Hindawi
Journal of Clinical Pharmacy and Therapeutics
Volume 2023, Article ID 4867817, 10 pages
https://doi.org/10.1155/2023/4867817

Research Article

Antimicrobial Resistance and Biofilm Production in Uropathogens from Renal Disease Patients Admitted to Tribhuvan University Teaching Hospital, Nepal

Anuja Dahal,[1] **Kamal Shrestha**,[2] **Rashmi Karki**,[3] **Saraswati Bhattarai**,[4] **Shiva Aryal**,[4] **Satish Kumar Deo**,[5] **Balmukunda Regmi**,[1] **Mark Willcox**,[6] **and Shyam Kumar Mishra**[2,6]

[1]*Department of Pharmacy, Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal*
[2]*Department of Microbiology, Tribhuvan University Teaching Hospital, Kathmandu, Nepal*
[3]*National Public Health Laboratory, Kathmandu, Nepal*
[4]*Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal*
[5]*Department of Pharmacology, Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal*
[6]*School of Optometry and Vision Science, University of New South Wales, Sydney, Australia*

Correspondence should be addressed to Anuja Dahal; anooza01@iom.edu.np

Received 7 April 2023; Revised 27 June 2023; Accepted 3 July 2023; Published 19 July 2023

Academic Editor: Keiko Hosohata

Background. Various antibiotics are prescribed empirically by physicians to cope with infections in renal disease patients. A urinary tract infection (UTI) is often caused by biofilm-forming multidrug-resistant (MDR) uropathogens. This study aimed to analyze the antibiogram of UTI strains from renal disease patients and the biofilm-forming ability of those strains. *Methods*. 102 patients clinically diagnosed with a UTI and renal disease were recruited into the study from August 2017 to January 2018. Clean-catch midstream urine samples were processed for the isolation and identification of the bacteria following standard methodologies. The antibiogram of the isolates ($n = 106$) was produced by the Kirby–Bauer disc diffusion method. Detection of biofilm formation was performed in tissue culture plates. *Results*. The incidence of a UTI in renal disease was 19.1%. Most patients were diagnosed with chronic kidney disease (18.63%), nephrotic syndrome (16.67%), and nephrolithiasis (14.71%). The commonest uropathogens were *Escherichia coli* (52.8%), *Klebsiella pneumoniae* (16%), and *Enterococcus* spp. (15.0%). Ceftriaxone was the most common antibiotic prescribed empirically (37%), whereas nitrofurantoin was the most prescribed antibiotic as adjusted therapy (36.1%). Among the first- and second-line antibiotics, most Gram-negative bacteria were sensitive to amikacin (70.7%), meropenem (70.7%), cefoperazone-sulbactam (70.0%), piperacillin-tazobactam (67.2%), gentamicin (66.7%), and nitrofurantoin (66.7%). Most Gram-positive bacteria were sensitive to doxycycline (90.0%), nitrofurantoin (72.2%), gentamicin (66.7%), and tetracycline (62.5%). All MDR Gram-negative uropathogens were susceptible to colistin sulfate and polymyxin B. Among the 106 isolates, 74.5% produced biofilms and 70.8% were MDR. In 67.0% of cases, including both MDR and biofilm-producing bacteria, the empirical therapy needed adjustment. *Conclusions*. Aminoglycoside, carbapenem, beta-lactam combination agents, and nitrofuran group of antibiotics may be the optimal first-line empirical therapies for uropathogens in hospitalized renal disease patients. Regular surveillance of resistance patterns and the study of biofilm formation in uropathogens must be performed to ensure effective management of the patients.

1. Introduction

Antimicrobials remain the mainstay of infectious disease treatment; however, the undiscerning use of antibiotics in many countries has resulted in the emergence of multidrug-resistant (MDR) microorganisms [1, 2]. Antimicrobial resistance (AMR) is a serious public health threat recognized by the World Health Organization (WHO) [3]. The spread of antimicrobial resistance and selection of MDR pathogens have most likely been caused by combinations of failure to

adherence to proper infection control techniques, irrational use of antibiotics, increased use of antibiotics in animals and plants, availability of antibiotics without a prescription, and counterfeit products of dubious quality [4]. Antibiotic resistance leads to higher medical expenses, prolonged hospital stays, and increased mortality rates [5]. There is an urgent need for change in the way antibiotics are prescribed and consumed. Even if new antibiotics are developed, without behavior change, antibiotic resistance will continue to be a major threat [6]. In the meantime, information about the antibiotic spectrum of activity against existing MDR strains may help reduce the rate of emergence and spread of antimicrobial resistance [7].

An additional factor contributing to antibacterial resistance is biofilm production by bacteria [8]. Biofilm is an association of microorganisms in which microbial cells adhere to each other on living or nonliving surfaces within a self-produced matrix of extracellular polymeric substances [9, 10]. Within biofilms, microbes are 10–100 times more resistant to antimicrobial agents [11]. Reasons for this increase in resistance can be due to the growth patterns of bacteria in biofilms, extracellular substances retarding antibiotic diffusion, and the upregulation of certain genes in bacteria in biofilms [11–14]. The National Institutes of Health (NIH) has estimated that approximately 65% of all microbial infections and 80% of all chronic infections are associated with biofilms [15, 16]. Human diseases in which biofilms have been associated include urinary tract infections (UTIs), catheter infections, middle ear infections, contact lens-associated infections, and less common but more lethal infections such as endocarditis and cystic fibrosis [17–19].

UTIs are commonly encountered by clinicians with an estimated annual global incidence of at least 250 million [20]. Furthermore, the prevalence of UTI increases in patients with preexisting renal disease [21]. This study examined the antibiogram patterns of common uropathogens in renal disease patients and the ability of the strains to form biofilms. This study also aimed to provide evidence for the rational use of antibiotics in patients with renal disease having UTIs.

2. Materials and Methods

A descriptive, hospital-based, cross-sectional study was conducted among renal disease patients diagnosed with UTI and admitted to the 750-bedded Tribhuvan University Teaching Hospital (TUTH), Nepal, from August 2017 to January 2018. The type of uropathogens and their antimicrobial resistance patterns along with biofilm-forming abilities were examined. The "universal sampling" technique was used to determine the sample size for this study. We had taken urine samples of 102 patients, but of them, 4 of the samples had polymicrobes, i.e., 2 uropathogens from each of 4 urine samples. So we treated them like individual isolates and performed antimicrobial susceptibility testing and biofilm analysis. This explains why the sample size varied between patient size (102) and their sociodemographic analysis and uropathogens isolated (106) and their further analysis. Clean catch mid-stream urine specimens ($n = 102$) were collected aseptically from the patients. Specimen collection, culture, and identification were performed according to standard guidelines [22–24].

The study conformed to the tenets of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board of the Institute of Medicine (IOM), Tribhuvan University (Ref. 256(6-11-6)2/074/075). Renal disease patients diagnosed with UTIs provided their approval by signing patient consent forms if ≥ 18 years or parents signing consent forms for younger subjects. Cases of any age, both males and females, were included in the study. Patients diagnosed with renal disease but with no signs of UTIs or who did not agree to sign the patient consent form were excluded from the study. Relevant clinical and epidemiological information was recorded from the patients. Patients with a history of recurrent UTI, kidney transplant patients, or patients under immuno-suppressing drugs were excluded from the study. Hemodialysis patients were also excluded from the study. We did take samples from catheterized patients too. A semistructured data sheet was pretested in the same study area, and pretesting bias was avoided. In the questionnaire, patients of age >16 years were considered legible for enquiring about marital status (children ≤16 years are not included in this variable) and occupation. Children and infants below primary education were also not included in the education level variable. Data entry, data checking, compiling, and editing were performed manually.

2.1. Antibiotic Susceptibility Testing (AST). All the isolates were subjected to antibiotic susceptibility testing (AST) by the Kirby–Bauer disc diffusion method on the Mueller–Hinton agar (HiMedia, India). The first-, second-, and third-line antibiotics (HiMedia, India) were chosen based on the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines [22] with some modifications based on the antibiotic testing policy in the microbiology laboratory of TUTH, Nepal. The results were reported as sensitive, intermediate, or resistant as described by CLSI [22]. Initially, all the isolates were subjected to first-line AST of the appropriate antibiotics for Gram-positive or Gram-negative bacteria. If the uropathogen was found to be resistant to more than 3 different classes of antibiotics, then it was tested with the appropriate second-line antibiotics. If any Gram-negative uropathogen was found to be resistant to meropenem or if the isolate was susceptible to only one antibiotic among the battery of second-line antimicrobials, it was further subjected to third-line antibiotic sensitivity testing. If an isolate showed resistance to ≥1 antibiotic from at least 3 different structural classes, it was considered to be multidrug-resistant (MDR) [25, 26].

Biofilm production: The biofilm production assay was performed using the tissue culture plate method [27]. A well-isolated colony of the organism isolated from the clinical specimen was inoculated in 2 mL of brain heart infusion (BHI) broth (HiMedia, India). The broth was incubated at 37°C for 24 h. The cultures were then diluted 1 : 100 with

fresh medium (BHI broth supplemented with 1% glucose) in the individual wells of the sterile well of flat bottom microtiter plates so that the final volume in each well was 200 μl. The plates were incubated at 37°C for 24 h. Then, the contents of each well were removed by gentle tapping. The wells were washed with phosphate-buffered saline (pH 7.2) three times and then were stained with 0.1% safranin. After drying the wells, the adhered dye was then dissolved by 100% ethanol. Finally, absorbance of released safranin from each well was measured using OD_{490nm}. Quantification was performed according to the criteria described by Stepanovic et al., as shown in Table 1 [28].

Here, OD is the average optical density of each isolate and OD_c is the cutoff OD for the microtiter-plate test as three standard deviations above the average optical density of the negative control. Previously identified biofilm-producing in-house clinical isolates were used as the positive control for biofilm production [27].

2.2. Data Analysis. Data analysis was performed using the 17.0 version of Statistical Package for Social Sciences (SPSS) software. Major variables analyzed in this study were sociodemographic data, the result of AST, biofilm production, and days of hospital stay. A descriptive type of analysis was performed to generate frequency and percentage. Chi-square/cross-tabulation was performed to test significance.

Table 1: Interpretation criteria of biofilm production by the microtiter-plate technique.

$OD \leq OD_c$	Nonadherent
$OD_c < OD \leq 2 \times OD_c$	Weakly adherent
$2 \times OD_c < OD \leq 4 \times OD_c$	Moderately adherent
$4 \times OD_c < OD$	Strongly adherent

Table 2: Distributions of samples based on the sociodemographic profiles.

		Number (*N*)	Percentage (%)
Age category of the subjects	<1 to 4 yr	9	8.8
	5 to 14 yr	8	7.8
	15 to 24 yr	14	13.7
	25 to 34 yr	13	12.7
	35 to 44 yr	15	14.7
	45 to 54 yr	13	12.7
	55 to 64 yr	18	17.6
	65 to 74 yr	6	5.9
	>75 yr	6	5.9
	Total	102	100.0
Gender	Female	50	49.0
	Male	52	51.0
	Total	102	100.0
Marital status	Single	26	25.5
	Married	76	74.5
	Total	102	100.0

3. Results

During the study period, a total of 10,404 urine samples were received by the microbiology laboratory. Among them, 534 samples were obtained from renal disease patients. A total of 102 renal disease patients who had a UTI were recruited to the study. The prevalence of a UTI in renal disease was 19.1%. A total of 106 uropathogens were isolated from the UTI-confirmed urines. A UTI was most seen in renal disease patients of age group 55–64 years (17.6%) followed by 35–44 years (14.7%) and 15–24 years (13.7%) (Table 2).

3.1. Patient Type and Different Wards to Which Patients Were Admitted. Of the 102 patients in the study, the majority were inpatients (95, 93.1%). Most of them were admitted to the nephrology (34, 33.3%) or pediatric (16, 15.7%) wards, whereas the lowest number of the patients was from neurology and surgery (4.9%). The majority of patients were diagnosed with chronic kidney disease (18.6%), followed by nephrotic syndrome (16.7%), nephrolithiasis (14.7%), and acute kidney disease (AKD) (11.8%), as shown in Table 3.

3.2. Types of Uropathogens. A total of 10 different bacterial species accounting for 106 different isolates from 102 urinary samples were isolated and identified. The majority were Gram-negative bacteria (87, 82.1%), and the remaining isolates were Gram-positive. The most common bacteria were *Escherichia coli* (52.8%), followed by *Klebsiella pneumoniae* (16.0%) (Table 4). Enterococci were the most common Gram-positive bacteria (Table 4).

3.3. Antibiotic Resistivity Pattern. Against Gram-negative bacteria (GNB), the antibiotics to which more than 50% of strains were resistant were amoxycillin (67, 88.2%), cotrimoxazole (55, 70.5%), ceftriaxone (53, 69.7%), ciprofloxacin (58, 66.7%), and ceftazidime (7, 63.6%) among the first-line antibiotics; amoxycillin-clavulanic acid (52, 98.1%), levofloxacin (44, 75.9%), and cefepime (52, 62.7%) among the second-line antibiotics; and imipenem (9, 60.0%), chloramphenicol (9, 56.3%), and doxycycline (9, 60.0%) among the third-line antibiotics (Table 5).

In Gram-positive bacteria, the first-line antibiotics to which more than 50% of strains were resistant were penicillin (14, 73.7%), ciprofloxacin (15, 79.0%), cotrimoxazole (2, 66.7%), and high-level gentamicin (10, 62.5%).

3.4. Antibiograms of Escherichia coli, Klebsiella, Pseudomonas, and Acinetobacter species. For *E. coli* isolates ($n = 56$), the antibiotics to which more than 50% of strains were resistant were amoxycillin (48, 85.7%), cotrimoxazole (38, 67.9%), ciprofloxacin (37, 66.1%), and ceftriaxone (40, 71.4%) among the first-line drugs. The antibiotics to which more than 50% of *Klebsiella pneumoniae* were resistant were cotrimoxazole (13, 76.5%), ciprofloxacin (11, 64.7%), ceftriaxone (11, 64.7%), and nitrofurantoin (12, 70.6%) among the first-line drugs. For the nonfermenting isolates, *Pseudomonas* and

Table 3: Patient distribution by type, wards to which they were admitted, and clinical diagnosis of renal disease ($n = 102$).

				Frequency	%
Patient type	Outpatient ($n = 7$)			7	6.9
	Inpatient ($n = 95$)	Ward	Female surgical ward	12	11.8
			Male surgical ward	11	10.8
			Nephro medicine ward	34	33.3
			Neuro medicine ward	5	4.9
			General surgical ward	5	4.9
			General medicine ward	6	5.9
			Pediatric ward	16	15.7
			Postoperative ward	6	5.9
	Total			102	100
Type of renal disease	Nephrolithiasis			15	14.7
	Glomerulonephritis			13	12.6
	Renal cyst			7	6.9
	Chronic kidney disease			19	18.6
	Acute kidney disease			12	11.8
	Acute kidney injury			5	4.9
	Hydronephrosis			4	3.9
	Isolated vesicoureteral reflux			8	7.8
	Nephrotic syndrome			17	16.7
	Nephritic syndrome			2	2.0
	Total			102	100

Table 4: Bacteriological profile of isolates from the urine specimens.

Organisms isolated	Frequency	Percent
Gram-negative bacteria	87	82.1
Escherichia coli	56	52.8
Klebsiella pneumoniae	17	16.0
Pseudomonas aeruginosa	9	8.5
Acinetobacter calcoaceticus baumannii complex	2	1.9
Citrobacter koseri	1	0.9
Citrobacter freundii	1	0.9
Morganella morganii	1	0.9
Gram-positive bacteria	19	17.9
Enterococcus faecium	8	7.6
Enterococcus faecalis	8	7.6
Staphylococcus aureus	3	2.8
Total	106	

Acinetobacter, the antibiotics which were found to be more than 50% resistant were ciprofloxacin (8, 72.7%), gentamicin (6, 54.5%), and ceftazidime (7, 63.6%) among the first-line drugs; levofloxacin (4, 66.6%) and meropenem (4, 66.6%) among the second-line drugs; and imipenem (1, 100%) among the third-line drugs.

3.5. Biofilm Formation Distribution among the Uropathogens. Among the uropathogens that formed biofilms ($n = 79$), the majority were *Escherichia coli* (51.9%), followed by *Klebsiella pneumoniae* (20.2%), *Pseudomonas aeruginosa* (10.1%), *Enterococcus faecium* (7.5%), and *Enterococcus faecalis* (3.7%) (Table 6). Among the 41 biofilms producing *Escherichia coli*, the majority (51.2%) produced moderate levels of biofilm, while out of the 16 biofilms producing *Klebsiella pneumoniae*, 43.8% produced high levels of biofilm (Table 6).

3.6. Biofilm Production and MDR. Among the 106 isolates, 70.8% were MDR, while 74.5% were biofilm producers (Table 7). The majority of MDR bacteria (54.3%) were found to produce biofilm but of different levels, and the relationship with multidrug resistance was statistically insignificant (p value >0.05).

3.7. Duration of Hospital Stay with Respect to MDR and Biofilm Production. Patients infected with MDR strains had a slightly higher duration of hospital stays (9.01 days; 95% CI (7.71, 10.32)) than patients with non-MDR (6.67 days; 95% CI (5.11, 8.22)). In the case of biofilm production, patients with strong biofilm-producing bacterial isolates had longer hospital stay (10 days) followed by patients with moderate biofilm producers (9 days). Patients having nonbiofilm producers had the least days of hospital stay (Figures 1 and 2).

Table 5: Resistance of bacterial isolates to antibiotics.

Gram-negative bacteria				Gram-positive bacteria			
Antibiotics	Number of tested isolates	Resistant (*n*)	Resistance percentage of those tested	Antibiotics	Number of tested isolates	Resistant (*n*)	Resistance percentage of those tested
First-line antibiotics							
Amoxycillin	76	67	88.2	Penicillin#*	19	14	73.7
Cotrimoxazole	78	55	70.5	Cotrimoxazole*	3	2	66.7
Ciprofloxacin	87	58	66.7	Ciprofloxacin#*	19	15	79.0
Gentamicin	87	29	33.3	High-level gentamicin#	16	10	62.5
Ceftriaxone	76	53	69.7	Gentamicin*	3	1	33.3
Ceftazidime	11	7	63.6	Tetracycline#	16	6	37.5
Nitrofurantoin	78	26	33.3	Nitrofurantoin#*	19	5	26.3
Ampicillin-sulbactam	2	1	50.0				
Second-line antibiotics							
Levofloxacin	58	44	75.9	Amoxycillin-clavulanic acid#	10	8	80.0
Amikacin	58	17	29.3	Levofloxacin#*	11	7	63.6
Piperacillin-tazobactam	64	21	32.8	Teicoplanin#*	13	3	23.1
Meropenem	58	17	29.3	Vancomycin#*	11	1	9.1
Cefoperazone-sulbactam	10	4	40.0	Doxycycline#	10	1	10.0
Cefepime	83	52	62.7	Meropenem#	10	7	70.0
Amoxycillin-clavulanic acid	53	52	98.1				
Third-line antibiotics							
Imipenem	15	9	60.0	Linezolid#	1	0	0.0
Chloramphenicol	16	9	56.3	Chloramphenicol#	1	0	0.0
Doxycycline	15	9	60.0	Erythromycin#*	1	1	100.0
Polymyxin B	15	0	0.0	Piperacillin-tazobactam#	1	1	100
Colistin sulfate	15	0	0.0				

* = antibiotics used against *Staphylococcus* sp., # = antibiotics used against *Enterococcus* sp.

Table 6: Categorization of biofilm formation among the biofilms producing uropathogens.

Uropathogens	Biofilm producers			Total biofilm production *n* (%)
	Weak *n* (%)	Moderate *n* (%)	Strong *n* (%)	
Escherichia coli	8 (19.5)	21 (51.2)	12 (29.3)	41 (51.9)
Pseudomonas aeruginosa	1 (12.5)	4 (50.0)	3 (37.5)	8 (10.1)
Acinetobacter calcoaceticus baumannii complex	1 (50.0)	0 (0.0)	1 (50.0)	2 (2.5)
Klebsiella pneumoniae	3 (18.8)	6 (37.5)	7 (43.8)	16 (20.2)
Enterococcus faecalis	1 (33.3)	2 (66.7)	0 (0.0)	3 (3.7)
Staphylococcus aureus	1 (50.0)	1 (50.0)	0 (0.0)	2 (2.5)
Enterococcus faecium	0 (0.0)	3 (50.0)	3 (50.0)	6 (7.5)
Citrobacter freundii	0 (0.0)	0 (0.0)	1 (100.0)	1 (1.2)
Total	15	37	27	79

Table 7: Biofilm production and MDR.

		MDR		Total
		Non-MDR	MDR	
Biofilm production	Yes	23 (21.6%)	56 (54.3%)	79 (74.5%)
	No	8 (7.5%)	19 (17.9%)	27 (25.47%)
Total		31 (29.2%)	75 (70.8%)	106 (100%)

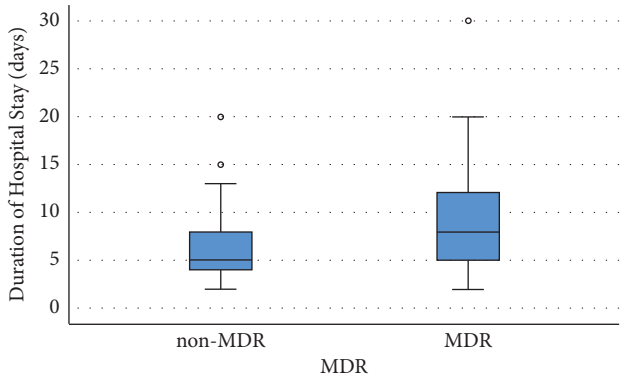

Figure 1: Box and whisker plot demonstrating duration of hospital stay in patients with and without MDR-isolates.

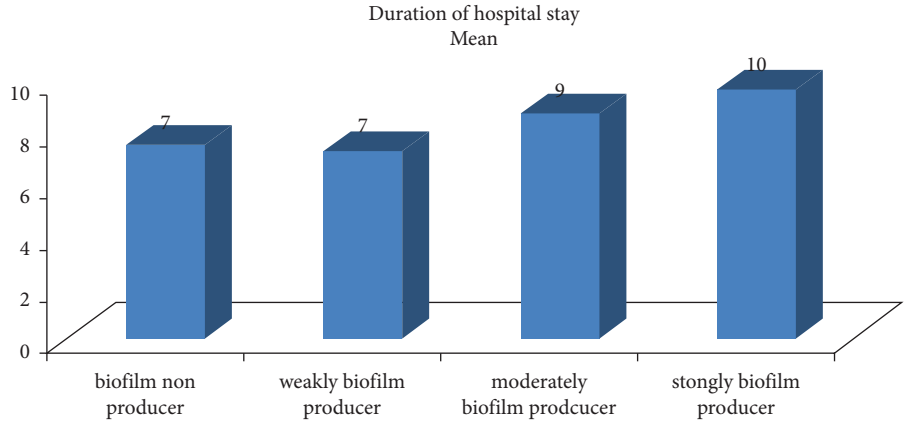

Figure 2: Mean duration of hospital stay of patients with respect to biofilm formation.

Table 8: Antibiotics used before and after culture and sensitivity (C/S) result.

S no.	Antibiotics	Before C/S (%)	After C/S (%)
1	Cefixime	11.0	6.9
2	Piperacillin/tazobactam	13.0	11.1
3	Ciprofloxacin	17.0	8.3
4	Ceftriaxone	37.0	4.2
5	Levofloxacin	1.0	1.4
6	Ofloxacin	10.0	8.3
7	Cloxacillin	1.0	2.8
8	Meropenem	6.0	4.2
9	Amikacin	5.0	6.9
10	Nitrofurantoin	1.0	36.1
11	Ceftazidime	1.0	0
12	Cefadroxil	3.0	0
13	Metronidazole	1.0	0
14	Norfloxacin	0	6.9
15	Imipenem	0	1.4
16	Teicoplanin	0	2.8
17	Linezolid	0	1.4
18	Doxycycline	0	1.4
19	Colistin	0	2.8
20	Chloramphenicol	0	4.2

3.8. The Change in Antibiotic Use after Sensitivity Assessment. The antibiotic use pattern for empirical therapy and adjusted treatment therapy was evaluated. Third-generation cephalosporins (cefixime, ceftriaxone, and ceftazidime) were the most frequently prescribed empirical antibiotics (49.0%), followed by fluoroquinolones which were prescribed in 28% of patients. Once the results of the culture and sensitivity were obtained, the most commonly prescribed antibiotic was nitrofurantoin (36.1%) followed by fluoroquinolones (24.9%) (Table 8).

3.9. Comparison of Empirical Therapy with MDR and Biofilm Production. In 67% of patients, the antibiotic used as an empirical therapy did not match the culture sensitivity results and so had to be prescribed alternative antibiotics. Most of the patients in which the empirically used antibiotic was changed were infected by MDR strains ($n = 53$) and biofilm-producing bacteria ($n = 50$) (Table 9).

4. Discussion

The prevalence of renal disease patients is increasing, and it has become a serious threat to the health sector of Nepal. Antimicrobial resistance (AMR) has further aggravated the prognosis of renal disease patients developing UTI. Our study highlights the alarming AMR threat to renal disease patients and why strict antibiotic stewardship practice should be enforced [29]. In this study, the prevalence of a UTI in renal disease was 19.1%. A previous study found a similar prevalence (17%) of a UTI during the first 6 months after renal transplantation [30, 31]. In Nepal, the prevalence of renal disease has been reported to approximately double in males compared to females with a ratio of 1.8 : 1 [32], although the study recruited approximately equal numbers of males and females. The estimated glomerular filtration rate (eGFR) declines in parallel with age [33], and this coincides with increasing trends of CKD prevalence from 7.4% for 18–39 years to 24.2% for 60–70 years [34]. This pattern is also reflected in the current study, with the highest number of renal diseases being seen in the age group of 55–64 years followed by 25–44 years. The current study found that a UTI was commonly caused by Gram-negative bacteria, with *E. coli* being the most common bacteria followed by *K. pneumoniae* then *Enterococcus* species. This is a similar bacteriological profile that has been reported by many others in CKD patients as well as studies of community acquired UTI [35–37].

Antimicrobial resistance is a looming threat to humankind, though its rate varies from place to place, and a common trend shows its rate is increasing. Similar to that, in our study, the resistance rate is high with 70.8% of MDR bacteria which is higher than findings from those in previously conducted studies. Baral found only 41.1% of MDR bacteria in their study in 2012 [38], but a more recent study conducted by Parajuli et al. [39] has reported 64.9% of MDR bacteria causing UTIs and in a more recent study by Shilpakar et al. [40] who have reported that more than 90% of Gram-negative bacteria were MDR. This highlights the difficulties that are likely to be encountered with the treatment of UTI patients in Nepal.

Resistance to amoxycillin, ceftriaxone, cotrimoxazole, and ciprofloxacin among the first-line antibiotics was ≈70% of all isolates [41]. Fortunately, no strains were resistant to the last-line drugs such as polymyxin B and colistin sulfate. Similar findings were reported by the other studies [42]. However, with the known problems of renal toxicity during therapy with polymyxin B and colistin sulfate, careful optimization of the polymyxin dose and drug monitoring is needed [43, 44]. Biofilm production was seen in 74.5% of isolates with the majority being *E. coli* (51.9%), followed by *K. pneumoniae* (20.2%) and *P. aeruginosa* (10.1%). These results agree with, although slightly higher than, those of another study from Nepal that reported biofilm formation in

Table 9: Empirical therapy with respect to multidrug-resistant bacteria and biofilm production.

Empirical therapy	MDR		Biofilm production	
	No	Yes	No	Yes
Therapy continued after AST	19	11	12	18
Therapy discontinued or changed after AST	8	53	11	50

58.7% of isolates, with strong biofilm formation in 36.5% and weak biofilm formation in 22.1% of isolates [27]. The current study found that biofilm-producing bacteria also tended to be MDR strains. Studies from Egypt and Iran [45, 46] found that the prevalence of MDR and XDR was higher in biofilm-producing strains. Of the biofilm-producing isolates from ventilator-associated pneumonia, 42.2% were MDR, but the relationship was statistically insignificant [47]. 67% of patients had their empirical therapy adjusted after the AST result, and this adjustment was higher in infections with MDR and biofilm-forming isolates. It would be useful in future studies to test the resistance of strains in biofilms. The numbers of ciprofloxacin-resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus strains* in biofilms could not be reduced even when four times the minimum inhibitory concentration of ciprofloxacin was used, whereas the numbers of bacteria in biofilms of ciprofloxacin-sensitive strains could be reduced by ≥ 60% by ciprofloxacin at its minimum inhibitory concentration [48, 49].

Infection with MDR bacteria results in longer days in the hospital [50], and the current study found that on average patients with MDR UTI remained in the hospital 2 days longer. Similarly, UTI patients infected with strong biofilm producers remained in the hospital for 3 days longer than patients infected with weak or nonbiofilm producers. This correlation between days of hospital stays with MDR and biofilm formation may be interconnected. It is possible that the greater number of days in the hospital had a selective pressure for the generation of MDR and/or strong biofilm-producing isolates. Regardless of the relationship between MDR and biofilm, both can add significant levels of burden to healthcare providers as well as patients, as well as costs to the hospital system.

5. Conclusion and Recommendations

Overall, the antibiotic resistance patterns in the current study showed most of the empirically used antibiotics were ineffective and over 50% of patients required therapy adjustment. A high percentage of uropathogens were found to be MDR and biofilm producers. The study reflects the crisis in resource-starved large hospital settings in developing countries without significant antibiotic stewardship strategies and the threat posed by increasing drug-resistant UTIs in renal disease. Strategies to inhibit or disperse biofilm formation by bacteria in vivo in renal disease patients should also be considered.

There is a need for the development of a protocol for rational use of antibiotics, and physicians and pharmacists must be aware of the rational use of antibiotics. There should be the use of a narrow spectrum of antibiotics when supported by clinical situations and culture reports. Furthermore, more thorough and routine studies are necessary to monitor for future changes in resistivity patterns. There must be provision for availability of trained pharmacists and microbiologists so that appropriate use of medicines and patient adherence to the treatment can be enhanced.

Abbreviations

UTI: Urinary tract infection
MDR: Multidrug resistant
CLSI: Clinical and Laboratory Standards Institute
MHA: Mueller–Hinton agar
BHI: Brain heart infusion
Mg: Milligram
Ml: Milliliter
μg: Microgram
L: Liter
IOM: Institute of Medicine
TUTH: Tribhuvan University Teaching Hospital
WHO: World Health Organization
CFU: Colony-forming units
CKD: Chronic kidney disease
AKD: Acute kidney disease
SLE: Systemic lupus erythematosus
DM: Diabetes mellitus
SPSS: Statistical Package for Social Sciences
OD: Optical density
GNB: Gram-negative bacteria
GPB: Gram-positive bacteria
AST: Antibiotic sensitivity test
HLG: High-level gentamicin
CLED: Cysteine lactose electrolyte-deficient agar
VUR: Vesico-ureteral reflux
FSW: Female surgical ward
MSW: Male surgical ward
POW: Postoperative ward
DNA: Deoxyribonucleic acid
ELISA: Enzyme-linked immunosorbent assay.

Data Availability

Data are available from corresponding author upon request.

Ethical Approval

Ethical approval was obtained from the Institutional Review Board of the Institute of Medicine (IOM), Tribhuvan University (Ref. 256(6-11-6)2/074/075).

Consent

All the patients in the study were informed in detail about the study for collection of clinical and epidemiological information. Confidentiality of all information was maintained. All patients signed the patient consent form prior to getting enrolled in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Anuja Dahal and Shyam Kumar Mishra conceptualized the study. Anuja Dahal, Shyam Kumar Mishra, Saraswati Bhatttarai, and Kamal Shrestha curated the data. Anuja Dahal, Shyam Kumar Mishra, Kamal Shrestha, and Shiva Aryal conducted formal analysis. Anuja Dahal, Shyam Kumar Mishra, and Kamal Shrestha carried out investigation. Anuja Dahal, Shyam Kumar Mishra, Kamal Shrestha, and Shiva Aryal designed the methodology. Shyam Kumar Mishra and Balmukunda Regmi supervised the study. Anuja Dahal, Shyam Kumar Mishra, and Mark Willcox were involved in validation. Anuja Dahal wrote the original draft. Anuja Dahal, Shyam Kumar Mishra, and Mark Willcox wrote, reviewed, and edited the manuscript. All the abovementioned authors contributed to interpretation of the study results, drafting and revision of the manuscript, and approved the final version of the manuscript. Anuja Dahal and Shyam Kumar Mishra contributed equally to this work.

Acknowledgments

We are grateful to the Department of Microbiology and Department of Pharmacy, Institute of Medicine, Tribhuvan University, and the Bacteriology Lab of Tribhuvan University Teaching Hospital for providing lab arrangement.

References

[1] K. P. Acharya and R. T. Wilson, "Antimicrobial resistance in Nepal," *Frontiers of Medicine*, vol. 6, p. 105, 2019.

[2] P. Solanki, Y. Sultana, and S. Singh, "Traditional medicine: exploring their potential in overcoming multi-drug resistance," in *Strategies to Overcome Superbug Invasions: Emerging Research and Opportunities*, pp. 118–129, IGI Global, Pennsylvania, USA, 2021.

[3] World Health Organization, *Antimicrobial Resistance Global Report on Surveillance*, WHO Library Cataloguing-in-Publication Data, Geneva, Switzerland, 2014.

[4] J. Premanandh, B. Samara, and A. N. Mazen, "Race against antimicrobial resistance requires coordinated action–an overview," *Frontiers in Microbiology*, vol. 6, p. 1536, 2015.

[5] P. D. Mauldin, C. D. Salgado, I. S. Hansen, D. T. Durup, and J. A. Bosso, "Attributable hospital cost and length of stay associated with health care-associated infections caused by antibiotic-resistant gram-negative bacteria," *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 1, pp. 109–115, 2010.

[6] D. C. Bean, D. Krahe, and D. W. Wareham, "Antimicrobial resistance in community and nosocomial escherichia coli urinary tract isolates, london 2005-2006," *Annals of clinical microbiology and antimicrobials*, vol. 7, no. 1, pp. 1–4, 2008.

[7] J. B. Sherchan, P. Gurung, P. Karkee, N. Ranabhat, N. Shrestha, and H. Ohara, "Microbiological and Clinical Profile of Uropathogenic Escherichia coli Isolates in Kathmandu University Hospital," *Journal of Nepal Health Research Council*, vol. 14, no. 1, 2015.

[8] S. Neupane, N. D. Pant, S. Khatiwada, R. Chaudhary, and M. R. Banjara, "Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic *Escherichia coli* isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal," *Antimicrobial Resistance and Infection Control*, vol. 5, no. 1, p. 5, 2016.

[9] S. Niveditha, S. Pramodhini, S. Umadevi, S. Kumar, and S. Stephen, "The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs)," *Journal of Clinical and Diagnostic Research: Journal of Clinical and Diagnostic Research*, vol. 6, no. 9, pp. 1478–1482, 2012.

[10] P. J. A. Di Martino, "Extracellular polymeric substances, a key element in understanding biofilm phenotype," *AIMS Microbiology*, vol. 4, no. 2, pp. 274–288, 2018.

[11] R. M. Donlan, "Biofilm Formation: a clinically relevant microbiological process," *Clinical Infectious Diseases*, vol. 33, no. 8, pp. 1387–1392, 2001.

[12] T. F. C. Mah, G. A. O'Toole, T.-F. C. Mah, and G. A. O'Toole, "Mechanisms of biofilm resistance to antimicrobial agents," *Trends in Microbiology*, vol. 9, no. 1, pp. 34–39, 2001.

[13] C. R. Kokare, S. Chakraborty, A. N. Khopade, and K. R. Mahadik, "Biofilm: Importance and applications," *Indian Journal of Biotechnology*, vol. 8, no. 2, pp. 159–168, 2009.

[14] J. G. Leid, "Bacterial Biofilms Resist Key Host Defenses," *Microbe*, vol. 4, no. 2, pp. 66–70, 2009.

[15] M. Jamal, W. Ahmad, S. Andleeb et al., "Bacterial biofilm and associated infections," *Journal of the Chinese Medical Association*, vol. 81, no. 1, pp. 7–11, 2018.

[16] J. Khan, S. M. Tarar, I. Gul, U. Nawaz, and M. Arshad, "Challenges of antibiotic resistance biofilms and potential combating strategies: a review," *3 Biotech*, vol. 11, no. 4, pp. 1–15, 2021.

[17] M. A. Mekni, O. Bouchami, W. Achour, and A. Ben Hassen, "Strong biofilm production but not adhesion virulence factors can discriminate between invasive and commensal Staphylococcus epidermidis strains," *Acta Pathologica, Microbiologica et Immunologica Scandinavica*, vol. 120, no. 8, pp. 605–611, 2012.

[18] S. Boase, "The Microbiome of Chronic Rhinosinusitis: Culture, Molecular Diagnostics and Biofilm Detection," *BMC infectious diseases*, vol. 13, pp. 1–9, 2013.

[19] A. K. Vijay and M. D. P. Willcox, "Adhesion of stenotrophomonas maltophilia, delftia acidovorans, and achromobacter xylosoxidans to contact lenses," *Eye and Contact Lens: Science and Clinical Practice*, vol. 44, no. 2, pp. S120–s126, 2018.

[20] D. A. Rosen, T. M. Hooton, W. E. Stamm, P. A. Humphrey, and S. J. Hultgren, "Detection of intracellular bacterial communities in human urinary tract infection," *PLoS Medicine*, vol. 4, no. 12, p. e329, 2007.

[21] Z. Tandogdu, T. Cai, B. Koves, F. Wagenlehner, and T. E. Bjerklund-Johansen, "Urinary tract infections in

immunocompromised patients with diabetes, chronic kidney disease, and kidney transplant," *European Urology Focus*, vol. 2, no. 4, pp. 394–399, 2016.

[22] S. James, "Performance standards for antimicrobial susceptibility testing," Clinical and Laboratory Standards Institute, Wayne, PA, USA, 33 edition, 2017.

[23] A. L. Leber, *Clinical Microbiology Procedures Handbook*, American Society of Microbiology, Washington, DC, USA, 2 edition, 2007.

[24] Palomar Health, "Specimen Collection and Handling Manual, Laboratory," 2014, https://www.palomarhealth.org/wp-content/uploads/2023/02/Specimen-Collection-and-Handling-Manual.pdf.

[25] M. Gajdács and F. J. A. Albericio, "Antibiotic Resistance: From the Bench to Patients," *Antibiotics*, vol. 8, no. 3, p. 129, 2019.

[26] D. F. Sahm, C. Thornsberry, D. C. Mayfield, M. E. Jones, and J. A. Karlowsky, "Multidrug-resistant urinary tract isolates of *Escherichia coli*: prevalence and patient demographics in the United States in 2000," *Antimicrobial Agents and Chemotherapy*, vol. 45, no. 5, pp. 1402–1406, 2001.

[27] S. K. Mishra, P. Basukala, O. Basukala, K. Parajuli, B. M. Pokhrel, and B. P. Rijal, "Detection of biofilm production and antibiotic resistance pattern in clinical isolates from indwelling medical devices," *Current Microbiology*, vol. 70, no. 1, pp. 128–134, 2015.

[28] S. Stepanović, D. Vuković, V. Hola et al., "Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci," *APMIS*, vol. 115, no. 8, pp. 891–899, 2007.

[29] I. J. Abbott, "Antibiotic management of UTI in the post-antibiotic era: a narrative review highlighting diagnostic and antimicrobial stewardship," *Clinical Microbiology and Infection: the Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 2022.

[30] K. C. Abbott, S. Swanson, E. R. Richter et al., "Late urinary tract infection after renal transplantation in the United States," *American Journal of Kidney Diseases*, vol. 44, no. 2, pp. 353–362, 2004.

[31] Z. Tandogdu, T. Cai, B. Koves, F. Wagenlehner, and T. E. Bjerklund-Johansen, "Urinary tract infections in immunocompromised patients with diabetes, chronic kidney disease, and kidney transplant," *European Urology Focus*, vol. 2, no. 4, pp. 394–399, 2016.

[32] M. Sigdel and R. Pradhan, "Chronic kidney disease in a tertiary care hospital in Nepal," *Journal of Institute of Medicine*, vol. 42, no. 1, 2018.

[33] M. Bartmanska and A. Wiecek, "Chronic kidney disease and the aging population," *Giornale Italiano di Nefrologia*, vol. 33, no. 66, p. 33, 2016.

[34] M. Tonelli and M. Riella, "Chronic kidney disease and the aging population," *African Journal of Nephrology*, vol. 17, no. 1, pp. 6–10, 2014.

[35] M. Shankar, S. Narasimhappa, and M. N.s., "Urinary tract infection in chronic kidney disease population: a clinical observational study," *Cureus*, vol. 13, no. 1, Article ID e12486, 2021.

[36] S. Pugalendhi and T. K. Dutta, "A Clinical and Microbiological Profile of Urinary Tract Infection in Patients with Chronic Kidney Diseases," *International Journal of Advances in Medicine*, vol. 6, no. 3, 2019.

[37] G. Gebremariam, H. Legese, Y. Woldu, T. Araya, K. Hagos, and A. GebreyesusWasihun, "Bacteriological profile, risk factors and antimicrobial susceptibility patterns of symptomatic urinary tract infection among students of Mekelle University, northern Ethiopia," *BMC Infectious Diseases*, vol. 19, no. 1, pp. 1–11, 2019.

[38] P. Baral, "High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal," *BMC Research notes*, vol. 5, no. 1, pp. 1–9, 2012.

[39] N. P. Parajuli, P. Maharjan, H. Parajuli et al., "High rates of multidrug resistance among uropathogenic *Escherichia coli* in children and analyses of ESBL producers from Nepal," *Antimicrobial Resistance and Infection Control*, vol. 6, no. 1, pp. 9–7, 2017.

[40] A. Shilpakar, M. Ansari, K. R. Rai, G. Rai, and S. K. Rai, "Prevalence of multidrug-resistant and extended-spectrum beta-lactamase producing Gram-negative isolates from clinical samples in a tertiary care hospital of Nepal," *Tropical Medicine and Health*, vol. 49, no. 1, pp. 23–29, 2021.

[41] H. J. Ho, M. X. Tan, M. I. Chen et al., "Interaction between antibiotic resistance, resistance genes, and treatment response for urinary tract infections in primary care," *Journal of Clinical Microbiology*, vol. 57, no. 9, pp. 10–1128, 2019.

[42] C. Dardi, "Biofilm formation and antimicrobial resistance pattern among uropathogens," *International Journal of Medical Research and Health Sciences*, vol. 4, no. 2, pp. 339–344, 2015.

[43] S. C. Nang, M. A. K. Azad, T. Velkov, Q. T. Zhou, and J. Li, "Rescuing the last-line polymyxins: achievements and challenges," *Pharmacological Reviews*, vol. 73, no. 2, pp. 679–728, 2021.

[44] R. L. Nation, M. Rigatto, D. Falci, and A. Zavascki, "Polymyxin acute kidney injury: dosing and other strategies to reduce toxicity," *Antibiotics*, vol. 8, no. 1, p. 24, 2019.

[45] A. M. Asaad, S. Ansari, S. E. Ajlan, and S. M. Awad, "Epidemiology of biofilm producing acinetobacter baumannii nosocomial isolates from a tertiary care hospital in Egypt: a cross-sectional study," *Infection and Drug Resistance*, vol. 14, pp. 709–717, 2021.

[46] E. Babapour, A. Haddadi, R. Mirnejad, S. A. Angaji, and N. Amirmozafari, "Biofilm formation in clinical isolates of nosocomial Acinetobacter baumannii and its relationship with multidrug resistance," *Asian Pacific Journal of Tropical Biomedicine*, vol. 6, no. 6, pp. 528–533, 2016.

[47] S. Baidya, S. Sharma, S. K. Mishra, H. P. Kattel, K. Parajuli, and J. B. Sherchand, "Biofilm Formation by pathogens causing ventilator-associated pneumonia at intensive care units in a tertiary care hospital: an armor for refuge," *BioMed Research International*, vol. 2021, Article ID 8817700, 10 pages, 2021.

[48] M. Yasir, D. Dutta, and M. D. J. M. Willcox, "Activity of antimicrobial peptides and ciprofloxacin against *Pseudomonas aeruginosa* biofilms," *Molecules*, vol. 25, no. 17, p. 3843, 2020.

[49] M. Yasir, D. Dutta, and M. D. P. Willcox, "Enhancement of antibiofilm activity of ciprofloxacin against *Staphylococcus aureus* by administration of antimicrobial peptides," *Antibiotics*, vol. 10, no. 10, p. 1159, 2021.

[50] R. H. Sunenshine, M. O. Wright, L. L. Maragakis et al., "Multidrug-resistant Acinetobacter infection mortality rate and length of hospitalization," *Emerging Infectious Diseases*, vol. 13, no. 1, pp. 97–103, 2007.