

Original Research Article

Antibiogram Typing of Urinary Pathogens Isolated from Patients Visiting Tertiary Care Hospital, Dharan, Sunsari, Nepal

Kumu Chaudhary¹, Pranita Dongol¹ and Hemanta Khanal^{2*}

¹Department of Microbiology, Sunsari Technical College, Dharan, Sunsari, Nepal

²Department of Microbiology, Central Campus of Technology, Dharan, Nepal

*Corresponding Author: Hemanta Khanal, Assistant Professor, Department of Microbiology, Central Campus of Technology, Dharan, Nepal
E-mail: khanal.hemanta@gmail.com

Abstract

Urinary tract infection (UTI) is commonest clinical complications that occurs in all populations and ages; however, most common in women. The aim of the study was to determine the prevalence of significant bacteriuria among the UTI suspected patients and to assess drug resistance pattern among positive isolates. The urine samples were collected aseptically from March to August 2014 and cultured in bacteriological media by semi quantitative standard loop method. Identification of the significant isolates was done by standard microbiological techniques. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. Data were analyzed by SPSS version 16. Out of 752 samples, significant growth was seen among 13.96% and patients with age group of 21-30 were most infected. Females had higher percentage of significant bacteriuria. Of the total antibiotics used, most (98.5%) gram negative isolates were sensitive to Chloramphenicol followed by Gentamicin and Tobramycin and all isolates were (100%) resistant to Amoxicillin. All the gram positive isolates were 100% sensitive to Gentamicin and Azithromycin. Multidrug resistance was observed in 89.52% of the total bacterial isolates. Higher resistance was seen to Amoxicillin.

Keywords: Midstream urine, significant bacteriuria, UTI, multi drug resistance

Introduction

Urinary tract infection (UTI) is one of the most common infections after respiratory infection in the human being (Kumari et al., 2005). It is one of the most common causes of mortality and morbidity and about 150 million people visit the doctor with the complaint of UTI (Orenstein et al., 1999). UTIs are the serious health problem. Negligence to asymptomatic infection leads to serious complications like renal failure. UTI have the greater likelihood of infection with the multidrug resistant organism and also have a greater risk of treatment failure (Puri et al., 2006). UTI includes pyelonephritis, urethritis, cystitis, and prostatitis. Symptoms include dysuria, frequency, lower back pain, urgency and fever (Cheesbrough, 2006).

The presence of bacteria in urine is bacteriuria and bacteriuria are considered significant when the urine contains 10^5 per mL of bacteria though the patients may be symptomatic or asymptomatic (Chakraborty, 2003). About 20% of women experience a single episode of UTI during their lifetime. *E. coli* is the main causative agent in more than 80% of uncomplicated UTI and *Staphylococcus* and *Citrobacter* is another more prevalent (Measly, 1991). The purpose of this study was to determine the prevalence of significant bacteriuria and to assess the level of multidrug resistance pattern of positive isolates among the patients visiting Bijayapur Hospital, Dharan, Nepal.

Materials and Methods

This cross-sectional study was conducted among UTI suspected patients visiting Bijayapur Hospital, Dharan, Sunsari from March to August 2014. A total of 752 midstream urine samples were collected from all age group patients for routine bacterial culture and for antibiotic sensitivity testing. The urine specimen was cultured on Cysteine Lactose Electrolyte Deficiency (CLED) Media by semi-quantitative

culture technique using flame sterilized standard nichrome wire loop. The inoculated plates, after overnight incubation at 37 °C, were observed for significant bacterial growth. Identification of significant isolates was done by using standard microbiological techniques (Chakraborty, 2003 and Frobes et al., 2007).

Antibiotic susceptibility test of the identified bacteria was performed by Kirby Bauer disc diffusion method as recommended by World Health Organization (Hugo et al., 1993). Data collected was transferred in Microsoft Office Excel 2007, descriptive and inferential statistics were analyzed

with SPSS version 16 for Microsoft Windows. Chi-square test was used to determine the significant association of dependent variables at 5% level of significance. The test is said to be significant if $p\text{-value} < 0.05$.

Results and Discussion

Out 752 UTI suspected patients, 415 (55.18%) were females and 337 (44.81%) were male patients. Among them, 105 were found to be infected. Among infected, 70 (66.60%) were female and 35 (33.33%) were males (Figure 1). Among 105 significant bacteriuric cases, 99 (94.28%) were due to Gram-negative

bacteria and 6 (5.71%) were due to gram-positive bacteria (Figure 1). Females had the higher percentage of significant bacteriuria compared to males and was statistically significant ($p=0.011$).

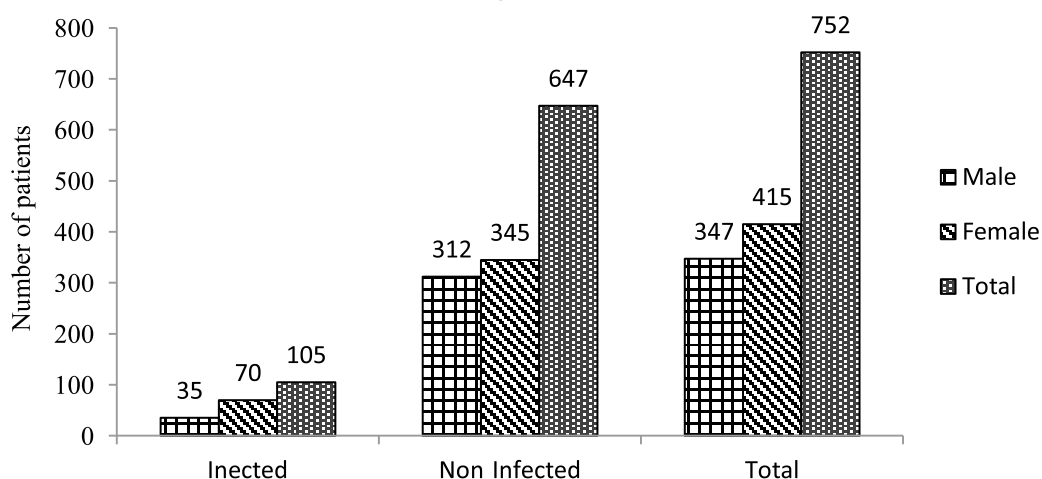


Figure 1: Number of infected and non-infected patients.

Altogether 13 different bacterial species were isolated among which *E. coli* (65.71%) was found to be predominant followed by *Klebsiella oxytoca* (10.47%). The other bacterial isolates were *Citrobacter freundii*, *Shigella* spp., *Serratia*, *Providencia*, *Morganella morganii*, *Proteus mirabilis*, *Enterobacter*, *Salmonella paratyphi*, among gram-negative isolates. The gram-positive isolates were *Staphylococcus aureus*, *Streptococcus faecalis* and *Sarcina*. *Staphylococcus aureus* constituted 3.80%, while both *Streptococcus faecalis* and *Sarcina* constituted 0.95% of the total gram positive isolates (Table 1).

Among the positive bacteriuric cases, the highest number of cases (i.e. 26) belonged to the age group 20-30 years followed by age group 1-10 years (i.e.17). In infants, out of 6 positive isolates, 4 were male child while 2 were female child. Females of age group 20-30 were found to have significant bacteriuria among 15 cases and in patients above 80 years the number of positive isolates were 2 and the result was statistically significant ($p\text{-value} 0.000$) (Table 1).

Table 1: Age and sex wise distribution of significant bacteriuric cases

Age groups	UTI positive male	UTI positive female	Total positive isolates	p-value
<1	4	2	6	0.000
1-10	6	11	17	
11-20	2	9	11	
21-30	11	15	26	
31-40	2	10	12	
41-50	3	5	8	
51-60	2	8	10	
61-70	2	5	7	
71-80	1	5	6	
Above 80	2	0	2	
Total	35	70	105	

Gram-negative bacteria were more prevalent than Gram-positive and this could be due to the presence of unique structure in Gram negative bacteria which help for attachment to the uroepithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion resulting in invasive infection (Agersew et al., 2012). Most of the UTI studies showed higher prevalence of gram negative isolates (Kumari et al., 2005; Orenstein et al., 1999; Puri et al., 2006; Cheesbrough, 2006). Females had higher percentage of bacteriuria probably due to anatomical characteristics of female as the urethra is proximity to perianal area and shorter urethra. A number of studies suggested that sexual activity has been reported to increase the prevalence of UTI in females (Oluremi et al., 2011 and Adedeji et al., 2009). This result corresponds to the number of other studies in which higher prevalence was seen in female (Amin et al., 2009 and Akbar et al., 2001) as described in table 1.

Higher prevalence of *E. coli* might be due to endogenous source of infection as they are the frequent isolates at hospital acquired and community acquired infections (Chhetri, 2011) and this study resembled with other similar researches by Paryani et al., (2012); Boyko et al., (2005) and Lin et al., (2008). *Klebsiella* species were the second common uropathogens (10.47%). Similar result was also reported by Turan et al.,(2008). *Sarcina* is a flora of gastrointestinal tract, however its presence in urine in significant number suggests that either the causative agents for UTI is varying or the urine was highly contaminated with stool as stated by Ratuapli et al., (2013) (Table 2).

Table 2: Pattern of bacterial isolates

Organisms	Frequency (%)
<i>E. coli</i>	69(65.71%)
<i>Klebsiella</i> species	11(10.47%).
<i>Citrobacter freundii</i>	4(3.80%)
<i>Shigella</i> spp.	5(4.76%)
<i>Serratia</i> spp.	3(2.85%)
<i>Providencia</i> spp.	3(2.85%)
<i>Proteus mirabilis</i>	2(1.90%)
<i>Enterobacter</i>	1(0.95%)
<i>Salmonella paratyphi</i>	1(0.95%)
<i>Staphylococcus aureus</i>	4(3.80%)
<i>Sarcina</i> spp.	1(0.95%)
<i>Streptococcus faecalis</i>	1(0.95%)

Table 3. Antibiotic sensitivity pattern of gram positive bacteria:

Antibiotic used	Antibiotic susceptibility pattern					
	<i>Staphylococcus aureus</i>		<i>Streptococcus faecalis</i>		<i>Sarcina</i>	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Chloramphenicol	4(100)	-	1(100)	-	-	1(100)
Cefazolin	1(25)	3(75)	-	1(100)	1(100)	-
Gentamycin	4(100)	-	1(100)	-	1(100)	-
Cefoxitin	-	4(100)	-	-	-	-
Carbenicillin	1(25)	3(75)	-	-	-	-
Azithromycin	4(100)	-	1(100)	-	1(100)	-
Amoxicillin	-	4(100)	-	1(100)	-	1(100)
Erythromycin	2(50)	2(50)	1(100)	-	1(100)	-
Tobramycin	1(100)	-	1(100)	-	1(100)	-

Of the total antibiotics used, most (98.5%) gram negative isolates were sensitive to Chloramphenicol followed by 97.9% Gentamicin and Tobramycin (97.9%). Gram positive bacteria showed 100% sensitivity to Gentamicin and Azithromycin where as 83.33% to Chloramphenicol. All theuopathogens were 100% resistant to Amoxycillin (Table 2 and Table 3).

Of the total antibiotics used for *E. coli*, Chloramphenicol, Tobramycin and Gentamycin showed 100% susceptibility followed by Nitrofurantoin and Nalidixic acid, Erythromycin with 62.31%, 37.68% and 26.08% respectively. Least susceptibility was shown to Cefazolin with 10.14%. All 69 isolates of *E.coli* were 100% resistance to Amoxycillin (Table 2). Multidrug resistance was observed in 89.52% (i.e. 94 out of 105). Out of 69 isolates of *E. coli*, 60 were MDR. Similarly out of 5 isolates of *Shigella* species, 3 were of MDR strain. Except these two, rest of all showed MDR (Table 4).

Table 4: Multi-drugs resistance pattern of bacterial isolates

Organisms	MDR	Total isolates
<i>E. coli</i>	60	69
<i>K. oxytoca</i>	11	11
<i>C. freundii</i>	4	4
<i>Shigella</i> species	3	5
<i>Serratia</i>	3	3
<i>Providencia stuartii</i>	3	3
<i>Proteus mirabilis</i>	2	2
<i>Sarcina</i>	1	1
<i>Enterobacter</i>	1	1
<i>Salmonella Paratyphi</i>	1	1
<i>Staphylococcus aureus</i>	4	4
<i>Streptococcus faecalis</i>	1	1
Total	94	105

Most gram negative isolates were sensitive to Chloramphenicol and all the isolates were resistant to Amoxycillin. Chloramphenicol has not been in use as a choice of drug since many years, as resistance to it has been documented to occur. However, in this study the lower resistance rate to Chloramphenicol was observed in all the isolates. Similar result was also reported by Nitzan et al., (2010).

In a similar study carried out by Agersew et al., (2012), 100% sensitive to Chloramphenicol and 100% resistance to Amoxicillin was found in gram negative urinary isolates (Table 4). In our study, higher sensitive to Gentamicin and Azithromycin whereas resistant to Amoxicillin were seen in all the gram positive isolates. Resistance to Amoxicillin may be due to the easy availability and indiscriminate use of commonly used drug such as Amoxicillin (Agersew et al., 2012).

The increasing resistance of pathogens against commonly used antimicrobial drugs is a serious issue and a major clinical problem to treating diseases. The rate of resistance among

pathogens varies from time to time and from place to place (Gales et al., 2001). A significant number of urinary isolates in this study indicates that the emergency of MDR strains is common in UTI patients of Eastern region. The antibiotic sensitivity pattern of organism 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, which is the main cause of MDR strains. Therefore, periodic evaluation of sensitivity pattern is essential for rational and appropriate use of antibiotics (Kumari et al., 2005).

Conclusions

The majority of patient visiting hospital with suggestive UTI may not have culture positive infections due to prior use of antibiotics. Multidrug resistance was observed in 100% of the total bacterial isolates. Chloramphenicol was found as the drug of choice against Gram negative isolates whereas Gentamicin and Azithromycin were against Gram positive isolates. But the

organisms are developing resistance towards antibiotic like Amoxicillin. A significant number of urinary isolates from the patients were MDR which can result in unavoidable treatment failure. These results suggest for a continual evaluation and regular monitoring of antimicrobial agents for proper treatment.

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