Bacteriological Profile and Antimicrobial Susceptibility Patterns of Urine Culture Isolates among Patients Attending to a Teaching Hospital, Jumla

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Introduction: Urinary tract infections (UTI) is applied to clinical conditions which range from asymptomatic bacteriuria to severe infection of the kidney with the development of sepsis. The pharmacotherapy of UTIs is grounded on the predictable spectrum of causative agents and their antimicrobial susceptibility patterns. Thus, this study aims to assess the bacterial profile and antimicrobial susceptibility pattern of isolated bacteria among patients with urinary tract infection.

Methods: A descriptive cross-sectional study was conducted from August to December 2021 among patients who came to various departments of Karnali Academy of Health Sciences (KAHS), Jumla with suspected urinary tract infection during the study period. Mid-stream urine specimens were collected from 365 individuals for bacteriological identification and antimicrobial susceptibility testing. Data on socio-demographic, clinical and risk factors were also collected using a structured proforma. Isolates were identified by standard microbiological methods and tested for in vitro antibiotic susceptibility by modified Kirby-Bauer disc diffusion method.

Results: Among the 365 Urine samples, 98 (26.8%) showed significant bacteriuria. Escherichia coli (67.3%), Enterococcus spp. (14.3%), Acinetobacter spp. (7.1%) were common bacterial isolates. The Gram-negative isolates were most resistant to Cefixime (67.4%), Ampicillin (65.06%) and most sensitive to Nitrofurantoin (98.7%). Gram-positive isolates were highly resistant to Ampicillin (66.6%) and most sensitive to Vancomycin (100%).

Conclusions: Our study concluded that there is pronounced increase in cases of UTIs and antimicrobial resistance with commonly prescribed antibiotics has been observed.

ABSTRACT

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INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections experienced by human. It is characterized by the presence of significant bacteria (more than $10^5$ bacteria) in a urine sample. Which is applied to a variety of clinical conditions ranging from asymptomatic bacteriuria to severe pyelonephritis with sepsis. UTI is the third most common infection with an estimated annual global incidence of at least 250 million in developing countries. Most common microorganisms causing UTI are Gram-negative enteric bacilli like Escherichia Coli (E.coli), Klebsiella species, Proteus species and Gram-positive organisms like Staphylococcus saprophyticus, Staphylococcus aureus, Enterococci. Among them Escherichia coli is the most common causative agent of UTI globally.

In Karnali province, antimicrobial susceptibility among pathogens involved in UTIs is poorly investigated. Hence, it is important to obtain essential information on local antimicrobial resistance rates at the Karnali region to provide adequate treatment to the community and hospital-acquired UTIs in our country, Nepal. Therefore, this study was undertaken to determine the foremost causative agents of UTIs and to understand their antimicrobial susceptibility patterns. Occurrence of UTI is very common in female due to shorter urethra, large bacterial load in uroepithelial mucosa, obstruction in the urinary tract, sexual activity and pregnancy. If untreated, UTI can lead to acute complications like sepsis and long-term complications like renal scarring, hypertension and chronic kidney diseases. The pharmacotherapy of UTIs is usually empirical, based on the predictable spectrum of etiologic agents and their susceptibility patterns. Due to the emanation of antimicrobial resistance among uropathogens, the effectiveness of empirical therapy has been affected.

METHODS

A descriptive cross-sectional study was conducted at Karnali Academy of Health Sciences. The study was done from August to December 2021. Ethical approval was obtained from IRC of Karnali Academy of Health Sciences, Jumla, Nepal and was granted accordingly with the reference number (078/079/02). Consent was taken from the patients before collection of urine samples from patients, attending various departments of the KAHS teaching hospital. The demographic profile like age, sex of patients, the organism isolated, and the antimicrobial susceptibility patterns were recorded using standard data collection form.

Inclusion criteria: All urine samples from different departments (inpatients and outpatients) to the laboratory department of KAHS for the culture and sensitivity test.

Exclusion criteria: Unlabelled or mislabelled urine samples, urine from catheter, urine samples collected till the rim of the container, urine samples received beyond 2 hours of collection at room temperature or beyond 24 hours of storage at $4^\circ C$ was excluded from the study.

Sample size determination and sampling technique:

The sample was determined by using single population proportion formula. With reference to a previous study, $p$-value = 30%. The margin of error (5%) and 95% level of confidence ($z=1.96$), the sample was calculated as follows:

$$n = \frac{z^2pq}{d^2}$$

$$z = 1.96, \ p = 0.30, \ q = 0.70, \ d = 0.05$$

$$n = 1.96 \times 1.96 \times 0.30 \times 0.70 / 0.05 \times 0.05 = 322 + 10\% \ (\text{non respondent})$$

$$n = 355 \ (\text{minimum sample size})$$

Therefore, a total of 365 UTIs suspected patient’s urine samples were included in the study from all the patients in outpatient department (OPD) and the inpatient department of Karnali Academy of Health Science, Teaching Hospital, Jumla. An enumerative sampling technique was used to select samples during the study period.

Cultivation and bacterial identification:

A freshly voided midstream urine sample (2-5ml) was collected in a wide-mouthed sterile container. Uncentrifuged uniformly mixed urine sample was inoculated using inoculating loop 0.001 ml of sample on cystine lactose electrolyte deficient media (Hi-Media, India) and incubated for 24 hours at $37^\circ C$. Growth on the media or without growth was recorded. The culture plate without growth was incubated further for more 24hrs. The sample with growth proceeded for gram staining and different biochemical test. Colony counts yielding bacterial growth of $10^5$ ml of urine were regarded as significant bacteriuria. All positive urine cultures with significant bacteriuria were further identified by their colony characteristics, Gram-stain and pattern of...
biochemical profiles using standard procedures. Gram negative bacteria were identified by \( H_2S \) production and carbohydrate utilization tests in Triple sugar iron agar, motility test, urease test, oxidase test, indole test and citrate test. The Gram-positive bacteria were identified using Bile Esculin catalase and coagulase tests. After the identification of organisms, an antimicrobial susceptibility test was done on Muller-Hinton agar and tested for in vitro antibiotic susceptibility by modified Kirby-Bauer disc diffusion method.

**Antimicrobial susceptibility testing:** Antimicrobial susceptibility test was performed using a modified Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antibiotics were used: Ampicillin (AMP: 10 \( \mu \)g), Cefotaxime (CTX; 30 \( \mu \)g), Amoxicillin (AMX: 20\( \mu m \)), Gentamicin (GEN:10 \( \mu \)g), Nitrofurantoin (NIT; 300 \( \mu \)g), Ciprofloxacin (CIP: 5\( \mu \)g), Cefixime (CFM:5\( \mu \)m), Ofloxacin(OF:5\( \mu \)m), Meropenem(MRP: 10\( \mu \)g), Vancomycin(VA:30\( \mu \)g). Isolates were classified as sensitive, intermediate and resistant consistent with the standardized table supplied by CLSI 2016.

**Data processing and analysis:** Data were entered in spreadsheet and edited then exported and analyzed using SPSS version 16.0. Descriptive statistics was used to determine frequency and percentage.

**RESULTS**

A total of 365 urine samples were obtained from patients age ranged from 1 month to 81 years. Among them 206 (56.4%) were females and 159 (43.60%) were males with a 1:1.29 male to female ratio.

**Prevalence of Urinary tract infection**

Out of 365 Urine samples, analyzed 98 (26.8%) had significant bacteriuria. The total prevalence of UTI was found to be higher in female patients. The age group with the highest susceptibility to UTI was found to be 0-20 years (45.92%) in both females and males. [Table 1].

**Bacterial Uropathogens:**

Among 98 culture-positive urine samples, Gram-negative and Gram-positive organisms were isolated in 83 (84.7%) and 15 (15.3%) respectively as shown in Figure 1.

**TABLE 2. Bacterial isolates.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Bacterial Isolates</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>E. coli</td>
<td>66</td>
<td>67.3 %</td>
</tr>
<tr>
<td>2.</td>
<td>Enterococcus sp.</td>
<td>14</td>
<td>14.3 %</td>
</tr>
<tr>
<td>3.</td>
<td>Acinetobacter sp.</td>
<td>7</td>
<td>7.1 %</td>
</tr>
<tr>
<td>4.</td>
<td>Klebsiella</td>
<td>6</td>
<td>6.1 %</td>
</tr>
<tr>
<td>5.</td>
<td>Citrobacter</td>
<td>2</td>
<td>2 %</td>
</tr>
<tr>
<td>6.</td>
<td>Proteus</td>
<td>1</td>
<td>6.1 %</td>
</tr>
<tr>
<td>7.</td>
<td>Salmonella</td>
<td>1</td>
<td>1 %</td>
</tr>
<tr>
<td>8.</td>
<td>S.saprophyticus</td>
<td>1</td>
<td>1 %</td>
</tr>
<tr>
<td>9.</td>
<td>Total</td>
<td>98</td>
<td>100 %</td>
</tr>
</tbody>
</table>

**Antimicrobial susceptibility pattern of bacterial isolates**

An antibiotic sensitivity test was performed on all bacterial isolates, based on the available antibiotic.

**Gram-negative bacteria:** Gram-negative isolates were most resistant to Cefixime (67.4%), Ampicillin (65.06%) and Ceftriaxone (49.39%). Antibiotics such as Nitrofurantoin (98.7%), Mer-
penem (95.1%), Amikacin (87%), and Gentamycin (83.1%) were the most effective and sensitive antibiotics against the gram-negative isolates. [Table 3]

**TABLE 3.** Antimicrobial Susceptibility Pattern of Gram-Negative Bacterial Isolates.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Antibiotics</th>
<th>NIT (98.4%)</th>
<th>CIP (63.6%)</th>
<th>GEN (83.3%)</th>
<th>AK (75.7%)</th>
<th>OF (43.9%)</th>
<th>CTX (95.4%)</th>
<th>MRP (22.7%)</th>
<th>AMP (25.7%)</th>
<th>CFM (71.2%)</th>
</tr>
</thead>
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<tr>
<td><strong>E. coli</strong></td>
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<td>65 (98.4%)</td>
<td>42 (63.6%)</td>
<td>55 (83.3%)</td>
<td>58 (87.7%)</td>
<td>47 (71.2%)</td>
<td>29 (43.9%)</td>
<td>63 (95.4%)</td>
<td>15 (22.7%)</td>
<td>17 (25.7%)</td>
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<td>3 (4.5)</td>
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<td>6 (9.09)</td>
<td>33 (50)</td>
<td>2 (3.03)</td>
<td>42 (63.6)</td>
<td>47 (71.2)</td>
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<td><strong>Acinetobacter</strong></td>
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<td>7 (100)</td>
<td>7 (100)</td>
<td>7 (100)</td>
<td>6 (85.7)</td>
<td>4 (57.1)</td>
<td>4 (57.1)</td>
<td>6 (85.7)</td>
<td>3 (42.8)</td>
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<td>1 (14.2)</td>
<td>3 (42.8)</td>
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<td>1 (50)</td>
<td>2 (100)</td>
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<td><strong>Proteus</strong></td>
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<td>2 (100)</td>
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<tr>
<td><strong>Klebsiella</strong></td>
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<td>6 (100)</td>
<td>6 (100)</td>
<td>5 (83.3)</td>
<td>5 (83.3)</td>
<td>5 (83.3)</td>
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<td>5 (83.3)</td>
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<td>1 (100)</td>
<td>0 (0)</td>
<td>1 (100)</td>
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</tr>
</tbody>
</table>

Key: AMP Ampicillin, CFM Cefixime, MRP Meropenem, AK Amikacin, OF Ofloxacin, NIT Nitrofurantoin, CTX Cefotaxime, GEN Gentamicin, CIP Ciprofloxacin

**Gram-positive bacteria:**

Out of the tested antibiotics, Gram-positive bacterial isolates were highly resistant to Ampicillin (66.6%), Ciprofloxacin (66.66%), Amoxicillin (66.66%). Among the tested antibiotics, Vancomycin (100%) and Nitrofurantoin (93.3%) were most effective to Gram-positive isolates [Table 4].

**TABLE 4.** Antimicrobial sensitivity of Gram-positive bacterial Isolates.

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Antibiotics</th>
<th>NIT (92.8%)</th>
<th>CIP (28.5)</th>
<th>GEN (42.8)</th>
<th>AMP (21.4)</th>
<th>CFM (28.5)</th>
<th>AMX (28.5)</th>
<th>VA (1400)</th>
<th>NV -</th>
</tr>
</thead>
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<td><strong>Enterococcus spp.</strong></td>
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<td>13 (92.8)</td>
<td>4 (28.5)</td>
<td>6 (42.8)</td>
<td>3 (21.4)</td>
<td>4 (28.5)</td>
<td>4 (28.5)</td>
<td>14 (100)</td>
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<td>I</td>
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<td><strong>S. saprophyticus</strong></td>
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<td>1 (100)</td>
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<td>1 (100)</td>
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</tbody>
</table>
Key: AMP Ampicillin, CFM Cefixime, NIT Nitrofurantoin, GEN Gentamicin, CIP Ciprofloxacin, AMX Amoxicillin, VA Vancomycin, NV Novobiocin

DISCUSSION

Urinary tract infection remains to be one of the most common infectious diseases diagnosed in the community of developing countries like Nepal. The overall prevalence of UTI in this study was found to be 26.8% which was in agreement with the findings of the previous studies conducted in Lalitpur, Nepal (25.24%) and Nigeria (25%) respectively. However, our finding was higher than other earlier studies reported (17.26%) in Lalitpur Nepal. But it was lower compared to other studies done in south eastern Nigeria (78%). This difference in the rate of UTI may be explained by variation in the methodology used, sexual behaviour (those sexually active individuals are more exposed to urinary tract infection), different environmental conditions, host factors, hygiene practices, lack of sanitary materials such as access of water, different socio-economic status. The prevalence rate was found to be higher in females (72.44%) than in males (27.56%) were similar to studies conducted in Nepal and other countries which support that ladies are at high threat for UTIs.

This high prevalence of UTIs among female participants may be due to ladies having shorter and wider urethra which is proximate to the anus and a probative terrain in the female genital tract for the optimal growth of bacteria compared to males. In our study, Escherichia coli was the most common bacteria isolated (67.3%) followed by Enterococcus sp. (14.3%) which was similar to the study conducted in Nepal and Ethiopia. The Gram-negative organisms were most resistant to Cefixime (67.4%) and Ampicillin (65.06%) which was similar to the study of Gunduz et al., Manandhar et al. The factors contributing to those resistance rates might be due to the irrational use /self-medication of antibiotics in the common study area. Most Gram-negative isolates of the present study were sensitive to Nitrofurantoin (98.7%), Meropenem (95.1%). This finding was in line with previous findings from Bangladesh and Nigeria where Ciprofloxacin and Nitrofurantoin were effective against Gram-negative isolates.

Gram-positive bacterial isolates were largely resistant to Ampicillin (66.6%), Ciprofloxacin (66.6%), Amoxicillin (66.6%). This high trend of resistance was similar with earlier proved results in Ethiopia, Ampicillin (87.5 – 100). This advanced resistance rate of Gram-positive organisms could be due to the production of penicillinase enzymes and other substitutes penicillin-binding proteins which helps the organism to become resistant to β-lactam antibiotics. This might be due to other resistance mechanism, inappropriate use and incorrect administration of those antibiotics in addition to other factors like strain and geographic variation.

Limitations of the study: A limited number of antibiotics were used to perform culture and sensitivity tests because of the rural location of the hospital. And Extended-Spectrum Beta-Lactamase (ESBL) producing bacteria were not identified. Molecular identification of bacterial isolates was not done because of limited resources.

CONCLUSIONS

Urinary tract infection is among the common causes of seeking medical treatment. In the present study, the overall prevalence of UTI was 26.8%. Escherichia coli was the most dominant isolate. Female had a significant association with the circumstance of UTIs. Gram-negative isolates were most resistant to Cefixime, Ampicillin and Ceftriaxone and were most sensitive to Nitrofurantoin and Meropenem. Gram-positive bacterial isolates were largely resistant to Ampicillin, Amoxicillin and were most sensitive to Vancomycin and Nitrofurantoin. Pronounced resistance has been observed with generally prescribed antibiotics.

Thus, from this study, there is a significant increase in UTIs and antibiotic resistance. Regular follow-ups are essential in each geographic area to establish original rates of uropathogen resistance to available antibiotics. Periodic modification of the protocol for empiric UTIs treatment is essential when changes are detected in the antibiotic’s vulnerability biographies of the bacteria.

Recommendations: To prevent the development of resistance, antibiotic susceptibility patterns must be continuously and periodically evaluated to select the appropriate regimen to treat UTI and to avoid complications. Institutional Antibiotic policy can be acclimatized to achieve a superior therapeutic outcome. Over and inadvisable use of antibiotics should be discouraged.

ACKNOWLEDGEMENTS

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