

Urinary Calculi: A Study On Bacteriological Profile And Its Association Between Patients With Co-Morbidities At A Tertiary Care Hospital

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Abstract

Background & Objectives: It is well-recognized and commonly observed that urinary calculi and urinary tract infections are related. However, little is known about the microbiological etiology of urinary calculi development. The study's goal was to identify the frequency of bacteria in urine and stone samples from nephrolithiasis patients who also had obesity, diabetes, and high blood pressure.

Methods: The research was carried out at SRM Medical College and Research Centre's Department of Urology. Each patient had pre-operative urine and post-operative stone samples taken.

Results: A total of 50 patients had their pre-operative urine and post-operative stone sample taken. It had a 36% culture positivity rate. 17 (or 34%) of 50 urine samples were positive for bacterial cultures. And 8 (16%) of the 50 calculi samples exhibited positive culture results. Seven of the 18 individuals showed growth in both the urine and stone samples. 85.7% of the organisms found in urine and stone samples were identical. *Escherichia coli* (52%) was the most common isolate followed by *Klebsiella pneumoniae* (24%), *Proteus mirabilis* (12%), *Enterococcus faecalis* (8%) and *Acinetobacter baumannii* (4%).

Conclusion: The selection of antibiotics by the clinicians is based on urine cultures and the efficiency of the treatment could be unreliable due to the organism present in the stone might be different in every case. Hence, if an infective stone is present, analyzing the microbial agent will help the patient in the prevention and recurrence of urinary calculi by giving them appropriate treatment.

Keywords: Urinary calculi, urinary tract infection, antibiotic resistance

INTRODUCTION:

Urinary calculi, also known as urolithiasis is a frequently encountered urological disorder in which mineral stones develop in the urinary tract i.e. ureter, pelvis and bladder. The excess saturation of minerals in urine is due to various etiological reasons which lead to stone formation. ⁽¹⁾

Urolithiasis has a prevalence of 12% and a recurrence rate of 50% according to several studies. And the overall prevalence of urinary calculi seems to be increasing. This may be due to the increase in sedentary lifestyles among people such as housewives, shopkeepers and drivers. Co-morbid conditions including obesity, diabetes, and hypertension are thought to put patients at higher risk. The risk of acquiring hypertension, chronic kidney disease (CKD), and end-stage renal disease is higher in persons who have urinary calculi (ESRD). ^(1,4)

In recent years, it has become clear that bacteria play a part in the creation of struvite stones, but less is known about how bacteria contribute to the more prevalent calcium-based stones ⁽²⁾

Proteus species, Staphylococcus aureus, Klebsiella species, Providencia species, Ureaplasma urealyticum species, and E. coli species, which do not generate urease, are the most often isolated organisms from infected stones ^(3,4).

Due to the uncertainty of the organism in the similarity of the organism present in the stone and urine, and the fact that these organisms are typically present within the interstices of a urinary stone, the effectiveness of treatment against the bacteria present in the stone must be determined. This is because the clinicians' selection of antibiotics is primarily based on the organism isolated from urine cultures.

Therefore, this study was created to determine the percentage of patients with hypertension, diabetes, and obesity who develop stones as well as the type of organisms isolated from the stone and urine as well as the consistency of the bacteria found in the stone and urine cultures.

MATERIALS AND METHODS:

A prospective cohort study was carried out on nephrolithiasis patients. Ethical approval was attained (2902/IEC/2021) from the Institute Ethical Committee (Human Studies) of SRM Medical College and Research Centre, Kattankulathur from August 2021 – February 2022. The patients who took part in the trial signed an informed consent form. 50 individuals with nephrolithiasis who also had diabetes mellitus, obesity, and hypertension had samples taken from them.

Inclusion Criteria:

Patients with nephrolithiasis who have type 2 diabetes mellitus, hypertension, and obesity are included in this study.

Exclusion Criteria

Participants with primary hyperparathyroidism, high vitamin D, renal tubular acidosis, and other secondary causes of nephrolithiasis are excluded from this study.

Patients who were receiving antibiotics at the time of the trial were not included.

Sample, preparation, isolation and identification of bacteria : (3,14)

Prior to surgical stone removal, each patient provided a midstream clean catch urine sample that was collected in a sterile, wide-mouth, screwcapped container. Additionally, post-operative stone samples were gathered in a saline-filled screw-cap bottle.

The urine samples were inoculated in 5% sheep Blood agar and MacConkey agar using a calibrated 0.001ml inoculating loop. 10⁵ colony-forming units per milliliter (CFU/ml) of urine is regarded as substantial bacteriuria by the semi-quantitative approach, and further processing was done to identify the organisms.

Preparation and Processing of Urinary Stone :

Stones that had been surgically removed were washed with sterile saline several times, ground into powder using a sterilized mortar and pestle, and then inoculated in Brain heart infusion (BHI) broth. These samples were then incubated at 35°C for 6 to 8 hours before being re-inoculated onto 5% sheep Blood agar and MacConkey agar.

Gram staining, Catalase test, Oxidase test, and Hanging drop were performed on the colonies. Gram-negative organisms were identified using biochemical tests such as Indole, Methyl Red, Voges Proskauer, Citrate, Mannitol Motility Test, Triple Sugar Iron Test, and Urease Test, whereas Gram-positive organisms were identified using Coagulase Test, Bile Esculin Test, and Mannitol Motility Test. The Kirby Bauer disc diffusion technique was used to evaluate the antibiotic susceptibility of all bacterial isolates. CLSI 2021 was used to interpret the zone size.

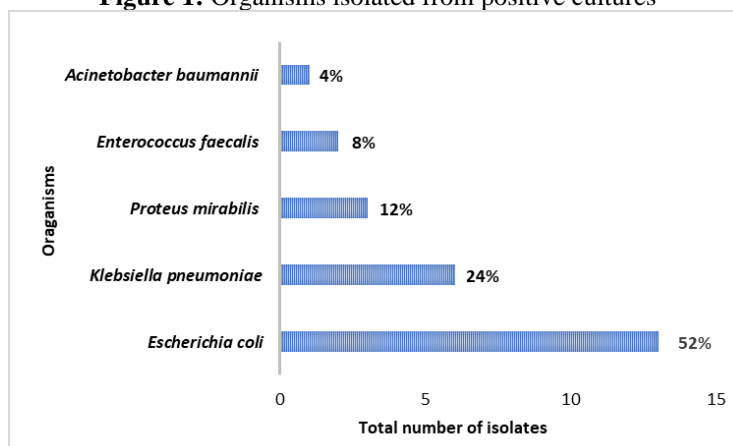
RESULT:

A total of 50 nephrolithiasis patients with obesity, type 2 diabetes mellitus, and hypertension had their urine and stones sampled. 18 of the 50 examples had successful cultural outcomes. The bulk of the group, consisting of 34 men and 16 women, were between the ages of 31 and 60.

Culture findings showed that 10 (20%) patients had growth only in the urine samples, and in 1 (2%) patient growth was isolated only from stone sample. Only 7 (14%) patients had growth in both urine and stone cultures. 32 (64%) patients had no growth in both urine and stone cultures.

Out of 25 positive cultures obtained from 18 patients, 13 (52%) were *Escherichia coli*, 6 (24%) were *Klebsiella pneumoniae*, 3 (12%) *Proteus mirabilis* and 2 (8%) *Enterococcus faecalis* and 1 (4%) *Acinetobacter baumannii* was found. (Figure 1)

Figure 1: Organisms isolated from positive cultures



Five of the 13 *Escherichia coli* that were isolated from urine and stone samples produced ESBLs, and two of them produced ampicillin. Furthermore, 2 of the 6 *Klebsiella pneumoniae* isolated from urine and stone samples produced amp C.

The similarity rate between urine culture and stone culture was 83% in our study (Table 1) which was nearest to the study done by H.Shafi *et.al* who had a rate of 70% [5] whereas other studies had various rates such as 16% [6], 48% [7], 50% [8].

Table 1: Similarity of organisms isolated from urine & stone samples

Total No. of patients who had growth in both samples	Similarity of organism	
	No. of Similar organism Isolated	No. of Different organisms isolated
7	6	1

All gram-negative bacteria isolated from urine samples were susceptible to Imipenem (93.7%), Nitrofurantoin (87.5%), Ciprofloxacin (81.25%), Amikacin (68.75%), Gentamicin (62.5%), Ceftazidime (56.25%), Cotrimoxazole (56.25%), Ofloxacin (50%) and Ampicillin (31.25%), according to the antimicrobial susceptibility pattern. Additionally, all of the isolated gram-positive bacteria were completely susceptible to the antibiotics Vancomycin, Linezolid, Ciprofloxacin, and Cotrimoxazole. (Table 2)

Table 2: Antibiotic susceptibility and resistant pattern of organisms from urine.

S.No	Antibiotics	Escherichia Coli (n=9)		Klebsiella pneumoniae (n=4)		Proteus Mirabilis (n=1)		Enterococcus faecalis (n=2)		Acinetobacter baumannii (n=1)	
		R	S	R	S	R	S	R	S	R	S
1.	Amikacin	4	5	1	3	-	1	-	2	-	1
2.	Ceftazidime	6	3	1	3	-	1	-	2	-	1
3.	Colistin	-	9	-	4	1	-	-	2	-	1
4.	Ertapenem	1	8	1	3	-	1	-	2	1	-
5.	Meropenem	-	9	1	3	-	1	-	2	-	1
6.	Cefipime	5	4	3	1	-	1	-	2	1	-
7.	Ampicillin	9	-	4	-	-	1	-	2	-	1
8.	Ciprofloxacin	9	-	3	1	-	1	-	2	1	-
9.	Cotrimoxazole	6	3	1	3	1	-	-	2	-	1
10.	Gentamicin	4	5	1	3	-	1	-	2	-	1
11.	Imipenem	-	9	1	3	-	1	-	2	-	1
12.	Nitrofurantoin	-	9	1	3	-	1	-	2	1	-
13.	Ofloxacin	5	4	1	3	-	1	-	2	-	1
14.	Linezolid	-	-	-	-	-	-	-	-	-	1
15.	Vancomycin	-	-	-	-	-	-	-	-	-	1
16.	Teicoplanin	-	-	-	-	-	-	-	-	-	1

The antimicrobial susceptibility pattern of gram-negative organism isolated from stone samples was similar to Pratima Shah *et al.* in that it was sensitive to Imipenem (87.5%), Gentamicin (75%) and Ceftazidime (75%) as well as Amikacin (62.5%), Ampicillin (62.5%), Ciprofloxacin (25%) and Cotrimoxazole (12.5%). (Table 3)

Table 3: Antibiotic susceptibility and resistant pattern of organisms isolated from stone.

S.No	Antibiotics	Escherichia Coli (n=4)		Klebsiella pneumoniae (n=2)		Proteus mirabilis (n=2)	
		R	S	R	S	R	S
1.	Amikacin	2	2	1	1	-	2
2.	Ceftazidime	2	2	-	2	-	2
3.	Colistin	-	4	-	2	2	-
4.	Ertapenem	-	4	-	2	-	2
5.	Meropenem	-	4	-	2	-	2
6.	Cefipime	3	1	2	-	-	2
7.	Ampicillin	3	1	-	2	-	2
8.	Ciprofloxacin	4	-	2	-	-	2
9.	Cotrimoxazole	3	1	2	-	-	2
10.	Gentamicin	2	2	2	-	-	2
11.	Imipenem	-	4	1	1	-	2
12.	Chloramphenicol	-	4	-	2	-	2

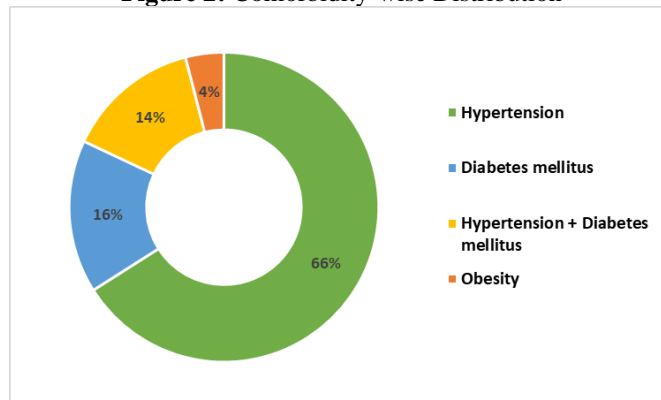
DISCUSSION:

In our study, the gender-wise distribution showed that males (68%) were predominant over females (32%) with nephrolithiasis. This showed that urinary calculi are formed in men more commonly than in women which was agreeing with other studies. Male to female ratio in our study was 2.12:1 which was the same as in the study conducted in Baghdad and several other studies had a similar ratio of 2.5:1 (9) and 2.86:1. (10)

This study demonstrates the mean age group of 31-40 (24%) which was similar to Pratima Shah *et.al.*,⁽¹⁴⁾ and the 51-60 (24%) age group had the highest nephrolithiasis patients followed by the 41-50 (20%) age group. And least was among the age group of 5-19 (4%) years.

In this study, comorbidities such as hypertension were seen in 68% of the patients, diabetes mellitus in 16% of the patients, 4% had obesity and 14% were diagnosed with both hypertension and diabetes. (Figure 2) In other studies, the comorbidity rate for hypertension was 78.3%⁽¹¹⁾ and 22.4% for diabetes mellitus.^(5, 12)

Figure 2: Comorbidity-wise Distribution



Because individuals with hypertension have aberrant renal calcium metabolism, which results in increased urine calcium excretion, several studies have shown that hypertension may be an independent risk factor for urolithiasis.^[6-8]

Subjects with diabetes have had urinary calculi more frequently than non-diabetic individuals, with uric acid stones predominating. Nerli *et al.*,⁽¹³⁾ explains this as insulin resistance impairs kidney ammonia genesis (a process in which the kidney produces ammonia to maintain the pH of the urine) which lowers the urine pH promoting uric acid stones. And his study also found a strong association between the uric acid stone in diabetic patients. Since in our study there is no biochemical analysis of the post-operative stone sample we couldn't analyze the concordance of stone type with the comorbidity mentioned.

Bacteria have been found to contribute to the production of struvite stones in recent years, but their significance in the formation of the more prevalent calcium-based stones has not been well examined. Several studies, however, suggest a probable link between urinary calculi and bacteria, owing to the high occurrence of urinary tract infections (UTI) in individuals with urinary calculi.^(2,4)

Stones with struvite and other components can also develop when urease-producing organisms infect other forms of stones, such as calcium-based or metabolically generated stones. These microorganisms are typically found in the spaces between urinary stones. Additionally, antibacterial drugs won't be able to enter the crevices between urinary tract stones. This results in the production of larger stones, which obstruct the renal pelvis and ureter over a period of weeks or months and cause consequences such as chronic urinary tract infections (UTI), which need for prolonged antibiotic therapy.

Additionally, in cases of infectious stones, immunocompromised individuals frequently have stone recurrence. Additionally, improper stone removal might result in additional mineral deposition or microbial contamination. The development of resistant organisms is made possible by the bacteria present in stones, which cause infection and hinder the complete eradication of urinary tract infection by traditional antibiotic therapy.

Additionally, due to the uncertainty of the organism in the similarity of the organism present in the stone and urine, the effectiveness of treatment against the bacteria present in the stone cannot be determined because the clinicians' primary basis for choosing antibiotics is the organism isolated from urine cultures.

Therefore, this study was created to determine the percentage of individuals with hypertension, diabetes, and obesity that develop stones as well as the kind of organisms isolated from the stone and urine as well as the consistency of the bacteria found in the stone and urine cultures.

CONCLUSION:

The current study emphasizes the value of microbiological stone analysis in patients with nephrolithiasis who have been diagnosed with hypertension, diabetes mellitus, and obesity in order to completely sterilize the urinary system and avoid stone recurrence. It is common for people with these comorbidities to develop metabolic stones rather than infected ones. In several investigations, including this one, the organism found in urine often correlates with the stone, with an 85.7% concordance rate. However, it is not required for the same organism to exist within the stone as it does in the urine. The therapy of patients with metabolic stones is consequently aided by the culture and analysis of all stones, which should be done.

In patients with nephrolithiasis who had been diagnosed with co-morbidity, this study has discovered several infectious stones, and comparable investigations have revealed that these individuals were mostly known to generate metabolic stones, highlighting the necessity of identifying the microbiological cause of stone formation and the antimicrobial sensitivity pattern in order to administer the proper antimicrobial therapy for the removal and prevention of recurrence of urinary calculi.

Authors Contribution:

Ishwarya contributed significant contributions to the conceptualization, data collection, and drafting of the work, as well as key revisions for important intellectual content. Ilamathy, Leela and Manjula completed Characterization investigations and final acceptance of the published version and agreed to be responsible for all parts of the project.

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