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SOME STUDIES ON BACTERIOLOGICAL PROFILE OF KIDNEY STONE

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Abstract: Most of the antimicrobial susceptibility surveillance studies focus on isolates from hospitalized patients. In the present investigation a retrospective analysis of microbiological data of antimicrobial susceptibility of bacterial Urinary Isolates of Urolithiasis from the hospitals in the Barshi town was performed. Such studies had not been undertaken earlier in this region. The bacteriological studies of urinary stone included samples from sixty six (66) cases of Urolithiasis. Thirty three (33) samples showed presence of bacteria namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Aeromonas* spp. and *Staphylococcus saprophyticus*. In twenty eight (28) infected stone cases the organisms isolated from crushed stone were same, while five (5) cases of culture positive stones were found different microorganisms. Biochemical identification study was performed as per the standard methods. The problem of Urolithiasis was found more in males than females. The relation between UTI & urinary stones was analyzed in present study, out of Sixty-six UTI (66) cases studied, in thirty-three (50%) cases an infection induced stones in which eight cases (12.12%) from female and twenty five (37.87%) cases from male. Infection induced stones were not detected in the female age group below fifteen. Three (3) cases of infection induced stones were detected in male group below 15. In case of middle age group (16 to 45) infection induced urinary stones were five (5) in female and seventeen (17) in male, while above 46 age group one (01) in female and five (05) in male. Sixty-six cases were reported including 18 female cases (27.27%) & 48 male cases (72.73%). The high incidence (50%) of infection-induced stones seen in the present study is in close agreement with observation of Vargas et al & Benu Dewan et al. The commonest pathogens recovered from pre-operative urine culture and stone cultures were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Aeromonas* spp, *Escherichia coli* and *Staphylococcus saprophyticus*. The isolation of urinary opportunistic pathogens among the UTI cases with urinary stones was found to be significant.

Keyword: Urinary stones, *Klebsiella*, *Pseudomonas*, *Proteus*, *Escherichia coli*, *Aeromonas* spp and *Staphylococcus saprophyticus*.

INTRODUCTION:

Historical and archaeological studies clearly reveal that ancient man suffered from urinary tract stone disease. The earliest evidence dates to before 4800 B.C. and was a bladder stone found among the pelvic bones of a young predynastic Egyptian. Another stone dating at about 4200 B.C. was probably of renal origin and analysis revealed it to be composed of calcium carbonate, calcium phosphate and calcium oxalate. The stone disease in ancient times was not uniform throughout all populations. It was relatively common particularly in children in ancient Persia but was rare in Turkey. Ancient records reveal that stone disease was also present in the Greek and Roman civilizations. Hippocrates can be credited with being the first to theorize about the etiological factors of urinary calculi. He observed that many patients having calculi in the bladder of kidneys has sandy sediment in their urine and suggested that the ingestion of muddy river water or water containing lime caused stone formation in the urinary tract. Galen, the great Roman physician also wrote about stone formation and

recognized such risk factors as heredity, race, climate, diet, drinking water, the ingestion of alcohol, and metabolic abnormalities.¹² Ancient Indian physician described four types of calculi that were formed by phlegm, vapor, bile, and semen. The cause and effect relationship between urinary infection and urinary stones has been known since antiquity.¹³ Bladder stones that we now recognize as infection induced have been found in Egyptian mummies dating back to 5000 B.C. Hippocrates recognize that putrefying joint abscesses were commonly associated with renal stones. Again in the early twentieth century several clinicians noted the relationship between urinary stone disease and infection.² The first laboratory study investigating the relationship of stone disease and infection was carried out in by Rosenow and Meisser (1923)¹⁵, who implanted *Streptococci* obtained from stone formers into the dental canal of dogs. Though the work has never been duplicated, a significant number of the animals studied developed urinary calculi.⁹ Shortly thereafter, other investigation instilled bacteria into rabbit bladders and

demonstrated the development of bladder stones.^{9,10} The association of urea splitting bacteria particularly of the *Proteus* species is well recognized but it is also noted that only few of the patients with *Proteus* infection have showed urinary stone.³ Certainly, infection enhances stone growth. Urea splitting organisms have been demonstrated to be embedded deep within the matrix of the stones. This points out the importance of removing all stone fragments at the time of surgery so that recurrence and infection may be prevented.¹⁴ In a recent report from Finland,¹¹ suggest that nanobacteria can produce calcium phosphate to initiate stone formation such as in nephrolithiasis and probably other foci of extra skeletal calcification.^{1,3,16,18} These stones frequently contain a higher percentage of matrix than metabolic stones, however, there has never been any relationship proven between the infection and matrix formation. A recent report by Musher and Griffith¹³ (1984) indicate that the use of urease inhibitors can impair stone growth in the presence of persistent infection. Since greater than 50% of patients with infected stag horn stones have treatable metabolic disorders, it is imperative to fully evaluate these patients preoperatively in order to reduce the chances of recurrent stone formation. However, patient's propensity to form stone is based on the presence or absence of more than one specific factor. a) Urinary super saturation. b) Presence or absence of natural inhibitors of crystallization. c) Urinary macromolecules d) Urinary infection. These have all been reported to be important predeterminants of stone formation. However, it is very likely that more than one of these factors are operating when an individual forms a stone. It is also very likely that the combination of predisposing factors will vary from individual to individual even though all form the same type of stone. Infection-induced stones refer to calculi composed of magnesium ammonium phosphate and carbonate and more than 45 different urease producing microorganisms are responsible for urinary stones. These are *Proteus*, *Klebsiella*, *Pseudomonas*, *Aeromonas* spp., *Staphylococcus* spp., *E. coli*, *Enterococci*, spp., *Streptococci* spp., *Lactobacilli* spp., *Gardnerella vaginalis* and *Bacteroides*,^{8, 11, 12}.

2. MATERIALS AND METHODS

2.1 Patient's survey: Sixty-six patients, 48 male and 18 female have been attending at Dr. J.M. Hospital and Research center, Barshi were enrolled in the study. All these patients were initially screened for significant bacteriuria. Detailed history was obtained from each patient that included information regarding name, age, sex, signs and symptoms of disease, duration of illness and history of recurrent urinary stone infection.

2.2 Collection and preservation of Sample: Male patients were asked to wash hands and the glands with soap. They were asked to pass urine after retracting the prepuce in the clean container. Female patient was asked to pass the sterile swab from anterior to posterior side after parting the labia. In case vaginal discharge was present an antiseptic solution savlon was used. The patient was then asked to pass the urine in clean container. Midstream urine samples were collected in sterile containers by taking all precautions to avoid the contaminations.

2.3 Preliminary study: The urine specimens were immediately brought to the laboratory and cultured within thirty minutes. Urinary stones were washed thrice with sterile normal saline and crushed in sterile pestle and mortar. Nidus was separated, crushed and emulsified in normal saline. One loopful of uncentrifuged urine samples was streaked on MacConkey's agar medium and Cysteine Lactose electrolyte deficient medium. Then the plates were examined after overnight incubation.

2.4 Bacterial characterization and identification: Bacterial growth was further processed as per the standard procedure for identification of bacteria. Bacteriological studies of pre-operative urine and stone performed in 66 cases of Urolithiasis. The crushed stone culture was performed in all cases. Identification was done by different biochemical reactions like, Acid and Gas from glucose, sucrose H₂S production NO₂ production. Indole production MR and VP reaction Citrate utilization i.e (IMViC test), Urease, Catalase and the final confirmation was done according to the Classification schemes^{4,5,6}.

RESULT AND DISCUSSION

The relation between UTI & urinary stones was analyzed in present study. Sixty-six cases were reported including eighteen female cases (27.27%) & forty eight male cases (72.73%). The high incidence (50%) of infection-induced stones seen in the present study is in close agreement with observation of Vargas et al (41.5%)¹⁷ & Benu Dewan et al (38.75%)². Similarly Sixty-six UTI cases with urinary stones were studied out of these thirty-three stones (50%) was identified as an infection induced stones in which eight cases (12.12%) from female and twenty five (37.87%) cases from male. Infection induced stones were not detected in the female age group below fifteen and three cases were detected in male group below 15. In case of middle age group (16 to 45) infection induced urinary stones were five in female and seventeen in male while above 46 age group they were found to be one and five respectively. (Table-1, Fig-1). Our reports indicate that infection induced urinary stones were found to be more common in all age groups of male as compared to female.

The relation between UTI & urinary stone was also analyzed in present study. Sixty-six cases were reported including eighteen female cases (27.27%) & forty eight male cases (72.73%). Chemical analyses of such stones are to be done so as to get biochemical profile of Urolithiasis. Out of thirty three cases in twenty eight (84.85%) infected stone cases, same common organisms were isolated from the crushed stones and in five (15.15%) cases of culture positive stones showed different microorganisms than pre-operative urine culture. The rate of infection accompanying stone formation was more in male in comparison with female. The commonest pathogens recovered from pre-operative urine culture and stone culture were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Aeromonas* spp and *Staphylococcus saprophyticus*. *Aeromonas* spp and *Staphylococcus saprophyticus* are the only bacteria isolated from crushed stones. (Fig-2A,

2B). Multiple antibiotic resistance among urinary isolates was also significantly variable 20, 21, 22 Thus detailed molecular study of multiple antibiotic resistance in urinary stone isolates is essential. In Previous study only the commonest pathogens recovered from pre-operative urine culture and stone culture was studied.

Table1. Patient profile under study, Occurrence of UTI in Urolithiasis

Age group in years	No. Of Urinary Tract Infections			
	Female		Male	
	Urinary Stones	Infection Induced Stones	UrinaryStones	Infection Induced Stones
Below 15	2	0	8	3
16-30	6	2	12	7
31-45	7	3	18	10
46 and above	3	1	10	5
Total	18	6	48	25

Fig1. Patient profile under study, Occurrence of UTI in Urolithiasis. X axis: Patients Age Groups in years, Y Axis: Number of Urolithiasis cases in UTI. Histogram I- Urinary Stones in female patient Histogram II- Infection Inducedones in female patient Histogram III- Urinary Stones in male patient Histogram IV- Infection Induced Stones in male patient

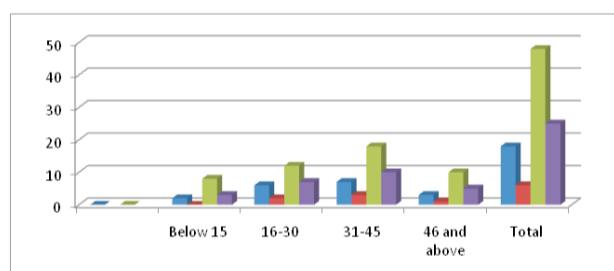


TABLE NO 2: Biochemical test for identification of Bacterial urinary isolates

Urinary isolates	Glucose	Sucrose	H2S production	NO3 production	Urease	Catalase	IMViC Test*			
							I	MR	Vp	C
<i>Escherichia coli</i>	+	-	-	+	-	+	+	+	-	-
<i>Klebsiella pneumoniae</i>	+	+	+	+	+	-	-	-	+	+
<i>Pseudomonas aeruginosa</i>	+ A	+	-	+	+	+	-	-	-	-
<i>Prot. vulgaris</i>	+	+	-	+	+	+	+	+	-	-
<i>Aeromonas sp</i>	+	+	-	+	-	+	+	+	+	-
<i>Staph. saprophyticus</i>	+	+	-	+	+	+	-	+	+	-

Fig.2A. Bacterial Profile in pre-operative with Urolithiasis

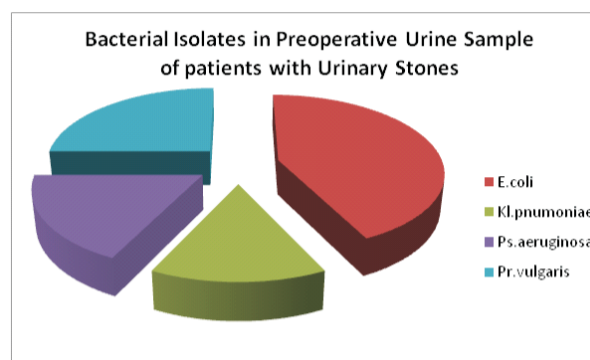
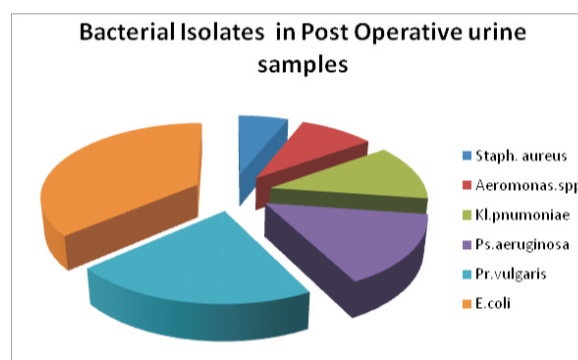


Fig.2B : Post-operative patients with Urolithiasis



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