

## BACTERIAL PREVALENCE, ANTIBIOTIC SENSITIVITY PATTERN AND PREDISPOSING FACTORS IN PATIENTS OF NOSOCOMIAL URINARY TRACT INFECTION (UTI) VISITED THE TERTIARY CARE HOSPITAL IN JAMNAGAR REGION, WESTERN GUJARAT, INDIA

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### ABSTRACT

#### BACKGROUND

Nosocomial UTI is the most common bacterial infection ranging from asymptomatic bacterial to septicaemia. Gram-negative bacteria contribute 80-85% of UTI and 15-20% by gram positive with major contribution by E. coli.

The aim of the study is to assess the bacterial prevalence, drug sensitivity pattern and predisposing factors in nosocomial UTI.

#### MATERIALS AND METHODS

778 midstream urine samples were tested by conventional methods of which 282 (36.25%) samples were identified as positive for bacteria. All the isolates were subjected to antibiotic sensitivity testing. Statistical analysis was done by Chi-square test.

#### RESULTS

Bacterial prevalence was 36.25%. 87.95% UTI were caused by gram negative while 12.05% cases were due to gram-positive bacteria. Most prevalent bacterium was E. coli (48.23%). Piperacillin+tazobactam were identified as most sensitive drug for all gram-negative isolates. Among the gram-positive isolates, coagulase-positive bacteria like Staph aureus were sensitive to all tested drugs while coagulase-negative bacteria were less sensitive to all exposed drugs and Enterococcus produced 75% sensitivity rate to vancomycin, teicoplanin and linezolid. UTI was common between 40-60 years (37.23%) with mean age 44.23±20.05 and P value was >0.05. High frequency observed in men (55.32%) than women (44.68%), (P >0.05). 53.19% cases had history of catheterisation (P <0.001).

#### CONCLUSION

Variable sensitivity pattern and increasing drug resistance observed in uropathogen, so study emphasise over antibiotic sensitivity testing before prescribing empirical therapy, understanding the risk factors helps to contain the UTI.

#### KEYWORDS

Bacterial Prevalence, Antibiotic Sensitivity Pattern, Predisposing Factors, Nosocomial UTI.

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#### BACKGROUND

Urinary system or urinary tract includes kidneys, ureters, bladder and urethra. It maintains the water and salt balance throughout the body and also voids the urine from the body. Urinary Tract Infection (UTI) is defined as an infection in one or more part of urinary system.<sup>1</sup> Anatomically, Urinary Tract Infection (UTI) is classified as an upper UTI, which encompasses kidneys and ureters while lower UTI encompasses the infection of bladder and urethra.

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Urinary tract infection is a common issue of health in hospital as well as in community. It accounts around 35% of all hospital-acquired infection and 2<sup>nd</sup> most common cause of bacteraemia in indoor cases.<sup>2,3</sup> It is one of the most important causes of morbidity in the general population.<sup>4</sup> Globally, it is estimated that 6 million outpatients visit and 3,00,000 indoor admissions are due to urinary tract infection.

An 80-85% of UTIs are due to gram-negative bacteria, while 15-20% are due to gram-positive bacteria.<sup>5,6</sup> Most frequent cause of UTI is enteric group of bacteria, however, it also produced by other group of bacteria.<sup>3</sup> Predominant pathogen among all isolates is E. coli in different categories of patients and it accounts about 80-85% of total cases,<sup>7-10</sup> however, UTI is also a disease due to other bacteria like Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter, Proteus species, Serratia, Enterobacter, etc. and from gram-positive list, it includes Staphylococcus

aureus, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Enterococcus* species, etc.<sup>10,11</sup> Rarely, UTI is also produced by viruses, fungus and parasites.

Around 95% of UTIs are produced by bacteria that typically multiply at the urethral opening and ascend up to the bladder, while rare cases are due to descending infection from the bloodstream into kidney.<sup>1</sup>

UTI can occur at any age. Almost, 10% of humans will have UTI at some part of their lives. Women are more prone to UTI than men because of shorter urethra, which is 1.5 inches compared to men with 8 inches urethra and bacteria have a shorter distance to travel to reach the bladder. The urethra is located close to the rectum in women and bacteria from the rectum are more likely to take entry into the urethra during wipe from back to front (instead of front to back) after a bowel movement. Sexual intercourse can also precipitate the UTI in women because bacteria can be pushed into the urethra. Use of antibiotics and menopause in women can alter the vaginal flora and increase the risk for UTI.<sup>12</sup> Pregnancy is also a contributing factor for UTI and about 5-10% of pregnant women have been diagnosed as UTI. About 80% of UTIs in the hospital are due to catheterisation. Structural and functional abnormalities in urinary tract, metabolic disorder like diabetes mellitus, local trauma and immunodeficiency diseases like HIV/AIDS are also contributory factors for development of UTI.<sup>1</sup>

Trend of UTI caused by bacteria is increased in recent years. Usually, empirical therapy is given to treat UTI before the laboratory reports of urine culture are available. The prevalence of drug resisting uropathogens is increasing globally.<sup>11,13,14</sup> Drug resistance to uropathogen is different in different environmental condition.<sup>15</sup> Very few data are available, which shows the common bacteria, their antimicrobial susceptibility and predisposing factors for UTI in our region. Present study is carried out to rule out all these.

## MATERIALS AND METHODS

**Study Duration and Sample Size-** It is a retrospective analysis of all samples collected and tested for routine diagnosis purpose from April 2015 to September 2015 at Department of Microbiology, Shri M. P. Shah Government Medical College and G.G.G. Hospital, Jamnagar. During this period, total 778 midstream urine samples were collected in sterile container and tested, of which 282 samples were identified as positive for bacteria. Complete histories of all patients were taken in a standard requisition form.

**Analysis to Determine the Sample Size-** A single proportion formula was used to calculate the sample size,  $n = Z^2 p (1-p)/d^2$ . Where-  $Z = Z$  score for 95% confidence interval = 1.96,  $p =$  prevalence,  $d =$  tolerable error = 5%. Thereby,  $n = (1.96)^2 0.8(1-0.8)/(0.05)^2 = 627$ , giving the final sample size of 627, but we enrolled the total 778 samples in our study.

**Sample Collection-** 778 early morning 5 mL of midstream urine specimens were collected in sterile, leak proof, plastic containers or test tubes under proper aseptic precautions.

**Isolation of Bacteria-** For the isolation of uropathogen, loop full of urine sample was streaked on nutrient agar and MacConkey's agar followed by incubation at 37°C for 24 hours. After overnight incubation, all the culture plates without any evidence of growth were considered as negative, while all the culture growth with colony counts yielding bacterial growth of more than  $10^5$ /mL of urine were regarded as significant bacteriuria,  $10^4$ - $10^5$ /mL of urine were regarded as doubtful and less than  $10^4$ /mL of urine were regarded as nonsignificant bacteriuria. Cases of significant bacteriuria were identified further to rule out gram-positive bacteria and gram-negative bacteria as a cause of UTI.

**Identification of Gram-Negative Bacteria-** Gram-negative bacteria were identified by culture characteristics, morphology under Gram stain, motility test and biochemical reaction like sugar fermentation tests, indole test, methyl red test, citrate test, urease test, Phenylpyruvic Acid (PPA) test, Triple Sugar Iron (TSI) test, oxidase test and catalase test.

**Identification of Gram-Positive Bacteria-** Gram-positive bacteria were identified by culture characteristics, subculture on mannitol salt agar and morphology under Gram stain and biochemical reaction like sugar fermentation tests, urease test, coagulase test, catalase test, phosphatase test and novobiocin sensitivity test.

**Antimicrobial Susceptibility Testing-** All gram-negative and gram-positive bacterial isolate were subjected to antimicrobial susceptibility testing according to the criteria of National Committee for Clinical Laboratory Standards (NCCLS), based on Kirby-Bauer disc diffusion principle.

All gram-negative isolates were tested with cotrimoxazole (1.25/23.75 µg), norfloxacin (10 µg), nitrofurantoin (300 µg), cefixime (5 µg), gentamicin (10 µg), ceftioxin (30 µg), ciprofloxacin (5 µg), carbenicillin (100 µg), amikacin (30 µg), tetracycline (30 µg), imipenem (10 µg) and piperacillin + tazobactam (100 µg + 10 µg) combination.

All *Staphylococcal* species were tested with ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), amikacin (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), clindamycin (2 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), ofloxacin (5 µg) and tobramycin (10 µg).

*Enterococcus* isolates were tested with ceftioxin (10 µg), vancomycin (30 µg), erythromycin (15 µg), teicoplanin (30 µg), clindamycin (2 µg), linezolid (30 µg), azithromycin (10 µg) and ceftioxime (30 µg).

**Quality Control-** All the tests of isolation, identification and susceptibility were performed under standard quality control techniques by using standard strains of *E. coli* ATCC 25922,

*P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603 and *Staph aureus* ATCC 25923.

**Inclusion criteria-** All the cases with following criteria were included in study.

- Patients without current antibiotics therapy.
- Culture with bacterial isolates.
- Indoor cases (hospitalised patients).

**Exclusion Criteria-** All the cases with following criteria were excluded from study.

- Patients with current antibiotics therapy.
- Culture with isolates other than bacteria like yeast cells or fungus.
- Repeated sample from same patients to avoid the duplication of data.
- Outdoor cases (Outpatient department cases).

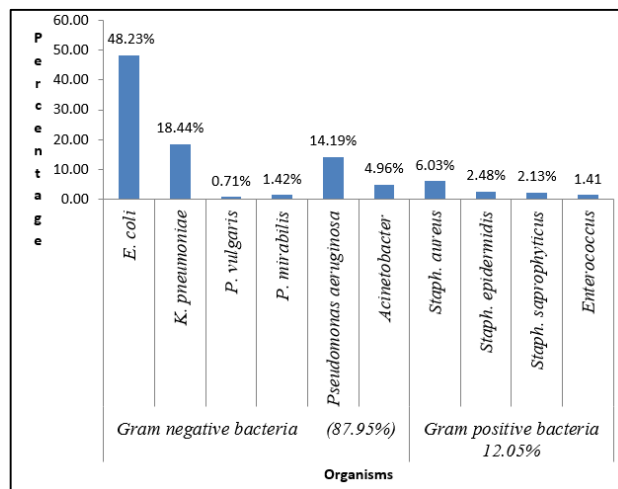
**Ethical Clearance-** It is a retrospective analysis of all the samples collected and tested for routine diagnosis purpose, so ethical consideration is not necessary.

**Statistical Analysis-** Statistical analysis was done by Chi-square test.

**RESULTS**

Total 778 samples were enrolled for final testing from April 2015 to September 2015 at Department of Microbiology, Shri M. P. Shah Government Medical College and G.G.G. Hospital, Jamnagar, of which 282 samples were identified as positive

for bacteria, which shows the 36.25% bacterial prevalence in urine.



**Figure 1. Distribution of Bacteria**

Figure 1 shows the distribution of bacteria causing UTI. 87.95% UTI were caused by gram-negative bacteria, while 12.05% cases of UTI were due to bacteria from gram-positive category. Among the gram-negative category, most prevalent pathogen was *E. coli* (48.23%) followed by *K. Pneumoniae* (18.44%), *Pseudomonas aeruginosa* (14.19%), *Acinetobacter* (4.96%), *P. vulgaris* (0.71%) and *P. mirabilis* (1.42%). *Staph aureus* (6.03%) was the commonly encountered organism in gram-positive category followed by *Staph. epidermidis* (2.48%), *Staph. saprophyticus* (2.13%) and *Enterococcus* (1.41%).

Bacteria	<i>E. coli</i> (n=136)	<i>K. pneumoniae</i> (n=52)	<i>P. aeruginosa</i> (n=40)	<i>Acinetobacter</i> (n=14)	<i>P. vulgaris</i> (n=2)	<i>P. mirabilis</i> (n=4)
Co-trimoxazole	42 (30.88%)	8 (15.38%)	11 (27.5%)	3 (21.43%)	1 (50%)	1 (25%)
Norfloxacin	18 (13.23%)	6 (11.53%)	18 (45%)	0	2 (100%)	1 (25%)
Nitrofurantoin	54 (39.70%)	7 (13.46%)	16 (40%)	2 (14.29%)	0	1 (25%)
Cefixime	28 (20.58%)	6 (11.53%)	11 (27.5%)	1 (7.14%)	0	0
Gentamicin	39 (28.67%)	6 (11.53%)	16 (40%)	8 (57.14%)	0	0
Cefoxitin	11 (30.88%)	3 (5.76%)	11 (27.5%)	0	0	0
Ciprofloxacin	18 (13.23%)	6 (11.53%)	16 (40%)	1 (7.14%)	0	0
Carbenicillin	14 (10.29%)	4 (7.69%)	11 (27.5%)	0	0	0
Amikacin	22 (16.18%)	3 (5.76%)	11 (27.5%)	2 (14.29%)	0	0
Tetracycline	32 (23.53%)	21 (40.38%)	11 (27.5%)	3 (21.43%)	0	0
Imipenem	23 (16.91%)	6 (11.53%)	22 (55%)	0	0	0
Piperacillin+Tazobactam	78 (57.35%)	26 (50%)	19 (47.5%)	10 (71.43%)	2 (100%)	4 (100%)

**Table 1. Antimicrobial Drug Susceptibility Pattern of Gram-Negative Bacteria**

Table 1 presents the antimicrobial drug susceptibility pattern of gram-negative bacteria. Piperacillin+Tazobactam was identified as most sensitive drug for all gram-negative bacteria, however, few exceptions were noticed in sensitivity pattern of *P. aeruginosa* where imipenem (55%) was

identified as most sensitive drug, while in *P. vulgaris*, norfloxacin (100%) was identified as equally sensitive drug. All other tested drugs produced very low sensitivity rate for particular uropathogen in our study.

Bacteria	<i>Staph. aureus</i> (n=17)	<i>Staph. epidermidis</i> (n=7)	<i>Staph. saprophyticus</i> (n=6)
Ampicillin	17 (100%)	3 (42.86%)	3 (50%)
Amoxicillin+Clavulanate	12 (70.59%)	3 (42.86%)	1 (16.67%)
Amikacin	12 (70.59%)	2 (28.57%)	2 (33.34%)
Cefuroxime	15 (88.24%)	4 (57.14%)	4 (66.67%)
Ceftriaxone	15 (88.24%)	3 (42.86%)	1 (16.67%)

Cefotaxime	15 (88.24%)	4 (57.14%)	4 (66.67%)
Clindamycin	12 (70.59%)	5 (71.43%)	3 (50%)
Ciprofloxacin	9 (52.94%)	5 (71.43%)	4 (66.67%)
Erythromycin	12 (70.59%)	1 (14.28%)	3 (50%)
Gentamicin	12 (70.59%)	3 (42.86%)	3 (50%)
Ofloxacin	9 (52.94%)	5 (71.43%)	1 (16.67%)
Tobramycin	9 (52.94%)	3 (42.86%)	3 (50%)

**Table 2. Antimicrobial Drug Susceptibility Pattern of Staphylococcal Species**

Table 2 gives the ideas about antimicrobial drug susceptibility pattern of Staphylococcus species. Among the gram-positive isolates, coagulase-positive bacteria like Staph aureus were sensitive to all tested drugs while coagulase-negative bacteria were less sensitive to all exposed drugs.

Enterococcus showed the 75% sensitivity rate to vancomycin, teicoplanin and linezolid, only 25% sensitivity rate to clindamycin and cefpirome and complete resistant to cefoxitin, erythromycin and azithromycin as shown in table 3.

Drug	No. (% <i>, n=4</i> )
Cefoxitin	0 (0%)
Vancomycin	3 (75%)
Erythromycin	0 (0%)
Teicoplanin	3 (75%)
Clindamycin	1 (25%)
Linezolid	3 (75%)
Azithromycin	0 (0%)
Cefpirome	1 (25%)

**Table 3. Antimicrobial Drug Susceptibility Pattern of Enterococcus**

In present research age, sex and catheterisation has been studied as predisposing factors for UTI as shown in Table 4. Majority of UTI cases were belong to 40-60 years (37.23%) followed by 20-40 (33.33%) years and male (55.32%) were more subjected to UTI than female (44.68%). P value for age and sex was >0.05, which was not significant and indicates that there was no relation between age/gender and UTI in present study. 53.19% of cases were identified with history of catheterisation. P value was <0.001, which indicates that catheterisation has significant role in production of UTI in present study.

Variable	Number (% <i>, n=282</i> )	P value
<b>Age</b>		
0-20	44 (15.61%)	>0.05
>20-40	94 (33.33%)	
>40-60	105 (37.23%)	
>60-80	35 (12.41%)	
>80	4 (1.42%)	
<b>Sex</b>		
Male	156 (55.32%)	>0.05
Female	126 (44.68%)	
Catheterisation	150 (53.19%)	<0.001

**Table 4. Predisposing Factors of UTI**

**DISCUSSION**

UTI is diagnosed as the most prevalent infection in clinical practise and almost 80% of UTI are caused by bacteria. One study performed in Davanagere, Karnataka, India, examined

71.72% bacterial prevalence in urine,<sup>11</sup> while one more study done in Lahore, Pakistan, examined 80.40% bacterial prevalence.<sup>16</sup> In present study, the bacterial prevalence in urine was 36.25%. This rate was almost half than the previous studies.<sup>11,16</sup> It indicates that the hospital infection control programme is going well in our hospital, however, efforts are still required to take this rate up to zero level.

In present study, 87.95% UTI were caused by gram-negative bacteria, while 12.05% cases of UTI were due to bacteria from gram-positive category. These data were almost in line with other studies<sup>5,17</sup> with 80% of UTI produced by gram negative and 20% UTI by gram-positive bacteria in a study of Gul N et al,<sup>5</sup> while 94% UTI were due to gram negative and 6% by gram-positive bacteria in a study of Khan IU et al.<sup>17</sup> E. coli (48.23%) was identified as predominant pathogen in our research. Highest prevalence of E. coli in UTI was also a part of other studies, however, the rates were variable, which includes 45.5%, 47.6%, 37.95%, 61.3% and 80% in study of Tasbakan MI et al,<sup>18</sup> Gul N et al,<sup>5</sup> Razzak SK et al,<sup>11</sup> Khan IU et al<sup>17</sup> and Sabir S et al,<sup>16</sup> respectively. Second most prevalent bacterial pathogen in our study (18.44%) and in study of Tasbakan MI et al (13.3%)<sup>18</sup> and Razzak SK et al (21.41%)<sup>11</sup> was K. Pneumoniae, which was Pseudomonas aeruginosa in study of Gul N et al (9.2%)<sup>5</sup> and Khan IU et al (12%),<sup>17</sup> while study of Sabir S et al<sup>16</sup> indicates Staph aureus (9.4%) as second most prevalent bacterial pathogen. Other microbes were almost in accordance to study of Gul N et al<sup>5</sup> and Khan IU et al.<sup>17</sup>

In our region, all the gram-negative isolates were most sensitive to Piperacillin+Tazobactam combination as depicted in Table 1, however, this rate was even low and near the range of 50%. Imipenem was identified as more sensitive drug for Pseudomonas aeruginosa and norfloxacin was identified as equally sensitive drug for Proteus species (in comparison to Piperacillin+Tazobactam combination); however, both imipenem and norfloxacin failed to control the growth of other isolates. Coagulase-positive bacteria like Staph aureus were almost sensitive to all tested drugs while Coagulase-negative bacteria like Staph epidermidis and Staph saprophyticus were less sensitive to all tested drugs as shown in Table 2. Enterococci were sensitive to vancomycin, teicoplanin and linezolid and less sensitive to other tested drugs as shown in Table 3. Different authors noticed the variable sensitivity pattern of uropathogen in different region. In one research of Bangalore (Bengaluru), India, trimethoprim was identified as most sensitive drug for gram-negative and gram-positive urinary isolates.<sup>19</sup> In a study done at Karachi, Pakistan,<sup>5</sup> gentamicin (69.2%) was identified as most sensitive drug followed by co-trimoxazole

with 55% efficacy and kanamycin with 50% efficacy for gram-negative isolates while gram-positive isolates were most susceptible to chloramphenicol (84.6%) followed by ofloxacin and gentamicin with 76.9% efficacy for each and norfloxacin with 69.2% efficacy. One more research performed at Bahir Dar, Ethiopia,<sup>20</sup> indicated 87% sensitivity of nitrofurantoin to gram-negative isolates while gram-positive bacteria had high sensitivity to gentamicin (83.3%), nitrofurantoin (83.3%) and amoxicillin-clavulanic acid (100%).

There are multiple risk factors responsible for UTI had been assessed in different studies.<sup>21</sup> In our study, we focused only on age, sex and use of urinary catheters (Table 4). Mean age in our study was 44.23±20.05 years, which was 59.1±18.3 years in study of Tasbakan MI et al.<sup>18</sup> Prevalence of UTI also depends on age and sex. During the 1<sup>st</sup> year of life, Male:Female ratio is 2.8-5.4:1, but after the 1<sup>st</sup> and 2<sup>nd</sup> year, there is a striking female preponderance with M:F ratio is 1:10. Study performed at Davanagere, Karnataka, India,<sup>11</sup> identified more cases of UTI in adult group between 20-40 years (54.98%) followed by younger or paediatrics population in <20 years (24.81%) age, while in our study, most cases were belonging to older age between 40-60 years (37.23%) followed by adult group between 20-40 years (33.33%). Exact reasons for more cases in older group were not known, however, statistically, it was not significant (P value >0.05). Normally, UTI is the disease of women.<sup>1</sup> More UTI in women (69.8%) than men (30.2%) was also a part of study performed at Rawalpindi, Pakistan,<sup>17</sup> while in current study, UTI was noticed more in men (55.32%) than women (44.68%) with Male:Female ratio 4:1 before 1 year and 1.22:1 after 1 year. Exact reasons for this was not known, but maybe due to more testing of male patients than female, however, statistically, it was not significant (P value >0.05). Urinary catheterisation is the major contributing factor for bacteriuria. It inoculates the organisms into the bladder causing mucosal irritation and providing a surface for bacterial adhesion. 10%-30% of patients who undergo short-term catheterisation (2-4 days) develop bacteriuria and 90%-100% of patients who undergo long-term catheterisation develop bacteriuria. About 80% of nosocomial UTIs are due to urinary catheterisation while only 5-10% is related to genitourinary manipulation.<sup>1,22</sup> Tasbakan MI et al<sup>18</sup> enrolled 63.98% UTI cases with history of urinary catheterisation, while in our study, about 53.19% of cases were exposed to catheter. Direct role of catheterisation in development of UTI was statistically significant (P value <0.001) in our study and it required careful monitoring of all catheterised patient.

## CONCLUSION

In the current study, the prevalence of UTI was 36.25%. Early and aggressive management of hospital-acquired urinary tract infection is recommended to take the rate up to zero mark, especially those in the high-risk group.

In our study, gram negative was identified as major group with predominance by *E. coli* followed by *K. pneumoniae*. Study revealed that gram-negative isolates like

*Proteus* species and *Acinetobacter* were rarely encountered, but produced good amount of sensitivity rate to Piperacillin+Tazobactam combination, while commonly encountered microbes like *E. coli*, *K. Pneumoniae* and *Pseudomonas aeruginosa* were produced poor sensitivity rate, near the range of 50% in our region. In gram-positive isolates, *Staph aureus* and *Enterococcus* produced good rate of sensitivity to all tested drugs, while coagulase-negative species produced poor response to all exposed drug. These results are indicating that resistance strains are start to emerge in urine in our region and requirement of periodic evaluation of sensitivity pattern of all such microbes before prescribing empirical therapy to UTI patients and also requirement of development of new drugs, which are orally effective, low cost and with less side effects.

UTI prevalence was positively associated with previous history of catheterisation in present study. Because of the risk of widening of UTI by catheter, it should be used only when required, under aseptic precautions, screened regularly for infection and removed as soon as possible. Understanding other risk factors like age and sex also help contains the UTI.

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