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Original Article

Pathogen distribution and risk factors of positive stone culture for patients with upper urinary tract stone

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Abstract: Background: Upper urinary tract stones are often complicated by perioperative infections. Bacterial culture analysis of calculi can reveal pathogen distribution on stone surfaces and help identify risk factors for positive culture outcomes, aiding in infection control. Methods: A retrospective analysis was conducted on 173 patients with upper urinary tract stones who underwent holmium laser lithotripsy. Clinical characteristics potentially influencing positive stone cultures were collected and used to establish a binary logistic regression model for identifying risk factors. Additionally, the correlation between positive stone cultures and postoperative infection markers was analyzed. Results: Among the 173 patients, 44 had positive stone cultures, and 47 pathogen strains were detected. Forty-six patients had positive urine cultures, with 49 pathogen strains identified. The main pathogens in stone cultures were *Escherichia coli* (22 strains, 46.81%), *Proteus mirabilis* (5 strains, 10.64%), *Pseudomonas aeruginosa* (4 strains, 8.51%), and *Klebsiella pneumoniae* (3 strains, 6.38%). In urine culture, the main pathogens were *Escherichia coli* (26 strains, 53.06%), *Klebsiella pneumoniae* (5 strains, 10.2%), and *Acinetobacter baumannii* (4 strains, 8.16%). Binary logistic regression identified the following risk factors for positive stone cultures: positive urine white blood cells (OR = 2.881, 95% CI = 1.070-7.760, P = 0.036), hydronephrosis (OR = 5.644, 95% CI = 2.168-14.696, P < 0.0001), struvite stones (OR = 7.512, 95% CI = 1.864-30.283, P = 0.005), and a history of diabetes (OR = 6.580, 95% CI = 1.820-23.791, P = 0.004). Spearman correlation analysis showed a strong correlation between positive stone cultures and postoperative fever (r = 0.666) and CRP failure to return to normal 48 hours post-surgery (r = 0.633), both of which were higher than the correlation with urine bacterial culture. Conclusion: *Escherichia coli* is the predominant pathogen in stone cultures. Risk factors for positive cultures include positive urine leukocytes, hydronephrosis, struvite stones, and a history of diabetes. Positive stone cultures are associated with a higher risk of postoperative infection compared to positive urine cultures.

Keywords: Upper urinary tract stones, stone culture, urine culture, pathogen distribution, risk factors

Introduction

Kidney and ureteral stones, collectively referred to as upper urinary tract stones, are the most common type of urinary stones. These stones can lead to upper urinary tract obstruction, hindering normal urine flow. Urinary obstruction can lead to infection, and perioperative infection is a serious complication. Pathogens causing the infection may either float in the urine or adhere to and colonize the stone surface. In individuals without urinary tract obstruction, bacteria in the urine are typically excreted, and the mucous membrane of the urinary tract possesses an antibacterial barrier, preventing symptoms of urinary tract infection. As a result, urine bacterial cultures are often negative. In contrast, in patients with upper urinary tract stones, the obstruction prevents normal urine expulsion, allowing bacteria to colonize the stone surface. These bacteria colonized on stones can be released into the urine, leading to asymptomatic bacteriuria. Ureteroscopic holmium laser lithotripsy is the primary method for treating upper urinary tract stones [1]. After stone fragmentation, bacteria colonized within the stones may be released into the urine, and due to increased intraluminal ureteral pressure during surgery, bacteria may retrogradely enter the bloodstream. Postoperatively, patients may experience fever and elevated infection markers, such as white blood cell count (WBC),

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Table 1. Comparison of patients' characteristics between the two groups

Group	Age (year)	Weight (Kg)	Height (m)	BMI	Disease classification (case)		Stone diameter (mm)	Operative time (min)
					Renal stone	Ureterolith		
Positive group (n = 44)	43.73±13.65*	64.84±14.05*	1.68±0.06*	22.95±4.09*	14	30	11.77±4.60*	54.32±12.26*
Negative group (n = 129)	42.03±17.23	64.42±12.36	1.67±0.08	22.91±3.36	54	75	11.05±4.20	51.67±14.81

*P > 0.05.

Table 2. Clinical features potentially associated with positive bacterial culture of stone

Clinical characteristics	Positive A (n = 44)	Negative B (n = 129)
Sex		
Male	30	70
Female	14	59
Macrohematuria		
Yes	9	41
No	35	88
History of diabetes		
Yes	17	7
No	27	122
History of Urinary calculus		
Yes	18	50
No	26	79
History of calculus surgery		
Yes	14	50
No	30	79
Bilateral upper urinary calculi		
Yes	26	49
No	20	6
Hydronephrosis		
Yes	28	28
No	16	101
Stone density		
Low	30	79
High	14	50
Infectious lithiasis		
Yes	16	7
No	28	122
White blood cells in urine		
Positive	31	45
Negative	13	84
Blood creatinine		
Normal	33	17
Abnormal	11	112

C-reactive protein (CRP), and procalcitonin (PCT) [2]. While some studies have explored the distribution of pathogens on stone surfaces, research on the relationship between stone culture results and clinical characteristics, such as the risk factors for positive stone cultures and the correlation with postoperative infections, remains limited [3-5]. This study aims to perform bacterial cultures on upper urinary tract stone specimens, investigate the types and distribution of pathogens, and identify the risk factors for positive stone cultures and their correlation with clinical outcomes. The findings may provide a practical basis for surgeons in managing surgical infections.

Patient and sample information

We retrospectively analyzed the clinical data of 173 patients with upper urinary tract stones who were hospitalized at Ningxiang People's Hospital in Hunan Province from January 2018 to September 2023. The study protocol was approved by the Ethics Committee of Ningxiang People's Hospital (Ethical approval number: 202325). Among the 173 patients (100 males, 73 females), the average age was 42.5±16.4 years. A total of 123 patients experienced renal colic before admission, while 50 patients had no pain. Based on the results of the stone culture, patients were divided into two groups: a positive culture group and a negative culture group. No statistically significant differences were found by t-test in baseline data between the two groups (**Table 1**). We also collected clinical characteristics potentially related to stone culture, including gender, presence of gross hematuria, history of diabetes, history of urinary stones, history of stone surgery, whether the stones were bilateral, presence of hydronephrosis, stone density, presence of struvite stones, presence of leukocytes in urine, and abnormal blood creatinine levels (**Table 2**).

Methods and materials

All patients were scheduled for ureteroscopic lithotripsy. The patients had no prior antibiotic use before admission and underwent routine urine tests and urine bacterial culture before receiving any antibiotics after admission. The urine specimen (5.0 mL) were collected after disinfecting the vulva for female patients and the glans for male patients with povidone-iodine. Urine samples were subjected to routine urine tests and bacterial culture within 30 minutes. Upon admission, patients also underwent routine blood, CRP, and PCT tests. Patients with abnormally elevated preoperative PCT were excluded in this study.

If routine urine tests showed negative results for both leukocytes and urine nitrites, and blood WBC count was < 10.0×10^9/L, no antibiotics were administered before surgery. Instead, a preventive infusion of 0.5 g levofloxacin was given 30 minutes before surgery. On the first postoperative day, if the patient did not have a fever and infection indicators (blood leukocytes) were lower than preoperative levels, antibiotic prophylaxis was not extended beyond 48 hours, even if urine leukocytes remained elevated. If the patient developed fever, persistent CRP elevation, or abnormally elevated PCT, antibiotic therapy was extended until both body temperature and PCT returned to normal.

If the routine urine test at admission showed positive leukocytes (> 10 WBC/μL) or positive urine nitrite (+), levofloxacin (0.5 g/d) was administered for anti-infection treatment until both urine leukocytes and urine nitrites turned negative, and blood WBC count was < 10.0×10^9/L, after which surgical treatment could proceed. Surgical instruments included rigid ureteroscopy (KARL STORZ, 9.5F) or flexible ureteroscopy (Olympus, 8F). A holmium laser (Lumenis, Power Suite 60w) was used to fragment the stones, followed by stone extraction with forceps (KARL STORZ, 5.0F, Germany). Stone samples were collected intraoperatively for bacterial culture. The stone culture procedure was as follows: After extraction, the stone samples were washed three times with sterile saline to remove any surface urine that might contain antibiotics. The stones were ground into powder, and 5.0 mL of saline was added, followed by thorough shaking. The resulting solution was subjected to bacterial culture within 30 minutes. Stone culture sampling was completed on the operating table to minimize contamination. Pathogen identification was conducted using the BD Phoenix-100 automated microbiology identification system (BD, Franklin Lakes, NJ, USA). Positive urine culture was defined according to the European Association of Urology guidelines: bacterial colony count > 10^5 colony-forming units/mL [6], and the same criteria applied to positive stone cultures [7].

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Measurement data were expressed as mean ± standard deviation and compared between groups using independent t-test. Count data were expressed as numbers or percentages and compared using the chi-square test. Spearman rank correlation analysis was conducted to assess the relationship between positive stone and urine culture results and postoperative infection-related indicators. A binary logistic regression analysis was conducted to identify the risk factors for positive stone culture results based on specific clinical characteristics. Risk factors were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A P-value < 0.05 was considered statistically significant.

Results

Among the 173 patients, 44 had positive stone culture results, yielding a positivity rate of 25.43%. A total of 47 pathogen strains were detected in these 44 patients (3 patients had 2 strains cultured). As shown in **Figure 1**, the predominant pathogens included *Escherichia coli* (22 strains, 46.81%), *Proteus mirabilis* (5 strains, 10.64%), *Pseudomonas aeruginosa* (4 strains, 8.51%), *Klebsiella pneumoniae* (3 strains, 6.38%), *Staphylococcus aureus* (3 strains, 6.38%), *Acinetobacter baumannii* (2 strains, 4.26%), *Enterococcus faecium* (2 strains, 4.26%), and other pathogens (6 strains, 12.77%, including 2 strains of fungi).

Among the same 173 patients, 46 had positive urine bacterial culture results, with a positivity of 26.59%. A total of 49 pathogen strains were detected in these 46 patients (3 patients had 2 strains cultured). As shown in **Figure 2**, the main pathogens included *Escherichia coli* (26 strains, 53.06%), *Klebsiella pneumoniae* (5 strains, 10.2%), *Acinetobacter baumannii* (4

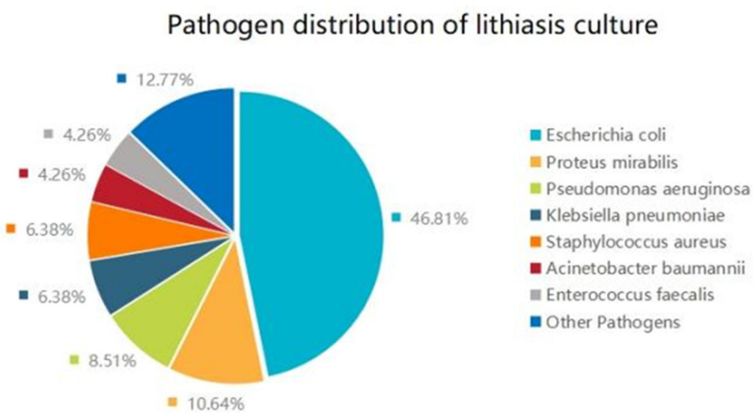

Figure 1. The predominant bacteria in stone culture included Escherichia coli (46.81%), followed by Proteus mirabilis (10.64%), Pseudomonas aeruginosa (8.51%), Klebsiella pneumoniae (6.38%), Staphylococcus aureus (6.38%), Acinetobacter baumannii (4.26%), Enterococcus faecalis (4.26%) and other pathogens (12.77%).

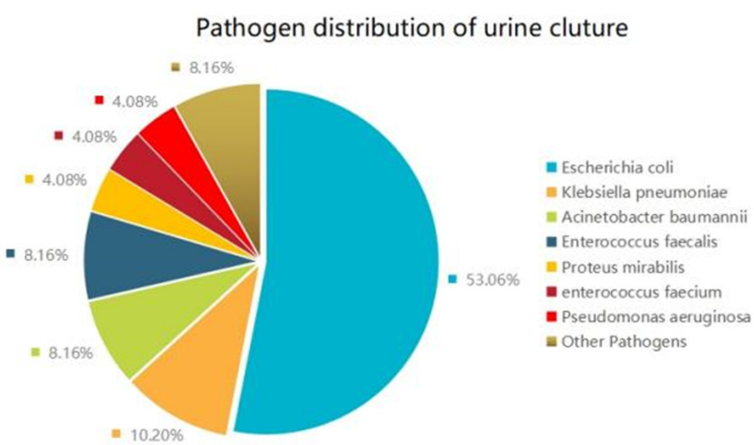

Figure 2. The predominant bacteria in urine culture included Escherichia coli (53.06%), followed by Klebsiella pneumoniae (10.20%), Acinetobacter baumannii (8.16%), Enterococcus faecalis (8.16%), Proteus mirabilis (4.08%), enterococcus faecium (4.08%), Pseudomonas aeruginosa (4.08%) and other pathogens (8.16%).

strains, 8.16%), *Enterococcus faecium* (4 strains, 8.16%), *Proteus mirabilis* (2 strains, 4.08%), *Enterococcus faecalis* (2 strains, 4.08%), *Pseudomonas aeruginosa* (2 strains, 4.08%), and other pathogens (4 strains, 8.16%, including 2 strains of fungi).

Binary logistic regression analysis revealed that the risk factors for a positive stone culture included: positive urinary leukocyte (OR = 2.881, 95% CI = 1.070-7.760, P = 0.036), hydronephrosis (OR = 5.644, 95% CI = 2.168-14.696, P < 0.0001), struvite stones (OR = 7.512, 95% CI = 1.864-30.283, P = 0.005), and a history of diabetes (OR = 6.580, 95% CI = 1.820-23.791, P = 0.004) (**Table 3**). Other clinical characteristics, such as gender, gross hematuria, a history of stones, bilateral upper urinary tract stones, history of stone surgery, stone density, and serum creatinine levels, were not identified as risk factors for a positive stone culture.

Spearman rank correlation analysis was performed to assess the relationship between positive stone and urine culture results and postoperative infection indicators. The results indicated that a positive stone culture was correlated with postoperative fever and elevated CRP levels that did not return to normal within 48 hours. This correlation was stronger than that observed for positive urine bacterial culture results (**Table 4**).

Discussion

With the advancement of microbiome research, it has been discovered that even urine with negative leukocytes in routine urinalysis can still harbor various bacteria. However, due to their low concentration, these pathogens are difficult to detect, and the patient may not exhibit symptoms of urinary tract infection [8]. For patients with upper urinary tract stones, bacteria in the urine may colonize the stone surface, rapidly proliferate, and intermittently release into the urine, leading to asymptomatic bacteriuria [9, 10]. Since colonized bacteria can produce biofilms and form a drug-resistant barrier, they are difficult to eliminate with short-term antibiotic application [11]. In this study, none of the patients had long-term antibiotic therapy. Before performing stone culture, we thoroughly rinsed the stones to remove residual antibiotics, then ground the stones into powder to release the colonized bacteria, and identified

Table 3. Risk factor for positive lithiasis culture

Factors	OR	95% CI	P
White blood cells in urine			
Positive vs Negative	2.881	1.070-7.760	0.036
Hydronephrosis			
Yes vs No	5.644	2.168-14.696	0.000
Infectious lithiasis			
Yes vs No	7.512	1.864-30.283	0.005
History of diabetes			
Yes vs No	6.580	1.820-23.791	0.004

Table 4. Correlation between stone or urine culture with specific clinical characteristics ($P < 0.05$)

	Fever after surgery	Unrecovered CRP after 48 h	Unrecovered blood WBC after 48 h	Abnormal Procalcitonin after surgery
Stone culture	0.666	0.633	0.413	0.501
Urine culture	0.385	0.443	0.229	0.259

the pathogens through bacterial culture. Therefore, the results of stone culture in this study are reliable.

In this study, *Escherichia coli* was the predominant pathogen in both stone and urine cultures, consistent with its role as the most common colonizing bacterium in the urinary tract and the leading cause of urinary tract infections [12, 13]. Our findings align with those of other researchers [14]. In stone culture, the proportions of *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* followed that of *Escherichia coli*. These opportunistic pathogens, typically colonizing the skin of the vulva, do not survive well in normal urine but can proliferate on the surface of stones, forming bacterial biofilms [15-17]. Proteus mirabilis, Pseudomonas aeruginosa, and Escherichia coli, are Gram-negative bacteria, suggesting that antibiotics targeting Gram-negative bacilli should be considered for empirical treatment. Additionally, *Proteus mirabilis* and *Staphylococcus aureus* are common pathogens in infectious stones [18]. In urine bacterial culture, the proportions of *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterococcus faecium* also followed that of *Escherichia coli*; these three bacteria are also common bacteria causing urinary tract infections [19-21]. Given our strict measures to avoid specimen contamination during urine collection, these results likely exclude contamination by vulvar bacteria. Fungi were detected in both stone and urine cultures, which is typically associated with dysbiosis [22]. Other researchers have also detected fungi in stone culture [23]. Since none of the patients in this study had long-term antibiotic use, we speculate that urinary tract obstruction by upper urinary tract stones may promote bacterial adherence, leading to dysbiosis and subsequent fungal proliferation, though further research is needed to support this. Therefore, when considering postoperative anti-infection treatment, the potential of fungal infection should be considered.

The results of stone bacterial culture can be influenced by many factors, and we explored the relationship between clinical characteristics and positive stone culture. The presence of leukocytes in the urine indicates potential urinary tract infection due to pathogen invasion, as bacteria can adhere to and colonize the surface of stones [24]. Our study also indicates that the presence of leukocytes in the urine is a risk factor for positive stone culture. Thus, it is important to ensure that urinary leukocytes are negative before performing lithotripsy. Diabetes is known to be a risk factor for multiple infections, as elevated glucose levels in the urine promote bacterial growth and reproduction [25]. Therefore, it is understandable that diabetes is a risk factor for positive stone culture. The results also suggest that controlling blood glucose before surgery may reduce the incidence of postoperative infection. Urinary obstruction leads to urine accumulation in the proximal ureter, which facilitates bacterial growth on the stone surface [26]. This study found that hydronephrosis is a risk factor for positive stone culture, supporting this explanation. For patients with severe preoperative hydronephrosis, increased vigilance regarding postoperative infection risk is necessary. Struvite stones, which account for approximately 10-15% of all stone types, are primarily composed of struvite and carbonate apatite [27]. These stones are loose in texture, have low density, and are often associated with recurrent urinary tract infections. They may harbor a large number of urease-producing bacteria [28], making struvite stones a risk factor for positive stone culture. If struvite stones are suspected before surgery, surgeons should be particularly cautious about the risk of postoperative infection. While previous studies have shown that female sex is a risk factor for positive urine culture [29], our study found that sex is not a risk factor for positive urine culture. The possible explanation is that women are more prone to lower urinary tract infections. However, when the upper urinary tract calculi are complicated by infection, gender factors do not appear to play a significant role.

The most common complication of upper urinary tract stones is infection, typically indicated by abnormally elevated infection markers before surgery or delayed normalization of these markers after surgery. Infection may lead to fever and abnormal elevations in CRP, which usually return to normal within 48 hours postoperatively. Prophylactic use of antibiotics before surgery can eliminate bacteria in the urine, but not those colonized on the stones. This study demonstrates that a positive stone culture is correlated with postoperative fever and delayed normalization of CRP levels, with this correlation being stronger than that observed with positive urine bacterial culture. These findings suggest that routine stone culture in patients with upper urinary tract stones can provide valuable guidance for postoperative anti-infection treatment.

Limitations

This study has several limitations. (1) Of the variety of bacteria adhering to the urinary tract and stone surface, only the dominant are detected by routine bacterial culture. 16s-rDNA sequencing, which can detect a broader range of strains, would be more suitable; (2) The sample size in this study is relatively small, and larger cohort studies are needed to validate these findings; (3) Some stone or urine samples were not subjected to drug sensitivity test, preventing drug resistance analysis. Since drug sensitivity tests take several days to obtain and bacterial resistance patterns can vary, immediate postoperative anti-infection measures were still implemented, minimizing the impact of this limitation on clinical practice. However, we acknowledge that drug susceptibility testing can provide valuable insights for surgeons when selecting appropriate antibiotics. We look forward to incorporating drug sensitivity testing in future studies.

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Disclosure of conflict of interest

None.

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