

Urinary tract etiology, antimicrobial susceptibility and resistance pattern of bacterial isolates in hemodialysis patients attending tertiary care hospital.

¹Dr Shipi Hora, Associate Professor, Department of Microbiology, Jhalawar Medical College, Jhalawar.

²Dr Mahesh Dan, PG Resident, Department of Microbiology, Jhalawar Medical College, Jhalawar.

Corresponding Author: Dr Mahesh Dan, PG Resident, Department of Microbiology, Jhalawar Medical College, Jhalawar.

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Abstract

Urinary tract infections are the most common bacterial infection. Patients with renal impairment and with underlying urological abnormalities are more susceptible to and are at high risk for urinary tract infections due to impaired immune defenses and lack of natural voiding to excrete urine. This study aims to determine the bacterial characteristics and antimicrobial susceptibility patterns of microorganisms that cause UTI in hemodialysis patients and to detect bacterial isolates that develop multi-drug resistance. A cross-sectional study was conducted from July 2021 to December 2021 on 180 patients with renal failure undergoing hemodialysis in Hemodialysis unit of the Jhalawar Government Hospital (Rajasthan). The prevalence of UTIs in hemodialysis patients was 29.4%, and the incidence of urinary tract infections in female participants (32.9%) was higher than that in male participants (26.5%). A significant increase in the prevalence of urinary tract infections has been observed in the elderly population over 40 years of age. E. coli is the most common pathogen of urinary tract infection in hemodialysis patients. Staph. hemolyticus had the highest

multidrug resistance (81.8%). Gram-negative bacteria (56.6%) were the main microorganisms isolated from urine samples. Studies have shown that Cefepime is the most effective drug against Gram-positive bacteria, and Imipenem is the most effective drug against Gram-negative bacteria. In addition, the Gram-positive isolates showed the highest resistance to Cotrimoxazole (69.6%) and Nitrofurantoin (60.9%), whereas the Gram-negative isolates showed the highest resistivity to Ciprofloxacin (60.0%) and Norfloxacin (56.7%). The findings of this study will be helpful in determining the treatment policies and reducing the risk of urinary tract infections in hemodialysis patients.

Keywords: Urinary tract infections, Hemodialysis, Antimicrobial susceptibility, Multi-drug resistance pattern, Bacteriuria.

Introduction

Urinary tract infection (UTI) is the invasion of any urinary tract tissue from the renal cortex to the opening of the urethra by microorganisms. The urinary tract includes organs such as the kidneys, ureters, bladder, urethra, and accessory structures, which collect and store

urine and release it from the body. UTIs may involve only the lower urinary tract or both the upper and lower urinary tract [1]. The lower urinary tract is called simple cystitis (bladder infection), and when the upper urinary tract is involved, it is called pyelonephritis (kidney infection) [2]. Urine formed in the kidney is a sterile liquid that can be used as a good medium for bacterial growth [3].

Bacteria are considered the most common cause of lower and upper urinary tract infections. Hemodialysis patients and patients with underlying urological abnormalities are more susceptible to bacterial UTI, and the risk of infection is high due to high disease severity, lack of natural voiding to excrete urine, impaired immune defenses, and the need to routinely puncture blood vessels to remove blood for hemodialysis [4]. Further, urination is the natural process of removing bacteria from the urinary tract [5], but hemodialysis patients urinate mechanically. This lack of natural urination and excretion of urine is often overlooked as a source of infection for hemodialysis patients. UTI is the second leading cause of morbidity and mortality in hemodialysis patients [6,7]. Therefore, timely routine monitoring and diagnosis of UTI is very essential for controlling infection and managing treatment [8].

There are many factors that affect the clinical manifestation of UTI. For instance, it depends on the severity of the infection, the affected urinary tract, the pathogen, and the patient's ability to produce a strong immune response. However, the present article aims to determine the bacterial characteristics and antimicrobial susceptibility patterns of microorganisms that cause UTI in hemodialysis patients and to detect bacterial isolates that develop multi-drug resistance.

Objectives

- To understand the Urinary tract etiology and study the prevalence of uropathogenic profile among hemodialysis patients.
- To determine the antimicrobial susceptibility pattern of bacterial isolates.
- To detect bacterial isolates that develops multi-drug resistance.

Materials and Methods

Study Domain: Hemodialysis patients with uropathogenic profile.

Study Design: Retrospective study.

Place of Study: Department of Microbiology, Government Hospital, Jhalawar, Rajasthan.

Period of Study: Six months (July 2021 to December 2021).

Study Population: Hemodialysis patients admitted in Hemodialysis unit with sign and symptoms of UTI.

Inclusion Criteria: Dialysis patients suspected of having a urinary tract infection.

Exclusion Criteria: Dialysis patients without urinary tract infections.

Sample Size: A total of 180 patients who agreed to participate in this study constituted the sample. Thus, the sample size (n) of this study is 180 patients.

Sample Collection Methodology: A cross-sectional study was conducted on 180 patients with renal failure undergoing hemodialysis in Hemodialysis unit of the Jhalawar Government Hospital (Rajasthan) from July 2021 to December 2021. As pathogens accumulate in the patient's bladder overnight, the patients were asked to collect 6-15 ml of the first midstream morning voided urine in a leak-proof sterile container. The samples were sent to the laboratory as soon as these were collected for further analysis.

Inoculation and Identification Procedure: The collected urine specimens were inoculated onto the Cystine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar, and Blood agar medium by the semi-quantitative culture techniques using a standard loop. Isolates were identified on the basis of standard operating procedures, such as biochemical properties, morphological appearance, staining reactions, serotyping, etc., as and when required [9,10].

Antimicrobial Susceptibility Test: Antimicrobial susceptibility testing of the isolates was done by the modified Kirby-Bauer disc diffusion method in Mueller-Hinton agar as per CLSI guidelines, 2014. The following antibiotics were used on the surface of Mueller-Hinton agar: linezolid, chloramphenicol, nitrofurantoin, cotrimoxazole, levofloxacin, ciprofloxacin, amoxicillin, ceftriaxone, cefepime, azithromycin, and amikacin. These antimicrobial agents were selected because of the frequent empirical treatment used by the clinicians. Based on the CLSI guidelines, the results were interpreted as resistant, intermediate, and sensitive.

Detecting Multi-drug Resistance: Depending on the susceptibility pattern of the isolates, bacterial resistance to two or more classes of the antimicrobials tested was considered as Multi-Drug Resistance [11]. Positive results from urine culture and antimicrobial sensitivity test results were reported to the attending physician for subsequent treatment and follow up.

Data Processing and Analysis: Quantitative data were cleaned, edited, and entered onto MS-Excel sheet and exported for further analysis to the statistical software, SPSS (version 23.0). Fisher’s exact test and binary logistic regression test results were used. A p-value not exceeding .05 was employed to declare the statistical significance.

Study Results

Demographic Characteristics: This study is based on a sample of 180 hemodialysis patients suspected of having a urinary tract infection and admitted in Hemodialysis unit of the Jhalawar Government Hospital (Rajasthan) from July 2021 to December 2021. The demographic factors of the patients participating in the study can be depicted in Table 1 below:

Table 1: Demographic Characteristics

Factors	Groups	Count	Percentage
Gender	Male	98	54.4
	Female	82	45.6
	Total	180	100.0
Age group	Below 20 years	23	12.8
	21 – 40 years	48	26.7
	41 – 60 years	65	36.1
	Above 60 years	44	24.4
	Total	180	100.0
Frequency of Dialysis per week	Once	0	0.0
	Twice	21	11.7
	Thrice	159	88.3
	Total	180	100.0
Cause of Renal Failure	Hypertension	118	65.6
	Diabetes Mellitus	27	15.0
	Other Chronic Disease (e.g. Glomerular disease, Polycystic disease, etc.)	35	19.4
	Total	180	100.0

As evident from the results of Table 1, 54.4% of the respondents were male and 45.6% were female, with ages ranging between 12 years to 73 years (mean age =

45.7 years, SD = 11.23). Majority of the patients (36.1%) belonged to the age group 41-60 years, followed by 26.7%, 24.4%, and 12.8% patients belonging to the age groups 21-40, above 60, and below 20 years respectively. No patient reported single dialysis per week. 21 (11.7%) patients reported of having dialysis twice a week. Most of the patients (88.3%) had three times dialysis per week. The collected data revealed that all the patients had chronic diseases, the most dominant (65.6%) cause being hypertension, followed by diabetes mellitus (15%), and other chronic diseases (19.4%).

Gender-wise growth pattern

Table 2: Gender-wise Growth Pattern

Gender	Count	Positive Count	Percentage	p-value
Male	98	26	26.5%	.127
Female	82	27	32.9%	
TOTAL	180	53	29.4%	

The statistical result of the growth pattern among both the genders as summarized in Table 2 reveals that among the total 180 urine specimens collected, 29.4% (53/180) showed significant bacteriuria. A marginal higher positive rate was seen in female patients (32.9%; 27/82) in comparison to male patients (26.5%; 26/98). The gender-wise growth pattern was not found to be statistically significant (p = .127).

Age-wise growth pattern

Table 3: Age-wise Growth Pattern

Age group	Male		Female		Total	
	Count	Growth +ve	Count	Growth +ve	Count	Growth +ve
Below 20 years	14	3 (21.4%)	9	2 (22.2%)	23	5 (21.7%)
21 - 40 years	26	7 (26.9%)	22	8 (36.4%)	48	15 (31.3%)
41 - 60 years	34	10 (29.4%)	31	11 (35.5%)	65	21 (32.3%)
Above 60 years	24	6 (25.0%)	20	7 (35.0%)	44	13 (29.5%)
TOTAL	98	26 (26.5%)	82	27 (32.9%)	180	53 (29.4%)

Table 3 summarizes the statistical result of the growth pattern in the identified age groups. Out of the total 180 hemodialysis patients, the highest growth positive rate was seen in the age group 41-60 years (32.3%; 21/65), followed by the age group 21-40 years (31.3%; 15/48) and the age group of above 60 years (29.5%; 13/44), with the least growth positive rate seen in the age group of below 20 years (21.7%; 5/23). The age-wise growth pattern was found to be statistically significant (p = .038). In male patients, the highest growth positive rate was seen in the age group 41-60 years (29.4%; 10/34) and the lowest growth positive rate was seen in the age group of below 20 years (21.4%; 3/14). Further, in female patients, the highest growth positive rate was seen in the age group 21-40 years (36.4%; 8/22) and the lowest growth positive rate was seen in the age group of below 20 years (22.2%; 2/9).

Bacterial Isolates and Multi-drug Resistance Strain

Table 4: Bacterial Isolates and Multi-drug Resistance Strains

Isolated Bacterial Species	Count	Multi-drug Resistance Strains
Escherichia coli	18	7 (38.9%)
Staphylococcus hemolyticus	11	9 (81.8%)
Enterococcus faecalis	9	4 (44.4%)
Staphylococcus aureus	7	4 (57.1%)
Staphylococcus saprophyticus	4	2 (50.0%)
Klebsiella oxytoca	2	1 (50.0%)
Citrobacter freundii	2	1 (50.0%)
TOTAL	53	28 (52.8%)

The present study of 180 hemodialysis patients isolated seven different bacterial strains in a total of 53 patients, as summarized in Table 4. Escherichia coli was the most dominant (18/53) causative agent of UTI, followed by Staphylococcus hemolyticus (11/53), Enterococcus faecalis (9/53), Staphylococcus aureus (7/53),

Staphylococcus saprophyticus (4/53), Klebsiella oxytoca (2/53), and Citrobacter freundii (2/53). In addition, out of the total 53 bacterial isolates, 28 (52.8%) were Multi-drug resistance strains. Among these Multi-drug resistance strains, Staphylococcus hemolyticus (81.8%) and Staphylococcus aureus (57.1%) isolates were the most predominant strains.

Antibiotic Sensitivity Pattern of Gram-positive Isolates

Table 5: Antibiotic Sensitivity Pattern of Gram-positive Isolates

Antibiotics used	Susceptibility Pattern (n=23)		
	Resistant	Intermediate	Sensitive
Linezolid	7 (30.4%)	3 (13.0%)	13 (56.5%)
Chloramphenicol	3 (13.0%)	5 (21.7%)	15 (65.2%)
Nitrofurantoin	14 (60.9%)	5 (21.7%)	4 (17.4%)
Cotrimoxazole	16 (69.6%)	3 (13.0%)	4 (17.4%)
Levofloxacin	10 (43.5%)	6 (26.1%)	7 (30.4%)
Ciprofloxacin	7 (30.4%)	11 (47.8%)	5 (21.7%)
Amoxicillin	13 (56.5%)	7 (30.4%)	3 (13.0%)
Ceftriaxone	12 (52.2%)	3 (13.0%)	8 (34.8%)
Cefepime	5 (21.7%)	2 (8.7%)	16 (69.6%)
Azithromycin	13 (56.5%)	6 (26.1%)	4 (17.4%)
Amikacin	6 (26.1%)	2 (8.7%)	15 (65.2%)

As indicated by the results summarized in Table 5, among the Gram-positive isolates, Cefepime (69.6%) was found to be the most effective drug, followed by Chloramphenicol (65.2%), Amikacin (65.2%), and Linezolid (56.5%). The Gram-positive isolates showed 69.6% resistance to Cotrimoxazole, 60.9% resistance to Nitrofurantoin, 56.5% resistance to Azithromycin and Amoxicillin, and 52.2% resistance to Ceftriaxone.

Antibiotic Sensitivity Pattern of Gram-negative Isolates

Table 6: Antibiotic Sensitivity Pattern of Gram-negative Isolates

Antibiotics used	Susceptibility Pattern (n=30)		
	Resistant	Intermediate	Sensitive
Nitrofurantoin	15 (50.0%)	6 (20.0%)	9 (30.0%)
Cotrimoxazole	12 (40.0%)	5 (16.7%)	13 (43.3%)
Ofloxacin	14 (46.7%)	6 (20.0%)	10 (33.3%)

Norfloxacin	17 (56.7%)	5 (16.7%)	8 (26.7%)
Levofloxacin	4 (13.3%)	8 (26.7%)	18 (60.0%)
Ciprofloxacin	18 (60.0%)	4 (13.3%)	8 (26.7%)
Imipenem	1 (3.3%)	2 (6.7%)	27 (90.0%)
Cephalexin	16 (53.3%)	2 (6.7%)	12 (40.0%)
Ceftriaxone	9 (30.0%)	5 (16.7%)	16 (53.3%)
Cefepime	8 (26.7%)	4 (13.3%)	18 (60.0%)
Azithromycin	15 (50.0%)	5 (16.7%)	10 (33.3%)
Amikacin	1 (3.3%)	4 (13.3%)	25 (83.3%)

As indicated by the results summarized in Table 6, among the Gram-negative isolates, Imipenem (90.0%) was found to be the most effective drug, followed by Amikacin (83.3%), Levofloxacin (60.0%), Cefepime (60.0%), and Ceftriaxone (53.3%). The Gram-negative isolates showed 60.0% resistivity to Ciprofloxacin, followed by 56.7% resistivity to Norfloxacin, and 53.3% resistivity to Cephalexin.

Discussion and Conclusion

The findings of this study showed significant bacteriuria in 29.4% of the samples, which indicated the cause of urinary tract infection in the participating patients. This finding is significantly higher than that observed in similar outstanding studies of Belete et al. [12] (15.8%) and Pradhan & Pradhan [13] (13.8%); and lower than that observed in the study of Singh et al. [14] (34%). However, Haider et al. [1] (32.3%) reported similar finding. In addition, female participants (32.9%) were slightly more culturally positive than male participants (26.5%). Past studies [15,16] have also reported a higher incidence of UTI in female patients. This may be mainly due to anatomical and behavioral differences between male and female patients.

The prevalence of urinary tract infections of the hemodialysis patients included in this study was 21.7% in patients under the age of 20, rising to 31.3% and 32.3% in those aged 21-40 and 41-60 respectively. However, the prevalence of UTI in patients over 60 years of age was slightly lower at 29.5%. Thus, the findings indicated a

significantly higher prevalence of urinary tract infections in the elderly group over 40 years. Haider et al. [1] also reported a higher prevalence of urinary tract infections in the elderly age group. This may be mainly due to the increased prevalence of risk factors in the elderly population such as hypertension, diabetes, and cardiovascular diseases [17].

Among the seven different bacterial strains isolated in this study, *E. coli* was the most predominant pathogen among hemodialysis patients. Overall multidrug-resistant strains were found to be 52.8% in this study. *Staph. hemolyticus* showed the highest degree of multidrug resistance (81.8%). Other studies have reported varying prevalence of MDR in hemodialysis, ranging from as low as 3% to as high as 59% [18,19]. This may be related to different risk factors, such as temporary dialysis, antibiotic exposure, nursing home stay, and previous hospitalization [19,20].

The study indicated that Gram-negative bacteria (56.6%) were the main microorganisms isolated from urine samples. Similar findings were reported by Karki et al. [21] and Kothari & Sagar [22]. Among Gram-negative bacteria, *E. coli* is found to be the predominant microorganism. This result is consistent with the results reported by Beyene & Tsegaye [23]. A major factor contributing to the high prevalence of uropathogenic *E. coli* is type 1 fimbriae, which enhances binding and invasion to superficial epithelial cells [24].

Among the Gram-positive isolates, Cefepime (69.6%) was found to be the most effective drug, whereas among the Gram-negative isolates, the most effective drug was Imipenem (90.0%). Further, the Gram-positive isolates showed highest resistance to Cotrimoxazole (69.6%) and Nitrofurantoin (60.9%), whereas the Gram-negative isolates showed highest resistivity to Ciprofloxacin (60.0%) and Norfloxacin (56.7%). These results are

consistent with those reported by Khatiwada et al. [15] and Miya et al. [25].

In conclusion, the prevalence of UTIs in hemodialysis patients was 29.4%, and the incidence of urinary tract infections in female participants (32.9%) was higher than that in male participants (26.5%). A significant increase in the prevalence of urinary tract infections has been observed in the elderly population over 40 years of age. *E. coli* is the most common pathogen of urinary tract infection in hemodialysis patients. *Staph. hemolyticus* had the highest multidrug resistance (81.8%). Gram-negative bacteria (56.6%) were the main microorganisms isolated from urine samples. Studies have shown that Cefepime is the most effective drug against Gram-positive bacteria, and Imipenem is the most effective drug against Gram-negative bacteria. In addition, the Gram-positive isolates showed the highest resistance to Cotrimoxazole (69.6%) and Nitrofurantoin (60.9%), whereas the Gram-negative isolates showed the highest resistivity to Ciprofloxacin (60.0%) and Norfloxacin (56.7%). The findings of this study will be helpful in determining the treatment policies and reducing the risk of urinary tract infections in hemodialysis patients.

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