MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

The concordance of the microbiologic profile of preoperative urine, pelvic urine, and renal stone culture in nephrolithiasis patients

Asmaa E. Ahmed[1], Hassan Abol-enein[2], Amira Awadalla[2], and Omar A El-Shehaby[1]*

[1]*Botany department, Faculty of Science, Mansoura University, Mansoura, 33516, Egypt.*

[2]*Center of Excellence for Genome and Cancer Research, Urology and Nephrology Center, Mansoura University, Mansoura, 33516, Egypt.*

**** Correspondence to:** dr-asmaaelsayed@hotmail.com, 01008256566.*

Received:5/7/2022
Accepted: 1/8/2022

Abstract: Background: The correlation between stone disease and bacteria was restricted to the association with struvite stones. Few studies shed light on the association between bacterial infection and non-infectious stones.

Objective: To identify the bacteria isolated from preoperative urine, pelvic and urine kidney stone samples.

Methods: Fifty preoperative urine, pelvic and urine kidney stone samples were collected from nephrolithiasis patients. The stones' chemical composition was tested by Fourier-transform infrared spectroscopy (FTIR). The three type of samples were subjected to microbiological analysis.

Results: The 50 patients consisted of 22 males and 28 females. The mean age of the patients was 48.7±14. The most common type of stone was uric acid in 27 (45%). *s.aureus* was the most predominant pathogen in stone and pelvic urine samples with 7 (35%) and 4 (66.6), respectively. *E-coli* was the most common pathogen in the preoperative bladder urine with 7 (50%).

Conclusion: Gram-positive bacteria were the predominant pathogen associated with stone and pelvic urine. The preoperative urine culture did not reflect the upper urinary tract infection.

keywords: Pelvic, stone, nephrolithiasis, Gram-positive.

1.Introduction

A kidney stone disease is considered the third most common urinary tract disorder. The precise cause of nephrolithiasis and relapse is unknown [1]. Nearly half of patients with renal stones can relapse within ten years [2].

There are multiple risk factors for kidney stone formation, such as metabolic, environmental, nutritional, obstructive uropathology, race, gender, and urinary tract infection [3].There is a significant risk of recurrence in patients with kidney diseases; it is critical to understand the risks that are etiologically important and hence predict the disease's future outcome [4]. Microbial infection can cause stone formation by enhancing crystal adhesion, which promotes the inflammation of tissue, the formation of crystal–matrix interaction, and an organic matrix [5]. Presence of urinary tract infection with urease non-splitting or splitting bacteria could be one of the risk factors in promotion in formation of renal stones [6].

The Stones infected with bacteria represent ~15% of all urinary stone disorders. A high incidence of growth of new stones is observed in patients with infected stones, mainly if there are any remaining stone fragments [7].

The European Association of Urology guidelines on urolithiasis recommends a preoperative bladder culture and perioperative antibiotic before stone removal to avoid infectious complications [8]. Although, a preoperative bladder culture is insufficient to predict the infections after stone removal, as it does not reflect the microbiological profile of the upper urinary tract system, mainly when there is an obstruction or an infected stone [9]. Some studies reported that stone

and pelvic urine cultures were good predictors for stone infections [10].

The current study investigated the microbiological status in pelvic urine, stone samples, and pre-operative urine samples.

2. Materials and methods

2.1.Patients

After obtaining approval from the local Institutional Review Board, a total of 50 patients with kidney stones who underwent a stone removal procedure were included. This prospective study was conducted at Urology and Nephrology center Mansoura University. Pre-operative urine samples, stone samples, and pelvic urine samples were obtained from each patient under aseptic condition. A part of extracted stone was sent for biochemical analysis with FT-IR. The other part of the stone, Pre-operative urine samples, and pelvic urine samples were sent for microbiological testing.

2.2.Biochemical analysis of the stone samples

The retrieved stones were cleaned with deionized water to remove any residuals attached with stone samples. The samples were thoroughly dried with filter paper, and then the samples were cut into small pieces. The stone samples were homognized to a fine texture using a pestel. The samples were analyzed by Perkin-Elmer Fourier Transformer Infrared Spectrophotometer (FTIR 2000) spectroscopy, with a wave number that varies from 7800 to 350 $cm-1$. Finally, the acquired spectrums were compared to those in the kidney stone library.

2.2.Microbiological analysis

Pre-operative urine samples were taken in a sterile container after cleaning the external genital organs. Pelvic urine samples were collected during the stone removal. The stones were prepared for bacteriological culture, according to Ogata, Akakura [11]. The stone samples were washed by sterile physiological saline. Then the stones were crushed with a clean mortar. The stones were cultured in 5 ml nutrient broth media that was incubated for 18–24 h at 37°C. Then, the stone suspension and urine (pre-operative & pelvic) samples were sub-cultured on blood agar and MacConkey's agar plate to isolate microbial agents. Colony morphology was employed to characterize pure isolates of bacterial pathogens. Biochemical methods and Gram stain were utilized to identify Gram-positive and Gram-negative bacteria.

2.4. Statistical analysis

Data were analyzed using SPSS version 14.2 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA). Data were statistically reported employing mean ± standard deviation (SD) or numbers and percentages when appropriate.

2.Results

The current study compressed 50 patients diagnosed with kidney stones, the group consisted of 22 males and 28 females. The mean age of the patients was 48.7±14. The preoperative urine samples, pelvic samples and stone samples were positive for bacterial culture in 14, 6, and 20 patients, respectively (Table 1).

Table 1: Patient characteristics

Items	**Number of patients , N=50**
Age (Mean±SD)	48.7±14
Sex	
Male (%)	22 (44%)
Female (%)	28(56%)
Urine sample n (%) Positive Bacterial culture Negative Bacterial culture	14 (28%)36 (72%)
Urine pelvic sample n (%) Positive Bacterial culture Negative Bacterial culture	6 (12%)44 (88%)
Stone samples n (%) Positive Bacterial culture Negative Bacterial culture	20 (40%)30 (60%)

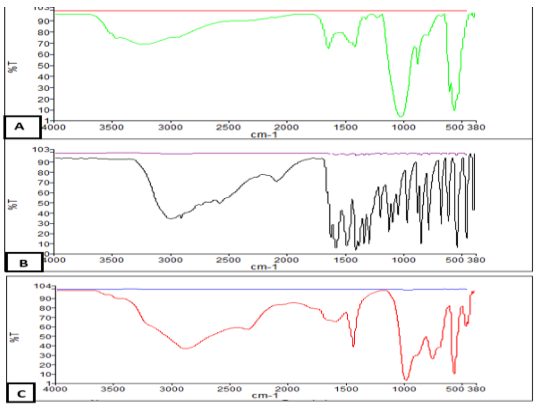

Plate 1: FTIR spectrum profile of kidney stones showing major peaks. a) FTIR spectrum

profile uric acid stone and b) FTIR spectrum profile Calcium oxalate stone.

The most common type of stone was uric acid in 27 (45%) of patients followed by Calcium oxalate in 17 (34%). Calcium phosphate, Magnesium ammonium phosphate, and Cysteine were represented 3 (6%), 2 (4%) and 1 (2%), respectively. The distribution of bacterial infection in relation to the type of stone is shown in table 2. Plate 1 and 2 represented the FT-IR spectra of the stone chemical composition.

Table 2: Stone infection in relation to their chemical composition

Type of the Stone	Total N=50	Stone positive bacterial cultureN= 20	Stone negative bacterial cultureN=30
Calcium oxalate	17(34%)	8 (40%)	9 (30%)
Calcium phosphate	3 (6%)	2 (10%)	1(3.3%)
Uric acid	27(54%)	7 (35%)	20 (66%)
Cystine	1 (2%)	1 (5%)	0 (0%)
Magnesium ammonium phosphate	2 (4%)	2 (10 %)	0 (0%)

Table 3: Distribution of pathogens between clinical samples

Bacteria	isolatesfrompreoperative urinesamplesn=14	isolates from pelvicurinesamplesn=6	isolates fromstone samplesn=20
Escherichia coli	7 (50%)	1 (16%)	2(10%)
Pseudomonas aeruginosa	0 (0%)	0	2(10%)
Klebsiella pneumoniae	1 (7%)	0(0%)	0 (0%)
Enterococcus faecalis	2 (14.5)	1 (16%)	5(25%)
Staphylococcus epidermidis	0	0(0%)	4(20%)
Staphylococcus aureus	4 (28%)	4 (66.6)	7(35%)

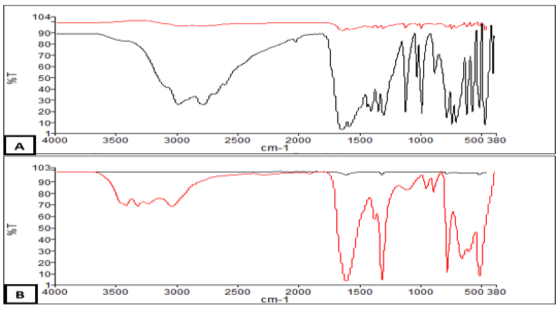

Plate 2: FTIR spectrum profile of kidney stones showing major peaks. a) FTIR spectrum profile Calcium phosphate stone and b) Fourier transform infrared spectrum profile Cysteine stone. C) FTIR spectrum profile of Magnesium ammonium phosphate stone showing major peaks.

In the stone samples the most predominant type of bacteria was *s.aureus* 7 (35%) followed by *Enterococcus faecalis* 5(25%), *Staphylococcus epidermidis* 4 (20%), *Escherichia coli* 2 (10%), and *Pseudomonas aeruginosa* 2(10%).

In pelvic urine samples *s.aureus* was the most common pathogen with 4 (66.6) followed by *Escherichia coli* and *Enterococcus faecalis* with 1 (16%)

E-coli was the predominant pathogen in the preoperative bladder urine with 7 (50%) followed by s.aureus with 4 (28%), Enterococcus faecalis 2 (14.5) and Klebsiella pneumoniae 1 (7%) (Table 3).

2.Discussion

Kidney stone is one of the most prevalent urological illnesses, with worldwide incidence rates ranging from 2 to 20% [12] [4].The stone production process was addressed by two theories: infection stones and stones associated with UTI. Infection stones are represented by struvite stones caused by urease-producing bacteria [13]. Stones presented with urinary tract infection can be occurred due to bacterial adhesion, obstruction, and indwelling catheters. Most studies believe that these two reasons interact closely, resulting in a vicious spiral, regardless of which occurs first.

This study analyzed the microbiological profile in preoperative urine, pelvic, and stone samples in patients undergoing stone removal.

In the present study, nephrolithiasis is more common in women (65%) than in males (44%) and mean of ages was 48.7years. These findings were similar to the findings of the study by Osman, Elshal [14] .They revealed that nephrolithiasis is more common in females (57%) than in males (43%) and the mean age was of 52.

The chemical composition of the stones was detected by Fourier-transform infrared spectroscopy (FT-IR). In the present study, uric acid calculi were the most observed type of stones and accounted for 48%, followed by pure calcium oxalate stones (26%).

These results were in agreement with the study of Mohamed, El-Shimy [15] as they concluded that uric acid stones was the most common type (40%). Meanwhile Shah, Baral [16] reported that calcium oxalate stones were the predominant stone type. The major differences in the ratios of the stone constituents, especially uric acid which may reflect specific life style nutrition and food habit.

In the current study, 20 percent of the renal stones were infected. This rate may be greater than that of Mariappan, Smith [17] (11.1%), but it may be equivalent to Mariappan and Loong [18] 28.08 percent. Geographical distribution, race, individual variance, socioeconomic position, and dietary effects might all have a role.

The present investigation showed that the bacteria isolated from stones are different from those isolated from pelvic and preoperative bladder urine samples[8]. Gram-positive bacteria was the predominant one in the stone and pelvic urine samples; however, the preoperative bladder samples were dominated by Gram-negative bacteria. In the present study, *E.coli* was the most common bacterial infection in the preoperative urine samples; however, *s.aureus* pathogen was the most prevalent bacteria in stone and pelvic urine samples. Dornbier, Bajic [19] reported that the dominant taxa in stone samples was *staphylococcus spp.* through studying the calcium stone bacterial population, and Kitano, Shigemoto [20] stated that the renal calculi are significantly correlated with *S. aureus* infection as it has the ability to be colonized in the stones. Liu, Zhang [21] reported that the *Staphylococcus spp.* is the dominated one in patients with kidney calculi.

These findings are in line with other studies showing that the microbiology of stone disease has shifted from Gram-negative to Gram-positive organisms in the recent generation [10]. There are several possibilities like decreasing frequency of struvite stones in the urological patient population [8, 22]. Treatment through the endoscopic procedures needs repeated episodes of instrumentation throughout the urinary tract that provide chances for introducing Gram-positive organisms.

Conclusions

This work showed that stone culture was technically viable, retrievable, and analyzable. Gram-positive bacteria were the predominant pathogen associated with stone and pelvic urine. The preoperative urine culture did not reflect the upper urinary tract infection. More extensive studies will be needed to investigate stone culture's clinical utility and cost-effectiveness during stone surgery.

4. References

1. Pearle, M.S., et al., (2005) Urologic diseases in America project: urolithiasis. The *journal of urology*,. **173(3)**: p. 848-857.
2. Nowfar, S., et al., (2011) The relationship of obesity and gender prevalence changes in United States inpatient nephrolithiasis. Urology,. **78**(5): p. 1029-1033.
3. Tabibian, J.H., et al., (2008). Uropathogens and host characteristics. *Journal of clinical microbiology*, **46(12)**: p. 3980-3986.
4. Shavit, L., P. Jaeger, and R.J. Unwin, (2015) What is nephrocalcinosis? Kidney international,. **88**(1): p. 35-43.
5. Al-Jebouri, M.M. and N. Atalah, (2012) A study on the interrelationship between renal calculi, hormonal abnormalities and urinary tract infections in Iraqi patients.
6. Tavichakorntrakool, R., et al., (2012) Extensive characterizations of bacteria isolated from catheterized urine and stone matrices in patients with nephrolithiasis. Nephrology dialysis transplantation,. **27**(11): p. 4125-4130.
7. Zisman, A.L., et al., (2015) Do kidney stone formers have a kidney disease? Kidney international,. **88**(6): p. 1240-1249.
8. De Lorenzis, E., et al., (2020) Feasibility and relevance of urine culture during stone fragmentation in patients undergoing percutaneous nephrolithotomy

and retrograde intrarenal surgery: a prospective study. *World journal of urology*,: p. 1-8.
9. Walton-Diaz, A., et al., (2017)Concordance of renal stone culture: PMUC, RPUC, RSC and post-PCNL sepsis—a non-randomized prospective observation cohort study. International urology and nephrology,. **49**(1): p. 31-35.
10. Korets, R., et al., (2011) Post-percutaneous nephrolithotomy systemic inflammatory response: a prospective analysis of preoperative urine, renal pelvic urine and stone cultures. *The journal of urology*,. **186(5)**: p. 1899-1903.
11. Ogata, T., et al., (2003) Annual changes of the incidence and clinical characteristics of magnesium ammonium phosphate urinary stones. International *journal of urology*,. **10(1)**: p. 1-5.
12. Zeng, G., et al., (2017) Prevalence of kidney stones in China: an ultrasonography based cross-sectional study. *BJU international*,. **120**(1): p. 109-116.
13. Abrahams, H.M. and M.L. (2003) Stoller, Infection and urinary stones. Current opinion in urology,. **13**(1): p. 63-67.
14. Osman, Y., et al., (2016). Stone culture retrieved during percutaneous nephrolithotomy: is it clinically relevant? Urolithiasis, **44**(4): p. 327-332.
15. Mohamed, H.I., et al., (2018) Identification of different bacterial species isolated from infected renal stones and evaluation of its uricolytic activity. *Journal of Medicine in scientific research*,. **1**(1): p. 35.
16. Shah, P., et al., Urinary Calculi: (2020.) A Microbiological and Biochemical Analysis at a Tertiary Care Hospital in Eastern Nepal. International *journal of microbiology*, **2020**.
17. Mariappan, P., et al., (2005) Stone and pelvic urine culture and sensitivity are better than bladder urine as predictors of urosepsis following percutaneous nephrolithotomy: a prospective clinical study. *The journal of urology*,. **173**(5): p. 1610-1614.
18. Mariappan, P. and C.W. Loong (2004), Midstream urine culture and sensitivity test is a poor predictor of infected urine proximal to the obstructing ureteral stone or infected stones: a prospective clinical study. *The journal of urology*,. **171**(6 Part 1): p. 2142-2145.
19. Dornbier, R.A., et al. (2020), The microbiome of calcium-based urinary stones. Urolithiasis,. **48(3)**: p. 191-199.
20. Kitano, H., et al., (2020:) Indwelling catheterization, renal stones, and hydronephrosis are risk factors for symptomatic Staphylococcus aureus-related urinary tract infection. *World journal of urology*, p. 1-6.
21. Liu, F., et al., (2020)Characteristics of the urinary microbiome in kidney stone patients with hypertension. J*ournal of translational medicine*,. **18(1)**: p. 1-13.
22. Viprakasit, D.P., et al., (2011) Changing composition of staghorn calculi. The *journal of urology*,. **186(6)**: p. 2285-2290.