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ORIGINAL RESEARCH

Bacteriological Analysis of Urine Cultures and Related Clinical Features in Patients with Infection Stones: A Single-Center Retrospective Cohort Analysis from Beijing, China

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Objective: To compare clinical and metabolic characteristics of infection stone patients according to urine culture results and to evaluate differences among *urea-splitting, non–urea-splitting*, and *urine culture–negative groups*.

Methods: A retrospective cohort study was conducted in 809 patients with infection stones, categorized as *urea-splitting, non–urea-splitting*, or *urine culture–negative*. Continuous variables were analyzed according to distribution: normally distributed data were compared using one-way ANOVA (with Bonferroni-adjusted post-hoc tests), or Welch's ANOVA when variances were unequal; non-normally distributed data were assessed using the Kruskal–Wallis test followed by Dunn's post-hoc test. Categorical variables were compared using Chi-square or Fisher's exact tests with Bonferroni-adjusted pairwise comparisons when appropriate. A two-sided $P < 0.05$ was considered statistically significant.

Results: Among 809 patients with infection stones, urine cultures were positive in 519 (64.1%), including 255 (31.5%) with *urea-splitting* bacteria and 264 (32.6%) with *non–urea-splitting* bacteria; 290 (35.8%) were culture-negative. *Ureaplasma urealyticum* was the most common *urea-splitting* organism, and *Escherichia coli* predominated among *non–urea-splitting* bacteria. Clinical features, stone characteristics, and 24-hour urine indices were similar between the *urea-splitting* and *non–urea-splitting groups*. In contrast, the *urine culture-negative group* had smaller stones, a higher proportion of mixed infection stones, and significantly higher 24-hour urinary potassium, calcium, phosphorus, and uric acid excretion.

Conclusion: *U. urealyticum* was the most frequently detected urea-splitting organism in this cohort. While *urea-splitting* and *non–urea-splitting groups* showed similar clinical and metabolic profiles, the *urine culture–negative group*s exhibited distinct metabolic abnormalities and stone characteristics, suggesting a potentially different pathophysiologic pattern. Further studies are warranted to clarify the relationship between culture negativity and metabolic disturbances in infection stone formation.

Keywords: infection stones, *Ureaplasma urealyticum*, urea-splitting bacteria, URINARY microbiology, host metabolic response

Introduction

Urolithiasis is a common urological condition that has been increasing in prevalence over the past few decades. Globally, kidney stone disease has become an increasingly significant health concern, placing a growing burden on healthcare systems. In the United States, over $2 billion is invested annually for treating urolithiasis.[1] The prevalence of urolithiasis among Chinese adults is 5.8% (6.5% for men and 5.1% for women), with an average of 1 in 17 Chinese adults suffering from the condition.[2] Infection stones, a specific subset of urolithiasis, represent approximately 10%–15% of cases with the condition. Infection stones are characterized as follows: 1. They are particularly prone to becoming renal staghorn stones (occupying the renal pelvis and branch to at least the renal infundibula); 2. They grow rapidly and can become

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stones in approximately 4 weeks; 3. They have a very high recurrence rate, with stone reformation within 1 year in approximately 36% of patients; and 4. They cause significant functional impairment to the kidney, and if not effectively treated, can cause renal abscesses, perinephric abscesses, and pyelonephritis xanthogranulomatous, leading to renal failure or death. A prior study showed that approximately 14% of patients with conservatively treated infection stones develop progressive renal failure, 9% require dialysis for deteriorating renal function, and the overall survival rate for patients with infection stones is only 41% over 15 years.[3]

Infection stone formation is associated with *urea-splitting* bacteria. The most common *urea-splitting* bacteria in the urinary system include *Proteus mirabilis* and *Pseudomonas aeruginosa*.[4] Urease enzymes split the urea in urine, producing ammonia and carbon dioxide. Ammonium ions increase the urine pH to form alkaline urine. Ammonia, magnesium, and phosphate in alkaline urine converge to form ammonium magnesium phosphate hexahydrate (struvite) and carbonate apatite, the most critical components of infection stones.[3] However, according to recent studies, detection of *non-urea-splitting* bacteria, such as *Escherichia coli*, in infection stones is increasing.[5] The high detection rate of *non-urea-splitting* bacteria in midstream urine cultures from patients with infection stones indicates that *non-urea-splitting* bacteria play an equally significant role in infection stone formation.[6] Given these observations, a clearer understanding of the clinical characteristics associated with non–urea-splitting bacteria in infection stones is needed.

Despite the well-recognized association between *Urea-splitting* bacteria and infection stones, several important clinical questions remain insufficiently addressed. First, recent studies have reported an increasing detection of *non–urea-splitting* bacteria in patients with infection stones, yet large cohort data characterizing their clinical features, stone composition patterns, and metabolic profiles remain limited, particularly in the Chinese population. Second, the relationship between urine culture findings (*urea-splitting, non–urea-splitting*, or culture-negative) and the clinical presentation of infection stones has not been clearly defined. Third, culture-negative infection stones—which are frequently encountered in routine practice—are poorly understood, and their metabolic characteristics have rarely been systematically examined. To address these gaps, the present study aimed to analyze, in a large retrospective cohort, the distribution of bacterial species in preoperative urine cultures of patients with infection stones and to compare the associated clinical characteristics, stone composition, and 24-hour urinary biochemical parameters across different microbiological groups. We hypothesized that patients with urea-splitting, non–urea-splitting, and culture-negative results may exhibit distinct clinical or metabolic profiles. This study seeks to provide updated bacteriological and clinical data to enhance understanding of infection stone phenotypes in contemporary clinical practice.

Materials and Methods

Patients and Study Design

A retrospective analysis was conducted on patients with infection stones who underwent percutaneous nephrolithotomy or ureterorenoscopy between January 2016 and December 2020. Intraoperative stone specimens were homogenized and analyzed for stone composition using Fourier transform infrared spectroscopy. Pure infection stones were defined as stones composed entirely of magnesium ammonium phosphate hexahydrate (struvite), with or without carbonate apatite. Mixed infection stones were defined as stones in which struvite and/or carbonate apatite were present but not at 100% composition.

A total of 809 patients with infection stones were included and categorized into three groups according to the first midstream urine culture obtained at admission: the *urea-splitting group*s, the *non–urea-splitting groups*, and the *urine culture–negative group*s (Figure 1). Admission urine culture is routinely performed in our institution for all patients scheduled for stone surgery and reflects the bacteriological status prior to the initiation of inpatient antimicrobial therapy. Contaminated midstream samples, identified by mixed flora, low colony counts, or laboratory contamination alerts, were excluded.

Positive urine cultures were defined as $>10^5$ CFU/mL for women and $>10^4$ CFU/mL for men. *U. urealyticum* was cultured using the Mycoplasma IST2 kit (bioMérieux, France), and other bacterial isolates were identified using the VITEK II Compact 60 analyzer. Clinical information collected included age, sex, body mass index (BMI), hypertension, diabetes mellitus, stone characteristics, urinalysis, the first urine culture obtained at admission, stone composition, and 24-hour urinary biochemical data.

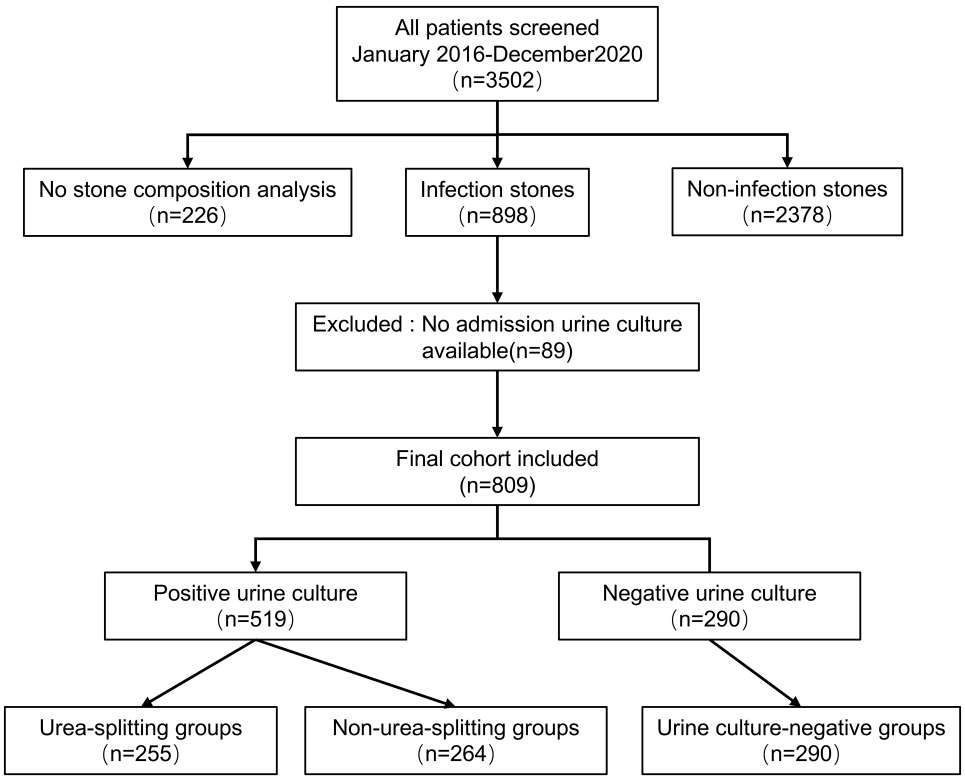

Figure 1 Flowchart of patient selection and final cohort composition.

To provide a contextual reference for urinary findings, we additionally summarized data from 100 individuals undergoing routine health examination at our center as a reference population (Supplementary Table S1).

Statistical Analysis

Measurement data from the *urea-splitting, non–urea-splitting*, and *urine culture–negative groups* were tested for normality using the Kolmogorov–Smirnov test. Homogeneity of variance was assessed using Levene's test. Normally distributed variables are presented as mean ± standard deviation and were compared using one-way analysis of variance (ANOVA). When the overall ANOVA was significant, Bonferroni-adjusted post-hoc pairwise comparisons were performed. For normally distributed variables with unequal variances, Welch's ANOVA was applied. Non-normally distributed variables are presented as median (interquartile range) and were compared using the Kruskal–Wallis test, followed by Dunn's post-hoc test for pairwise comparisons when appropriate. Categorical variables are presented as frequencies and percentages and were first compared using the chi-square test or Fisher's exact test when required. When an overall difference was observed, pairwise 2×2 comparisons were conducted with Bonferroni correction to adjust for multiple testing. A two-sided P-value <0.05 was considered statistically significant. All analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

Results

We identified 3502 patients in our stone database from January 2016 to December 2020, including 898 with infection stones, 2,378 with non-infection stones, and 226 who did not undergo stone composition analysis. Following exclusion of patients without urine culture, 809 patients with infection stones were included in the study population, including 374 females (46.2%) and 435 males (53.8%). A total of 228 (28.2%) cases had pure infection stones, including 47 (5.8%) struvite stones, 33 (4.1%) pure carbonate apatite stones, and 148 (18.3%) pure infection stones containing struvite and carbonate apatite. The remaining 581 (71.8%) cases contained one or more additional components (Table 1). The distribution of infection stones according to location was as follows: renal (502/809, 62.1%), renal and ureteral (177/

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Table 1 Comparison of Clinical Features Between the *Urea-Splitting, Non-Urea-Splitting*, and *Urine Culture-Negative Groups* with Infection Stones

	Urea-Splitting Groups	Non-Urea-Splitting Groups	Urine Culture-Negative Groups	P-value
Patients	255(31.5%)	264(32.6%)	290(35.9%)	
Age	47(37, 57)[a]	53(39, 61)	49(35, 58)	0.010
Gender				<0.001
Male(n)	130(51.0%)[a,b]	87(33.0%)[c]	218(75.2%)	
Female(n)	125(49.0%)	177(67.0%)	72(24.8%)	
Body Mass Index	24.87(22.1, 27.4)	24.49(22.3, 27.0)	24.46(22.5, 27.3)	0.569
Stone Events				0.722
Yes	167(65.5%)	176(66.7%)	184(63.4%)	
No	88(34.5%)	88(33.3%)	106(36.6%)	
Underlying Diseases				
Hypertensive	56(22.0%)	56(21.2%)	85(29.3%)	0.048
Diabetes	29(11.4%)	27(10.2%)	32(11.0%)	0.911
Biochemical testing				
Creatinine	70.9(56.3, 94.6)	67.2(54.2, 85.9)[c]	75.1(61.6, 88.7)	0.002
Uric Acid	344(273.5, 394.0)[b]	333(275.0, 396.0)[c]	363(302.0, 432.0)	0.001
eGFR	99.32(68.0, 111.1)	98.11(75.3, 108.9)	98.27(84.2, 110.7)	0.872
Parathyroid Hormone	50.17(36.8, 66.0)	50.61(35.3, 64.2)	48.59(36.7, 66.5)	0.768
Serum Calcium	2.24(2.2, 2.3)	2.22(2.2, 2.3)[c]	2.27(2.2, 2.3)	0.002
Surgery time	99.5(70.8, 133.0)[b]	97.0(74.0, 128)[c]	80.0(62.0, 114.0)	<0.001
Urine Analysis				
Nitrite				<0.001
Positive	54(21.2%)[a,b]	82(31.1%)[c]	5(1.7%)	
Negative	201(78.8%)	182(68.9%)	285(98.3%)	
Leukocyte				<0.001
Negative	47(18.4%)[a,b]	22(8.3%)[c]	119(41.0%)	
±	24(9.4%)	16(6.1%)	53(18.3%)	
1+	25(9.8%)	24(9.1%)	37(12.8%)	
2+	29(11.4%)	34(12.9%)	40(13.8%)	
3+	130(51.0%)	168(63.6%)	41(14.1%)	
Protein				<0.001
Negative	110(43.1%)[a,b]	106(40.2%)[c]	174(60.0%)	
±	42(16.5%)	51(19.3%)	50(17.2%)	
1+	86(33.7%)	93(35.2%)	59(20.4%)	
2+	11(4.3%)	11(4.2%)	7(2.4%)	
3+	6(2.4%)	3(1.1%)	0(0%)	
Urine pH	6.5±0.7[b]	6.4±0.6	6.3±0.6	0.007

Notes: [a]P < 0.0167 for Urea-splitting groups vs Non-urea-splitting groups; [b]P < 0.0167 for Urea-splitting groups vs Urine culture-negative groups; [c]P < 0.0167 for Non-urea-splitting groups vs Urine culture-negative groups.

809, 21.8%), and ureteral (130/809, 16.1%). In total, 75% of patients (607/809) had unilateral infection stones and 25% (202/809) had bilateral infection stones (Table 2).

Bacteria were cultured from 519 (64.1%) of 809 patients with infection stones, including 255 (31.5%) cases with *urea-splitting* bacteria and 264 (32.6%) cases with *non-urea-splitting* bacteria. A total of 290 (35.8%) patients were urine culture-negative. The most prevalent *urea-splitting* bacteria was *U. urealyticum* (77/809, 9.5%), followed by *P. mirabilis* (54/809, 6.7%), and *Staphylococcus spp*. (35/809, 4.3%). The most common *non-urea-splitting* bacteria were *E. coli* (149/809, 18.4%), *Enterococcus spp*. (60/809, 7.4%), and *Streptococcus spp*. (35/809, 4.3%), as shown in Figure 2.

Table 2 Comparison of Stone Characteristics Between the *Urea-Splitting, Non-Urea-Splitting*, and Urine Culture-Negative Groups with Infection Stones

	Urea-Splitting Groups	Non-Urea-Splitting Groups	Urine Culture-Negative Groups	P-value
Patients	255(31.5%)	264(32.6%)	290(35.9%)	
Number of stones				0.001
Single	54(21.2%)[a]	51(19.3%)[b]	92(31.7%)	
Multiple	201(78.8%)	213(80.7%)	198(68.3%)	
Laterality of stones				0.329
Left	97(38.0%)	98(37.1%)	131(45.2%)	
Right	92(36.1%)	98(37.1%)	91(31.4%)	
Left and Right	66(25.9%)	68(25.8%)	68(23.4%)	
Location of stones				<0.001
Renal	180(70.6%)[a]	185(70.1%)[b]	137(47.2%)	
Ureteral	25(9.8%)	16(6.1%)	62(21.4%)	
Renal and Ureteral	50(19.6%)	63(23.8%)	91(31.4%)	
Maximum stone diameter	28.0(18.0, 50.5)[a]	28.0(20.0, 60.0)[b]	20.0(15.0, 48.0)	<0.001
Stone composition				<0.001
Pure Infection Stone	101(39.6%)[a]	95(36.0%)[b]	32(11.0%)	
Mixed infection stones	154(60.4%)	169(64.0%)	258(89.0%)	
Pure Infection Stone composition				0.058
Struvite	23(22.8%)	14(14.7%)	10(31.3%)	
Carbonate apatite	16(15.8%)	10(10.5%)	7(21.9%)	
Mixed	62(61.4%)	71(74.8%)	15(46.8%)	

Notes: [a]P < 0.0167 for Urea-splitting groups vs Urine culture-negative groups; [b]P < 0.0167 for Non-urea-splitting groups vs Urine culture-negative groups.

Clinical Characteristics

Sex distribution differed significantly among the three groups ($P < 0.001$). Age also differed significantly among the groups ($P = 0.010$). The *urea-splitting groups* showed a nearly balanced sex composition (130 males and 125 females), whereas the *non–urea-splitting groups* demonstrated a female predominance (177 females and 87 males), and the *urine culture-negative groups* exhibited a marked male predominance (218 males and 72 females). Bonferroni-adjusted pairwise comparisons confirmed significant differences in sex distribution among all three groups (all adjusted $P < 0.0167$). Regarding age, the *non–urea-splitting groups* (53 years [39–61]) was significantly older than the *urea-splitting groups* (47 years [37–57]) after Bonferroni correction (adjusted $P < 0.0167$), while no significant age differences were observed between either group and the *urine culture-negative groups*. Serum creatinine levels also differed among the three groups ($P = 0.002$). Post-hoc comparisons revealed that the only significant difference occurred between the *non–urea-splitting groups* and *urine culture-negative groups* (adjusted $P < 0.0167$). Serum uric acid levels were significantly higher in the *urine culture-negative groups* than in both the *urea-splitting* and *non–urea-splitting groups*, consistent with the pairwise comparisons (adjusted $P < 0.0167$ for both). In contrast, no significant differences were detected in BMI, diabetes, or eGFR among the three groups. Routine urinalysis showed pronounced differences. The *non–urea-splitting groups* had the highest rates of nitrite positivity and leukocyturia, whereas the *urine culture-negative groups* had the lowest levels (all $P < 0.001$). Urinary protein positivity was significantly more frequent in the *urea-splitting* and *non–urea-splitting groups* than in the *urine culture-negative group*s, as confirmed by post-hoc testing (adjusted $P < 0.0167$). Urine pH was 6.5 ± 0.7 in the *urea-splitting group*s, 6.4 ± 0.6 in the *non–urea-splitting group*s, and 6.3 ± 0.6 in the *urine culture-negative groups*, with a significant difference observed only between the *urea-splitting* and *urine culture-negative groups* after Bonferroni adjustment (adjusted $P < 0.0167$) (Table 1). The mean urine pH in health examination participants was 5.7 ± 0.6, which was lower than that in the urea-splitting, non–urea-splitting, and urine culture-negative groups (Supplementary Table S1).

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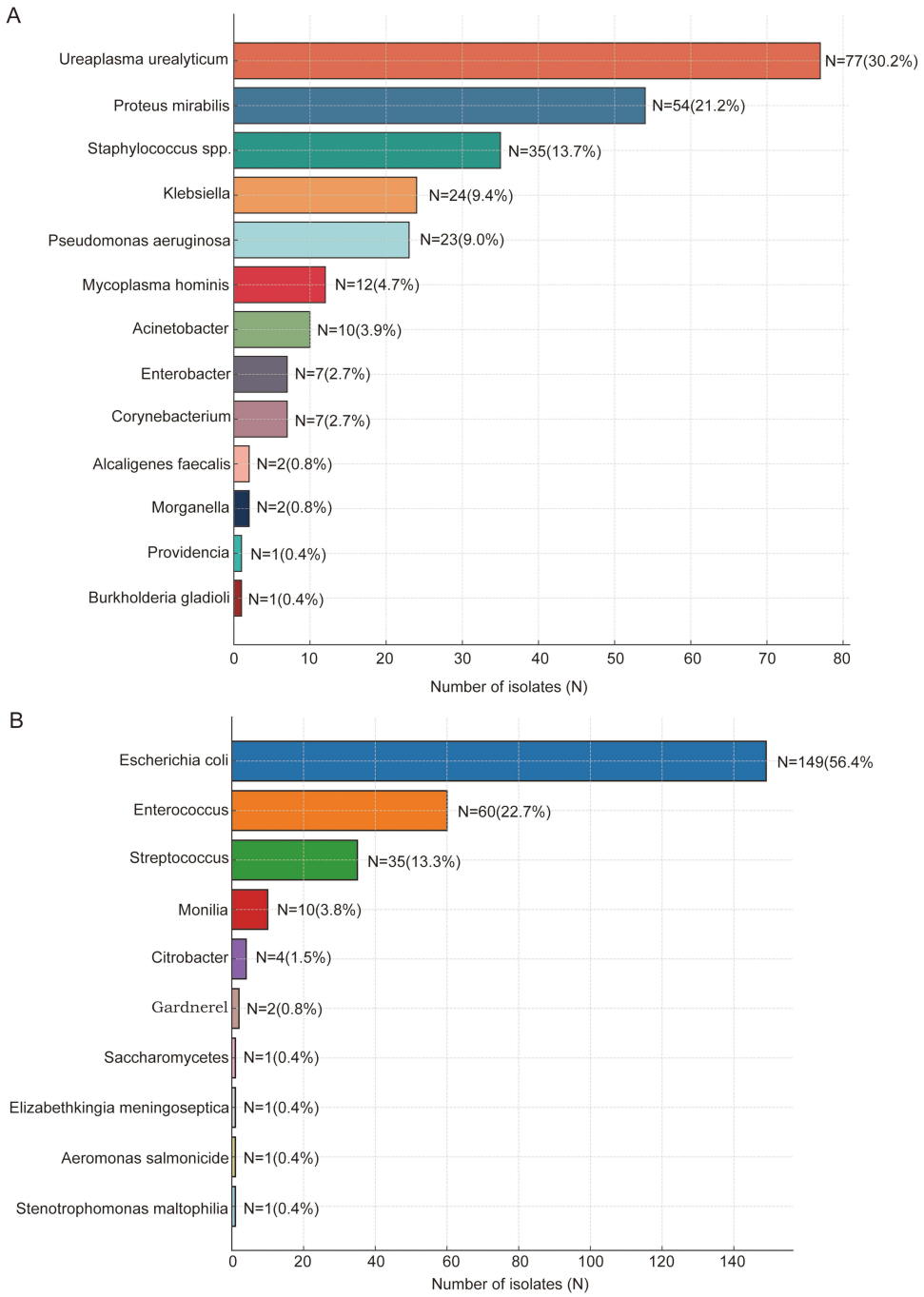

Figure 2 Urine culture results in patients with infection stones. (**A**) Distribution of urea-splitting bacteria. *U. urealyticum* was the most frequently identified urea-splitting species. (**B**) Distribution of Non–urea-splitting bacteria. *Escherichia coli* was the most commonly detected non–urea-splitting species.

Stone Characteristics

The number and distribution of stones differed significantly among the three groups. Multiple stones were present in 78.8% of the *urea-splitting groups* and 80.7% of the *non–urea-splitting groups*, compared with 68.3% of the *urine culture-negative groups* ($P = 0.001$). Post-hoc Bonferroni-adjusted comparisons confirmed that the lower prevalence of multiple stones in the urine culture-negative group was responsible for the overall difference (adjusted $P < 0.0167$), whereas no significant difference was observed between the *urea-splitting* and *non–urea-splitting groups*. The laterality of stones showed no significant differences among groups ($P = 0.329$). Left-sided stones accounted for 38.0% in the *urea-splitting group*s, 37.1%

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Table 3 Comparison of 24-h Urine Composition Between the *Urea-Splitting, Non-Urea-Splitting*, and Urine Culture-Negative Groups with Infection Stones

	Urea-Splitting Groups	Non-Urea-Splitting Groups	Urine Culture-Negative Groups	P-value
Patients	255(31.5%)	264(32.6%)	290(35.9%)	
24-hour urinalysis				
Volume (L/D)	2.1(1.6, 2.6)[a]	2.1(1.6, 2.6)[b]	2(1.5, 2.4)	0.008
Chlorine (mmol/D)	110.2(84.0, 150.7)	113.4(79.8, 148.5)	119.7(90.0, 160.6)	0.332
Sodium (mmol/D)	142.05(100.3, 192.9)	140.94(99.6, 185.0)	157.80(115.4, 214.0)	0.063
Potassium (mmol/D)	28.48(20.5, 36.2)[a]	28.10(22.3, 36.4)	31.80(23.8, 40.4)	0.009
Calcium (mmol/D)	3.11(1.7, 4.6)[a]	3.48(2.4, 5.0)	3.89(2.8, 5.7)	0.001
Phosphorus (mmol/D)	12.50(9.0, 17.1)[a]	12.81(9.1, 17.1)[b]	15.96(12.1, 21.5)	<0.001
Uric acid (μmol/D)	2493.2(1972.9, 3060.3)[a]	2675.0(2072.3, 3120.0)[b]	2826.9(2283.6, 3678.4)	0.001

Notes: [a]P < 0.0167 for Urea-splitting groups vs Urine culture-negative groups; [b]P < 0.0167 for Non-urea-splitting groups vs Urine culture-negative groups.

in the *non–urea–splitting group*s, and 45.2% in the *urine culture-negative groups*. Similar distributions were observed for right-sided and bilateral stones. In contrast, stone anatomical location showed marked differences ($P < 0.001$). Renal stones were the most common type in the *urea-splitting* (70.6%) and *non–urea-splitting groups* (70.1%), but were significantly less frequent in the *urine culture-negative groups* (47.2%). Bonferroni-adjusted post-hoc testing confirmed significant differences between the *urine culture-negative groups* and both the *urea-splitting* (adjusted $P < 0.0167$) and *non–urea-splitting groups* (adjusted $P < 0.0167$), while no significant differences were found between the two culture-positive groups. Conversely, combined renal-and-ureteral stones were most commonly observed in the *urine culture-negative group*s (31.4%), significantly higher than in the *urea-splitting* (19.6%) and *non–urea-splitting groups* (23.8%). Maximum stone diameter differed significantly among the groups ($P < 0.001$). Stones were significantly larger in both the *urea-splitting* (28.0 mm [18.0–50.5]) and *non-urea-splitting groups* (28.0 mm [20.0–60.0]) than in the *urine culture-negative group*s (20.0 mm [15.0–48.0]), as confirmed by post-hoc comparisons (both adjusted $P < 0.0167$). Stone composition also differed substantially among the groups ($P < 0.001$). Pure infection stones were more frequent in the *urea-splitting* (39.6%) and *non–urea-splitting groups* (36.0%) compared with the *urine culture-negative group*s (11.0%). Bonferroni-adjusted pairwise comparisons showed significant differences between the *urine culture-negative groups* and both the *urea-splitting* (adjusted $P < 0.0167$) and *non-urea-splitting groups* (adjusted $P < 0.0167$). However, no significant difference was observed between the two culture-positive groups. Among patients with pure infection stones, the distribution of struvite, carbonate apatite, and mixed struvite–carbonate apatite stones did not differ significantly among the three groups ($P = 0.058$), although the borderline P-value warrants cautious interpretation (Table 2).

24-Hour Urine Analysis

The 24-hour urine volume was similar between the *urea-splitting* and *non–urea-splitting groups* (2.1 [1.6–2.6] L for both), but both were significantly higher than the *urine culture-negative groups*(2.0 [1.5–2.4] L), as confirmed by Bonferroni-adjusted post-hoc comparisons (adjusted $P < 0.0167$ for both). Although 24-hour urinary chloride and sodium excretion tended to be higher in the *urine culture-negative group*s, neither difference reached statistical significance (chloride: $P = 0.332$; sodium: $P = 0.063$). By contrast, several solute excretion parameters showed significant group differences. The *urine culture-negative groups* exhibited significantly higher 24-hour urinary potassium and calcium excretion compared with the *urea-splitting groups* (both adjusted $P < 0.0167$). Phosphorus and uric acid excretion were also highest in the *urine culture-negative groups*, with post-hoc analysis confirming significant differences between the *urine culture-negative groups* and both the *urea-splitting* and *non–urea-splitting groups* (all adjusted $P < 0.0167$). No significant differences in any 24-hour urine solute were observed between the *urea-splitting* and *non–urea-splitting groups* (Table 3).

Discussion

Urolithiasis is a disease that is prone to recurrence. It is mostly of noninfectious etiology, such as low fluid intake, hot climate, and a combination of certain metabolic diseases (hypertension, gout, obesity).[7] Infection stones represent a distinct subgroup of urolithiasis, traditionally associated with *Urea-splitting* bacteria such as Proteus mirabilis. Although infection stones account for approximately 10–15% of all urinary stones,[8] the proportion in our center was higher (25.6%). This likely reflects the characteristics of a tertiary referral hospital that manages complex or recurrent stone disease rather than a true epidemiologic increase. Patients with infection stones also tend to present with concomitant urinary tract infection and higher surgical complexity, further contributing to increased representation in specialized centers.

In early studies, infection stone formation was closely associated with *Urea-splitting* bacteria as represented by *P. mirabilis*, which hydrolyzes urea to ammonium and bicarbonate to form an alkaline environment and promotes the formation of infection stones.[6,9] However, recent studies have shown that *non–urea-splitting* bacteria contribute more substantially to infection stone formation than previously understood. Razi et al[10] and Li et al[11] highlight that diverse bacterial species—including *E. coli*—can participate in stone pathogenesis through biofilm formation, virulence factors, or cooperative interactions with *Urea-splitting* bacteria. A multicenter European cohort reported that only 30% of infection stones were associated with *Urea-splitting* bacteria.[12] Our results align with these contemporary findings: only 31.6% of infection stones were associated with *urea-splitting* bacteria, whereas *E. coli* was detected in 18.4% cases. These observations suggest that infection stones are not exclusively driven by classical urease-mediated alkalinization but instead involve a broader spectrum of bacterial communities, consistent with emerging models of polymicrobial infection and microbial–metabolic interactions.[11]

A key observation in our study was the substantial similarity in stone characteristics between the *urea-splitting* and *non–urea-splitting* groups. These two groups showed no significant differences in stone composition (struvite, carbonate apatite, or mixed struvite–carbonate apatite stones), stone diameter, stone location, or number of stones. Only the urine culture-negative group differed significantly, exhibiting smaller maximum stone diameters, a higher proportion of mixed infection stones, and markedly higher 24-hour urinary excretion of potassium, calcium, phosphorus, and uric acid. Together, these findings suggest that *non–urea-splitting* bacteria may contribute to infection stone formation through mechanisms beyond classical urease-mediated alkalinization. However, stone composition in this study was analyzed mainly at a broad categorical level, and more detailed compositional characterization beyond these categories was not available in the current retrospective dataset. These clinical observations raise the possibility that infection stone formation may not rely solely on classical urease-mediated alkalinization. Recent molecular studies support a more nuanced understanding of urease activity in the urinary tract. Fitzgerald et al[13] demonstrated that urease gene expression in *Proteus mirabilis* is strongly dependent on the urinary environment: UreR activates urease genes in a urea-dependent manner, and the maturation of urease requires nickel import through the Ynt transport system, which is preferentially active during urinary tract infection. These findings indicate that urease activity varies with urinary conditions such as urea availability and metal ion homeostasis, suggesting that different bacterial species may contribute to local alkalinization to different extents. Another possibility is that some *non–urea-splitting* bacteria may acquire urease-related functions through genetic exchange. Prior studies have shown that urease genes can reside on plasmids in *urea-splitting* bacteria and, theoretically, may be transferred between species through mechanisms such as genomic transformation or conjugation. While our study did not include molecular assays to assess gene transfer, the structural organization of urease loci reported in recent genomic studies—some of which are linked to mobile genetic elements—supports the biological plausibility of this hypothesis.[14,15] In addition, polymicrobial infection may also play a role. *E. coli*, the most common organism among *non–urea-splitting* bacteria in our cohort, replicates rapidly and can outgrow slower *Urea-splitting* bacteria in routine culture, especially under alkaline conditions.[16] This may lead to under-detection of *Urea-splitting* bacteria and over-representation of *non–Urea-splitting* isolates in urine cultures. Biofilm formation further supports interactions between bacterial species: recent studies indicate that *Urea-splitting* and *non–urea-splitting* bacteria can coexist within biofilms, enabling metabolic cooperation and creating localized alkaline microenvironments that promote crystallization even when urease activity is limited.[10] Our urine pH findings are compatible with these possibilities. Previous studies have shown that urine pH in healthy adults is physiologically variable and is influenced by factors such

as sex, diet, and systemic acid–base balance.[17] In addition, reference studies in healthy populations have generally reported urine pH values around 6.0,[18] providing a useful clinical context for interpretation. In our supplementary reference cohort, the mean urine pH among individuals undergoing routine health examination at our center was 5.7 ± 0.6, which is broadly consistent with these published data. The slightly lower value in our cohort may be related to the use of random urine samples, which are more susceptible to variation due to timing of collection, dietary intake, and other short-term physiological factors. Against this background, all three infection-stone subgroups showed higher urine pH values than the health examination participants (6.5 ± 0.7 in the urea-splitting group, 6.4 ± 0.6 in the non–urea-splitting group, and 6.3 ± 0.6 in the urine culture-negative group vs 5.7 ± 0.6 in health examination participants). Notably, even the urine culture-negative group maintained a higher urine pH than the healthy reference population. Although the *urea-splitting groups* had a slightly higher mean urine pH than the *non–urea-splitting groups* (6.5 vs. 6.4), the difference did not reach statistical significance. Meanwhile, the urine culture-negative group showed the lowest urine pH but the highest metabolic abnormalities, reinforcing the potential importance of metabolic–microbial interactions in infection stone formation. While our retrospective clinical data cannot directly validate these molecular mechanisms, the convergence of stone phenotypes between the *urea-splitting* and *non–urea-splitting groups* suggests that infection stone formation may arise from complex interactions involving polymicrobial colonization, horizontal transfer of urease-related genes, biofilm-associated metabolic cooperation, and host metabolic factors. Further clinical and experimental studies are needed to clarify the extent to which these mechanisms operate in infection stone pathogenesis.

Our study also provides new insights into the metabolic characteristics of patients with infection stones. Traditionally, metabolic evaluation has not been emphasized in infection stone management, as earlier reports suggested a relatively low prevalence of metabolic abnormalities in this population, and major U.S. and European guidelines do not routinely recommend 24-hour urine testing for infection stone formers.[3,19] However, emerging evidence suggests that metabolic factors may still influence stone growth and recurrence. Iqbal et al[20] reported that although pure and mixed infection stones showed no differences in 24-hour urine parameters, patients who received metabolic evaluation and targeted intervention experienced lower rates of stone-related events, highlighting the potential clinical relevance of metabolic abnormalities even in infection stone disease. In contrast to earlier studies, our results demonstrated distinct metabolic patterns among the three bacterial groups. Notably, patients in the *urine culture-negative groups* exhibited substantially higher 24-hour urinary excretion of potassium, calcium, phosphorus, and uric acid compared with both the urea-splitting and *non–urea-splitting groups*, with most differences remaining significant after pairwise comparison. These findings suggest that infection stones in culture-negative patients may be more strongly influenced by metabolic disturbances than by bacteriological factors. Conversely, the relatively lower metabolic burden in the *urea-splitting* and *non–urea-splitting groups* may help explain the similarity in stone characteristics between them, despite different microbiological profiles. Taken together, our findings raise the possibility that infection stone formation may involve variable combinations of microbial activity and metabolic imbalance, rather than a uniform pathophysiologic pathway across all patients. Future studies integrating microbiological, metabolic, and molecular analyses are needed to clarify how these factors interact and to determine whether targeted metabolic management could benefit selected patients with infection stones.

A notable finding in our study was the high detection rate of *U. urealyticum* (30.2%) among urea-splitting bacteria—an observation rarely reported in the past two decades. Early studies demonstrated that *U. urealyticum* can contribute to infection stone formation: as early as 1984, increasing colony counts of *U. urealyticum* were shown to alkalinize urine and promote infective stone development[21] and a 1998 clinical study reported that *U. urealyticum* was cultured from 30% (43/145) of patients with infection stones. However, in the years since, most reports of infection stones have identified *P. mirabilis* as the predominant Urea-splitting bacterium, with *U. urealyticum* rarely detected. In contrast, *U. urealyticum* was the most common urea-*splitting* bacterium in our cohort (77/255, 30.2%), followed by *P. mirabilis* (21.2%). This unexpectedly high detection rate warrants methodological clarification. Unlike routine aerobic urine culture, *U. urealyticum* requires specialized conditions for isolation—including the use of dedicated A7/A8 agar or PPLO media, rapid sample processing, and cultivation at 37 °C in a humid environment with 5–10% CO_2—because its lack of a cell wall renders it highly sensitive to desiccation, pH fluctuations, and temperature changes,[22,23] Many hospitals do not routinely perform *U. urealyticum* culture, leading to substantial under-detection in standard clinical practice. Our center routinely incorporates expanded-spectrum urine cultures that include *U. urealyticum* testing, which likely

contributed to the higher detection frequency observed in this study. Nevertheless, this finding should be interpreted cautiously and not inferred as reflecting its true national prevalence. Further multicenter studies using standardized microbiological methods are needed. Additionally, our study relied exclusively on midstream urine cultures; pelvic urine and stone-core cultures, which more accurately reflect upper urinary tract microbiology and increase detection of fastidious or low-abundance organisms,[24,25] were not performed. This methodological limitation may have influenced the bacteriological profile observed. Given the potential pathogenic role of *U. urealyticum* in infection stones and the higher detection rate observed in our population, screening for *U. urealyticum* may be considered in selected patients with suspected infection stones, particularly in centers with the capacity for expanded-spectrum culture. Appropriate antimicrobial therapy guided by susceptibility testing may help reduce recurrence risk, although our study did not include postoperative follow-up to evaluate clinical outcomes. Future prospective studies are required to determine whether targeted antimicrobial treatment for *U. urealyticum*–positive patients can improve long-term outcomes in infection stone disease.

This study has several limitations. First, it was a single-center retrospective study, and the characteristics of the enrolled patients may have been influenced by regional healthcare-seeking patterns and referral practices, resulting in potential single-center referral bias that may limit the generalizability of our findings. In addition, as with all retrospective studies, the completeness of medical records may have affected the availability of some clinical variables. Second, we analyzed the first urine culture obtained at admission, and some patients may have taken antibiotics before hospitalization; therefore, the influence of prior antibiotic exposure on culture results—particularly the suppression of low-abundance *Urea-splitting* bacteria—cannot be completely excluded. Detailed data on prior infection history were also not consistently available in the current retrospective dataset. Third, this study relied solely on the first midstream urine culture obtained at hospital admission, without pelvic urine cultures or stone-core microbiological testing, which may have resulted in missed detection of localized upper urinary tract infections or mixed infections. Fourth, stone composition was analyzed mainly at a broad categorical level, and more detailed compositional characterization beyond these categories was not available in the current retrospective dataset. Fifth, the hypotheses that *non-urea-splitting* bacteria may contribute to stone formation through acquisition of urease genes or coexistence with *Urea-splitting* bacteria are based solely on clinical observations in this study and remain speculative without molecular validation; no urease gene assays or sensitive detection methods such as metagenomic sequencing or qPCR were performed. In addition, variations in 24-hour urine testing methods and reference ranges across laboratories may have influenced the metabolic comparisons, and our exploration of metabolic abnormalities was limited by the retrospective design and the available testing framework. Finally, postoperative follow-up, including stone recurrence and antibiotic treatment outcomes (eg., in *U. urealyticum*-positive patients), was not available, limiting further assessment of the clinical implications of pathogen screening and metabolic evaluation. Despite these limitations, the relatively large sample size allowed us to identify several meaningful patterns in the bacteriological profiles and metabolic characteristics of patients with infection stones. Future multicenter, prospective studies incorporating pelvic urine or stone-core microbiology, molecular mechanism studies, and long-term follow-up are needed to further clarify the roles of *Urea-splitting* and *non-Urea-splitting* bacteria in infection stone formation and to better evaluate the clinical utility of pathogen screening and metabolic assessment.

Conclusion

In this retrospective cohort, we observed a notably high detection rate of *U. urealyticum* among urea-splitting bacteria in patients with infection stones, a finding that underscores the importance of expanded microbiological testing in selected clinical settings. The *Urea-splitting* and *non–Urea-splitting* groups showed similar stone characteristics, suggesting that infection stone formation may involve mechanisms beyond classical urease-mediated alkalinization. In contrast, patients in the urine culture–negative group demonstrated pronounced metabolic abnormalities and a higher proportion of mixed infection stones, indicating that metabolic disturbances may play a greater role in this subgroup. These findings highlight the heterogeneous nature of infection stones and suggest that both microbial and metabolic factors should be considered in their clinical evaluation and management.

Data Sharing Statement

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

This retrospective cohort study was approved by the Ethics Committee of Beijing Tsinghua Changgung Hospital (Beijing, China) (Approval No. 25976-6-01). The study protocol was reviewed and approved in accordance with the ethical standards of the institutional research committee and with the principles of the Declaration of Helsinki. Given the retrospective nature of the study and the use of anonymized clinical data, the requirement for written informed consent was waived by the Ethics Committee.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no relevant conflicts of interest in this work.

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