International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 11-08-2018; Accepted: 14-09-2018
www.medicalsciencejournal.com
Volume 4; Issue 10; October 2018; Page No. 70-75

The pattern of microbial infection in patients with upper urinary tract stone disease: A prospective study

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Abstract
Introduction: The contribution of bacteria in the pathogenesis of stone formation is a well-established fact. The incidence of SIRS increases when there is both positive urine culture and positive stone culture in comparison when one of them is absent.
We aimed to analyse and find a correlation between stone composition, stone microbial profile and urine microbial profile in these patients.
Materials and methods: It was a prospective study conducted from March 2015 to February 2018. Preoperative urine cultures were done routinely in all the patients. The stone analysis was done using FTIR (Fourier Transform Infrared Spectroscopy). Urinary microbial floras, stone microbial pattern and stone composition were correlated.
Results and observation: The average age was 45.8 years and male to female ratio of 2.53: 1. Preoperative urine culture was positive in 60.34% of female patients and 46.94% of male patients (p=0.084). Infected stones were higher in female patients (p=0.0295). Preoperative urine culture and stone culture were positive in 50.73% and 39.5% of patients respectively. In urine culture *E.coli* was the most common bacteria (40.38%) (p=0.0029). The most common bacteria in stone culture in our series were *Pseudomonas spp.* (40.74%).
Conclusion: Is it important to culture stones? We observed that more than one-tenth of patients had only positive stone culture but sterile urine culture. We found *E.coli* as most common organism isolated in preoperative culture while *Pseudomonas* was the most common in stone culture. This discrepancy should be in mind while treating a stone patient of postoperative urosepsis, who is not responding to sensitive antibiotics according to urine culture.

Keywords: urine culture, stone culture, stone composition, upper urinary tract stone disease

Introduction

Urolithiasis is an expanding health problem. Nearly 10% of people worldwide will have stone disease in their lifetime [1]. The contribution of bacteria in the pathogenesis of stone formation is a well-established fact. Urinary tract infection with urease-producing bacteria is a cause for Magnesium-ammonium-phosphate (Struvite) stone formation [2]. In a study in Taiwan, urinary tract infection (UTI) was present in the 34% of children with newly diagnosed urolithiasis while childhood UTI prevalence is only 8% demonstrating a clear association between UTI and stone disease [3]. A similar finding was seen among the adult population in a Swedish study where 28% of the adult patients with the stone disease had a positive urine culture [4].

Bacteria can be cultured from urinary stones. Many studies have found bacteria in approximately 15-70% of urinary stones [5-8]. Among them, in CaOx stones 13-44% of cultures had *E.coli* and *Pseudomonas species* as the most common bacteria whereas, among the struvite stones, urease splitting organisms were most common [9, 7, 8]. Dajaivi *et al,.* in their study found that 14.5% of the stones were infected. Out of them, 65% of patients had infected urine. In 45% of these infected stones, same bacterial species were found in the urine of these patients [10].

Systemic inflammatory response syndrome may occur following surgical manipulation of stone during surgery due to the release of a large number of bacteria and its endotoxins. The incidence of SIRS increases when there is both positive urine culture and positive stone culture in comparison when one of them is absent [11, 12].

We here conduct a prospective study in patients with the urinary stone disease who were operated from March 2015 to February 2018 in the Dept of Urology, Gauhati Medical College Hospital. Our aim was to analyse and find a correlation between stone composition, stone microbial profile and urine microbial profile in these patients.

Materials and Methods

We had prospectively studied 205 patients of urinary tract stones who had undergone surgical intervention in the Department of Urology, Gauhati Medical College Hospital from March 2015 to February 2018.

All patients of renal stone or ureteric stone disease who had undergone surgery for stone removal were included. Age ranged from 15 to 70 years.

Out of the total of 205 patients, 150 patients had renal calculi and 55 patients had ureteric calculi. Of the patients with renal calculi, 129 patients underwent PCNL, 14 underwent pyelolithotomy and 7 underwent nephrolithotomy. Among the patients with ureteric calculi, 47 patients had undergone ureteroscopic lithotripsy (URSL) and 8 patients laparoscopic ureterolithotomy.

Preoperative urine cultures were done routinely in all the patients. Those patients with positive urine cultures were given the course of sensitive antibiotic for 2 weeks before the operation.

In half an hour, the urine sample collected was transported or if a delay was inevitable it was refrigerated at 4˚C for up to 4 hours. Urine culture was performed in the Department of Microbiology, Gauhati Medical College. The urine sample was delivered using calibrated loop technique delivering 0.001ml of urine and then was placed on Blood Agar and MacConkey Agar plates.

The stone analysis was done using FTIR (Fourier Transform Infrared Spectroscopy). The stones were classified as calcium oxalate (CaOx) if they had a majority (> 50%) of CaOx with or without any CA (carbonate apatite). Stone was grouped as CA (carbonate apatite) if they contained a majority (>50%) of CA with or without any CaOx. They were grouped as uric acid (UA) stones if they contained any uric acid component and as struvite if magnesium ammonium phosphate (MAP) were found.

The patients were grouped according to as upper tract calculi (renal and ureteric) and lower tract calculi (vesical calculi). Stone compositions were grouped as Calcium oxalate (CaOx), Uric acid (UA), Carbonate apatite (CA) or magnesium ammonium phosphate (MAP). Urinary microbial floras were correlated with the stone microbial pattern.

Statistical analyses were performed using Pearson chi-square analysis (IBM SPSS 24). p-value < 0.05 were taken as significant.

Results and Observation

The average age of patients with upper tract stones was 45.8 years. There were 58 female patients and 147 male patients with a male to female ratio of 2.53: 1.

Among the 58 female patients, preoperative urine culture was positive in 35 patients (60.34%) whereas among the 147 male patients it was positive in 69 patients (46.94%). Though there was an increased incidence of positive preoperative urine culture in female patients, this difference was not statistically significant (p= 0.084). Among the female patients, the stone culture was positive in 29 patients (50%) and among the male patients, it was positive in 53 patients (36.05%). This higher incidence of infected stone in female patients had statistical significance (p=0.0295)

Among the 205 patients of upper tract calculi (renal and ureteric calculi), 144 patients (70.24%) had calcium oxalate stones, 21 patients (10.24%) had stones composed of carbonate apatite (calcium phosphate), 35 patients (17.07%) had uric acid calculi and remaining 5 patients (2.4%) had struvite stones (magnesium ammonium phosphate).

The incidence of positive urine culture and positive stone culture according to stone composition has been depicted in Figure 1 and Figure 2. We observed in preoperative urine culture that MAP has the highest incidence of positive culture and the other compositions (CaOX, CA and Uric acid) showed a similar incidence with each other which was not statistically significant (p=0.443). In stone culture too, the MAP showed the highest incidence of positive culture which does not have statistical significance, due to a low number of MAP patients (p=0.278).

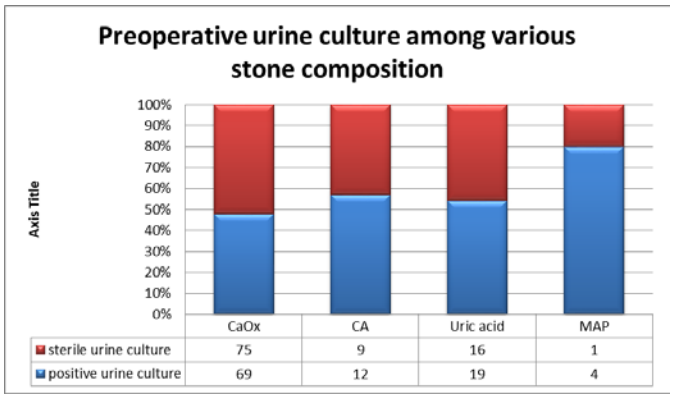

Fig 1: Incidence of positive urine culture among various stone compositions.

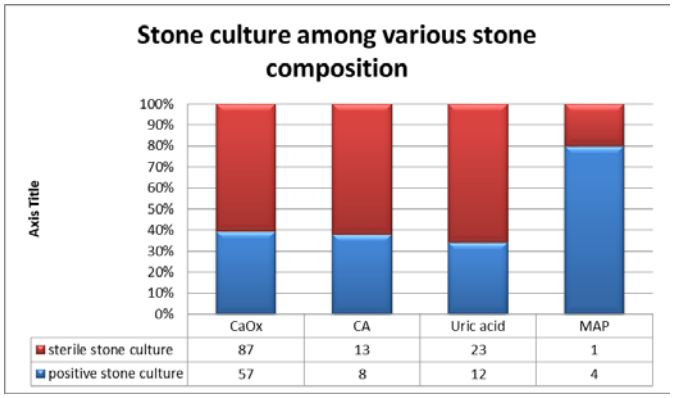

Fig 2: Incidence of positive stone culture among various stone composition.

Preoperative urine cultures were positive in 104 patients (50.73%). *E.coli*, *Klebsiella*, *Pseudomonas*, *Proteus*, *E.faecalis* and *Staphylococcus aureus* were among the microorganisms present and are depicted in Figure 3. We found that *E.coli* is the most common bacteria followed by *Klebsiella* and *Pseudomonas* in preoperative urine culture and are statistically significant (p= 0.0029)

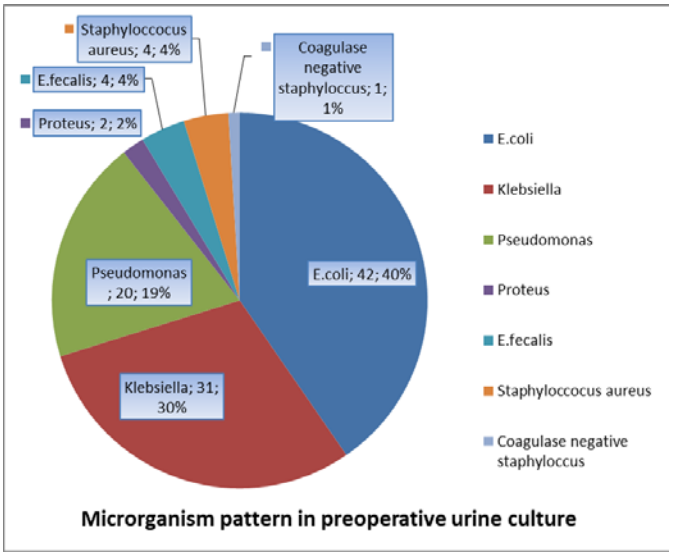

Fig 3: Microorganism pattern in preoperative urine culture

Among the upper urinary tract stones, stone cultures were positive in 81 patients (39.51%). The bacterial species that were found in stone culture are depicted in Figure 4. The most common bacteria identified among positive stone cultures were *Pseudomonas spp*. followed by *E.coli* and *Klebsiella* and it is statistically significant. (p=0.025)

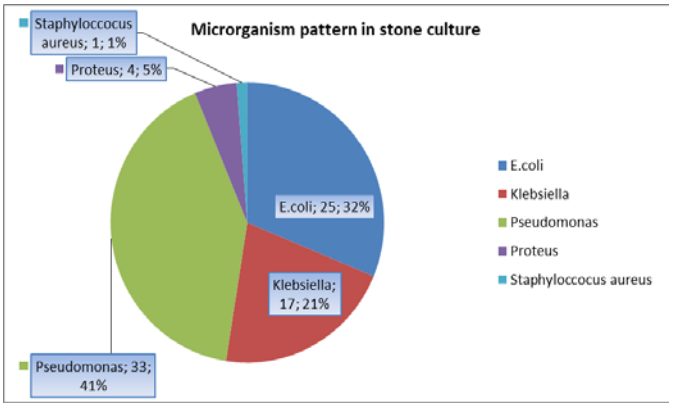

Fig 4: Microorganism pattern in stone culture

Among 144 patients with calcium oxalate (CaOx) stones, preoperative cultures were positive in 69 patients (47.92%). Out of these most common bacteria, *E.coli* was present in 28 patients (40.6%), *Klebsiella* in 21 patients (30.4%), *Pseudomonas* in 14 patients (30.4%), *E.faecalis* in 3 patients (4.3%), *Staphylococcus aureus* in 3 patients (4.3%). When *E.coli*, *Klebsiella* and *Pseudomonas* were compared *E.coli* was found to have statistically significant correlation (p=0.03).

Among the 21 patients with carbonate apatite (CA) stone, preoperative cultures were positive in 12 patients (57.14%). *E.coli* was present in 6 (50%), *Klebsiella* in 4 patients (33.33%), *Pseudomonas* in 1 patient (8.33%) and *Staphylococcus aureus* in 1 patient (8.33%). Among the patients with CA, *E.coli* was the most common bacteria followed by *Klebsiella* bearing a significant correlation [p= 0.046].

In the uric acid group of 35 patients, preoperative urine cultures showed significant growth in 19 patients (54.28%). *E.coli* was present in 6 patients (31.58%), *Klebsiella* was present in 6 patients (31.58%), *Pseudomonas* was present in 5 patients (26.32%), *E.faecalis* in 1 patient (5.26%) and coagulase negative *Staphylococcus aureus* in 1 patient (5.26%).When *E.coli*, *Klebsiella* and *Pseudomonas* were compared there was no statistical significance association between various organism to uric acid (p=0.92).

Five patients had MAP stones. Out of these preoperative urine cultures was positive in 4 patients (80%). Two patient (50%) had *E.coli* and another 2 patients (50%) had *Proteus*. Among the 4 groups of stone, preoperative urine culture positivity was highest among MAP stones (80%) as depicted in Figure 5. Although, struvite stones had a higher rate of positivity it was not statistically significant (p=0.44). This result might be due to an extremely low number of patients with MAP stones.

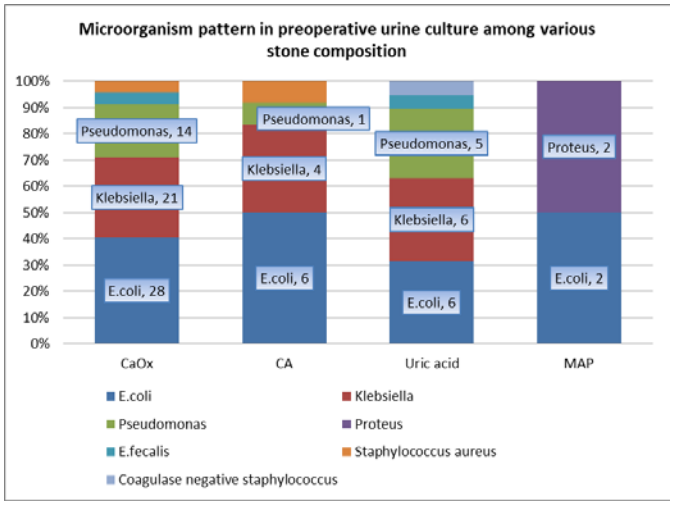

Fig 5: Microorganism pattern in preoperative urine culture among various stone composition.

Among 144 patients with calcium oxalate (CaOx) stones, stone cultures were positive in 57 patients (39.58%). Out of these most common bacteria was *Pseudomonas* which was present in 26 patients (45.61%), *E.coli* in 17 patients (29.82%), *Klebsiella* in 12 patients (21.05%), *Staphylococcus aureus* in 1 patient (1.75%)and Coagulase negative *Staphylococcus* in 1 patient (1.75%). We found *Pseudomonas* to be the most common bacteria associated with calcium oxalate (CaOx) stones followed by *E.coli* and *Klebsiella* (p=0.017).

Among the 21 patients with carbonate apatite (CA) stone, stone cultures were positive in 8 patients (38.09%). *E.coli* was present in 2 (20%), *Klebsiella* in 3 patients (37.5%) and *Pseudomonas* in 3 patient (37.5%) (p= 0.829).

In the uric acid group of 35 patients, stone cultures showed significant growth in 12 patients (34.28%). *E.coli* was present in 6 patients (50%), *Klebsiella* was present in 2 patients (16.67%), *Pseudomonas* was present in 4 patients (33.33 %). When *E.coli*, *Klebsiella* and *Pseudomonas* were compared there was no statistical significant correlation detected (p=0.223) in their difference.

Out of the 5 patients who had MAP stones, the stone culture

was positive in 4 patients (80%). All the 4 patients had *Proteus* spp. in stone culture.

The comparison of incidence among the pattern of microorganism in stone culture in various stone compositions is depicted in Figure 6

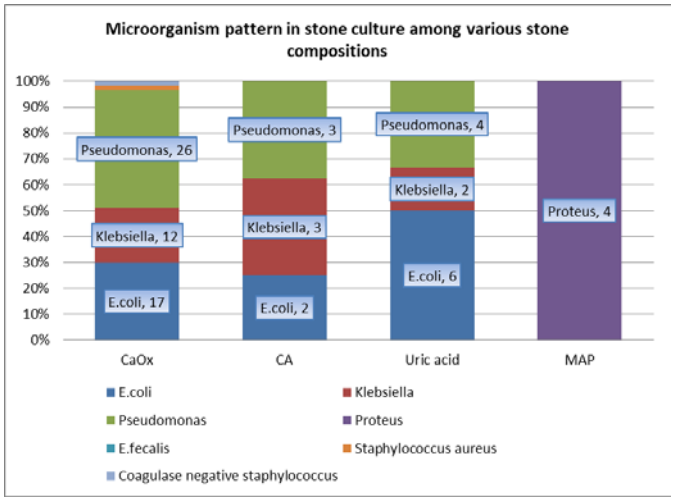

Fig 6: Microorganism pattern in stone culture among various stone composition.

We divided the entire group of 205 patients into 4 groups (Figure 7) according to stone composition: Group I – Positive urine and positive stone culture; Group II – Only positive stone culture; Group III – Only positive preoperative urine culture; Group IV – Both urine and stone culture sterile. We found that 18 patients (12.5%) having CaOx stones, 4 patients (19.05%) having CA stones, 4 patients (11.43%) having uric acid stones and 1 patient (20%) having MAP stone, had only positive stone culture (Group II).

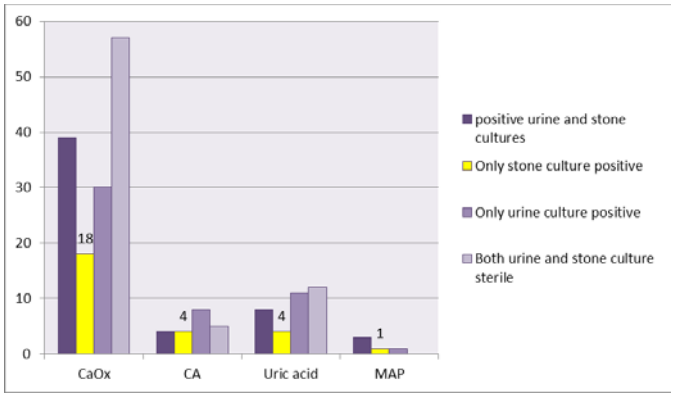

Fig 7: Chart showing four group of patients: Group I – Positive urine and positive stone culture; Group II – Only positive stone culture; Group III – Only positive preoperative urine culture; Group IV – Both urine and stone culture sterile.

Discussion

This study was conducted in 205 patients with upper urinary tract stones to find a correlation among causative microorganisms from preoperative urine, crushed stone culture and chemical composition of the stones. In our series, the male to female ratio in our series is 2.53:1.

Ahmad S *et al*,. have found an increased incidence of stone formation in male than female in the ratio of 4.9:1 [13]. Lieske JC *et al*,. found that male to female ratio increased mildly with age, with a male to female ratio of 2.09:1 in the age group 70-79 years [14].

The incidence of UTI and infected stone was more in female patients than male which has been documented in many studies [15, 8, 16, 17]. In our series, preoperative urine culture was positive in 35 (60.34%) of 58 female patients and 69 (46.94%) of 147 male patients (p=0.084). We found a higher incidence of infected stone during stone culture, in female patients (male – 36.04%; female – 50%; p=0.0295). Simon J *et al*,. found that female had increased incidence of infectious renal stones than males [15]. Solanki *et al*,. in their 100 patients, found a preponderance of infection stones in females (1:1.8) [8]. UTI in females occurs more often than males at the ratio of 8:1. Approximately 50-60% of the female population report UTI once in their lifetime [18, 16]. Increased UTI in female patients may be due to frequent bacterial contamination along the short urethral canal from the perineum. The change in genitourinary tract mucosa after menopause may cause colonisation by coliform organisms, which may be a factor for recurrent UTI in females [17].

Many studies have reported that *E.coli* is the most common bacteria isolated from urine culture of patients [19-22]. In our series we found preoperative urine culture to be positive in 50.73% patients.; *E.coli* is the most common bacteria (40.38%) isolated, followed by *Klebsiella* (29.81%) and *Pseudomonas* (19.23%) (p=0.0029)

While correlating preoperative urine culture according to the stone composition in our study we found that 80% of patients with the MAP were associated with preoperative urine culture positivity (p=0.44). For CaOx stones *E.coli* was the most common organism (40.6%) in preoperative urine culture; followed by *Klebsiella* (30.4%) and *Pseudomonas* (30.4%) (p=0.03); similarly for CA stones *E.coli* (50%) is found to be the most common organism (p=0.046). Solanki found that *E.coli* was the most predominant microorganism from preoperative urine culture overall in their 100 patients (21.73% and 32.25%) [8].

In our series, we found positive stone culture in 81 patients (39.5%). Thompson *et al*,. found a very high incidence of 76.74% of positive stone culture in their 86 patients [7]. Studies from across the world have demonstrated that bacteria can be grown from approximately 15-70% of urinary tract stones following culture [5, 9, 7]. Bratell *et al*,. found the incidence of stone having a positive culture was 51% [23]. Hugosson *et al*,. reported an incidence of 31% [24]. This much variation of positive stone culture incidence can be due to a variable incidence of highly infectious MAP (struvite) stones in many previous studies.

We have found that the most common bacteria in stone culture in our series is *Pseudomonas* (40.74%) followed by *E.coli* (30.86%) and *Klebsiella* (20.99%) (p=0.025). Similarly, in CaOx stones *Pseudomonas* was the most common bacteria identified followed by *E.coli* and *Klebsiella*. (p=0.017). Among the CaOx stones, 13%-44% of stone culture was positive. *E.coli* and *Pseudomonas spp*. were the most common organisms isolated from CaOx stones followed by *Proteus*

typically associated with struvite stones [8, 7, 9]. Solanki *et al*,. concluded that *E.coli* was the most common bacteria identified from the stone culture in his patients [8]. Songra *et al*,. and Gault *et al*,. found *Pseudomonas* to be the most common bacteria in stone culture [25, 26] Even many earlier studies have found *E.coli* to be the most common bacteria in CaOx stones [27, 28].

Most of the MAP stones are infectious and most common bacteria is *Proteus* spp. In our series 4 of 5 patients (80%) of the MAP had an infectious stone. All were positive for *Proteus* spp. Thompson *et al*,. observed that 47 of 49 stones that were struvite were infected. *Proteus* mirabilis was the most common bacteria in 80% of their infected struvite stones [7].

The more frequent occurrence of struvite stones in female population and in paraplegic patients with indwelling catheters suggest that infection with urea-splitting bacteria is vital in the formation of struvite calculi since both these groups have increased the susceptibility of infection with urea-splitting bacteria [29-31].

We found that overall 27 out of 205 (13.17%) patients had only positive stone culture and sterile urine culture. 12.5% of patients having CaOx stones, 19.05% of patients having CA stones, 11.43%of patients having uric acid stones and 20% having MAP stone, had an only positive stone culture (Group II as in Figure 8). Songra MC *et al*,. observed that chances of sepsis were very high in the group where both cultures were positive, followed by a group in which only stone culture was positive (p= 0.001478). Isolated urine culture had the least predictive value for sepsis than stone culture and lowest chance of sepsis was observed among the patients where both cultures are negative (p=0.026) [11].

Jairam R. Eswara & Ahmad Sharif *et al*,. observed that 73% (8/11) of the patients who developed sepsis following surgical treatment for stones had positive stone cultures, while 1% (3/232) with negative stone cultures developed sepsis (P = 0.003). They found that the pathogen causing infection had a significantly higher correlation with the organism grown on stone culture than the preoperative urine culture suggesting the importance of crushed stone culture [32].

Conclusion

The preliminary observation from our study revealed that upper urinary tract stones are most common in males and most of the patients present in their 4th decade. Female patients had more predilections towards urine infection in stone bearing patients. *E.coli* is the most common bacteria isolated from preoperative urine culture overall, and also among CaOx and CA stone formers. While *Pseudomonas spp.* is the most common bacteria cultured from crushed stone culture overall and CaOx stones. MAP stones are associated with the highest incidence of positive urine and positive stone culture although a larger study with more number of MAP stone patients is needed to statistically confirm it. Female patients are associated with positive stone culture more than male patients. Is it important to culture stones? We observed that more than one-tenth of patients had only positive stone culture but sterile urine culture. The importance has been suggested because of this limited correlation between stone culture and preoperative urine culture. Again, we found *E.coli* as most common organism isolated in preoperative culture while *Pseudomonas* was the most common in stone culture. This discrepancy should be in mind while treating a stone patient of postoperative urosepsis, who is not responding to sensitive antibiotics according to urine culture.

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