MICROBIOLOGICAL STUDY OF URINARY CALCULI IN PATIENTS WITH URINARY INFECTIONS

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Abstract: *Purpose: the investigation of the urinary calculi bacteriology in relation to urinary tract infection, the study of the role of urinary tract infection in urolithiasis and the study of the antimicrobial sensitivity pattern of bacteria isolated from urine and calculi specimens. Material and methods: urine samples and urinary calculi originating from 320 patients were processed. Bacteriological study was conducted on pre-operative urine and operated renal calculi. The isolated organisms were identified by standard techniques and the antibiotic susceptibility was performed according to CLSI standards. Results: a number of 252 bacterial strains were isolated from urocultures and 188 from urinary calculi. Escherichia coli was the most frequent isolated species both from urine and urinary calculi. The comparative antibiotic resistance analysis of the isolated strains from urine and calculi showed a lower susceptibility to most of the tested antibiotics for the bacterial strains isolated from urinary calculi. Conclusions: the preponderant etiology of urinary infections in patients with urolithiasis was given by E. coli, Proteus spp., Enterococcus spp. and Pseudomonas aeruginosa. The preponderant microorganisms found in calculi were: E. coli, P. aeruginosa, Enterococcus spp. and Proteus spp. There is a statistically significant association regarding the presence of urinary calculi and the development of urinary infections. Bacterial isolates from urinary calculi were less sensitive to most of the tested antibiotics than the isolates from uroculture.*

Cuvinte cheie: *infecţie de tract urinar, calculi urinari, microorganisme*

Rezumat: *Scop: Scopurile acestui studiu sunt: analiza bacteriologică a calculilor corelată cu infecţiile de tract urinar, studiul rolului infecţiilor de tract urinar în litiaza urinară, precum şi studiul sensibilităţii la antibiotice a bacteriilor izolate din probele de urină şi calculi. Material şi metodă: S-au procesat probe de urină şi calculi urinari provenite de la 320 pacienţi. S-a efectuat studiul bacteriologic al probelor de urină recoltate preoperator şi al calculilor urinari extraşi. Microorganismele izolate s-au identificat folosind tehnici standard, sensibilitatea la antibiotice determinându-se conform standardelor CLSI. Rezultate: Un număr de 252 de tulpini bacteriene au fost izolate din uroculturi şi 188 din calculi urinari. Escherichia coli a fost cea mai frecvent izolată specie, atât din probele de urină cât şi din calculii urinari. Analiza comparativă a rezistenţei faţă de antibiotice a tulpinilor izolate din urină şi din calculi arată o sensibilitate scăzută la majoritatea antibioticelor testate a tulpinilor bacteriene izolate din calculii urinari. Concluzii: Etiologia preponderentă a infecţiilor urinare la pacienţii cu litiază urinară a fost dată de E. coli, Proteus spp., Enterococcus spp. şi Pseudomonas aeruginosa. Microorganismele izolate preponderent din calculi sunt: E. coli, P. aeruginosa, Enterococcus spp. şi Proteus spp. Există o asociere statistic semnificativă în ceea ce priveşte prezenţa calculilor urinari şi evoluţia infecţiilor urinare. Tulpinile bacteriene izolate din calculi au avut o sensibilitate mai scăzută faţă de majoritatea antibioticelor decât tulpinile izolate din uroculturi.*

INTRODUCTION

The evidence of urinary calculi has been found in a 7000 years old Egyptian mummy.(1) The association of stones and putrefaction has been known since Hippocrates.

More than 600,000 patients are seen in the emergency department in the United States each year due to urolithiasis and the complications of this disease.(2) A recent U.S. study indicated that up to $2 billion is being spent on the diagnosis and treatment of urolithiasis.(3) Therefore, new approaches in treatment and prevention could have a huge economic effect over and above benefits in terms of reduced morbidity.(4) Numerous risk factors responsible for contributing to stone formation have been identified, including environmental, metabolic, dietary, racial, gender, obstructive uropathology and urinary tract infection.(5) Due to the high risk of recurrences in patients with urolithiasis, it is necessary to identify the risk factors that might be of etiological importance and thus get some clues for predicting the further course of the disease.

Urinary tract infections with certain bacteria play an important role in the synthesis of renal stone.(6) The bacteria isolated from urine and those from calculi differed in their susceptibility to antimicrobial agents.(7)

The continuous modifications the 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.

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PURPOSE

Our study was conducted in order to evaluate the chemical composition and bacteriological spectrum of renal stones and of the pre-operative urines; we also investigated the correlation between the antibiotic susceptibility of the bacteria isolated from urolithiasis and urinary tract infection.

METHODS

The present study was conducted on 320 patients of urolithiasis, admitted in the Urology and Surgical departments of Tîrgu-Mureş Hospital for the management of renal calculi.

From December 2008 to December 2010, 320 patients diagnosed by the urologist as having urinary calculi were included in this study. Ethical clearance to conduct the research was obtained from the hospitals. Informed consent was obtained from all participants. There were no refusals to participate. A questionnaire was administered to patients to collect demographic data and information on congenital anomalies, previous urinary stone, family history of urolithiasis and dietary habits.

Patients were examined by a physician; those with renal stone disease with renal failure and renal tumours were excluded.

A specially designed proforma, containing general information about the patient, urinary symptoms and signs, was filled out for every patient included in this study. After the clinical examination, every patient was investigated in the same manner: urine analysis, imagistic methods, blood examination, and biochemical composition of the calculi and bacteriological culture of calculi.

Bacteriological study was conducted from pre-operative urine and from surgically extracted renal calculi. Pre-operative urine samples were collected aseptically for macroscopic and microscopic examination. Both pre-operative urine and operated renal calculi were processed for bacteriological culture.

The morning urine's midstream was collected in sterile containers, after a rigorous cleaning of the external genital organs. The urine analysis was performed using Uric 3.V strips, Uricon Biotech Korean.

The bacteriological analysis of the urine (uroculture, the isolation and identification of microorganisms, antibiotic susceptibility) was performed using regular bacteriological methods. The presence of more than 2 bacterial species was the ground of assuming a urine contamination during emission.

Processing of calculi for bacteriological culture was done as described by Ogata T.(8) The calculi were thoroughly rinsed in sterile physiological saline and then crushed with a sterile grinding mortar. The crushed calculi core was cultured in 5ml thioglycolate broth which was incubated at 37°C for 18-24 hours, and then subcultures were made on blood agar and MacConkey's agar plate for isolation of etiological agents.

The isolated organisms were identified by standard techniques.(9)

Chemical analysis of renal stones for oxalate calcium magnesium, ammonium and phosphate were performed as described by Bradley.(10)

The antibiotic susceptibility testing of the isolated strains was performed by the standard Kirby Bauer diffusimetric method, and the interpretation of the results according to CLSI protocol.

For *E. coli, Proteus, Klebsiella and Enterobacter,* we followed the antibiotic susceptibility to ampicillin, gentamycin, amikacin, amoxicillin-clavulanate, ceftazidime, cefepime, tetracycline, trimethoprim-sulfamethoxazole, nalidixic acid, ciprofloxacin and imipenem.

For *Pseudomonas,* we followed the antibiotic susceptibility to gentamycin, amikacin, piperacillin/tazobactam, cefepime, ciprofloxacin and meropenem.

For *Staphylococcus* spp., we followed the antibiotic susceptibility to cefoxitin, cefepime, trimethoprim-sulfamethoxazole, vancomycin, gentamycin, ciprofloxacin, tetracycline, ampicillin, erythromycin, clindamycin and penicillin.

For *Enterococcus* spp., we followed the antibiotic susceptibility to ampicillin, ciprofloxacin, vancomycin, erythromycin, gentamycin, tetracycline and cefepime.

RESULTS

From a total of 27326 urocultures sampled between December 2008 and December 2010, 320 were included in our study, corresponding to the 320 urinary stones sampled after aseptic surgical interventions; in both cases, the microbiological analysis was performed.

The patients had ages between 26-74 years old. The mean age of patients with urinary stones was 58.4 with a median of 58 while mean age of patients with urinary infection disease was 51.4 with a median of 55.6. A high frequency of urolithiasis was found in the 30-49 years age group.

In the study group, significant bacteriuria was found in 252 cases (78.75%), for these, the microbiological analysis being conducted. A higher frequency of positive urocultures was found in females (187; 74.2%) that in males (65; 25.8%), especially in the over 30-49 age group.

The incidence of urinary calculi was higher in males (182; 56.88%) compared to females (138; 43.12%), with the ratio of 1.3:1.

The urocultures identified *E. coli* as the predominant microorganism (149 strains; 59.1%, from which 13; 5.15% were ESBL producing), followed by *Proteus* spp. (29 strains; 11.5%), *Enterococcus* spp. (28; 11.11%), *Pseudomonas* spp. (14; 5.5%) and others (table no. 1).

Out of 320 patients with urinary calculi, 42 patients were having symptomatic urinary infection. 188 calculi (58.75%) originated from kidneys, 48 (15%) from urinary bladder and 84 (26.25%) from ureters. From kidney calculi, 175 (93.08%) were unilateral. From these, 54 (30.85%) were on the left side, while 121 (69.15%) were on the right side. Bilateral kidney calculi were present only in 6.92%.

132 (41.25%) calculi were sterile, whereas 188 (58.75%) were infected. *E. coli* was the predominant microorganism isolated from urinary calculi (81; 43%), from which 11 (5.85%) were ESBL producing, followed by *Pseudomonas aeruginosa* (27; 14.36%), *Enterococcus* spp. (26; 13.82%), *Proteus* spp. (25; 13.29%) and others (table no. 1).

Out of the 320 investigated cases we obtained the following results:

1. 252 (78.75%) positive urocultures and 68 (21.25%) negative
2. 188 (58.75%) infected stones and 132 (41.25%) sterile calculi

Therefore we had:

a. 180 cases (56.25%) with positive bacteriological examination, both for urine and urinary calculi
b. 72 cases (22.5%) with positive urocultures and sterile calculi
c. 60 cases (18.75%) where both the urocultures and the calculi were sterile
d. 8 cases (2.5%) with negative urocultures and infected calculi

Table no. 1. Microorganisms isolated from urine and urinary stones

Microorganism	Uroculture		Urinary stone culture		p
	No.	Percentage of total positive urocultures	No.	Percentage of total positive calculi	
Gram negative	214	84.9%	148	78.72%	0,129
Escherichia coli	149	59.1%	81	43.08%	0,020
E. coli ESBL+	13	5.1%	11	5.85%	1,064
E. coli ESBL -	136	53.9%	70	37.23%	0,023
Proteus spp.	29	11.5%	25	13.29%	1,158
P. mirabilis	21	8.3%	21	11.17%	1,246
P. vulgaris	8	3.17%	4	2.12%	0,918
Klebsiella spp.	9	3,5%	6	3.19%	0,974
K. pneumoniae	8	3.17%	5	2.65%	0,957
K. oxytoca	1	0.39%	1	0.53%	1,012
P. aeruginosa	14	5.5%	27	14.36%	1,604
*Other **	13	5.15%	9	4.70%	0,962
Gram positive	34	13.5%	35	18.61%	1,436
Enterococcus spp.	28	11.11%	26	13.82%	1,237
E. faecalis	24	9.5%	24	12.76%	1,280
E. faecium	4	1.6%	2	1.06%	0,958
Staphylococcus spp.	6	2.38%	9	4.78%	1,188
S. aureus	6	2.38%	6	3.19%	1,068
S. epidermidis	0	0%	3	1.59%	-
Yeasts *(Candida spp.)*	4	1.58%	5	2.65%	1,087
** Citrobacter, Morganella, Acinetobacter*					

There is a statistically significant association regarding the presence of urinary calculi and the development of urinary infections ($p<0.0001$, RR=1.755; 1.498 to 2.057 at 95% confidence interval).

Out of the 180 cases with positive uroculture and infected stones, 127 (70.55%) revealed the same microorganisms, while 53 cases (29.45%) showed different micro-organisms than the preoperative urine culture.

The similarity between the microbial agents involved in urinary infections and those isolated from urinary calculi according to their antibiotic susceptibility can be followed in table no. 2.

Table no. 2. The similarity between the microbial agents involved in urinary infections and those isolated from urinary calculi

Isolated strain	Positive urinary calculi cultures n=188	Positive urocultures with the same bacterial species n=127	Identical antibiotic susceptibility pattern n=46
Escherichia coli	81	68 (83.95%)	23 (28.39%)
Proteus spp.	25	20 (80%)	18 (72%)
Pseudomonas spp.	27	13 (48.14%)	8 (29.62%)
Klebsiella spp.	6	4 (66.66%)	1 (16.66%)
Enterococcus spp.	26	14 (53.84%)	11 (42.3%)
S. aureus	6	3 (50%)	2 (33.33%)
Other	17	5 (29.41%)	-

Alteration in urinary pH also plays an important role in the synthesis of renal stones, because in certain stones, the pH is specific. Urinary pH was high (>7) in 232 cases (72.5%), while in 88 cases (27.5%) the urine was acidic (4-5). Also, in 241 (75.31%) cases, both macroscopic and microscopic hematuria was present. Associated bacterial pyuria was present in 230 cases (71.87%) and crystallurea in 209 cases (65.31%).

Chemical analysis of urinary stones revealed that 31.87% were phosphate stones, 15.93% oxalate dihydrate, 6.56% urate stones, 2.5% oxalate dihydrate and urate, 1.25% calcium carbonate, 0.31% oxalate monohydrate and 0.31% cystine.

The proportions of sterile renal stones compared to the infected stones can be followed in table III. A significant difference of proportions between infected and sterile calculi was found in phosphate type.

Table no. 3. Proportions of sterile renal stones and infected stones

Chemical composition	Infected calculi n=188	Sterile calculi n=132	p
Phosphate	102 (54.25%)	27 (20.45%)	0.002
Oxalate dihydrate	51 (27.12%)	39 (29.54%)	1.200
Urate	21 (11.17%)	31 (23.48%)	1.738
Oxalate dihydrate and urate	8 (4.25%)	11 (8.33%)	1.276
Calcium carbonate	4 (2.12%)	14 (10.6%)	1.404
Oxalate monohydrate	1 (0.53%)	10 (7.57%)	1.208
Cistine	1 (0.53%)	0	-

Due the fact that the enterobacteriaceae are the most frequent isolated strains, we analyzed their antibiotic susceptibility (table no. 4).

Table no. 4. Antibiotic susceptibility of the enterobacteriaceae isolated from urine and urinary calculi

Antibiotic	Urine		Calculi		p
	Nr	%	Nr	%	
AMP	19	9,79%	8	7,14%	0,826
GM	125	65,10%	64	57,14%	0,285
AMC	106	55,21%	33	29,46%	**0,010**
CAZ	130	67,71%	53	47,32%	**0,010**
FEP	148	77,08%	62	55,36%	**0,002**
TET	89	46,35%	34	30,36%	0,108
SXT	115	59,90%	43	38,39%	**0,016**
NA	116	60,42%	42	37,50%	**0,011**
CIP	48	25,00%	27	24,11%	0,931
AK	179	93,23%	91	81,25%	**0,003**
IPM	190	98,96%	108	96,43%	0,130

There are significant differences regarding the susceptibility for β-lactam antibiotics, sulfonamides, quinolones and aminoglycosides between the enterobacteriaceae isolated from urine and urinary calculi.

The antibiotic susceptibility of the enterobacteriaceae isolated from urine and urinary calculi can be followed in figures no. 1 and 2.

Figure no. 1. Antibiotic susceptibility of enterobacteriaceae isolated from urine samples

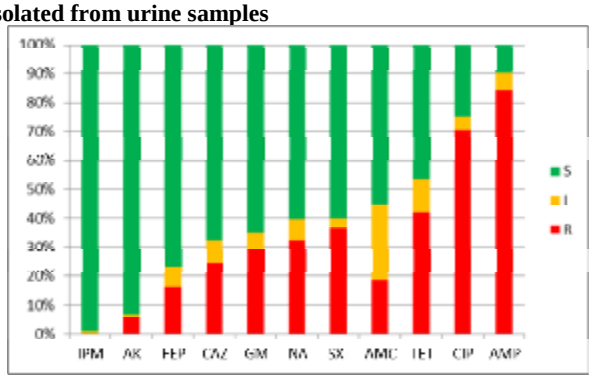
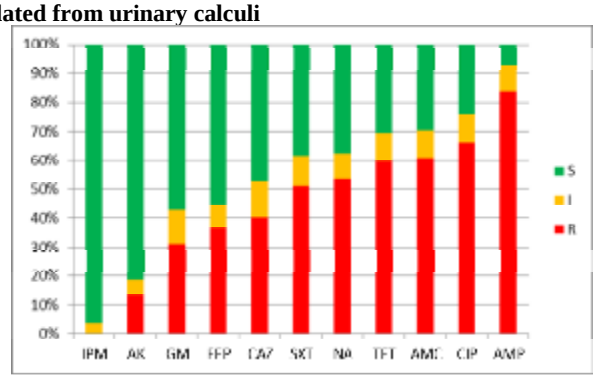


Figure no. 2. Antibiotic susceptibility of enterobacteriaceae isolated from urinary calculi



DISCUSSIONS

Despite modern antibiotic therapy and technological advances in lithotripsy, the presence of infection in urinary stone patients as well as infectious stones is still a significant cause of morbidity and mortality. Not many new investigations are focusing on the pathogenesis and treatment of these difficult cases. Recent findings lend more theories as to how infection leads to stone formation.

The main objective of the present study was to describe the distribution of bacteria isolated from urinary tract infections and urinary infected stones, especially if there are significant statistical differences in antibiotic susceptibility pattern.

In our study, female patients were affected with UTI more often than male patients, but the urolithiasis was more frequent in males, in a ratio of 1.3:1. This results are in agreement with other studies, which observed similar ratios.(11) This may be related to hormonal effects high inhibitory activity, lower food intake and lower body size.(12) In addition, Weshman and McGeown demonstrated increased citrate concentrations in the urine of women. It has been postulated that this may aid in protecting females from calcium urolithiasis since citrate inhibits nucleation of calcium oxalate crystals.(13)

Regarding the age distribution, our results showed a higher frequency of urolithiasis in the age group of 30-49 years. This results is in agreement with other studies which show urolithasis occurs more frequently in the third decade of life.(14)

Although much has been learned regarding the pathogenesis of kidney stone, the bacteria implicated in forming calculi remains incompletely understood.(15) *E. coli* is not a urease producing organism and is not considered to be a calculus producing microorganism. However the present study revealed that *E.coli* was the predominant microorganism recovered from mixed calculi. The present findings are consistent with the study of Sahkaee and Bratell et al.(7,16)

In our study, most of the calculi were on the left side, bilateral being present only in 6.92%. Similar observations were made by Bucholz et al, which showed that most stones were in the left kidney.(17)

Regarding the antimicrobial sensitivity pattern of bacteria we have found that there were differences between urinary isolates. We observed that the enterobacteria isolated from calculi were less sensitive than urinary bacteria to most of the tested antibiotics. This observation can be explained by the presence of bacteria below the surface of the stone, which are not reached by antibiotics although the urine and stone surface were cleared with appropriate antibiotic therapy.(18) If the antibiotic therapy is stopped while he infected stone fragment is still present, the original infection will recur.(19)

Generally we have found that the most efficient antimicrobial drugs against the enterobacteria isolated from urocultures were the β-lactam antibiotics and aminoglycosides. The antimicrobial drugs most efficient on enterobacteria isolated from urinary calculi were the same as in those isolated from urine, although the sensibility degree was lower.

It appears that the bacteriological findings from urine do not always reflect the bacteriology of urinary tract calculi, which is in agreement with the result of previous studies.(6,20) The 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 presence of bacteria within the calculi may be due to insignificant intermittent bacteriemia 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.(11) Thus, a vicious cycle starts, infection bringing about calculi formation and calculi causing infection.(21,22)

Most of the current literature on the subject focuses on pathogenesis of infectious urinary stones. The remaining literature highlights difficult cases, outcomes of treatments, and overall reviews of the subject.(23)

Recent findings lend more theories as to how infection leads to stone formation. Further investigation is critically needed to improve the outcomes of patients suffering from infections with urinary stones and infectious stones.

For many patients, clues to the calculi formation are obtained with an extensive search for risk factors. Such an outcome most certainly reflects our incomplete understanding of the stone formation or the way we usually collect and analyze urine.

Despite the obvious shortcomings, it is important to reveal a correlation between the various risk factors by a careful medial history with a radiographic examination as well as an analysis of stone, blood and urine composition and an effective individualized treatment.

CONCLUSIONS

Our study revealed the following aspects:

- The incidence of urolithiasis was slightly higher in men compared to women, with a higher rate of infectious calculi compared to sterile ones.
- Urinary infection frequency was higher in women compared to men.
- The preponderant etiology of urinary infections in patients with urolithiasis was given by the following microorganisms: *Escherichia coli, Proteus* spp., *Enterococcus* spp. and *Pseudomonas aeruginosa*.
- The preponderant microorganisms found in calculi were: *Escherichia coli, Pseudomonas aeruginosa, Enterococcus* spp. and *Proteus* spp.
- There is a statistically significant association regarding the presence of urinary calculi and the development of urinary infections
- Bacterial isolates from urinary calculi were less sensitive to most of the tested antibiotics than the isolates from uroculture.

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