

## Antibiogram of bacterial isolates from urine at Okhaldhunga Community Hospital: a rural setting in Nepal

Anjila Pal<sup>1</sup>, Rabin Bom<sup>2</sup>

<sup>1</sup>MSc Medical Microbiology, <sup>2</sup>MDGP Consultant, Okhaldhunga Community Hospital, Nepal

### ABSTRACT

**Introduction:** Infection of urinary tract is the second most common site of infection after respiratory tract worldwide. The objective of the study is to study the prevalence of positive urine samples and antibiotic susceptibility pattern of the bacterial isolates in Eastern part of Nepal.

**Method:** A total of 210 Mid-Stream Urine (MSU) samples collected from both inpatient and outpatient departments of Okhaldhunga Community Hospital (OCH) were included in the study. Each urine samples were cultured and the antibiogram of isolates were determined as per CLSI guideline.

**Result:** The prevalence of positive culture was found to be 40.9%. Out of which, 82.5% were identified bacteria. The age group with the greatest number of bacterial isolates was 20-30 years. Altogether five different bacteria were identified. Among these, *E. coli* was the most predominant isolate (76.1%), followed by *S. aureus* (16.9%) and *K. pneumoniae* (4.2%). Nitrofurantoin was found to be the most effective drug for the Gram-negative bacilli with a sensitivity of 86.3% while Amoxicillin was least effective showing 87.7% resistance. For Gram positive cocci, Chloramphenicol and Cloxacillin were the most effective and Penicillin was least effective drug showing 84.6% sensitivity and 92.3% resistance respectively.

**Conclusion:** The prevalence of positive urine culture was 40.9%. The most effective antibiotic was Nitrofurantoin. Since the isolates showed high resistance towards Amoxicillin and Doxycycline, antibiotic susceptibility testing of the isolates should be performed time to time to monitor the resistance pattern of the bacteria.

**Keywords:** *E. coli*, OCH, MSU, nitrofurantoin

### CORRESPONDENCE

Ms. Anjila Pal

Dept. of Medical Microbiology, Okhaldhunga Community Hospital, Nepal

Email: anjilapal789@gmail.com

## INTRODUCTION

Urine, in absence of urinary tract infection (UTI), emerges from kidney and bladder free from microbes. During passage through distal urethra, a small number of bacteria may enter the urine as contaminants. Normally, human urine contains 96% water, 2% urea, and the remaining 2% consists of uric acid, creatinine, ammonia, sodium and potassium ions, etc. Therefore, urine can prove to be an excellent medium for the growth of microorganisms. Infection of urinary tract is the second most common site of infection after respiratory tract infection.<sup>1</sup>

UTI is defined by the presence of  $\geq 10^5$  organisms per ml in a midstream sample of urine (MSU).<sup>2</sup> Microbiologically, a UTI exists when pathogenic microorganisms are detected in the urine, urethra, bladder, kidney, or prostate.<sup>3</sup> In developing countries, UTIs are one of the most commonly diagnosed disease among the patient seeking medical service.<sup>4</sup> UTIs can be categorized as acquired or nosocomial with *Escherichia coli* as the most predominant organism. *Klebsiella* and *Proteus* are other responsible pathogens in community acquired infection and in nosocomial are *Pseudomonas*, *Proteus*, and *Enterobacter*.<sup>5</sup>

UTI is an extremely common condition that occurs in both genders of all ages. However, the prevalence and incidence of infection are higher in women than in men, likely due to the shorter urethra and its proximity to perianal region.<sup>6</sup>

In Nepal, a number of studies have been done on the prevalence and antimicrobial resistance patterns of the isolates from UTIs. However, no data has been reported from the present area of study. Therefore, this study was done to determine the prevalence of positive urine samples and the susceptibility pattern of the isolated bacteria to the most commonly used antimicrobial drugs at Okhaldhunga Community Hospital (OCH), a remote rural hospital in the Eastern part of Nepal.

## METHOD

A retrospective study was carried out from December 1<sup>st</sup> 2018 to May 29<sup>th</sup> 2019 at Laboratory of OCH. Data was taken from a register maintained and intended for sample entry. All the urine samples were inoculated onto MacConkey Agar (MA) and Blood Agar (BA) by semi-quantitative method using calibrated loop, and incubated aerobically at 37°C for 24-48 hours. Growth of more than 10 bacterial colonies in a culture plate

was considered as positive urine culture. Growth of 100 colonies or more i.e.  $10^5$  colony forming units (CFU/ml) of urine, was considered as significant bacteriuria. Identification of isolates were done following their cultural characteristics, Gram staining and biochemical properties.

Antibiotic susceptibility testing of all isolates was performed on Mueller Hinton Agar (MHA) using Kirby-Bauer's disc diffusion method. Antibiotic discs used for susceptibility tests were obtained from HiMedia. Amoxicillin/Clavulanic acid (30µg), Amoxicillin (10µg), Azithromycin (15µg), Ceftriaxone (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Cloxacillin (10µg), Cotrimoxazole (25µg), Doxycycline (30µg), Erythromycin (15µg), Gentamycin (10µg), Nalidixic acid (30µg), Nitrofurantoin (300µg), and Norfloxacin (10µg). Interpretation as 'Sensitive', 'Intermediate' or 'Resistant' was done on the basis of the diameters of zones of inhibition of bacterial growth as recommended by Clinical Laboratory Standard Institute (CLSI) guideline.

Data entry and analysis was carried out using Excel 2016 and the Statistical Package for Social Sciences (SPSS) version 17.

## RESULT

A total of 210 non-repetitive MSU samples collected from patients for routine urine culture were analyzed. Of the 210 urine samples, 86 gave positive culture report with 71 identified bacteria. Out of these 71, the most predominant isolate was *E. coli* (76.10%) followed by *Staphylococcus aureus* (16.90%) and *Klebsiella pneumoniae* (4.20%) as shown in table 1.

In the present study, UTI occurred more in females (83.10%) than in males. Most isolates were from age group 20-30 years as shown below in figure 1.

Nitrofurantoin was found to be the most effective antibiotic (86.30%), followed by Chloramphenicol and Gentamycin (83.56%) for Gram negative bacterial isolates. Most isolates were resistant to Amoxicillin (87.67%) and Doxycycline (72.60%) as shown below in table 2.

Chloramphenicol and Cloxacillin were found to be the most effective drugs with a sensitivity of 84.62% for Gram positive isolates. Most pathogens were resistant to Penicillin and Erythromycin with a resistance of 92.31% and 84.62% respectively as shown below in table 3.

**Table 1. Percentage of identified bacterial isolates in positive urine culture**

Organisms	Total isolates (N= 71)
<i>E. coli</i>	76.10%
<i>S. aureus</i>	16.90%
<i>K. pneumoniae</i>	4.20%
<i>Proteus spp.</i>	1.40%
<i>Enterococcus faecalis</i>	1.40%

**Figure 1. Age and gender-wise distribution of identified bacterial isolates****Table 2. Antibiotic susceptibility pattern of overall Gram-negative bacilli**

Antibiotic	Sensitive %	Intermediate %	Resistant %
Amoxicillin/Clavulanic acid	28.77	8.22	63.01
Amoxicillin	10.96	1.37	87.67
Azithromycin	23.29	12.33	64.38
Ceftriaxone	54.79	2.74	42.47
Chloramphenicol	83.56	1.37	15.07
Ciprofloxacin	54.79	4.11	41.10
Cotrimoxazole	43.84	1.37	54.79
Doxycycline	13.70	13.70	72.60
Gentamycin	83.56	8.22	8.22
Nalidixic acid	30.14	6.85	63.01
Nitrofurantoin	86.30	0	13.70
Norfloxacin	42.47	8.22	49.31

**Table 3. Antibiotic susceptibility pattern of Gram-positive isolates**

Antibiotic	Sensitive %	Intermediate %	Resistant %
Amoxicillin	23.08	0	76.92
Azithromycin	30.77	7.69	61.54
Ceftriaxone	76.92	7.69	15.38
Chloramphenicol	84.62	0	15.38
Ciprofloxacin	38.46	15.38	46.15
Cloxacillin	84.62	7.69	7.69
Cotrimoxazole	15.38	7.69	76.92
Doxycycline	38.46	15.38	46.15
Erythromycin	0	15.38	84.62
Penicillin	7.69	0	92.31

## DISCUSSION

This is the study to evaluate the antibiotic susceptibility pattern of bacterial isolates to the commonly prescribed antibiotics to treat UTI in Okhaldhunga, Nepal.

The total growth positive rate of the study (40.95%) was higher than the study done by

Khatiwada S, et al<sup>7</sup> in which total growth positive rate was 23%. However, this was in agreement with the study conducted by Rijal A, et al (49%).<sup>8</sup> The age group with highest positive culture results was 20-30 years (30.2%). Similar result was obtained in a study conducted by Bhatt CP, et al.<sup>9</sup> This is the reproductive age group where the

couples in this age are sexually active and their chance of getting UTIs is also high. Leigh had found that the prevalence of UTI is higher in married women compared to nuns and unmarried women.<sup>10</sup>

Out of total 210 patients, majority of patients were females (77.6%) with a male to female ratio of 1:3.5. Similar male to female ratio of 1:3.2 was found in a study conducted by Rijal A, et al.<sup>8</sup> *E. coli* was the predominant isolate in both the genders. Higher frequency of *E. coli* isolation in urine is in accordance with various studies conducted inside Nepal and in different parts of the world.

Among the identified bacterial isolates, *E. coli* was the predominant isolate (76.06%) followed by *Staphylococcus aureus* (16.90%) and *Klebsiella pneumoniae* (4.22%). Other less common isolates were *Proteus* spp (1.41%) and *Enterococcus faecalis* (1.41%). This finding is similar to the study done by Kattel HP, et al<sup>11</sup> and Yadav K, et al.<sup>2</sup>

Of the 86 culture positive samples, 58 (67.4%) had a significant bacterial growth. Thus, prevalence of UTI was 27.6% including 79.3% in females and 20.7% in males. This finding was similar with the finding of Kumari N et al (25.7%)<sup>12</sup> but greater than the finding of Raza S, et al (19.7%)<sup>13</sup> and less than the finding of Paudel L, et al (36.9%).<sup>14</sup>

Nitrofurantoin was found to be the most effective drug with 86.30% sensitivity, similar to a study done by Rijal A, et al. But, in a study conducted by Panday DR, et al<sup>15</sup>, Nitrofurantoin was found to be second most sensitive antibiotic after Amikacin and followed by Imipenem. Though Imipenem was found to be the most effective drug, the study recommended using Gentamycin or Nitrofurantoin instead as carbapenems are considered the drug of last resort. On the other hand, 87.67 % isolates were resistant to Amoxicillin followed by 72.60% resistant to Doxycycline. *E. coli* and *K. pneumoniae* showed high percentage of resistance to Amoxicillin in a similar study conducted by Al-Mijalli SH.<sup>16</sup>

In case of Gram-positive isolates, Chloramphenicol (84.62%) and Cloxacillin (76.92%) were the most effective drugs while Penicillin (92.31%) and Erythromycin (84.62%) were the least effective. This was similar with the study conducted by Raina S, et al<sup>17</sup> and Thass N, et al<sup>18</sup> in which majority of the GPC were resistant to Penicillin.

The study was done on the urine samples collected over a period of six months. As the hospital is situated in rural setting where the population is

less, study done over a period of at least one year will have more samples and thus, yield more reliable result. 15 bacteria isolated on culture were unidentified ones. Thus, proper identification of the bacterial isolates must be done which further helps to study and understand the resistance pattern of the isolates in that area.

## CONCLUSION

Overall, Nitrofurantoin, Chloramphenicol and Cloxacillin were found to be the most effective antibiotics. But the bacterial isolates showed high resistance towards Amoxicillin, Doxycycline, Penicillin and Erythromycin. Thus, it is recommended to conduct such studies to keep an eye on the resistance pattern of the bacterial isolates and emergence of multi-drug resistant bacteria.

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## Conflict of Interest

None

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