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Research

Bacteriological profile and antibiotic sensitivity pattern of uropathogens in hemodialysis patients attending tertiary care hospital, Kathmandu, Nepal

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Abstract: *Hemodialysis (HD) patients are more susceptible to urinary tract infection (UTI) and UTIs are an important cause of morbidity and mortality in these patients. This study was conducted to find out the prevalence of UTI and multidrug-resistant (MDR) patterns of isolates among HD patients. A cross-sectional study was carried out from February 2018 to August 2018 at the National Kidney Center (NKC), Banasthali, Kathmandu. A total of 200 (108 male and 98 female) mid-stream urine samples were collected from patients with renal failure undergoing hemodialysis. Of them, 26% of samples showed significant bacteriuria. 22.2% and 30.4% of samples showed significant bacteriuria among males and females, respectively (p=0.19). Among the age-group, bacteriuria was ranked top at the age group >70 years (35.3.8%) followed by 51-70 years (34.1%) (p=0.046). Nine different bacteria were isolated. Among them, the most predominant organism was Esch. coli (32.7%) followed by Staph. aureus (26.9%), Staph. saprophyticus (13.5%) and others. For the Gram-negative isolates, Imipenem (96.8%) was found to be the most effective drug followed by Amikacin (83.9%) and Cefepime (64.5%). For Gram-positive isolates, Cefepime (76.1%) was found to be the most effective drug. The organisms showed 71.4% resistance to Cotrimoxazole and 57.1% resistance to Amoxicillin, Azithromycin, and Nitrofurantoin. The overall prevalence of MDR was found to be 57.7% in HD patients. Amikacin and Imipenem were found to be the drug of choice to treat UTI in HD patients. This study will be beneficial for making treatment policy and reducing the risk of UTI in HD patients.*
Keywords: *Hemodialysis, UTI, bacteriuria, multidrug-resistant, Nepal*

Introduction

Hemodialysis (HD) patients are more susceptible to bacterial urinary tract infection (UTI) and UTIs are an important cause of morbidity and mortality in these patients [1]. HD patients are at high risk for infections, which is attributed to impaired immune defenses, a high severity of illness, and the need for routine puncture of a vascular access site to remove blood for hemodialysis [2]. Moreover, urine voiding is a natural process of bacterial clearance in the urinary tract [3]. Usually, HD patients are subjected to excrete urine mechanically and lack of natural voiding to excrete urine is often overlooked as a source of infection in HD patients [4]. UTI is the second most common cause of death in patients dependent on HD [5]. Therefore, routine monitoring and diagnosis of urinary tract infection (UTI) on time is very crucial that help in infection control and therapeutic management [4, 6].

Mounting evidence has already demonstrated that HD patients are more vulnerable to UTI. The prevalence of pyuria in dialysis patients from different studies ranges between 28% and 72% and the prevalence of documented UTI in those studies varied between 11% and 70% [7]. A very high incidence of UTI was reported by Jadav et al (1977) [8] both in acute renal failure (73.0%) and chronic renal failure patients (57.5%) in Mumbai (India). Likewise, in a study conducted in Nepal in chronic HD patients, Gram-negative organisms were isolated at a rate of 84.6% [9]. In another study, 21.1% were found to have a final diagnosis of UTI on urine discharge [10]. Amikacin, Imipenem, Ceftazidime, and Gentamicin are antibiotics of choice for the treatment of UTI in HD patients.

This study was aimed to determine the bacterial profile and antimicrobial susceptibility pattern of organisms causing urinary tract infections in hemodialysis patients and detect Multi-Drug Resistant (MDR) producing strains of isolates.

Materials and methods

Study area and sample size

A cross-sectional study was conducted in the National Kidney Center´s Laboratory, Kathmandu, Nepal from Feb 2018 to Aug 2018. The sample size was determined by Fisher's Formula. 200 urine samples were taken from patients with renal failure undergoing hemodialysis. 5-10 ml clean-catch midstream urine was collected in a leak-proof, wide-mouthed, properly capped, sterile container in the hospital on the day of dialysis. The urine sample was transported to the laboratory

as soon as possible for further process. The urine was cultured onto the MacConkey agar and blood agar medium by the semi-quantitative culture techniques using a standard loop. Isolates were identified on the basis of standard morphological appearance of the colonies, staining reactions, biochemical properties and serotyping if required in specific cases [11, 12]. *Staph. saprophyticus* was identified by Novobiocin test.

Bacterial Antibiotic Sensitivity Test

Antimicrobial susceptibility testing of the isolates towards various antimicrobial disks was done by the modified Kirby-Bauer disk diffusion method as recommended by CLSI 2014 using Mueller Hinton Agar. The antibiotics used were amikacin, azithromycin, cefepime, ceftriaxone, amoxicillin, ciprofloxacin, levofloxacin, cotrimoxazole, nitrofurantoin, chloramphenicol, and linezolid. Based on the susceptibility pattern of isolates, bacteria resistant to at least more than 2 classes of antibiotics were considered as Multi-Drug Resistant [13].

Ethical approval

Ethical clearance was obtained from the Institutional Review Committee of Shi-Gan Health Foundation and National Institute of Tropical Medicine, Kathmandu prior to the research (IRC Reference No: 04/2071/08/26).

Results

Growth pattern in two genders:

Out of the total 200 urine samples, 26% (52/200) of samples showed significant bacteriuria. A marginally higher positive rate was seen in females (30.4%; 28/98) compared to their male counterparts (22.2%; 24/108) (p=0.19) (Table 1).

Table 1: Bacterial growth positivity in hemodialysis patients of two genders.

Sex	Total count (n)	Positive count (n)	%	P-value
Male	108	24	22.2%	0.19
Female	98	28	30.4%	
Total	**200**	**52**	**26.0**	

Among HD patients of different age groups, highest growth positive rate was observed in the age-group of more than 70 years followed by 51 – 70 years (35.3%; 6/17) and 31 – 50 years (34.1%; 30/88) and the least growth positive rate was seen in the age group of 11-30 years (15.4%; 4/26).

This increasing trend with age was statistically significant (p=0.046) (Table 2). In males, the highest rate of growth positive was seen in the age-group of >70 (33.3%) and lowest in the age-group 11.30 (6.7%) while in females, highest significant bacteriuria was seen in the age-group 51-70 (47.1%) and the least in the age-group 31-50 (13.9%) (Table 2).

Table 2: Bacterial growth positivity in HD patients of different sex and age-groups.

Age group	Male		Female		Total	
	Total (n)	Growth positive	Total (n)	Growth positive	Total (n)	Growth positive
11-30	15	1 (6.7%)	11	3 (27.3%)	26	4 (15.4%)
31-50	33	7 (21.2%)	36	5 (13.9%)	69	12 (17.4%)
51-70	54	14 (25.9%)	34	16(47.1%)	88	30 (34.1%)
>70	6	2 (33.3%)	11	4 (36.4%)	17	6 (35.3%)
Total	**108**	**24 (22.2%)**	**92**	**28 (30.3%)**	**200**	**52 (26.00%)**

Bacterial profile and multi-drug resistant strain

A total of 52 bacterial strains were isolated from 200 HD patients. *Eschirichia. coli* was the most frequently isolated bacterial spp. Of 52 bacterial isolates, 30 (57.7%) were MDR. Among MDR strains, *Staph. aureus* (78.6%) isolates were most predominant MDR (Table 3).

Table 3. Multidrug-resistant strain isolated from HD patients.

Organisms	Total isolates (n)	MDR strains (%)
Escherichia coli	17	7 (41.2%)
Staphylococcus aureus	14	11 (78.6%)
Staphylococcus saprophyticus	7	5 (71.4%)
Klebsiella oxytoca	5	2 (40.0%)
Citrobacter freundii	4	2 (50.0%)
Others	5	3 (60.0%)
Total	**52**	**30 (57.7%)**

Antibiotic sensitivity pattern of Gram-positive isolates

For Gram-positive isolates, Cefepime (76.1%) was found to be a more effective drug. The Gram-positive isolates showed 71.4% resistance to Cotrimoxazole and 57.1% resistance to Amoxicillin, Azithromycin, and Nitrofurantoin (Table 4).

Antibiotic sensitivity pattern of Gram-negative isolates

Among the Gram-negative isolates, Imipenem (96.8%) was found to be the most effective drug followed by Amikacin (83.9%) and Cefepime (64.5%). The Gram-negative isolates showed 54.8% resistivity to Cephalexin and Ciprofloxacin (Table 5).

Table 4: Antibiotic sensitivity pattern of Gram-positive isolates.

Antibiotics used	Susceptibility pattern		
	Resistant n (%)	Intermediate n (%)	Sensitive n (%)
Amikacin	5 (23.8%)	1 (4.8%)	15 (71.4%)
Azithromycin	12 (57.1%)	6 (28.6%)	3 (14.3%)
Cefepime	4 (19.1%)	1 (4.8%)	16 (76.1%)
Ceftriaxone	11 (52.4%)	2 (9.5%)	8 (38.1%)
Amoxicillin	12 (57.1%)	6 (28.6%)	3 (14.3%)
Ciprofloxacin	8 (38.1%)	9 (42.9%)	4 (19.1%)
Levofloxacin	9 (42.8%)	6 (28.6%)	6 (28.6%)
Cotrimoxazole	15 (71.4%)	3 (14.3%)	3 (14.3%)
Nitrofurantoin	12 (57.1%)	6 (28.6%)	3 (14.3%)
Chloramphenicol	2 (9.5%)	5 (23.8%)	14 (66.7%)
Linezolid	6 (28.6%)	2 (9.5%)	13 (61.9%)

n = 21

Table 5: Antibiotic sensitivity pattern of Gram-negative isolates

Antibiotics used	Susceptibility pattern		
	Resistant n (%)	Intermediate n (%)	Sensitive n (%)
Amikacin	0 (0.0%)	5 (16.1%)	26 (83.9%)
Azithromycin	16 (51.6%)	6 (19.4%)	9 (29.0%)
Cefepime	7 (22.6%)	4 (12.9%)	20 (64.5%)
Ceftriaxone	10 (32.3%)	6 (19.4%)	15 (48.3%)
Cephalexin	17 (54.8%)	1 (3.2%)	13 (41.9%)
Imipenem	0 (0.0%)	1 (3.2%)	30 (96.8%)
Ciprofloxacin	17 (54.8%)	6 (19.4%)	8 (25.8%)
Levofloxacin	4 (12.9%)	9 (29.0%)	18 (25.8%)
Norfloxacin	14 (45.2%)	7 (22.6%)	10 (32.1%)
Ofloxacin	13 (41.9%)	7 (22.6%)	11 (35.5%)
Cotrimoxazole	13 (41.9%)	5 (16.2%)	13 (41.9%)
Nitrofurantoin	14 (45.2%)	7 (22.6%)	10 (32.2%)

n = 31

Discussion

In this study, one-fourth of the samples showed significant bacteriuria indicating the cases of UTI. This finding is lower than the finding of Singh et al (2016) [14] which was 34.0% and higher than the finding of Pradhan and Pradhan (2017) [15] (13.8%). However, similar finding has been reported by Dhakal et al (1999) [16] (25.2%). A marginally higher culture-positive rate was observed in females (30.4%) than in their male counterparts (22.2%). The higher rate of UTI in females was also reported by Pardeshi (2018), Daoud and Afif (2011), Khatiwada et al (2018) and Jha and Bapat (2005) [17, 18, 19, 20]. This might be mainly due to the anatomical and behavioral differences among females.

UTI in HD patients included in this study showed an increasing trend with age (15.4% in the age group of 11-30 years to 35.3% in the age group of >70 years) and this increment was significantly higher. Haider et al (2017) also reported a higher prevalence of UTI in the elderly groups [21]. This might be mainly due to the increasing prevalence of risk factors such as diabetes, hypertension and cardiovascular disease among older groups [22]. In the United States, chronic kidney disease was reported to be about 10% in patients of age >65 years, in contrast to 1.5% of the younger employed population [23].

Of the different kinds of pathogens isolated in this study *Esch. coli* was the commonest (17/52; 32.7%) followed by *Staph. aureus* (14/52; 26.9%) and *Staph. saprophyticus* (7/52; 13.5%) and others. The overall multi-drug resistance in this study was found to be 57.7% (30/52). *Staph. aureus* showed the highest degree of MDR (78.6%) followed by *Staph. saprophyticus* (71.4%). Different rates of prevalence of MDR ranging as low as 3% to as high as 59% in hemodialysis have been reported by other investigators [9, 24, 25, 26, 29]. This might be associated with different risk factors such as prior hospitalization, temporary dialysis access, residence in nursing homes, and antimicrobial exposure [24, 25, 27, 28].

Gram-negative bacilli (59.6%) were the predominant organisms isolated from urine samples than the Gram-positive cocci (40.4%). This finding is in agreement with those of Moges et al (2002) [30]; Kothari and Sagar (2008) [31]; Puri et al (2006) [32] and Karki et al (2004) [33]. Among the Gram-negative bacteria, *Esch. coli* was found as the most predominant organism. This result is in agreement with those of Beyene and Tsegaye (2011) [34]; Daoud and Afif (2011) [18]; Elkehili et al (2010) [35]; Aypak et al (2009) [36] and Rai et al (2008) [37]. The major factor responsible for

the high prevalence of uropathogenic *Esch. coli* (UPEC) is the type 1-pilli which enhances binding and invasion to the superficial epithelial cells [38].

Staph. aureus was found as the second most predominant organism in this study. This finding is different than the findings of Chaudhary et al (2016) [9]. The presence of this organism in urine often indicates pyelonephritis acquired via a hematogenous route or descending route. This finding, however, is in agreement with the findings of Nicholas et al (2018) [39] where *Staph. aureus* is the predominant organism in HD patients to cause septicemia.

In this study, Imipenem (96.8%) was found to be the most effective drug against Gram-negative bacterial isolates followed by Amikacin (83.9%) which is similar to the study done by Khatiwada et al [19]. All *Esch. coli* isolates were susceptible to Imipenem followed by Amikacin (94.1%). Quinolone/ Fluoroquinolone (Ciprofloxacin, Norfloxacin) were found least active. A study done in Senegal has shown that the increasing resistance to these drugs is hypothesized for the generalized use of fluoroquinolones in animal feed and subsequent transmission of resistant strains from animals to humans [40]. *Acinetobacter* spp. showed 100% sensitivity to aminoglycosides, macrolide and β-lactam groups of antibiotics used. *Ps. aeruginosa* showed 100% resistivity to Nitrofurantoin and Cephalexin. Several factors are responsible for the rise of resistance rate of bacterial uropathogens including misuse of antibiotics, frequent oral use of wide-spectrum antimicrobials that may change intestinal flora (which is usually common cause of UTI) and inappropriate dosage and duration of treatments [41]. Though aminoglycosides are effective against Gram-negative bacteria in this study, the use of aminoglycosides in HD patients should be assessed against their ototoxicity and nephrotoxicity [42].

Conclusion

The study findings showed that *Esch. coli* were the most predominant pathogens among the HD patients followed by *Staph. aureus* with an increasing trend with age. This study also showed a marginally higher prevalence of UTI in female HD patients than in their male counterparts. The study showed that Imipenem and Amikacin were the most effective drug for Gram-positive bacteria and Imipenem and Amikacin for Gram-negative bacteria with the overall prevalence of MDR of 57.7%. Our data will be beneficial for making treatment policy and reducing the risk of UTI in HD patients.

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Author's contribution

Miya, A.K. designed and performed experiments. **Ansari, M.** designed the study, analyzed the data and wrote the manuscript. **Rai, G.** revised and approved the work plan, supervised the overall work and helped in data analysis. **Pant, A.D.** helped in experimental design and

supervised the lab work. **Rai, K.R** wrote and revised the manuscript and helped in data analysis. **Rai, S.K.** revised the work plan, experimental design, and manuscript. All authors read and approved the final manuscript.

Dedication

Not mentioned

Conflict of Interest

There are no conflicts to declare.

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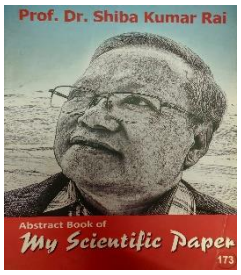

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