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RESEARCH ARTICLE

BACTERIOLOGICAL PROFILE OF RENAL STONES IN A TERTIARY CARE CENTER IN NORTH EAST INDIA

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ABSTRACT

Background and Objectives: Urolithiasis is a frequently occurring urological disorder associated with multiple etiological factors, out of which bacterial infection is one .A definite relationship is seen between the presence of bacteria and activity of bacterial urease in development of stones .This study was thus undertaken to analyse the renal stones and their association with different bacteria.
Materials and Methods: The study was conducted in a tertiary care center in North East India. 95 stones were collected intraoperatively. Upon removal, the stones were placed immediately in a sterile container and bacteriological analysis was performed by standard conventional methods.
Results: Among the 95 stones analysed from 95 patients and 35% belong to age group 21-30 years with male: female ratio was 1.2:1 and 60% showed growth of organism and from one stone 2 organisms were isolated. Preoperative urine analysis showed growth in 52% of the patients and among these renal stone culture was positive in 82.2%. .Urease splitting organisms isolated from pre operative urine was 42.2% and that from renal stones was 60%.
Conclusion: Infection of renal calculi with multiple drug resistance strains might reflect treatment failure. Bacteria present within the calculi may not be detected by urine culture, and may act as foci of persistent infection of the urinary tract.

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INTRODUCTION

Renal stone disease has been recognized in many parts of the world since antiquity as one of the most painful and commonest urological disorders. The evidence of calculi has been found in a 7000 year old Egyptian mummy. Its incidence has increased considerably during the 20th century (Sami-ullah *et al*., 2007). The prevalence of renal stone formation is approximately 2-3% in the general population (Anderson, 1996). Renal stones are not usually fatal, although some primary conditions that produce kidney stones can lead to death from problems associated with the primary disease or complications of renal failure (Halstead *et al.,* 1967). There are numerous risk factors responsible for or contributing to stone formation like environment, metabolism, dietary, race, sex, obstructive uropathy and infections of urinary tract etc. Out of these, urinary tract infection (UTI) is one of the most common causes of stone formation (Drach *et al*., 1992). The occurrence of infected stones varies widely, between 3 and 34% (Grenabo *et al*., 1985). Microorganisms can influence the process in many ways, the best known is the infection stones by urease producing bacteria (Hedelin *et al.*, 1984). Brown was the first to advance the theory that bacteria split urine and thus caused and facilitated stone formation (Brown, 1901). In 1925, Hager and Magath suggested the enzyme 'urease' to be the cause of hydrolysis of urine thus increasing the ph (Hager *et al.,* 1925). In the alkaline urine, phosphate is precipitated either in the form of calcium phosphate or as magnesium phosphate. Non-urease producing microorganisms could be involved in the formation of infected stones too by facilitating crystallization. (Hans Hedelin, 2000). Further more, infected stones can lead to urosepsis and death (Halstead *et al.,* 1967). Although there is some relation between UTI and the formation of urinary stones, but this is not the only factor involved, as many patients with chronic pyelonephritis do not have kidney stones. Moreover in many cases of nephrolithiasis no infection was found (Straffon *et al*., 1970). Urinary tract infections with certain bacteria play an important role in the synthesis of renal stone (Jan *et al.*, 2008). The bacteria isolated from urine and those from calculi differed in their susceptibility to antimicrobial agents (Sakhaee, 2009).

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The continuous modifications of microbes' antibiotic susceptibility leads to the increase in mortality and morbidity by infectious diseases in general, and by urinary tract infections in particular, resulting in the increase of expenses for patient care (Tudor Bianca *et al.*, 2013). Till date there is no documented their study available on bacteriological profile of renal stones and association with UTI from North East India. In view of this, the present study was done to investigate the bacteriology of renal calculi in relation to urinary tract infection, to study the role of UTI in stone formation and to study the antimicrobial pattern of bacteria isolated from urine and calculi specimens.

MATERIALS AND METHODS

The study population included 95 patients who were diagnosed with renal stones and underwent surgical intervention for stone removal in NEIGRIHMS, Shillong (A tertiary care centre in NE India). Ethical clearance to conduct the study was obtained from the Institute,

Stone analysis

The stones were removed intra operatively and placed immediately in a sterile container containing 5ml normal saline. A portion of the stone was crushed in sterile normal saline with a sterile grinding mortar under aseptic conditions The crushed stone core was inoculated in 5ml BHI Broth which was incubated aerobically at 37°C for 18-24 hours, and then subcultures were made on Blood agar and Mac Conkey's Agar media.

Urine analysis

Preoperative urine was collected and culture was done by semi-quantitative technique on Cysteine Lactose Electrolyte Deficient Medium (CLED). Bacterial isolates were identified on the basis of cultural, morphological, and biochemical characteristics. The presence of more than two bacterial species was the ground of assuming contamination (Collee, 1996). The antimicrobial susceptibility testing was performed by the standard Kirby Bauer disc diffusion method, and the interpretation of the results was done according to Clinical Laboratory Standard Institutes Guidelines (CLSI, 2014).

Statistical analysis

Significance was evaluated by Fisher's exact test and/or $\chi 2$ test and 'p' value less than 0.05 was considered as significant

RESULTS

The study was done over a period of 1 year, where 95 stones from 95 patients were analyzed. Out of the 95 stones analyzed, 57 stones (60%) showed growth of organism and from one stone 2 organisms were isolated. Thirty (32%) stones showed no growth of organism and 8 stones (8%) showed growth of contaminants (Table 1). The incidence of renal stones was highest in the age group of 21-30 years (34 stones, 35%) followed by 31-40 years (18 stones, 19%) and 11-20 years(15 stones,16%).Overall the prevalence of infection of renal stones was more in age group of >50 years where 13 out of the 14 stones analysed showed infection (93%) followed by 11-20 years where 10 out of 15 stones analysed showed infection (67%, Table 1).

Table 1. Age and sex wise distribution of 95 renal stones

Age wise distribution	No growth		Infection		Contamination		Total
	M	F	M	F	M	F	
<10 yrs	1	-	-	-	-	-	1 (1%)
11-20 yrs	1	3	2	8	1	-	15 (16%)
21-30 yrs	8	4	8	9	3	2	34 (35%)
31-40 yrs	1	5	9	2	-	1	18 (19%)
41-50 yrs	4	2	5	1	-	1	13 (14%)
>50 yrs	1	-	9	4	-	-	14 (15%)
Total	16	14	33	24	4	4	95 (100%)

Table 2. Comparision of Pre operative urine culture and stone culture

	Stone culture positive	Stone culture negative	Total
Pre operative urine Culture Positive	37	8	45
Pre operative urine Culture Negative	20	22	42
Total	57	30	87

Table 3. Microorganisms isolated from Pre-operative Urine and Renal Stones

Microrganisms	Pre operative urine culture (n)	Renal stone culture (n)	P value
Escherichia coli	20	9	0.0031
Klebsiella species	11	5	0.0598
Enterobacter species	0	1	0.933
Acinetobacter baumanii	0	2	0.5841
Psuedomonas aeruginosa	5	16	0.633
Enterococcus species	6	9	0.9445
Staphylococcus aureus	3	11	0.1209
CONS	0	2	0.5841
Candida species	0	3	0.3314
Total	45	57	

Incidence of renal stones as well as infection of renal stones were more among males 54% and 58% respectively as compared to females The ratio of incidence and infection of renal stones among males and females were 1.2:1 and 1.5:1 respectively (Table 1). Pre operative urine samples of 95 patients were collected and analyzed. The comparison of the urine and renal stone cultures were done for 87 stones as 8 stones which showed growth of contaminants were excluded. Pre operative urine analysis showed growth in 45 cases (52%) and among these renal stone culture was positive in 37 cases (82.2%) Among these 37 cases, same organisms were isolated in 14 cases (37.5%). Pre operative urine was sterile in 42 cases (48%) and among these 20 cases (35%) showed growth in renal stones Table 2. The association of preoperative urine culture and renal stone culture was found to be statistically significant (p=0.008)

Most common organism isolated in preoperative urine was *Escherichia coli* 20 cases (45%) followed by *Klebsiella s*pecies 11 cases (25%). On the other hand the most common organism isolated in stone culture was *Pseudomonas aeruginosa* 16 cases (28%) followed by *Staphylococcus aureus* 11 cases (19%) (Table 3). Urease splitting organisms isolated from pre operative urine was 42.2% and that from renal stones was 60%. Pre operative urine analysis revealed that all organisms were 100% resistant to Ampicillin followed by Cephalosporin 85% and Amoxicillin clavulanic acid 81%.

Multi Drug Resistance (Resistant to > three groups of antimicrobials) was seen in 68% isolates. 25% of the Gram Positive isolates were resistant to Nitrofurantoin whereas none of the Gram Positive isolates showed resistant to Vancomycin.

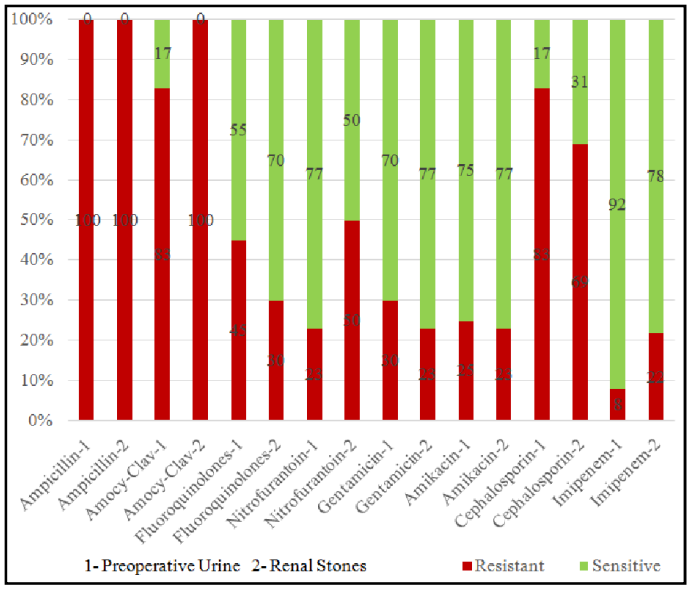

Figure 1. Antibiotic Profile of Gram negative bacteria of Preoperative Urine and Renal Stones

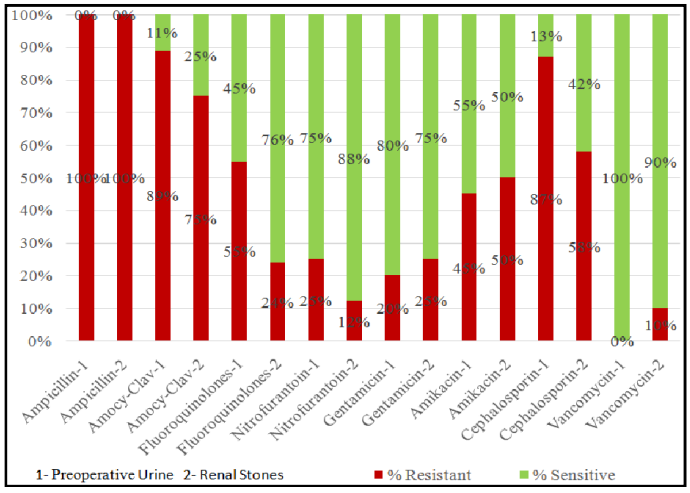

Figure 2. Antibiotic Profile of Gram positive bacteria of Preoperative Urine and Renal Stones

Among the Gram Negative organisms 23% were resistant to Nitrofurantoin and 8% shows resistance to Imipenem. Similarly, in case of renal stones the isolates showed highest resistance (96%) to Ampicillin followed by Amoxicillin clavulanic acid (87%) and Cephalosporin (67%). Multi Drug Resistance was seen in 58% isolates. Gram Positive isolates showed the least resitance to Vancomycin (10%) followed by Nitrofurantoin (12%) (Figure 1) whereas the Gram Negative isolates showed least resistant to Imipenam (22%) and Fluroquinolones 30% (Figure 2).

DISCUSSION

In our study the prevalence of infected renal stones was 60% which was similar to the findings by Tudor Bianca *et al.* (59%). (Tudor Bianca *et al*, 2013) and Bralett *et al.* (53%) (Bratell *et al.*, 1990). The male preponderance of renal stones in our study was similar to that of other studies (Golechha *et al.,* 2001; Tudor Bianca *et al.*, 2013). This may be attributed to hormonal effects, high inhibitory activity, lower food intake and lower body size (Robertson *et al*., 1980; Ryall *et al*., 1987). Moreover, Welshman and Mc Geown demonstrated increased citrate concentrations in the urine of women (Welshman *et al.*, 1977). It has been postulated that this may aid in protecting females from calcium urolithiasis since citrate inhibits nucleation of calcium oxalate crystals. (Nicar *et al.,*1987). Various studies (Golechha *et al*., 2001; Tudor Bianca *et al.*, 2013) showed that the infected renal stones was more among females which, may be due to increased incidence of recurrent urinary tract infection in them which is due to close proximity of urethra to anus (Baron *et al.*, 1994). But in our study infection of renal stones were found to be more in males than females. Further studies with larger sample size are required to justify the significance of this finding.

In our study, the incidence of renal stones was highest in patients in their second decade of life. This may be due to dietary change with increase in protein intake (non vegetarian diet) in this part of the country (Robertson *et al.*, 1981). 56% of the cases showed culture positive in preoperative urine samples. This was in concordance with various studies (Golechha *et al*., 2001 and Tudor Bianca *et al*., 2013). *Escherichia coli* was the most common organism isolated in preoperative urine and in renal stone culture *Pseudomonas aeruginosa* was the commonest organism isolated. These findings were found to be in concordance with many studies (Golechha *et al*., 2001; Naas *et al*., 2007; Gault *et al*, 1995). The isolation rate of *Escherichia coli* in culture of preoperative urine when compared to culture of renal stones was found to be statistically significant (p=0.0031)

Urease producing organisms were detected in 42.2% in preoperative urine and 60% in renal stones suggesting that they might play an important role in stone formation (Naas,*et al*., 2007). It is observed that the bacteriological findings from urine do not always correlate exclusively with the bacteriology of renal stones. Such findings might be due to intermittent release of small number of microorganisms from the calculi which may or may not be isolated from urine .The explanation for the presence of bacteria within the calculi may be due to insignificant intermittent bacteraemia from where the bacteria are excreted in renal pelvis, and may act as a nidus for deposition of crystals either by damaging the mucous coat or perhaps also by acting as a nidus for crystallization of salts. Thus, a vicious cycle starts, infection bringing about calculi formation and calculi causing infection (Tudor Bianca *et al*., 2013). It was seen in our study that the renal calculi isolates were more resistant than urinary isolates to most of antimicrobial agents tested. This can be explained by the presence of bacteria below the surface of the stone which are not easily accessible to antimicrobials. Although the antibiotics although the urine and stone surface were cleared with antimicrobial therapy (Nickel *et al.*, 1985). If the antibiotic therapy is stopped while the infected stone fragment is still present, the original infection may recur (Orson Moe, 2006).

Therefore the study highlights that bacteria present within the calculi may not be detected by urine culture, and may act as foci of persistent infection of the urinary tract and renal stones thereof. Stones, when treated by any method, leads to their disruption and release of the bacteria leading to bacteraemia or endotoxaemia. Prophylactic antibiotic therapy should be given to the patients in the pre-operative period. Manipulating the stone during attempts at removal and fragmentation may liberate these organisms into the bloodstream, heightening the risk of sepsis or systemic inflammatory response syndrome (SIRS). Since majority of the calculi isolated showed Multi-drug resistance, prescribing antimicrobial therapy will eradicate the organisms in the urine and provide broad-spectrum coverage for the potentially different bacteria harboured within the stone. Furthermore, performing a stone culture may identify organisms that are not covered by initial antimicrobial therapy, providing an opportunity to institute earlier targeted therapy.

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