

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 type. 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 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

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BACKGROUND

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.¹ 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.¹ 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

Cysteine 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, TSI test, Urease 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 by lawn culture method with the help of sterile swab stick. Antibiotic discs were put into plate and incubated for 24 hrs. By Kirby Bauer disc diffusion method⁶ antibiotic sensitivity test was performed. All the media and antibiotics were purchased from HiMedia, Mumbai.

Antibiotic Panel

Antibiotic for GNB were Amikacin (30µ), Cefoperazone (75 µ), Cefoperazone sulbactam (75/30 µ) Piperacillin 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

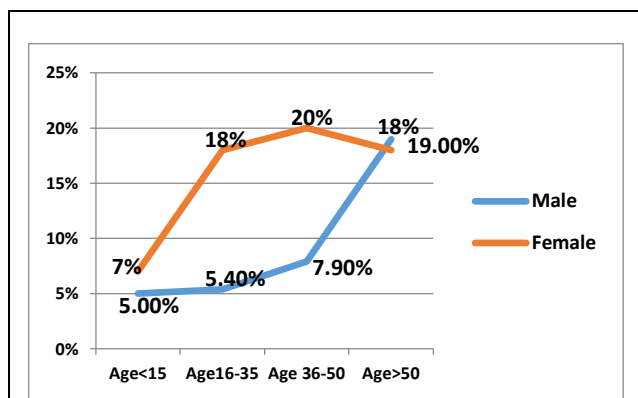


Figure 1. Age Wise Distribution of Significant Bacteriuria

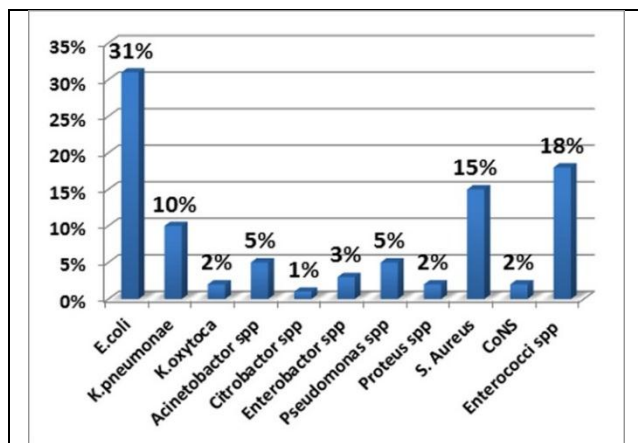


Figure 2. Distribution of Different Bacteria in Positive Growth

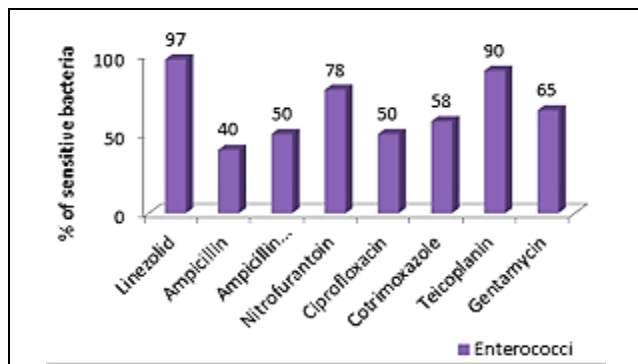


Figure 3. Enterococci Antibiotic Sensitivity Pattern

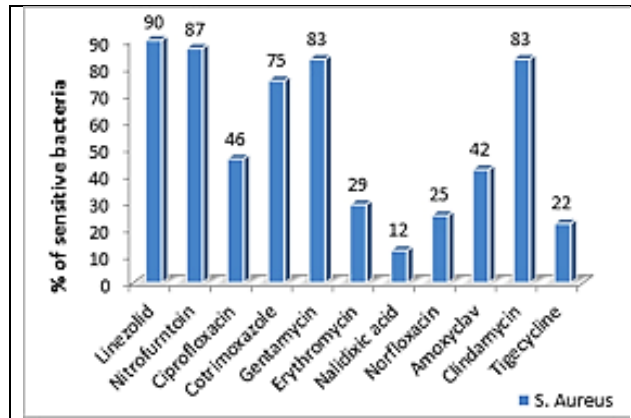


Figure 4. Staphylococcus aureus Antibiotic Sensitivity Pattern

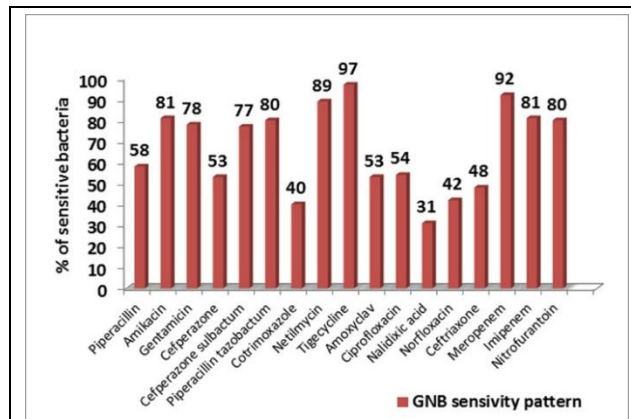


Figure 5. Gram Negative Bacteria (GNB) Antibiotic Sensitivity Pattern

Antibiotic	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>K. oxytoca</i>	<i>Acinetobacter</i>	<i>Citrobacter</i>	<i>Enterobacter</i>	<i>Proteus</i>	<i>Pseudomonas</i>
Pi	44	79	43	80	67	89	80	60
Ak	85	79	71	73	67	100	80	67
Gen	78	82	71	73	67	89	80	67
CPZ	42	73	0	60	67	67	80	94
CFS	78	70	57	80	34	78	80	100
PIT	82	79	71	100	34	89	80	67
COT	63	76	57	80	34	67	60	94
NET	92	76	86	87	100	100	80	100
TGC	100	94	100	94	67	89	100	100
AMC	45	64	0	80	67	67	20	87
CIP	46	76	0	73	34	67	60	77
NA	20	45	0	47	34	44	40	80
NX	32	48	0	73	34	56	40	87
CTR	36	67	0	73	34	56	60	94
MRP	95	82	100	94	67	89	100	94
IPM	79	82	100	94	34	89	60	53
NIT	93	74	100	67	100	67	60	60
GAT	NA	NA	NA	NA	NA	NA	NA	67
CAZ	NA	NA	NA	NA	NA	NA	NA	47
CAC	NA	NA	NA	NA	NA	NA	NA	47
AT	NA	NA	NA	NA	NA	NA	NA	60

Table 1. Percentage (%) of Sensitive Strains of Different Gram-Negative Bacteria to Different Antibiotics

AK=Amikacin, Pi=Piperacillin, Gen=Gentamycin, CPZ=Cefoperazone CFS=Cefoperazone sulbactam, PIT=Piperacillin tazobactam, COT=Cotrimoxazole Net=Netilmicin, TGC= Tigecycline, AMC= Amoxiclav, CIP=Ciprofloxacin, NA=Nalidixic acid. NX=Norfloxacin, CTR=ceftriaxone, MRP=Meropenem, IPM=Imipenem, NIT=Nitrofurantoin, GAT=Gatifloxacin, CAZ=Ceftazidime, CAC=Ceftazidime clavulanic acid AT= Azithromycin

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 colonisation¹. Acidic pH, osmolarity of urine, high concentration of toxic waste of body, organic acid concentration of urine is inhibitory to many bacteria.¹ Constant flushing of urine, antibacterial substance released from uroepithelium, valve like mechanism in junction of ureter and bladder, TNF, IFN gamma, released due to stimulation by lipopolysaccharide, Tamm-Horsfall protein or uromucoid from uroepithelium that binds with type 1 fimbriae of *E. coli* are protective for host and prevent UTI development.¹ 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)^{7,8} that express type 1 fimbriae or type P fimbriae, invade urinary tract and cause UTI.⁷ *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 uroepithelium and cause UTI in sexually active young women. According to Anderson⁹ '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 Afroza et al (6.87%)¹⁰, Arshi et al (30%)¹¹, Angamiet et al (28.1%)¹² but lower than Nzalie et al (58.8%)¹³, Maheswary et al (63.51%)¹⁵, Prakash et al (53.82%)¹⁶, Taye 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%)¹⁸ 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 Afroza et al,¹⁰ Angamiet et al,¹² Prakash et al,¹⁵ Dash et al,¹⁹ Taye et al,¹⁷ Khan et al,¹⁷ Nzalie et al,¹³ and Jubina et al,²¹ 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 fluid^{24,25}. 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 al¹⁹ (68.8% *E. coli* followed by 9.7% Enterococci), Manjunath et al (60.7% *E. coli* followed by 12.1% Enterococci species)¹⁸ and Arshi et al (56.7% *E. coli* followed by 13.1% Enterococci species)¹¹ but not consistent with the most of the studies worldwide. *Klebsiella* was the 2nd most common bacteria in Nzalie et al¹³, Angamiet et al,¹² Prakash et al,¹⁵ Taye et al,¹⁶ Jubina et al,²¹, Khan et al,¹⁷ Mahajan et al.²⁸ Akochere ET al²⁹ showed that *Klebsiella* was the least prevalent (1.2%) bacteria in UTI. Ehinmidu ET al³⁰ 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%), Netilmicin (89%), Imipenem (81%), Amikacin (81%), Nitrofurantoin (80%) Piperacillin 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,¹⁴ Afroza et al,¹⁰ Mahajan et al,²⁸ Prakash et al,¹⁵ Dash et al,¹⁹ Jubina et al,²¹ Khan et al,¹⁷ Angamiet et al.¹² 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,¹⁵ Dash et al,¹⁹ Khan et al¹⁷ and Mahajan et al.²⁸ 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. 3rd 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.

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