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Original Research Article

Prevalence, Biofilm-Forming Capacity, and Antimicrobial Resistance Patterns of Uropathogens in Patients with Kidney Stone Disease

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Abstract:

Introduction: The most prevalent urological condition is the kidney stone disease, which is associated with urinary tract infections, due to the formation of biofilm, antimicrobial resistant uropathogen. Infection and stone formation were scoexisted complicated treatment regimen, which enhances the risk of recurrence. The study objective was to assess the prevalence, biofilm-forming capacity, and antimicrobial resistance patterns of the uropathogens among the kidney stone patients.

Method: The cross-observational study was conducted among 80 patients with kidney stones, diagnosed clinically and radiologically. The culture of the Midstream urine samples was processes y=under standard microbiological methods. Congo red agar and microtitre plate assays were performed to evaluate the biofilm formation, and the Kirby–Bauer disc diffusion was done to determine the antimicrobial susceptibility.

Result: Result findings revealed that 80 kidney stone disease patients had the most predominant calcium oxalate–based stones, which accounted for 58 cases (72.5%), 32 patients (40%) showed calcium oxalate monohydrate, and 26 patients (32.5%) showed mixed COM + COD stones. Struvite stones were observed among 16 patients (20%), while the rare one was the uric acid stones. The most frequent pathogen was Escherichia coli.

Conclusion: The study concludes the predominance of calcium oxalate stones, highlighting the role of infection, specifically the urease-producing and biofilm-forming uropathogens like Escherichia coli and Proteus mirabilis.

Keywords: Kidney stone disease; Uropathogens; Biofilm formation; Antimicrobial resistance; Calcium oxalate stones.

Introduction

The kidney stone disease, also termed urolithiasis, is a common health problem that impacts the community, and approximately 1 out of 15 individuals has been diagnosed with kidney stones. The combination of climate change results in global warming and dietary changes as well as altered lifestyle, rise the occurrence of kidney stones. There are several risk factors, or complications contributed to the formation of kidney stones, such as urinary system infections, diabetes, obesity, use of medication, renal tubular acidosis and hyperparathyroidism [1]. Urease-producing bacteria like Proteus mirabilis are common pathogens causing urinary tract infections, contributing to the kidney stones. Another reason for the formation of a kidney stone is the biofilm layer consisting of various microorganisms. The pool of bacteria gets attached to the inanimate surface layer, such as the kidney stone, which forms a burden for treatment. Infection causing pathogen exhibited strong resistance to treatment [2]. The association regarding the co-existence of UTIs and urolithiasis pose a challenge for management. Several longitudinal studies have demonstrated that patients with renal calculi are 6 times more likely to form UTIs, compared to those without kidney stones. Urease-producing bacteria facilitate the formation of extracellular polymers for biofilm formation, providing antimicrobial resistance to traditional antimicrobial treatment regimens. These protective biofilms reduce the efficacy of antibiotics, thus promoting consistent infection and kidney stone-like disorder [3]. Several studies have demonstrated that biofilm formation by uropathogens can be found among non-catheterised KSD patients. Microbiological assessment highlighted that positive culture urine isolates were present in around 64.9% of KSD patients, which were characterised by high multidrug resistance and strong activity of biofilms [4]. The crucial element to maintain the infectious cycle was the development of biofilm by uropathogens. Biofilm containing bacteria showed enhanced antibiotic resistance, against the immunological reactions, thus driving towards more chronic and recurrent periodic infection [5].

The urological diseases are associated with many urinal complications, like glycosuria, hematuria,

proteinuria, dysuria, albuminuria, and urinary incontinence. Individuals with long-term urological disorders and metabolic complication which are very common to bacterial infections, affects all ages. Also, untreated UTIs can impair renal function, resulting in sepsis, septic shock and death [6]. The example of some prevalent disease-causing pathogens was Escherichia coli, Klebsiella spp, Pseudomonas spp, Staphylococcus spp, Proteus spp, Enterococcus spp and Enterobacter spp. The prevalence of uropathogens depends on age, sex, catheterisation, hospitalisation, and antimicrobial exposure [7]. Major bacterial strains along with their specific adaptation strategies vary according to other urological disorders, based on the basis of the metabolites [8]. The study aims to assess the relationship between the glycaemic control and the severity of diabetic retinopathy and macular involvement among diabetic patients.

Method

Research design: This is a cross-sectional observational study to evaluate the prevalence, biofilm-forming capacity and the pattern for antimicrobial resistance of uropathogens among patients with kidney stone disease. The study was conducted for a duration of one year in a tertiary care hospital. Total 80 patients with kidney stone disease based on clinical and radiological findings were selected for the study. Samples of midstream urine were collected from all patients by clean-catch midstream technique aseptically, and well-written and informed consent was taken for the study. The cultures of urine samples were standardised by microbiological methods for the isolation of the uropathogens. The isolated organisms were evaluated for their ability to form the biofilm, by standard phenotypic methods, while antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for identifying the pattern for the antimicrobial resistance.

Inclusion Criteria

1. Patients who were diagnosed with the renal calculi were included in the study.
2. Both male and female patients were enrolled.
3. Informed and written consent were required for the study.

Exclusion Criteria

1. Those who have received the antibiotic therapy for two weeks before the sample collection, was excluded.
2. Pregnant women were not included in the study.
3. Patients with immune-compromised condition were not included in the study.
4. Individuals diagnosed with the urinary tract abnormalities were excluded.
5. Contaminated urine samples or inadequate sample of urine were not included in the study.

Procedure: The urine sample was collected from 80 patients who were diagnosed with renal calculi, using the use of clean-catch midstream technique and were transported to the laboratory for processing. Cystine Lactose Electrolyte Deficient (CLED) agar medium was used to culture the urine sample, and incubation was performed at 37 °C for 24 hours**.** The bacteriuria was evaluated on the basis of the morphological feature of the colony and colony count, and isolates of bacteria were detected by standard microbiological methods**,** consisting of Gram staining and biochemical tests**.** The Kirby–Bauer disc diffusion method was performed to measure the susceptibility according to the CLSI 2025 guidelines**.** Biofilm-forming capacity of the isolates was assessed by the Congo Red Agar method, while the microtitre plate assay was performed for quantitative evaluation, which employs the crystal violet method**.** Sub-analysis was performed for the renal stone sample, obtained from the percutaneous nephrolithotomy. 15 positive urine samples were obtained, which enabled the stone–urine analysis. Fourier Transform Infrared Spectroscopy (FTIR) was performed to determine the composition of the stone.

Statistical Analysis: Descriptive statistics was performed for data analysis. All experiments were performed in triplicate, mean optical density (OD) values were used for data analysis. Microsoft Excel was used for data entry and analysis. The correlation for the biofilm-forming ability of the isolates with the antimicrobial resistance property was observed and the interpretation of the result findings was done on the basis of the standard criteria for microbiological procedure. Data analysis was done by SPSS version 27.

Result

Table 1 outlines the socio-demographic profile of patients with kidney stone disease and demonstrates a clear predominance of middle-aged adults. The highest proportion of patients belonged to the 31–40 year age group (27.5%), followed closely by those aged 41–50 years (25%). Collectively, more than half of the cases were concentrated between 31 and 50 years of age, indicating that kidney stone disease was most frequent during the economically productive years. Younger adults aged 18–30 years constituted 17.5% of cases, while patients older than 60 years represented the smallest proportion at 10%, suggesting a relatively lower burden at the extremes of age.

Sex distribution showed a marked male predominance, with males accounting for 65% of cases compared to 35% among females. This finding reflects a higher susceptibility or exposure to risk

factors for stone formation among males. In terms of residence, a greater proportion of patients were from urban areas (57.5%) compared to rural areas (42.5%), indicating a possible association with urban lifestyle factors, dietary patterns, and environmental exposures. Educational status revealed that the majority of patients had at least secondary-level education, with 40% falling into this category. Patients with primary education or less and those with graduate-level education each accounted for 30% of the study population, suggesting a relatively even educational distribution. Occupational status demonstrated that nearly half of the patients (47.5%) were engaged in sedentary occupations, while 32.5% were involved in manual labor and 20% were unemployed or homemakers. The higher proportion of sedentary workers may indicate reduced physical activity as a contributing factor to stone formation. Socioeconomic status showed that most patients belonged to the middle socioeconomic group (45%), followed by the lower socioeconomic group (35%), with a smaller proportion from the upper socioeconomic group (20%). This distribution suggests that kidney stone disease was more prevalent among individuals from middle and lower socioeconomic strata. Additionally, a history of urinary tract infection was present in 41.3% of patients, underscoring a substantial association between prior infection and kidney stone disease, while 58.7% reported no previous history of urinary tract infection.

Table 1: Socio-Demographic Characteristics of Patients with Kidney Stone Disease (n = 80)

Variable	Category	Number of Patients (n)	Percentage (%)
Age group (years)	18–30	14	17.5
	31–40	22	27.5
	41–50	20	25
	51–60	16	20
	>60	8	10
Sex	Male	52	65
	Female	28	35
Residence	Urban	46	57.5
	Rural	34	42.5
Educational status	Primary education or less	24	30
	Secondary education	32	40
	Graduate and above	24	30
Occupation	Sedentary occupation	38	47.5
	Manual labor	26	32.5
	Unemployed / Homemaker	16	20
Socioeconomic status	Lower	28	35
	Middle	36	45
	Upper	16	20
History of UTI	Present	33	41.3
	Absent	47	58.7

Table 2 demonstrated that the 80 patients with kidney stone disease, the most prevalent were the calcium oxalate–based stones, which accounted for 58, for 72.5%. The largest proportion of 32 cases (40%) was observed with Calcium oxalate monohydrate (COM) stones, which was followed by the mixed calcium oxalate monohydrate and dihydrate (COM + COD) stones among 26 patients (32.5%). 16 (20%) were observed with the Struvite stones, which contributed to the infection related to the calculi. The uric acid stones were uncommon among 6 patients. Escherichia coli was the most frequently associated organism, specifically for the calcium oxalate–based stones and struvite stones. Klebsiella spp. and Acinetobacter spp were related to the COM stones, while association with the calcium oxalate and uric acid stones was observed by Enterococcus spp. Struvite stones showed a relationship with the urease-producing organisms, such as Proteus mirabilis, alongside E. coli and Acinetobacter spp.

Table 2: Distribution of cases based on the type of stone and the associated organisms

Stone Type	No. of Cases (n = 80)	Percentage (%)	Associated Organisms
Calcium oxalate monohydrate (COM)	32	40	E. coli, Klebsiella spp., Acinetobacter spp.
Calcium oxalate monohydrate + calcium oxalate dihydrate (COM + COD)	26	32.5	E. coli, Enterococcus spp.
Uric acid	6	7.5	Enterococcus spp.
Struvite	16	20	Proteus mirabilis, E. coli, Acinetobacter spp.

Table 3 demonstrates that Escherichia coli was the predominant uropathogen isolated from patients with kidney stone disease, accounting for nearly half of all isolates at 47.5%. This finding indicates that E. coli remains the principal microbial contributor in stone-associated urinary tract infections, reflecting its strong colonization ability and adaptability to the urinary environment. Proteus mirabilis constituted 15% of isolates, highlighting a clinically relevant proportion of urease-producing organisms that are known to facilitate stone formation, particularly infection-related calculi. Klebsiella species and Enterococcus species contributed 11.3% and 10% of isolates, respectively, suggesting a mixed spectrum of Gram-negative and Gram-positive organisms in this population. The presence of Pseudomonas aeruginosa (8.8%) and Acinetobacter species (7.5%) indicates a notable proportion of opportunistic and potentially multidrug-resistant pathogens, emphasizing the complexity of microbial involvement in kidney stone disease.

Table 3: Distribution of Isolated Uropathogens among Kidney Stone Patients (n = 80)

Uropathogen	Number of Isolates	Percentage (%)
Escherichia coli	38	47.5
Proteus mirabilis	12	15
Klebsiella spp.	9	11.3
Pseudomonas aeruginosa	7	8.8
Enterococcus spp.	8	10
Acinetobacter spp.	6	7.5
Total	**80**	**100**

Table 4 illustrates that biofilm formation was a common phenotypic characteristic among the isolated uropathogens. Strong biofilm production was observed in 27.5% of isolates, while an additional 22.5% demonstrated moderate biofilm-forming capacity. Collectively, half of the isolates exhibited moderate to strong biofilm formation, indicating a substantial burden of organisms capable of persistent adherence and survival within the urinary tract and on stone surfaces. Weak biofilm producers constituted 17.5% of isolates, suggesting partial adherence potential, whereas 32.5% were non-biofilm producers. The predominance of biofilm-forming isolates underscores the role of biofilm in chronic infection, therapeutic failure, and recurrence of stone-associated urinary tract infections.

Table 4: Biofilm-Forming Capacity of Isolated Uropathogens (Phenotypic Assessment)

Biofilm Formation Grade	Number of Isolates	Percentage (%)
Strong biofilm producer	22	27.5
Moderate biofilm producer	18	22.5
Weak biofilm producer	14	17.5
Non-biofilm producer	26	32.5
Total	80	100

Table 5 shows a high level of antimicrobial resistance among the uropathogens isolated from kidney stone patients. Resistance was most pronounced against ampicillin, with 57.5% of isolates demonstrating resistance, followed closely by amoxicillin–clavulanic acid at 52.5% and co-trimoxazole at 48.8%. These findings indicate limited effectiveness of commonly prescribed first-line oral antibiotics in this patient population. Resistance to ciprofloxacin was observed in 38.8% of isolates, reflecting a concerning decline in fluoroquinolone susceptibility. In contrast, comparatively lower resistance rates were noted for nitrofurantoin (22.5%), amikacin (11.3%), and

imipenem (7.5%), suggesting that these agents retain higher efficacy. Overall, the resistance pattern highlights a substantial burden of multidrug resistance and reinforces the need for culture-guided antimicrobial therapy in patients with kidney stone disease complicated by urinary tract infection.

Table 5: Antimicrobial Resistance Pattern of Major Uropathogens Isolated (n = 80)

Antimicrobial Agent	Resistant Isolates (n)	Resistance (%)
Ampicillin	46	57.5
Amoxicillin–clavulanic acid	42	52.5
Co-trimoxazole	39	48.8
Ciprofloxacin	31	38.8
Nitrofurantoin	18	22.5
Amikacin	9	11.3
Imipenem	6	7.5

Discussion

The study had demonstrated that 41.0% of cases were detected with culture-confirmed infection, while the 34.3% showed low agreement between the two procedures by dipstick analysis. Elder adults had 5 fold high ODDS of UTI, and females were more predominant than males. The most common predominant uropathogen was Escherichia coli, which accounted for about 47% of the isolates. This was followed by the 17% of Pseudomonas aeruginosa, 14.2% of Proteus mirabilis and Klebsiella pneumoniae (11.4%). About 51% of isolates showed multidrug resistance. Low rate of MDR was demonstrated by P. aeruginosa, with high susceptibility to amikacin and imipenem. 51.5% of E. coli isolates showed the formation of biofilm, and the strain causing the biofilm provides high resistance to co-trimoxazole and amoxicillin–clavulanic acid [9]. Another study had revealed that UPEC isolates showed high prevalence of multidrug resistance, among most of the ESBL strains, forming the blaCTX-M. A strong biofilm producer was low resistant towards drugs, highlighting the inverse relationship between the density of biofilm and drug resistance. Frequent stages of virulence, such as hemolysis, siderophore production, and motility, were observed to vary with the phylogenetic group. BP was associated with the formation of biofilm and adverse [10]. The findings of the paper demonstrated the high challenge of multidrug-resistant UPEC, which was carried by the SBL gene carriage. The strong biofilm formation was related to the antimicrobial resistance. Phylogroup B2 showed association with virulence, resistance and genetic display among UPEC isolates [11]. The study showed that 16.3% of patients had ultrasound-confirmed urinary stone disease, and the most predominant uropathogen is E. coli. Young adults were in the age of 20 to 29 years contributed to the major cases. High resistance has been shown by gram-negative isolates, ampicillin and amoxicillin-clavulanate, while the efficacy was observed with nitrofurantoin and ciprofloxacin. Multidrug resistance was observed among 32.7% isolates, and ESBL producers showed 34.6% of Enterobacteriaceae, specifically the E. coli and K. pneumonia. The female sex, pre-history of UTI and the use of recent drugs were independently related to the UTIs [12].

Conclusion

The study has concluded that calcium oxalate–based stones constituted the predominant form of kidney stone disease, affecting nearly three-quarters of the study population, with calcium oxalate monohydrate emerging as the most common subtype. The substantial proportion of mixed calcium oxalate stones further indicates the multifactorial metabolic basis of stone formation. The presence of struvite stones in one-fifth of patients highlights a clinically important infection-related component, particularly associated with urease-producing organisms, while uric acid stones were relatively uncommon. Microbiological analysis established Escherichia coli as the leading uropathogen, accounting for almost half of all isolates, thereby reinforcing its central role in stone-associated urinary tract infections. The detection of Proteus mirabilis, Klebsiella spp., Enterococcus spp., Pseudomonas aeruginosa, and Acinetobacter spp. reflects a diverse microbial spectrum with potential implications for disease persistence and recurrence. A notable finding was the high prevalence of biofilm formation, with half of the isolates demonstrating moderate to strong biofilm-forming capacity, underscoring the contribution of biofilm to chronic infection, antimicrobial tolerance, and stone recurrence. Furthermore, the antimicrobial resistance profile revealed considerable resistance to commonly used first-line antibiotics, particularly beta-lactams and co-trimoxazole, alongside a concerning level of fluoroquinolone resistance. In contrast, lower resistance rates to nitrofurantoin, amikacin, and imipenem suggest retained therapeutic efficacy of these agents. Collectively, these findings emphasize the close interplay between stone composition, uropathogen profile, biofilm formation, and antimicrobial resistance, thereby highlighting the necessity for integrated diagnostic evaluation and culture-guided antimicrobial management in patients with kidney stone disease.

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