibiotic sensitivity pattern of uropathogens. We wanted to study the etiological profile and antibiotic sensitivity pattern of urinary isolates in a tertiary care hospital of Western Odisha.

METHODS
Early morning mid-stream urine samples of 730 clinically suspected UTI patients were collected by clean catch technique and sent to microbiology department. Cysteine lactose electrolyte deficient (CLED) agar media was seeded with urine with the help of 0.01 ml (4 mm) loop. After incubation for 24 hrs at 37°C growth was observed and identified by Gram stain and biochemical tests. Antibiotic sensitivity was performed by disc diffusion method as per CLSI guidelines. Antibiotic sensitivity was performed for all Gram-negative bacteria, Enterococci and Staphylococcus.

RESULTS
Among 730 samples, 238 (33%) showed significant bacteriuria and 63% of significant bacteriuria samples were from female. Middle age females (36-50 yrs) were more affected (38%) followed by old age (>50 yrs) male (19%) and old age (>50 yrs) female (18%). E. coli was the most common bacteria (31%) followed by Enterococci (18%). Fluoroquinolones like nalidixic acid and norfloxacin showed high resistance rate (31%, 42% in case of Gram-negative bacteria and 12%, 25% in case of Staphylococcus species respectively). Nitrofurantoin showed excellent sensitivity to both Gram-positive cocci and Gram-negative bacilli. (80% for gram-negative bacilli and 87% for Staphylococcus species and 78% for enterococci species). Aminoglycoside and carbapenem showed excellent sensitivity to Gram-negative bacteria (81% and 92% respectively). Third generation cephalosporins showed poor sensitivity (48% to 53%).

CONCLUSIONS
Enterococci rather than Klebsiella species was the 2nd most common uropathogen in our study. Aminoglycoside was still useful for UTI. Nitrofurantoin was the best option for empirical therapy.

KEY WORDS
UTI, Aetiology, Antibiogram, Bacteria, Sensitivity
Urinary tract infection is classified clinically as urethritis, asymptomatic bacteriuria, cystitis, acute urethral syndrome and pyelonephritis.1 Clinical symptoms of urethritis is dysuria and frequency. Asymptomatic bacteriuria means patients is symptomless but excreting bacteria more or equal to 10^5 CFU/ml. Most common type of infection is cystitis which is manifested as dysuria, frequency, urgency and tenderness over bladder area and sometimes bloody urine. As cystitis is a localised infection, fever and other signs of a systemic illness are absent.1 Acute urethral syndrome is manifested as dysuria, frequency and urgency in young sexually active woman who excrete bacteria fewer than 10^5 CFU/ml in urine. Almost 50% of all women who complain of burning micturition fall into this group. Pyelonephritis is a systemic infective condition involving kidney calices, pelvis and manifested by fever and flank pain.1 Global annual incidence of UTI is 150 million costing about 6 billion dollar per year.2 Approximately 10% of human will have a UTI at some times during their lives. UTI is also a common nosocomial infection.3 UTI is important complication of Diabetes, renal transplantation, renal disease, structural and neurological abnormality that interfere with urinary flow. Escherichia coli is the most frequent causative agent of community acquired UTI. Other bacteria frequently causing UTI are Klebsiella spp., Citrobacter spp., Enterobacter spp., Acinetobacter spp., Coagulase negative Staphylococcus, Staphylococcus aureus and Enterococci. Pseudomonas, Klebsiella and Enterobacter are responsible for complicated UTI.1 The hospital environment is the source of organisms involved in nosocomial UTI. Bacteria can invade and cause UTI via two routes: ascending and haematogenous pathway.1 In ascending pathway responsible bacteria first colonize the vaginal cavity and periurethral area and then enter into the bladder, multiply in the bladder and then pass up to ureters to the kidney.3 UTI may also occur by the haematogenous route in less than 5% of cases,4 the exact prevalence of UTI is dependent on age and sex. In the neonatal period, UTI are less than 2% in male and female.1 the incidence of UTI among males remains relatively low after neonatal period and until 60 years of age when BPH obstruct the urine flow, therefore UTI is more prevalent in female. Recurrence and persistence of infection is also common in female. Sexual activity and hormonal changes are two important causes of high incidence of UTI in female of young age group. Culture sensitivity is the gold standard method of laboratory diagnosis of UTI. Any colony count more or equal to 10^5 CFU/ml is significant bacteriuria. (by KaaS concept). If the organism is Staphylococcus specie, or patient is pregnant/diabetic, or patient is already on antibiotic therapy low colony count is also significant. An early morning mid-stream urine collected by clean catch technique is the best sample for culture sensitivity provided that the sample is collected in sterile container and processed within 2 hrs. of collection. Physician of developing country like India usually prescribe empirical antibiotic therapy just after getting complain of burning micturition and positive RE/ME report. So local microbial profile and antibiotic sensitivity should be known to physician in every region. Keeping it in mind we have conducted research to find out etiological profile of urinary isolates of UTI and their sensitivity in patients attending a tertiary care hospital of Western Odisha.

**METHODS**

This is a cross-sectional study carried out in the Department of Microbiology of a tertiary care hospital of Western Odisha for a period of 3 months from Sep 2019 to Nov 2019. Urine samples from 730 patients (clinically suspected for UTI) both from IPD and OPD were collected. Patient who were already in antibiotic therapy were excluded from the study.

**Sample Collection**

Patient was explained and instructed to collect early morning mid-stream urine into a 20 ml sterile container after proper cleaning of the genitalia with soap water. The samples were immediately transported to microbiology laboratory and processed within 2 hrs. of collection.

**Sample Processing**

Cystine lactose electrolyte deficient (CLED) agar media was streaked (t streaking method) with the help of a nichrome wire loop of 4 mm (0.01 ml).The plates were incubated at 37°C in incubator for 24 hrs. Next day growth was observed and Gram stain of colony was performed to identify it as GPC or GNB colony. Catalase test and slide coagulase test were performed in any GPC colony for presumptive identification of Staphylococcus. Oxidase test was performed in NLF (non-lactose fermenting) colony to rule out Pseudomonas. Indole test, TS1 test, Urase test and Citrate test were put in any GNB growth. Mannitol and tube coagulase were put for any GPC colony. Bile esculin test was put in GPC colony which showed diplococci in angle on gram stain. All biochemical tests were incubated in incubator at 37°C for 24 hrs. Motility was checked for any GNB by hanging drop preparation. Colony count of growth was determined by multiplying 100 to number of colony as CFU/ml. Any count more than 10^5 CFU/ml was considered significant. Inoculum of growth in peptone water was made and adjusted to 0.5 McFarland as per CLSI (clinical and laboratory standard institute) guideline.5 Then the inoculum was seeded into Muller Hinton agar plate. The plates were incubated at 37°C for 24 hrs. After 24 hrs the plates were checked and taken for further analysis.

**Antibiotic Panel**

Antibiotic for GNB were Amikacin (30µ), Cefoperazone (75 µ), Cefoperazone sulbactam (75/30 µ) Pipericillin tazobactam, (100/10µ) Piperacillin (100 µ), Cotrimoxazole, Netilmicin (30µ) Tigecycline, Amoxiclav (20/10µ) Ciprofloxacin (5µ), Nalidixic acid (30 µ), Norfloxacin (10µ), Ceftriaxone (30µ) Gentamycin (10µ), Meropenem(1 µ), Imipenem (10 µ) Nitrofurantoin (100µ) and special antibiotic for Pseudomonas were Ceftazidime (30µ) Ceftazidime clavulanic acid (30/10µ), Azithromycin (15µ), Linezolid (30µ), Nitrofurantoin (100 µ) Ciprofloxacin (5µ) Cotrimoxazole, Gentamycin (10µ) Erythromycin (15µ), Clindamycin (2µ), Tigecycline, Nalidixic acid (30µ)and Norfloxacin (10µ) were put for any catalase positive GPC.
growth (Staphylococcus). Ampicillin (10µ), Ampicillin sulbactam (10/10µ), Teicoplanin, Linezolid (30µ), Nitrofurantoin (100µ) Ciprofloxacin (5µ), Cotrimoxazole and Gentamycin (10µ) were put for any catalase negative GPC growth (enterococci).

**Quality Control**

E. coli ATCC 25922, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853, E. faecalis ATCC 29212 strains were used for quality control of biochemical test and antibiotic sensitivity test.

**Data Analysis**

Data analysis was done by SPSS software version 17.

### RESULTS

![Figure 1. Age Wise Distribution of Significant Bacteriuria](image)

![Figure 2. Distribution of Different Bacteria in Positive Growth](image)

![Figure 3. Enterococci Antibiotic Sensitivity Pattern](image)

![Figure 4. Staphylococcus aureus Antibiotic Sensitivity Pattern](image)

![Figure 5. Gram Negative Bacteria (GNB) Antibiotic Sensitivity Pattern](image)

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<th>Table 1. Percentage (%) of Sensitive Strains of Different Gram-Negative Bacteria to Different Antibiotics</th>
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<td>Ak=Amikacin, Pi=Piperacillin, Gen= Gentamicyn, CPZ=Cefoperazone CFS= Cefoperazone sulbactam, PIT=Piperacillin tazobactam, COT=Cotrimoxazole Net=Nalidixic acid, TGC= Tigecycline, AMC= Amoxiclav, CIP=Ciprofloxacin, NA=Nalidixic acid, PXN=Norfloxacin, AT= Azithromycin</td>
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Out of 730 sample 53% (390/730) showed no growth, 33% (238/730) showed significant bacteriuria. 12% (88/730) showed insignificant bacteriuria and 2% (14/730) showed growth of budding yeast cell (BYC). Among 238...
patients of significant bacteriuria 63% were female and 37% were male. Among male patients with significant bacteriuria 5% were in age group of less than 15, 5.4% in age group of 16-35, 7.9% in age group of 36-50 and 19% in age group of more than 50. Among female patients with significant bacteriuria 7% were in age group of less than 15, 18% in age group of 16-35, 20% in age group of 36-50 and 18% in age group of more than 50. (figure 1).

Among 326 positive growth 31% was E. coli, 10% was Klebsiella pneumoniae, 2% was Klebsiella oxytoca, 5% was Acinetobacter spp., 1% was Citrobacter spp., 3% was Enterobacter, 5% was Pseudomonas spp., 2% was Proteus spp., 15% was S. aureus, 2% was CoNS, 18% was Enterococci. (figure 2) Antibiotic sensitivity pattern of gram positive bacteria were showed in figure 3 (enterococci) and figure 4 (S. aureus) Antibiotic sensitivity pattern of Gram negative bacteria were showed in figure 5. Table 1 showed antibiotic sensitivity pattern of individual Gram negative bacteria.

**DISCUSSION**

All women who have been colonised in the vaginal or periurethral area by uropathogen do not develop UTI. Complex interplay of host and microbial factors determines the outcome of the colonisation. Acidic pH, osmolality of urine, high concentration of toxic waste of body, organic acid concentration of urine is inhibitory to many bacteria. Constant flushing of urine, antibacterial substance released from uropathogen, valve like mechanism in junction of ureter and bladder, TNF, IFN gamma, released due to stimulation by lipopolysaccharide, Tamm-Horsfall protein or uromucoid from uropathogen that binds with type 1 fimbrae of E. coli are protective for host and prevent UTI development. However mechanical obstruction resulting from kidney stone, stricture impairment of valve action between ureter and bladder, hormonal changes during pregnancy all can impair host protective mechanism leading to UTI. Most cases of UTI are caused by only a few organisms although any uropathogen can cause UTI. For example, a limited number of serogroups of E. coli (UPEC) that express type 1 fimbrae or type P fimbrae, invade urinary tract and cause UTI? Proteus and Klebsiella increase pH of urine and promote UTI. Some uropathogen strain produce more K antigen and inhibit phagocytosis. Staphylococcus saprophyticus (CoNS) has more attraction than S. aureus to uropathogen and cause UTI in sexually active young women. According to Anderson4 'PODS' formed by intra cellular bacteria is responsible for persistent infection and repeated recurrence.

In our study out of 730 samples, 238 (33%) showed significant bacteriuria which was higher than Arshi et al (6.87%) Arshi et al (30%),11 Angamiet et al (28.1%)12 but lower than Nzalie et al (58.8%),13 Manjunath et al (63.51%),15 Prakash et al (53.82%),16 Tave et al (36%),16 Khan et al (54.4%),17 Manjunath et al (42.37%)18 it was very similar to Dash et al (34.5%)19 most probably due to similar environmental niche. BYC was positive in 2% growth and it was lower than Manjunath et al (4.5%)19 and Venkatesh et al (6%),20 among significant bacteriuria 63% were in female and 37% in male. Female were more susceptible to UTI and it was almost universal finding. It was showed in study done by Manjunath et al,18 Maheswary et al,14 Arshi et al,11 Prakash et al10 Manjunath et al, Prakash et al,12 Dash et al,19 Tave et al,16 Khan et al,7 Nzalie et al15 and Juina et al11 Oladeinde et al,22 Kashef et al.23 Females were more affected due to proximity of urethral meatus to the anus, shorter urethra, less acidic Ph of the vaginal fluid24,25. Males outnumbered female only in elderly (>50) age group (19% vs 18%) in our study which was similar to Khan et al (38.1% vs 10.8%),17 Shankel et al (23% vs 19%),26 Prakash et al(43% vs 22.4%)15 and Sood et al (20.7% vs 17.34%).27 Elderly male were more susceptible due to neurogenic bladder and BPH (Benign prostatic hyperplasia) than younger male.19 In our study Most prevalent bacteria was E. coli (31%) followed by Enterococci (18%), S. aureus (15%) Klebsiella pneumoniae (10%). The least common bacteria was Citrobacter (1%). This finding was very similar with Dash et al (68.8% E. coli followed by 9.7% Enterococci), Manjunath et al (60.7% E. coli followed by 12.1% Enterococci species)10 and Arshi et al (56.7% E. coli followed by 13.1% Enterococci species) but not consistent with the most of the studies worldwide. Klebsiella was the 2nd most common bacteria in Nzalie et al, Angamiet et al, Prakash et al, Tave et al, Juina et al, Khan et al, Mahajan et al, Akochere ET All showed that Klebsiella was the least prevalent (1.2%) bacteria in UTI. Ehinmidu ET al showed that Pseudomonas was the most common (32%) bacteria in UTI.

In antibiotic sensitivity test of GNB overall highest sensitivity was showed by Tigecycline (97%) followed by Meropenem (92%), Netilimicin (89%), Imipenem (81%), Amikacin (81%), Nitrofurantoin (80%) Pipericillin Tazobactam (80%), Gentamycin (78%) Low sensitivity was showed by Nalidixic Acid (31%), Cotrimoxazole (40%), Norfloxacin (42%), Ceftriaxone (48%), Cefoperazone (53%). High sensitivity to carbapenem, aminoglycoside, nitrofurantoin and low sensitivity to 3rd generation cephalosporin, fluoroquinolones and cotrimoxazole were showed by Maheswary et al,7,14,19,20,24,25,28,29 Afroz et al,10 Mahajan et al,21 Prakash et al,12 Dash et al19 Juina et al22 Khan et al,17 Angamiet et al In case of Staphylococcus high sensitivity was seen in Linezolid (90%) Nitrofurantoin (87%) Gentamycin (83%). Clindamycin (83%). Cotrimoxazole (73%). Low sensitivity was seen in nalidixic acid, norfloxacin, erythromycin, tigecycline and ciprofloxacin. Enterococci also showed high sensitivity to linezolid, teicoplanin and nitrofurantoin and aminoglycoside but lower sensitivity to ampicillin, ampicillin sulbactam, cotrimoxazole and fluoroquinolones and this finding was similar to Prakash et al,12 Dash et al,19 Khan et al17 and Mahajan et al,21 Cotrimoxazole showed sensitivity only to Staphylococcus but showed poor sensitivity to Enterococcus and gram negative bacteria so it is not appropriate to use it as antibiotic of choice in empirical therapy. Fluoroquinolones like nalidixic acid, norfloxacin, ciprofloxacin is of no use today for UTI as more than 50% of bacteria were resistant to it and it was a universal finding all over the world. 2nd generation cephalosporin was used so much not only for UTI but also for another infection that it was now resistant to almost all uropathogen. Aminoglycoside was still fighting against all uropathogen, most probably due to less use in community acquired infection. As it was an injectable antibiotic, it could not be selected as empirical agent. So the remaining oral.
option was one and only Nitrofurantoin. In spite of high sensitivity, Carbapenem should not be used in uncomplicated UTI otherwise within decade it will be useless like fluoroquinolones.

**CONCLUSIONS**

Enterococci rather than Klebsiella spp. was the 2nd most common uropathogen in our study. Cotrimoxazole can be used if only Staphylococcal infection is suspected or confirmed but not for Enterococci or Gram negative bacteria. 3rd generation cephalosporin and fluoroquinolones should be avoided in UTI due to high resistance rate. Aminoglycoside was still useful for UTI in our study. Nitrofurantoin was the best option for empirical antibiotic therapy.

**REFERENCES**


