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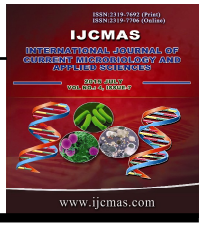


**Original Research Article**

# Bacteriological Profile and Antibiotic Resistance Pattern of Urinary Tract Infections in Kumaun Region

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Urinary tract infection, Antibiotic sensitivity,

**A B S T R A C T**

Urinary tract infection represents one of the most common diseases encountered in medical practice today and occurs neonate patients to the geriatric age group patients.UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections. Despite the widespread availability of antibiotics, it remains the most common bacterial infection in the human beings. Area specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for urinary tract infection and their resistance patterns may help clinicians prescribing the most effective empirical treatment. Therefore, the aim of this study was to determine the type and antibiotic resistance patterns of the urinary pathogens isolated from patients attending Government Medical College, Haldwani from January 2012 to March 2013. A hospital based cross sectional study was conducted and urine samples were collected using the mid stream "clean catch" method from 1823 clinically-suspected cases of urinary tract infections and tested bacteriological using standard procedures. Antimicrobial susceptibility test was performed for the isolated pathogens using Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines. Significant bacteriuria was found in 371/1823 (20.35%) of urine specimens. Gram-negative bacteria were more prevalent 78.16% than Gram-positive bacteria 21.83%. Of the 12 isolates, the most commonly isolated bacteria were *Escherichia coli* 63.35%. The overall p value for the occurrence of bacteria is statistically significant ($1.26199 \times 10^{-81}$, $p < .05$). Interestingly, this significance does not change across outpatient ($7.23345 \times 10^{-97}$, $p < 0.05$) and inpatient ($3.58258 \times 10^{-58}$, $p < 0.05$) populace i.e., outpatients were as likely to have been infected with *E. coli* as inpatients. The second most prevalent isolate was *Enterococcous sp.* (13.48%) followed by *Klebsiella sp.* (6.19%), *Cons* (3.23%), *Proteus mirabilis* (2.69%), *Mrcons* (2.42%), *Citrobacter* (2.15%), *S.aureus* (2.15%), *Pseudomonas sp.*(2.15%), *Acinetobacter sp.*(1.34% ), *Mrsa* (.53%) and *Alcalegenes f.* (.26%). The majority, 57.41%, of the positive cases were females while the remaining 42.59% were males. These results indicated that the prevalence of UTI was higher in female patients than in males. The isolated pathogens showed resistance to ampicillin /sulbactum, co-trimoxazole, norfloxacine, ciprofloxacine, linezolid cephoxitin and sensitivity to nitrofurantoin, ceftrioxone and ceftrioxone /sulbactum. In comparison to other geographies these results carried some stark differences e.g., high degree of resistance of Ampicillin /sulbactum while it also corroborated a general trend of increased resistance to antibiotics in other studies. One of the drugs Cotrimoxazole was found to be almost ineffective in most cases. The presence of bacterial isolates with very high resistance to the commonly prescribed drugs leaves few alternatives options of drugs for the treatment of UTIs. This study highlights the increased resistance to certain drugs in the studied geography that are found to be highly effective in other geographies of the world.

## Introduction

Urine in the human bladder is normally sterile. A bacterial Urinary tract infection (UTI) is most common kind of infection affecting the Urinary Tract. Urinary tract infection represents one of the most common diseases encountered in medical practice today and occurring from the neonate to the geriatric age group (Kunin, 1994; Raju *et al.*, 2004). Among the most common infectious diseases, urinary tract infections (UTIs) is a commonly encountered disease by clinicians in developing countries with an estimated annual global incidence of at least 250 million (Ronald *et al.*, 1991; Baris *et al.*, 2003). It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually(Wilson and Gaido,2004). UTI is one of the most important causes of morbidity in the general population, and is the second most common cause of hospital visits (Ronald and Pattulo, 1991). UTI is the third most common cause of admission to hospitals in India ( Bano *et al*., 2012). In the community, women are more prone to UTI. About 20% of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per year (Gebre, 1998). Pregnancy also makes them more susceptible to infection (Pastore *et al*., 1999).With advancing age, the incidence of UTI increases in men due to prostate enlargement and neurogenic bladder. UTI occurs in about one percent of boys and three to five percent of girls (Elder, 2007).

The relative frequency of the pathogens varies depending upon age, sex, catheterization, and hospitalization (Sefton, 2000). Despite the widespread availability of antibiotics, UTIs remain the most common bacterial infections in human populations. In recent years, widespread use of antibiotics has resulted in an increasing incidence of antibiotic resistance among the urinary tract pathogens all over the world. Worldwide, emerging of antibiotic resistance is increasing among the urinary pathogens (Kahlmeter, 2003). The increase in resistance of microorganisms to antimicrobial agents, especially in hospitalized patients, demands rapid identification of the pathogen (Ashkenizi *et al*., 1991). Early information enables the selection of appropriate antibiotic prior to the results of susceptibility tests and may there by prevent outbreaks ( Schaberg *et al*., 1991).

Thus, the aim of this study was to determine bacterial etiologic agent of uropathogens and evaluate their in vitro susceptibility pattern to commonly used antimicrobial agents.

### Materials and methods

The study population was drown from patients attending or admitted to various specialties of Government Medical College, Haldwani from January 2012 to March 2013.

### Sample collection and processing

Early morning mid-stream urine samples were collected by clean-catch method by using sterile wide mouthed glass bottles with screw cap tops. The specimens were transported to the bacteriology laboratory immediately or if there was a delay, refrigerated for 4 hours before processing. A calibrated loop method was used for the bacterial pathogens from urinary samples. A sterile 4.0 mm platinum wired calibrated loop was used which delivered 0.001ml of urine. A loopful urine sample was plated on

Cystine-Lactose-Electrolyte- Deficient (CLED) agar, MacConkey agar and blood medium (Hi Media Laboratories, Mumbai, India). All inoculated plates were incubated at $37^0$c aerobically for 24 hours. Colonies were counted and multiplied by 1000 which results in $>10^5$ colony forming unit (CFU)/ml of urine sample. Bacterial count less than this were considered insignificant. Growth of 3 or more organisms was considered contamination. Subculture of the colonies was done on blood agar and MacConkey agar to characterize the isolate. Bacterial pathogens were identified by conventional biochemical methods according to standard microbiological techniques (Collee *et al.,* 1996). For susceptibility testing Muller Hinton agar plate was swabbed with the suspension using sterile cotton swab and the antibiotic discs were placed over the agar and left for 30 minutes for diffusion of the antibiotics in the disc. The zones of inhibition were then read as resistant and sensitive using calibrated ruler and compared with the standard chart (Baurer *et al.,* 1966). Antibiotics agents employed for susceptibility testing were ampicillin(10µg), ceftriaxone(30µg), cotrimoxazole(25µg), gentamicine(10µg), Levofloxacin(5µg), Lenozolid(30µg), Nitrofurantoin(300µg), Norfloxacine(10µg) and ciprofloxacin (5µg). Each batch of culture media used was tested for sterility. Standard control strains of *E. coli* ATCC 11774, *S.aureus* ATCC43300, *Enterococcus faecalis* ATCC 51299, *Klebsiella* ATCC 13883, *pseudomonas aeruginosa* ATCC 15442 and *Proteus mirabilis* ATCC 7490 were used during culturing and antibiotics susceptibility testing as a control throughout the study.

**Data analysis**

Data was checked for completeness, cleaned manually, entered and analyzed using SPSS version 16 statistical software. The chi-square test ($X^2$) was used to measure the association and a p value less than 0.05 was considered statistically significant.

**Results and Discussion**

Of the 1823 urine samples received for culture during the 14 month period significant bacteriuria was found in 371(20.35%) samples, while 1452 (79.65%) samples were culture negative (either non-significant bacteriuria or very low bacterial count or sterile urine). Distribution of positive and negative cultures shows in Fig1.

Out of 371 isolates, 116 were indoor patients (71 females, 45 males) and 255 were outpatients (142 females, 113 males). The majority 213 (57.41%) of the positive cases were females while the remaining 158(42.58%) were males. These results indicated that the prevalence of UTI was higher in female than in males. This is due to the proximity of the genital tract and urethra (Schaeffer *et al*., 2001) and adherence of urothelial mucosa to the muco-polysaccharide lining (Gales, 2002). Distribution of males and females are shown in table 1.

A total of 371 bacterial uropathogens comprised of 290 (78.16%) gram negative and 81 (21.83%) gram-positive were isolated from positive urine samples shown in fig 2.

*Escherichia coli* was found to be the dominant bacteria among all isolated uropathogens with the prevalence rate of (63.34%). The second most prevalent isolate was *Enterococcus sp.* (13.48%) followed by *Klebsiella sp.* (6.19%), *Cons* (3.23%), *Proteus mirabilis* (2.69%) *Mrcons* (2.42%),

*Citrobacter sp.*(2.15%),*Staphylococcus aureus* (2.15%), *Pseudomonas sp.* (2.15%), *Acinetobacter sp*.(1.34%), Mrsa (.53%) and *Alcalegenes f*.(.26%).A comparative proportion of organisms in patients with UTI list of uropathgens isolated is shown in Table 2.

The overall p value for the occurrence of bacteria is statistically significant ($1.26199 \times 10^{-81}$, $p < .05$). Interestingly, this significance does not change across outpatient ($7.23345 \times 10^{-97}$, $p < 0.05$) and inpatient ($3.58258 \times 10^{-58}$, $p < 0.05$) populace i.e., outpatients were as likely to have been infected with *E. coli* as inpatients.

Majority of cases were of less than 30. Maximum number of cases was seen in age-group 21-30 years of age, where females were 65 the main factors which make females more prone to UTI in this age group are pregnancy and sexual activity(Arul *et al.,* 2012). In pregnancy, the physiological increase in plasma volume and decrease in urine concentration develop glycosuria in up to 70% women which ultimately leads to bacterial growth in urine. Also in the non pregnant state the uterus is situated over the bladder whereas in the pregnant state the enlarged uterus affects the urinary tract. Sexual activity in females also increases the risk of urethra contamination as the bacteria could be pushed into the urethra during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during child birth (Ebie *et al.,* 2001; Kolewale *et al.,* 2009). Majority of male culture positive cases were 33 in age group 0-10 years this is due to in infants boys have higher incidences of obstructive anomalies of urinary tract than in girls (Bano *et.al*, 2011). Table 3 shows the Age and sex wise distribution of all cases.

Of the 81 gram positive isolates, Methcilline resistance coagulase negative *S. aureus* shows 77.77% resistance to Cotrimoxazole and Norfloxacin. MRSA shows 100% resistant to Ampicillin/Sul, Nitrofurantoin and Levofloxacine , while showed sensitive to Linozolid. CONS were sensitive to Ampicillin/Sul and resistant to Nitrofurantoin83.3%. *S. aureus* showed75% resistance to Norfloxacin, ciprofloxacin and Levofloxacin while showed75% sensitivity to Nitrofurantoin. Table no 4 shows the Antibiotic resistance pattern of Gram positive organism.

In the 290 gram negative isolates, the three highest observed resistances were for Ampicilline /sulbactum77.24%, Norfloxacine 72.41%, and Ciprofloxacine70.68%, indicating over prescription of these drugs. The various observed drug sensitivities were as follows: Nitrofurantoin71.03%, Ceftrioxone /Sulbactum 65.86%, Cotrimoxazole38.96%. Among the isolates, *E.coli* 235 (63.34%) was the most common isolated organism. 184/235 (78.29%) *E.coli* strains were resistant to Ampicillin/Sul while 5/10(50%) of *Proteus Mirabilis* were sensitive to it. *Enterococcus* species was resistance to Norfloxacin 82%, Linezolid 78% and Ciprofloxacin 74% respectively. Table 5 shows Antibiotic resistance pattern of Gram negative organisms.

Urinary tract infections are one of the most common infectious diseases worldwide (McLaughlin and Carson, 2004; Blair, 2007). Bacterial infection of the urinary tract is one of the common causes for seeking medical attention in the community (Tessema, 2007). Microorganisms causing UTI vary in their susceptibility to antimicrobials from place to place and time to time (Orrett, 1998). So identification of the etiological agent and selection of an effective antibiotic agent to the organism in

question is very important for effective management of patients suffering from bacterial UTIs. Although the spectrum of agents causing UTI is relatively constant, their antibiotic susceptibility patterns are different in different geographical locations.

Gram-negative bacteria were more prevalent 290 (78.16%) than Gram-positive bacteria 81 (21.83%). Gram-negative bacteria have several properties to attach and invade urothelium in comparison to gram-positive pathogens. The majority, 213(57.41%) of the positive cases were females while the remaining 158(42.59%) were males. These results indicated that the occurrence of UTI was higher in female patients than in males. The high occurrence of infection in females is usually associated with anatomical and pathogenic factors e.g., the short length of the urethra hence lesser distance to bacteria ascending up the tract. Maximum number of cases was seen in age-group 21-30 years of age, where females were 65 and majority of mail culture positive case were in age group 0-10 years the main factors which make females more prone to UTI in this age group are pregnancy and sexual activity (Arul *et al*., 2012).

The present study shows that *E. coli* is the significant cause of UTI amongst the outpatients as well as inpatients. High percentage of isolates showed resistance to sulfa drugs such as Cotrimoxazole that is line with previous finds (Tambekar *et al*., 2005). The pathogens causing UTIs are almost always predictable with *Escherichia coli* being the primary etiological agent among both outpatients and inpatients (Hooton Stamn, 1997; Gupta et al., 1999; Gales et al., 2000). *E. coli* was the most common bacteria (63.34%) in UTI patients in this study. Interestingly, this differs from finds of other geographies e.g., U.S.A. study (75.5% - 87%) (Maji *et al*., 2012) and general population of India (68.69% and 83.0%) (Rayan *et al 1978.*).

The commonly used antibiotics such as Nitrofurantoin, Ampicillin/sulbactum and Co-trimoxazole were poorly effective against majority of the organisms isolated in this study. This starkly differs from the studies and findings in Caucasian women where Ampicillin and Co-trimoxazole remains the most useful antimicrobial agents (Ronald, 1991) and the findings by Ebie et al., 2001 among patients in Military Hospital, Jos, Nigeria where the isolates were highly susceptible to Nitrofurantoin and that of Olaitan, 2006 which found Co-trimoxazole to be very effective. The resistance shown in this study to Ampicillin, Cotrimoxazole, Norfloxacin, Ciprofloxacin, Levofloxacine antibiotics corroborates similar finds by many workers around the world: Mbata Theodore, 2007; Barnett BJ and Stephens, 1997; Gupta *et al*., 2002). Cotrimoxazole in present study was no longer found to be effective for UTI as most of uropathogens showed high degree of resistance to it. The antibiotic sensitivity pattern of organisms changes rapidly over a short period. It is especially true for developing countries where antibiotics are prescribed irrationally not only by the medical practitioners but the antibiotics are also purchased directly from the chemists (medicine shop keepers) without prescription (Palikhe, 2004). The presence of bacterial isolates with very high resistance to the commonly prescribed drugs leaves few alternatives options of drugs for the treatment of UTIs.

**Table.1** Distribution of males and females included in the study

| | IPD | OPD | Total |
|---|---|---|---|
| Male | 45 | 113 | 158(42.58%) |
| Female | 71 | 142 | 213(57.41%) |
| | 116(31.26%) | 255(68.73%) | 371(100%)) |

**Table.2** Comparative proportion of organisms in patients with UTI

| S.NO. | Isolated organisms | Outpatients | Inpatients | Percentage (%) |
|---|---|---|---|---|
| 1 | *Escherichia coli( n =235)* | 176 | 59 | 63.34 |
| 2 | *Klebsiella sp. (n=23)* | 14 | 9 | 6.19 |
| 3 | *Proteus mirabilis(n=10)* | 9 | 1 | 2.69 |
| 4 | *Pseudomonas sp.(n=8)* | 6 | 2 | 2.15 |
| 5 | *Enterococcus sp.(n=50)* | 20 | 30 | 13.48 |
| 6 | *Acinetobacter sp.(n=5)* | 2 | 3 | 1.34 |
| 7 | *Citrobacter sp.(n=8)* | 4 | 4 | 2.15 |
| 8 | *Staphylococcus aureus (n=8)* | 6 | 2 | 2.15 |
| 9 | *MRSA (n=2)* | 1 | 1 | .53 |
| 10 | *MRCONS (n=9)* | 7 | 2 | 2.42 |
| 11 | *CONS (n=12)* | 10 | 2 | 3.23 |
| 12 | *Alcalgenes(n=1)* | 0 | 1 | .26 |
| | Total no of isolates (n=371) | 255(68.73%) | 116(31.23%) | 100 |

**Table.3** Age and sex wise distribution of all cases

| Age-group | Male | Female | Total |
|---|---|---|---|
| 0-10 | 33 | 25 | 58 |
| 11-20 | 13 | 19 | 32 |
| 21-30 | 16 | 65 | 81 |
| 31-40 | 15 | 35 | 50 |
| 41-50 | 16 | 35 | 51 |
| 51-60 | 22 | 16 | 38 |
| 61-70 | 26 | 9 | 35 |
| 71-80 | 16 | 4 | 20 |
| 81-90 | 3 | 3 | 6 |
| Total | 160 | 211 | 371 |

**Table.4** Antibiotic resistance pattern of Gram positive organism

| **Antibiotics** | ***MRCONS (n=9)*** | ***MRSA (n=2)*** | ***CONS (n=12)*** | ***S. aureus (n=8)*** | ***Enterococcus spp. (n=50)*** |
|---|---|---|---|---|---|
| Ampicilin/sulbactum | 8(88.88%) | 2(100%) | 1(8.33%) | 5(62.5%) | 32(64%) |
| Cotrimoxazole | 7(77.7%) | 1(50%) | 6(50%) | 5(62.5%) | 28(56%) |
| Norfloxacin | 7(77.77%) | 0(0%) | 6(50%) | 6(75%) | 41(82%) |
| Nitrofurantoin | 4(44.44%) | 2(100%) | 10(83.3%) | 2(25%) | 27(34%) |
| Ciprofloxacin | 5(55.55%) | 2(100%) | 6(50%) | 6(75%) | 37(74%) |
| Levofloxacin | 5(55.55%) | 2(100%) | 6(50%) | 6(75%) | 36(72%) |
| Cephoxitin | NT | 1(50%) | NT | NT | NT |
| Linozolid | 2(22.22%) | 0(0%) | 5(41.66%) | 5(62.5%) | 39(78%) |
| Cephalexin | 8(88.88%) | 1(50%) | 6(50%) | 5(62.5%) | 31(62%) |

CONS: Coagulase negative *Staphylococci*, MRCONS: Methcilline resistant coagulase negative *Staphylococci*, *S. aureus*: *Staphylococcus aureus*, n = number of isolates, NT= not tested

**Fig.1** Distribution of positive and negative samples

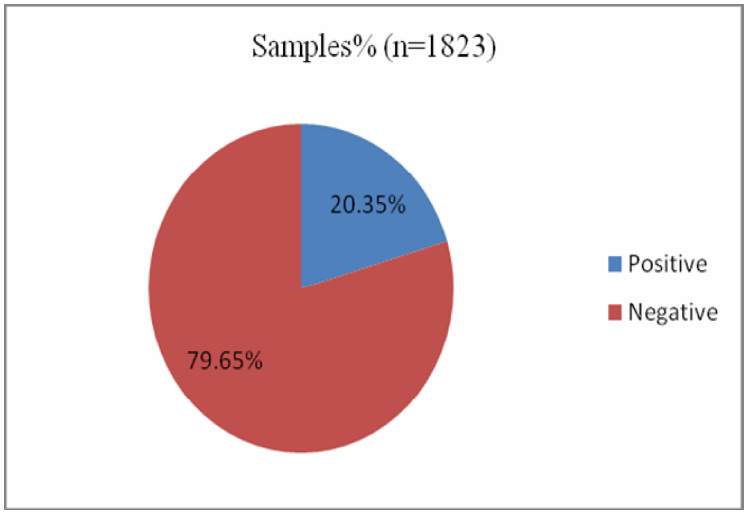
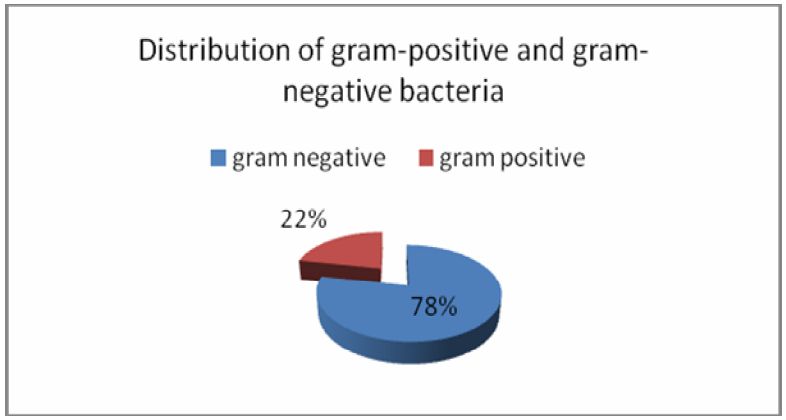


**Table.5** Antibiotic resistance pattern of Gram negative organisms

| **Antibiotics** | ***E. coli* (n=235)** | ***Klebsiella* spp.(n=25)** | ***P.mirabilis* (n=10)** | ***Pseudomonas* spp.(n=8)** | ***Citrobacter* spp.(n=8)** | ***Acinetobacter* spp. (n=5)** | ***Alcalegens* f. (n=1)** | **Total** |
|---|---|---|---|---|---|---|---|---|
| Ampicilin/sulbactum | 184(78.29%) | 18(78.26) | 5(50%) | 7(87.5%) | 6(75%) | 3(60%) | 1(100%) | 224(77.24%) |
| Ceftriaxone | 150(63.82%) | 17(73.91%) | 8(80%) | 6(75%) | 4(50%) | 5(100%) | 1(100%) | 191(65.86%) |
| Ceftriaxone/sulbactum | 59(25.10%) | 11(47.82%) | 10(100%) | 6(75%) | 4(50%) | 4(80%) | 1(100%) | 99(34.13%) |
| Cotrimoxazole | 138(58.72%) | 15(65.21%) | 5(50%) | 7(87.5%) | 6(75%) | 5(100%) | 1(100%) | 177(61.03%) |
| Nitrofurantoin | 30(12.76%) | 13(56.52%) | 10(100%) | 7(87.5%) | 5(62.5%) | 3(60%) | 0(0%) | 68(23.44%) |
| Norfloxacin | 178(75.74%) | 16(69.56%) | 2(20%) | 4(50%) | 5(62.5%) | 4(80%) | 1(100%) | 210(72.41%) |
| Ciprofloxacin | 172(73.19%) | 15(65.21%) | 3(30%) | 5(62.5%) | 5(62.5%) | 4(80%) | 1(100%) | 205(70.68%) |
| Levofloxacin | 158(67.23%) | 14(60.86%) | 1(10%) | 4(50%) | 5(62.5%) | 3(60%) | 1(100%) | 185(63.7%) |

N= number of isolates, *E. coli- Escherichia coli, P. mirabilis - Proteus mirabilis*

**Fig.2** Distribution of gram-positive and gram-negative bacteria



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