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**Research Article**

# TRENDS IN ANTIMICROBIAL RESISTANCE AMONG COMMON ISOLATES OF URINARY TRACT INFECTION IN TERTIARY CARE HOSPITAL OF NEPAL

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**Abstract**: *Background:* Urinary Tract Infection (UTI) is the most common infection in both community and hospital setting, in all age group with frequently occurring in female. The increasing pattern of the Multi Drug Resistance (MDR) among urinary isolates posses the significant problem in public health worldwide. Therefore, this study aims to assess the MDR and Antimicrobial Susceptibility Test (AST) pattern of bacterial isolates in our setting. *Methods:* Retrospective study of 2080 patients suspected for UTI attending Manmohan Memorial Community Hospital from 1st March 2011 to 30th February 2013 was done. Specimens were collected aseptically, cultured, identified and AST was done by standard methodology. *Results:* Of total, (557, 26.25%), with female to male ratio of about 3:1 showed the significant growth. Majority of the organism were Gram negative (478, 87.54%) with the most common pathogen *Escherichia coli* (78.63%). In total 305 (55.86%) of urinary isolates were MDR. Among the top three common Gram negative urinary isolates the maximum MDR was found in *Proteus spp.* (100%) followed by *Klebsiella spp.* (73.53%). Among Gram positive isolates 46.99% MDR was found in *Staphylococcus spp.* In comparison of tested antibiotic gentamicin followed by ciprofloxacin for the top three Gram negative and gentamicin followed by nitrofurantoin for the Gram positive urinary isolates were the most effective antibiotics. *Conclusions:* The most common causative organism for UTI was *E. coli* with maximum MDR in *Proteus spp.* and the appropriate antibiotic in vitro was gentamicin in this study. Higher resistance was found in antibiotics such as amipicllin, cotimioxazole, cephalexin, nalidixic acid and norfloxacin.

Key**words:** Antibiotic Susceptibility Test, *Escherichia coli*, Multi Drug Resistance, Urinary Tract Infection.

## INTRODUCTION

Urinary Tract Infection (UTI) is a condition in which the urinary tract is infected with uropathogens causing inflammation which is a common, distressing and occasionally life threatening condition usually requiring urgent treatment. These are the most common infections worldwide both in males and females in the community and hospital settings, occurring in all age groups.[1-2] UTIs may be presented as an asymptomatic or symptomatic and it may be uncomplicated or complicated.[3] Most of these infections are caused by retrograde ascend of bacteria from the fecal flora via the urethra to the bladder and kidney[4]. The most episode of UTIs are caused by *Escherichia coli*.[5]

Antimicrobial resistance is a serious public health problem because of emergence and rapid dissemination of resistant mutant strains.[6] Bacterial antimicrobial resistance is exacerbated by the diminishing number of new antimicrobial drugs in the pharmaceutical pipeline.[7] Now, antimicrobial resistance among urinary tract isolates has been reported with an increased frequency all over the world.[8-9]

Thus, this study is aimed to understand the MDR and susceptibility patterns of the uropathogens which helps to treat the patient with appropriate antimicrobials as well as to provide information to choose the empirical therapy where culture facility is not available.

## METHODS

A retrospective study was performed on suspected patients with history of symptoms of UTI attending Manmohan Memorial Community Hospital from 1st March 2011 to 30th February 2013. A total of 2080 specimens both from inpatients and outpatients including clean catch midstream urine and straight catheter urine samples were collected in a sterile container from the patients suspected of having UTI complication and not receiving antimicrobials. There were 1355 (65.14%) samples from female patients and 733 (35.26%) from male patients. The samples were inoculated on Blood agar and Mac Conkey agar within one hour of collection and incubated at $37^0$C. Then the bacterial uropathogens were isolated and tested for antimicrobial drug resistance patterns. Isolation of uropathogens was performed by a surface streak procedure on both Blood Agar and Mac Conkey Agar (Himedia, India) using semi quantitative method by using standard loop technique and incubated aerobically at $37^0$C for 24 hours. The culture plates were read for bacterial growth to decide whether the growth was pure growth or mixture. Further, if growth was found to be pure, whether it was significant growth or low count significant or insignificant growth. Growth was considered as a significant when there was $\geq 10^5$cfu/ml of middle stream urine following Kass, Marple and Sandford criteria. Bacterial identification was done using phenotypic characters such as study of colony characteristics and biochemical tests, namely catalase test, oxidase test, indole

test, citrate utilization test, $H_2S$ production, lactose fermentation, urea hydrolysis, gas production, motility test, cogulase test, manitol fermentation and novobiocin susceptibility test.[10]. Antimicrobial susceptibility test of isolates was performed by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guide lines.[11] The antibiotic discs used for the study were obtained from Himedia, India. *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) were parallel used as a part of quality control.

## RESULTS

Out of 2080 urine sample, only 546 (26.25%) showed the significant bacterial growth. Polymicrobial growth was not found. Culture was positive from all age group except from infant age group. Female were found more affected then male with the ratio of about 3:1. Summary of age, gender and MDR wise isolation of urinary isolates is shown in table 1.

**Table 1. Age, gender and MDR wise distribution of culture positive cases**

| Age in years | Gender | | Total |
|---|---|---|---|
| | Male, MDR | Female, MDR | |
| ≤1 (Infant) | - | - | - |
| 2-15(Child) | 22, 4 | 11, 4 | 33 (6.04%), 8 (24.24%) |
| 16-60(Adult) | 87, 36 | 340, 222 | 427 (77.20%), 258 (60.42%) |
| >60(Old) | 33,13 | 53, 26 | 86 (15.75%) 39 (45.34%) |
| Total | 142 (26.01%), 53 (37.71%) | 404 (73.99%), 252 (62.39%) | 546, 305 |

Frequency and MDR pattern of urinary isolates are shown in table 2. Gram negative bacteria were more common then Gram positive bacteria with the ratio of about 7:1. Among Gram negative pathogen, the most common organism isolated were *Escherichia coli* (76.66%) followed by *Klebsiella spp.* (6.10%), *Proteus spp.* (0.90%) and others (1.06%). Among Gram positive bacteria, *Staphylococcus aureus* (7.9%) followed by *Staphylococcus saprophyticus* (3.41%) and others (1.06%). The less frequently isolated Gram negative organism included *Morganella morganii*, *Enterobacter* spp., *Citrobacter freundii*, *Pseudomonas aeruginosa, Acinetobacter baumannii, Salmonella Typhi* and among Gram positive isolates included *Enterococcus faecalis* and Cogulase Negative Staphylococcus (CONS). *Escherichia coli* were the predominant organism among both Gram positive and Gram negative isolates.

**Table 2. Frequentcy and MDR patterns of frequently isolated pathogen from urine sample**

| Organism | Number and percentage of isolates (n, %) | MDR (n, %) |
|---|---|---|
| *E. coli* | 427 (76.66%) | 232(54.33%) |
| *Staphylococcus spp.* | 66 (11.85%) | 31 (46.99%) |
| *Klebsiella spp.* | 34 (6.10%) | 25 (73.53%) |
| *Proteus spp.* | 5 (0.90%) | 5 (100.00%) |
| Others | 15 (2.51%) | 12 (80.00%) |

Table 2 shows the distribution of MDR strains of urine isolates. About 56% uropathogen were found to be resistant to >2 antimicrobial in invitro AST and labeled as MDR. Most of the less frequently isolated organisms were multidrug resistance. About fifty five percentage of *E. coli* isolates were MDR. Similarly, 73.53% of *Klebsiella spp.* was found MDR. Among *Klebsiella spp.*, about sixty percentage of *Klebsiella pneumoniae* was MDR whereas 93.33% *Klebsiella oxytoca* was MDR. All isolated Proteus strains were multidrug resistance. All strains of Acinetobacter *baumannii*, *Citrobacter spp.*, *Pseudomonas aeroginosa*, *Salmonella Typhi*, *Enterococcus faecalis* were multi drug resistant but not analyzed because of their less number. *Staphylococcus spp.* was comparatively showed less drug resistance among common isolated organism in our study. Among *Staphylococccus spp*., 52.27 % of isolated *S. aureus* were MDR and 47.36% of isolated *S. saprophyticus* were MDR and MDR was not found in Coagulase Negative Staphylococcus.

**Table 3. Antibiotics resistant pattern of *E. coli* (n=427), *Klebsiella spp.* (n=34) and *Proteus spp.* (n=5)**

| Antibiotics | *E. coli* (n, %) | *Klebsiella spp.* (n, %) | *Proteus spp.* (n, %) |
|---|---|---|---|
| Ampicillin | 260 (60.89%) | - | 4 (80.00%) |
| Cephalexin | 223 (52.23%) | 24 (70.59%) | 4 (80.00%) |
| Cotrimoxazole | 170 (39.85%) | 21 (61.77%) | 5 (100.00%) |
| Nalidixic acid | 267 (62.53%) | 25 (73.53%) | 4 (80.00%) |
| Norfloxacin | 143 (33.49%) | 20 (58.83%) | 5 (100.00%) |
| Gentamicin | 66 (15.46%) | 9 (26.48%) | 1 (20.00%) |
| Cefexime | 64 (15.00%) | 16 (47.06%) | 2 (40.00%) |
| Nitrofurantoin | 23 (5.40%) | - | - |
| ciprofloxacin | 74 (17.35%) | 10 (29.42%) | 2 (40.00%) |

Nitrofurantoin was the most sensitive antibiotics followed by cefexime, gentamicin and ciprofloxacin for *E. coli*. *E. coli* was most resistant to nalidixic acid (62.53%) followed by ampicillin (60.89%) and cephalexin (52.23%) While *Klebsiella spp.* was most resistant to nalidixic acid (73.53%) followed by cephaleaxin (70.59%) and the most sensitive antibiotics was gentamicin followed by ciprofloxacin, nitrofurantoin and cefexime. In case of *Proteus spp.* gentamicin, cefexime, ciprofloxacin were sensitive antibiotics (Table 3).

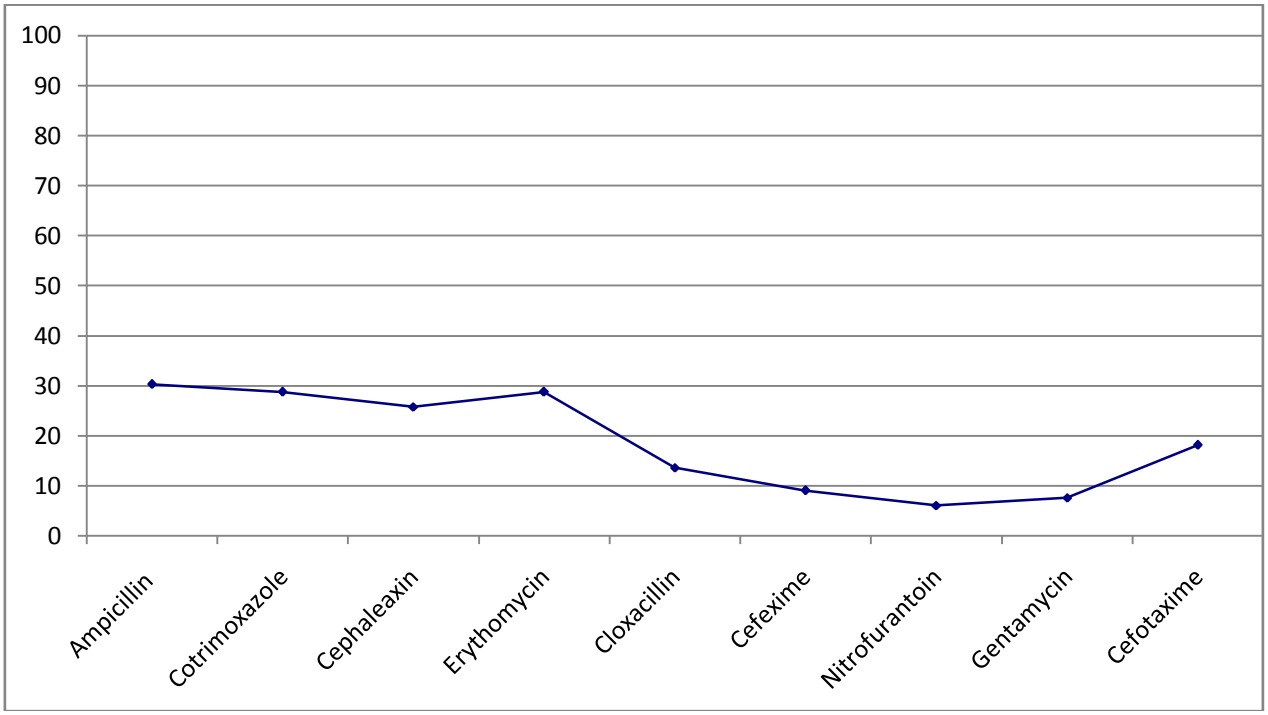


**Figure 1. Resistance of *Staphylococcus spp.***

Figure 1 shows the resistance patterns of Staphylococcus isolated from the urine samples. Nitrofurantoin was most sensitive antibiotics for *Staphylococcus spp.* followed by gentamicin and cefexime. Staphylococcus was most resistant to ampicillin(30.3%) followed by cotrimoxazole (28.75%) and erythromycin (28.75%).

## DISCUSSION

This study shows the distribution and antimicrobial resistance patterns of bacterial species isolated from patients clinically suspected of UTI in Microbiology Department of Manmohan Memorial Community Hospital. Out of total 2080 urine samples, 546 (26.25%) samples showed the growth of significant bacteriuria. Kattel *et al.*[12] Acharya *et al.*[13] and Baral *et al.*[14] also observed low rate isolation at national level and also in Cambodia 22.53%[15] and Latin America 29.9%[16] at the international level. Further our study showed higher rate of isolation of pathogens than in India 10.86%[17], 9.17%[18] and 9.2% Ethiopia[2] but lower than the Cameron[19] towns which observe 58.3% and in Nigeria 39.68%.[20]

Usually UTI is originated from colonic bacteria, which comprises mainly Gram negative bacteria. Among the Gram negative, the member of enterobacteriaceae was the most predominant organism. In this study, it was observed that 12.38% of UTI is caused by Gram positive bacteria and 87.62% is caused by Gram negative bacteria. Similar type of finding is observed by Akram *et al.*[21] and previous study conducted at same hospital.[22]

Like others reports from different countries including Nepal[12, 14, 22] *Escherichia coli* (78.63%) is the most common cause of urinary tract infection too in our study. Other microorganisms includes *Enterobacter spp*., *Serratia spp*., *Pseudomonas aeruginosa* and other *Enterococci spp*., *Staphylococcus saprophyticus, S. aureus*, *Acinetobacter spp.*[23]. Our findings are harmony with these reports.

Antimicrobial resistance is now an emerging public health problem globally. Unlike the scenario in the early seventies, in the study during the past two decades MDR associated with intergrons present in the isolates has pose resistance to the commonly used first line drug used in the treatment of UTI. [24] In our study, 55.86% of isolates were MDR strains. All *Proteus spp.* were MDR whereas low MDR was found in *Staphylococcus spp.* among the frequently isolated strains. Our these finding are compatible to other reports of world including previous studies done from Nepal.[13,14,25,26] The probable cause of high MDR in our study may be due to the empirical system of treatment and indiscriminate use of antibiotics in the developing and least developed countries.

Though fluoroquinolones are preferred as initial agents for empiric therapy of UTIs because of their unique characteristic of broad antibacterial spectrum, unique mechanism of action, good absorption from the

gastrointestinal tract, excellent tissue distribution as well as low incidence of adverse reaction, inhibition of DNA topoisomerase (gyrases)[27] but in our study, among Gram negative bacteria the most effective drug found was gentamicin, cefexime and nitrofurantoin among the tested antimicrobial agents. Unlike our finding, Das[28] found ampicillin to be most effective antimicrobial agent whereas our result is in harmony with others reports.[12] [24] Nitrofurantoin showed the greatest effectiveness against *E. coli* (94.6%) and gentamicin and cefexime at the rate of 84% but lower than in Greece (95.6%) and United kingdom (93.0%).[29, 30] In our study *E. coli* was resistant to ampicillin (59.59%) similar to one study conducted at Poland (56.8%) and resistant to Nalidixic acid is 61.55% i.e slightly lower than one study at Nepal.[31]

For kelbsiella spp. gentamicin showed the greatest effectiveness (73.53%) and ciprofloxacin (70.59%) which is much less that of Sharma *et al.* study conducted on 2011.[31] *Klebsiella spp.* were resistant to nalixidic acid about 75% which is much lower than Sharma *et al.*[31]

All *Proteus spp.* are resistant to cotrimoxazole and norfloxacin where as 60.0% sensitive towards ciprofloxacin, which is the third common Gram negative isolate in our study. In our setting the best choice of drug would be gentamicin for proteus (80%) in invitro AST.

In Gram positive isolates gentamicin and nitrofurantoin antimicrobial agents were the most effective. Thus, gentamicin and nitrofurantoin could be appropriate for empiric treatment of UTI in Nepal. Lastly, like a number of literature[34] description that the female are more frequently affected by UTI then male.

## CONCLUSIONS

The susceptibility and resistance profile of all Isolates in this study have shown that Gentamicin and Nitrofurantoin possess the higher efficacy in comparison to other antimicrobial agents used. In this study isolates identified were resistant to commonly used antibiotics in our setting. The emerging resistance to commonly used antibiotics alerts the immediate necessity of a continuous epidemiologic surveillance in primary health care facilities in Nepal.

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