Original Research Article

Evaluation of bacteriological profile of bladder calculi and its association with urinary tract infection in a tertiary health care centre

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Abstract

Background: The occurrence of urinary tract infection in presence of urolithiasis (urinary calculi) is frequent. Bladder calculi comprise approximately 5-10% cases of renal or urinary calculi. However, microbial agents of urolithiasis and their association with urinary tract infection are under investigated.Objectives: This study was aimed to evaluate the bacteriological profile of bladder calculi and its association with urinary tract infection.**Material & Methods:** This descriptive study was conducted in the Department of Microbiology at a tertiary care hospital from September 2018 to September 2019. A sample size of 100 (61 males and 39 females, presenting with bladder calculi) was calculated at 95% confidence interval at 5% acceptable margin of error by Epi Info software version 7.2. Preoperative urine culture and postoperative stone culture were performed. Isolation and identification of bacteria was done using standard microbiological techniques.**Results:** Urinary tract infection was present in 53% cases which included 26 males and 27 females. The mean age of patients was 42 ± 6.2 years. In majority of cases the reaction of urine was acidic (76%). The most common organism isolated in urine culture was *E. coli* (28%), followed by *Staphylococcus aureus*(8%), *Pseudomonas aeruginosa* (4%), *Klebsiellaaerogenes* (3%), *Proteus mirabilis (*2%) and mixed organisms (8%). On bladder stone core culture growth was observed in 50% cases including 22 males and 28 females. The most common organism isolated was *E. coli* (28%), followed by *Staphylococcus aureus* (6%), *Klebsiellaaerogenes* (8%) and mixed organisms (8%). *E. coli* was the predominant organism found both in urine and core culture of stone.**Conclusion:** The present study showed that urinary infection was present among majority of cases that presented with bladder calculi and *E. coli* was the most common type of organism associated. The association of microorganisms isolated from urine and stone core culture was considerable and can predict the source of infective stone. This study highlights the importance of microbiological analysis of stones for complete sterilization of urinary system and prevention of recurrence.

Keywords: Urolithiasis, urinary tract infection, bladder calculi, Urea-splitting bacteria.

Introduction

Urinary calculi (Urolithiasis) have been reported as the most common renal disease in India and worldwide. Its prevalence ranges from 7%-13% in North America, 5%-9% in Europe, and 1%-5% in Asia [1-3].They are amongst the most painful conditions and generally acute in onset requiring urgent medical care [4]. Bladder stones (bladder calculi) are hardened clumps of minerals that form in the bladder. Bladder calculi have been reported in approximately 5-10% cases of renal or urinary calculi among various studies [5]. The association between urolithiasis and urinary tract infections (UTIs) is well known and is frequently detected. Their interrelationship can be defined in two ways: urolithiasis following UTIs, i.e.,"infection-induced stones" or urinary stone with subsequent UTIs as its complications [6]. Previous studies have reported that in the human body, urinary tract is the most frequent site for calculus formation among all the other potential sites. The most common etiopathology behind bladder calculi are recurrent urinary tract infections, urinary obstruction, strictures, urinary stasis, inadequate water intake, medical procedures like catheterization and uncorrected anatomical abnormalities [7,8]. Many studies have documented that urinary stagnation results in deposition of minerals from urine in the form of various crystals leading to urinary bladder calculus formation. Some studies have stated that almost 10% individuals with urinary calculi report for medical care [9]. Stones may pass on their own, or may need a procedure to remove them. If left untreated, can lead to urinary tract infections and trouble urinating. The types of urinary stones most commonly reported are calcium oxalate and calcium phosphate [10]. The association between urinary tract infection and urinary calculi is common and the incidence varies from 7% to 60% as documented in previous studies [11-21].

Urea-splitting bacteria like *Proteus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Providencia* spp., and *Urea plasma urealyticum* are commonly responsible for struvite stones [10]. The pre-dominant bacteria found in the nuclei of urinary calculi are *Staphylococcus* and *Escherichia coli*. Urea splitting bacteria lead to hydrolysis of urea increasing the concentration of carbonate, bicarbonate and ammonium ions, thereby increasing the urinary pH rendering the urine alkaline and hence promoting stone formation in both clinical and experimental studies [15,16,22,23]. The antimicrobial agents are unable to invade, where these bacteria lie within the interspaces of stones in urinary tract. This causes progressive expansion of stones

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due to persistent infection over a period of weeks or months [6,10,24]. Thus the present study was conducted to assess and evaluate the bacteriological profile of bladder stones and find the concordance between urine and stone bacteria.

Material & Methods

The present descriptive study was conducted between September 2018 to September 2019 in the Department of Microbiology, Metro Mas Hospital, Jaipur, Rajasthan, India. A sample size of 100 (61 males and 39 females) was calculated at 95% confidence interval at 5% acceptable margin of error by Epi Info software version 7.2. Samples were collected from the enrolled patients presenting with bladder calculi by simple random sampling. Ethical clearance from the Institutional Ethics Committee was taken before the start of the study. Written informed consent was obtained from all the patients.

Preoperative urine culture and postoperative stone culture were performed.

Urine culture

Midstream urine specimen was collected from each patient before surgical stone removal and cultured using calibrated (4mm dia.) loop providing fixed quantity on CLED agar, MacConkey agar (MA) and Blood agar plate (BA) (HiMedia Laboratories) for isolation of bacterial pathogens. Cultures were incubated at 37ºC for 24 hours. Also microscopy and Gram stain of urine samples were performed. Using the semi-quantitative method, 10^5 colony-forming units per milliliter (CFU/ml) of urine was considered as significant bacteriuria. If no growth was observed after 24 hours of incubation, the samples were considered sterile. The bacterial pathogens grown in culture were identified up to species level by standard microbiological techniques like colony morphology, Gram staining, and several biochemical tests [11,13,17,20, 25-29]. Urinary pH was noted using pH test strip and color change was documented comparing with the provided color chart [30].

Stone culture

Stones were also collected from the same patient after surgery. The bladder stones were thoroughly rinsed in physiological sterile saline and then crushed for culture with a sterile hack-saw. Then the crushed stone cores were sent for culture in 5ml thioglycolate or brain heart infusion (BHI) broth which was further incubated at 37°C for approximately 18-24 hours and then subsequent subcultures were done on CLED agar, MacConkey agar (MA) and Blood agar plate (BA) for isolation of bacterial pathogens. Then the isolated pathogens were further subcultured on nutrient agar (NA) plate and incubated at 37ºC overnight. The typical colonies were identified on the basis of lactose fermenting and non-lactose fermenting on MA, type of hemolysis produced on BA, and pigment production on NA. Then, the strains were identified up to species level by standard microbiological techniques like colony morphology, Gram staining, and several biochemical tests [6,10].

All the data was recorded on Microsoft excel spread sheet and data analysis was done at 5% alpha and 95% confidence interval using SPSS v22 software. Test of significance was applied on collected and organized data and p-value less than 0.05 was considered as statistically significant association between study variables.

Results

In the present study, we enrolled 100 patients presenting with bladder calculi. The male (61%) to female (39%) ratio was 1.56:1. The mean age of patients was 42 ± 6.2 years. In majority of the cases, the reaction of urine was acidic (76%) and in remaining (24%) was alkaline (Table 1).

Table 1: Distribution of study participants according to study parameters.

Study parameters		No. of participants
Mean age (years)	42 ± 6.2 years	
Gender	Male	61
	Female	39
Urine pH	Acidic	76
	Alkaline	24

Bacterial growth was observed in the urine of 53 patients which included 26 males (49.05%) and 27 females (50.95%) (Table 2). However, no growth was observed in the remaining samples and were considered sterile. The most common bacterial species isolated was *E. coli* (28%), followed by *Staphylococcus aureus* (8%), *Pseudomonas aeruginosa* (4%), *Klebsiella aerogenes* (3%), *Proteus mirabilis* (2%) and mixed organisms (8%) (Table 3, Figure 1).

Table 2: Sex wise distribution of infection

	Sex		p-value
	Male	Female	
Urinary tract infection (53)	26	27	>0.05
Stone core culture infection (50)	22	28	>0.05

Bacterial growth was observed in the stone culture of 50 patients including 22 males (44%) and 28 females (56%) (Table 2). The most common bacterial organism isolated was *E. coli* (28%), followed by *Staphylococcus aureus* (6%), *Klebsiella aerogenes* (8%) and mixed organisms (8%) (Table 3, Figure 1). *E. coli* was the predominant organism found both in urine and core culture of stone.

Table 3: Pattern of bacteria isolated from urine and stone culture.

Bacteriological profile	Urine culture	Stone core culture
E. coli	28%	28%
Staphylococcus aureus	8 %	6%
Klebsiella aerogenes	3%	8%
Mixed organisms	8%	8%
Pseudomonas aeruginosa	4%	-
Proteus mirabilis	2%	-

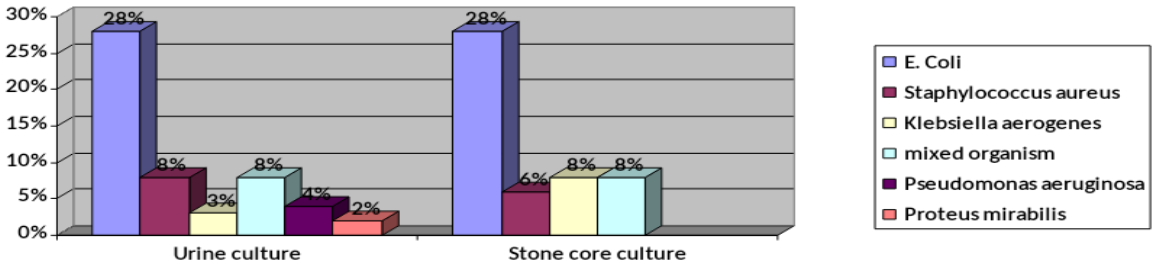

Fig 1: Pattern of bacteria isolated from urine and stone culture.

Discussion

In the present study, 100 patients diagnosed with bladder calculi were enrolled and samples were collected by simple random sampling. The male (61%) to female (39%) ratio was 1.56:1. The mean age of patients was 42 ± 6.2 years. In most of the cases the reaction of urine was acidic (76%) and in remaining (24%), it was alkaline (Table 1). Urinary tract infection was present in 53% cases (26 males and 27 females) (Table 2). Similar results were obtained in a study conducted among 50 patients diagnosed with renal calculi by Singh P. (2018). Study reported that the majority of study participants were males. Urinary pH was acidic in majority of cases and urinary infection was present in 50% of the cases [31]. Culture positivity of 50% and 55.20% has been reported in studies conducted in Nepal and Maharashtra respectively which is similar to our study [10,29]. A lower culture positivity of 48% and 45.67% were reported by Lewi, White et al. (1984) [21] and Hugosson, Grenabo et al. (1990) [32] respectively.However in a study conducted by Jan, Akbar et al. (2008) infection was present in 79% of cases which was higher compared to our study. The most common organisms isolated were E. Coli (30%), Proteus (19%), Klebsiella (11%), Pseudomonas (7%) and Staphylococcus aureus (3%). The frequency of renal stone disease in patients with urinary tract infection was however 18.98% (12.6% in male and 6.3% in female) whereas in our study it was found in 53% cases (26% males and 27% females). Mean age of patients with renal stones was 31.26 years and male to female ratio was 1.5:1 which was quite similar to our study [33].In the present study, urine culture showed that the most common bacterial organism was E. coli (28%), followed by Staphylococcus aureus (8%), Pseudomonas aeruginosa (4%), Klebsiella aerogenes (3%), Proteus mirabilis (2%) and mixed organisms (8% cases) (Table 3, Figure 1). E coli was found to be the most common isolate in various studies [10, 29, 32]. In our study, E. coli was present in 28% cases while Kore, Singh et al. (2011) have reported a prevalence of 24.48% [29]. Bladder stone core culture showed the most common organism was E. coli (28%), followed by Staphylococcus aureus (6%), Klebsiella aerogenes (8%) and mixed organisms (8% cases) (Table 3, Figure 1). In the present study E. coli was the predominant organism found both in urine and core culture of stones. Similar findings have been reported in other studies also [10,24,29]. Similar study by Bratell, Brorson et al. (1990) reported majority of urinary calculi to be of infectious etiology and the most common type of organism associated as E. coli [11]. In the present study infective stones were found in 50% cases which is higher as compared to 34.1% infective stones in a study by Shah, Baral et al (2020) [10]. It has been hypothesized that alteration in urinary enzymes, i.e., decreased urokinase and increased sialidase in urine, leads to the formation of mineralizable matrix. Microorganisms like Proteus mirabilis and Escherichia coli associated with infection-induced stones inhibit the urokinase and stimulate the sialidase activity leading to matrix formation, in turn causing increased crystal adherence to the renal epithelium [33]. An alternative explanation for the presence of bacteria within stone and urine is that of secondary ascending infection from the bladder urine. Penetration of bacteria in the stone prevents complete eradication of urinary tract infection by conventional antibiotic therapy, allowing the development of resistant organisms with intermittent shedding in urine. It is a vicious cycle of infection bringing about stone formation and stone formation causing infection [10,14].

Conclusion

We conclude from the present study that urinary tract infection was present among majority of the patients diagnosed with bladder calculi. The most common type of organism associated was E. coli. This study highlights the importance of microbiological analysis of stones for complete sterilization of urinary system and prevention of recurrence. More so, a further work on microbiological analysis of stones along with the correlation of various metabolic and dietary factors may provide better insights regarding the origin of the stones in the urinary tract.

Limitations of the study: The study enrolled patients from a single tertiary health care center; however, to generalize the results multi-centric studies are required.

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