Urinary calculi: bacteriological and chemical association

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الحصيات البولية: الارتباط الجرثومي والكيميائي

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الخلاصة: استهدفت هذه الدراسة استقصاء بكتريولوجية الحصيات البولية في ما يتعلق بعدوى الجهاز البولي، وتكوُّن الحصيات، وتركيبها الكيميائي، والحساسية للمضادات الحيوية. وقد شملت الدراسة اثنين وخمسين مريضاً (37 منهم من الذكور و15 من الإناث) من المصابين بالحصيات البولية، أخذت منهم عينات من البول والمصل ومن الحصيات البولية، وأجريت لهم اختبارات كيميائية حيوية مصلية لتحرّي حمض اليوريك والكلسيوم والفسفور، إضافة إلى تحليل البول والزرع. وتبيَّن إصابة 19 مريضاً من هولاء المرضى الاثنين والخمسين (أي 37%) بعدوى مرافقة في الجهاز البولي، وكانت الإشريكية القولونية *E. coli* والمتقلبة الرائعة *Proteus mirabilis* أكثر الجراثيم المسبِّبة لها شيوعاً. وكان هناك اختلاف بين المستفرَدات الجرثومية المأخوذة من البول والمستفرَدات الجرثومية المأخوذة من الحصيات من حيث حساسيتهما لمضادات الجراثيم. ونخلص من ذلك إلى أن زرع البول يمكن، في أكثر من 50% من المرضى الحصيات البولية، أن يؤدي إلى اكتشاف الجراثيم المُعْدية المرافقة لتكوُّن الحصيات والجراثيم الموجودة في الحصيات البولية.

ABSTRACT We investigated the bacteriology of urinary calculi in relation to urinary tract infection, stone formation, chemical composition and antibiotic sensitivity. Fifty-two patients (37 males, 15 females) with urolithiasis were studied. Urine, serum and urinary calculi specimens were taken and serum biochemical tests to detect uric acid, calcium and phosphorus were performed. Urine analysis and culture were also performed. Of the 52 patients, 19 (37%) had associated urinary tract infection, with *Escherichia coli* and *Proteus mirabilis* being the most common causative microorganisms. The bacterial isolates from urine and those from calculi differed in their susceptibility to antimicrobial agents. We conclude that in over 50% of patients with urolithiasis, urine culture can detect the infecting organisms associated with stone formation and the organisms within urinary calculi.

Calculs urinaires : association bactériologique et chimique

RESUME L'objectif de cette étude était d'examiner la bactériologie des calculs urinaires en association avec les infections urinaires, la formation des calculs, la composition chimique et la sensibilité aux antibiotiques. Cinquante-deux patients (37 hommes, 15 femmes) atteints d'urolithiase ont fait l'objet de l'étude. On a procédé à des prélèvements d'échantillons urinaires et sériques et de calculs urinaires et on a effectué des dosages sériques pour détecter l'acide urique, le calcium et le phosphore. Une analyse et une culture d'urine ont également été réalisées. Sur les 52 patients, 19 (37 %) avaient une infection urinaire associée, *Escherichia coli et Proteus mirabilis* étant les micro-organismes pathogènes les plus courants. Les bactéries isolées dans l'urine et celles des calculs différaient dans leur sensibilité aux agents antimicrobiens. Nous concluons que la culture d'urine peut détecter l'organisme infectant associé à la formation de calculs et les organismes contenus dans les calculs urinaires chez plus de 50 % des patients atteints d'urolithiase.

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Introduction

Urolithiasis is one of the commonest urological disorders affecting humans. This ancient health problem has afflicted people throughout history: scientists have found evidence of kidney stones in an Egyptian mummy estimated to be more than 7000 years old [*1*].

Struvite or infection-induced stones are the most dangerous of the urinary stone diseases, because of the potentially life-threatening complications of such infections. These stones are found mainly in women with recurrent urinary tract infections (UTI), and frequently complicate other types of urinary stones. They are composed of magnesium ammonium phosphate and/or carbonate apatite and account for 15%–20% of all urinary calculi [*2*].

Urea-splitting bacteria (i.e. *Proteus* spp., staphylococci, *Klebsiella* spp. or, more rarely, *Ureaplasma urealyticum)* metabolize urea in urine, resulting in the deposition of carbonate-apatite and struvite in a friable matrix, the so-called mixed phosphate stones. Non-urease-producing bacteria, such as *Escherichia coli*, are the most common organisms found in association with secondarily infected stones. However, sometimes the bacteria secondarily infecting a metabolic stone are urease-producing, in which case further stone growth is composed of struvite and carbonate apatite [*3*].

Although there is some relation between UTI and the formation of urinary stones, this is not the only factor involved, for many patients with chronic pyelonephritis do not have kidney stones, or stones may present in the kidney only. Furthermore, in many cases of urolithiasis, no infection is found [*4*].

The aims of this study were to investigate the bacteriology of urinary calculi in relation to urinary tract infection; to study the role of UTI in stone formation, and the chemical composition of the urinary calculi in relation to urinary tract infection; and to study the antimicrobial sensitivity pattern of bacteria isolated from urine and calculi specimens.

Methods

The study included 52 patients who presented with variable symptoms secondary to stones in the urinary tract and who had been admitted to the Department of Urology in Tripoli Medical Centre, El Zahra Centre for Kidney Disease Therapy and Elafia Clinic for surgical removal of the urinary stones. The age of the patients ranged from 15 years to 90 years (mean = 47 years) (Table 1). Of these patients, 37 were males and 15 females (male to female ratio of 2:1). Diagnosis was made by intravenous urogram or, in patients allergic to contrast material, a combination of renal ultrasonography and plain abdominal X-ray film.

Samples of serum, urine and urinary stones were collected from the study patients. The serum and urine specimens were collected pre-operatively and before any antibiotic therapy was given. Serum levels of calcium, phosphorus and uric acid were measured by standard biochemical methods in the laboratory of the hospital using an automatic device. The urine specimens were subjected to routine microscopic and biochemical analysis and bacteriological culture. The urinary stone specimens were subjected to bacteriological culture and qualitative chemical analysis.

Bacterial isolates from urine specimens and calculi were identified by standard microbiological procedures [*5*], and by the API 20E System (bioMérieux, France). The susceptibility of the bacterial isolates to antimicrobial agents was measured *in vitro* by the method of Kirby and Bauer [5].

The results were analysed statistically using the chi-squared test and Fisher exact test where appropriate.

Results

We found that 37 (71%) male patients had urolithiasis, compared with 15 (29%) female patients. Nearly half (48%) of the patients were aged 30–49 years (Table 1). Six (16%) of the 37 male patients had a history of previous urinary stone disease (either they spontaneously passed a stone or it was extracted surgically), while the remainder had no previous history of urinary stone disease. Three (20%) of the 15 female patients had a previous history of urinary stone disease (Table 2).

Hyperuricaemia, defined as a serum uric acid level higher than 5.7 mg/dL, was found in 7 (13%) of the patients (Table 3). Hypercalcaemia, defined as a serum calcium level higher than 2.6 mmol/L, was found in 3 (6%) of the patients. Hyperphosphataemia, defined as a serum phosphorus level higher than 1.45 mmol/dL, was found in 2 (4%) of the patients, and a combination of hyperuricaemia and hyperphosphataemia was found in 3 (6%) of the patients.

Table 1 **Age and sex distribution of the 52 patients with urolithiasis**

Age group (years)	Males No.	Males %	Females No.	Females %	Total No.	Total %
10–15	1	3	0	0	1	2
16–29	6	16	5	33	11	21
30–49	19	51	6	40	25	48
≥50	11	30	4	27	15	29
Total	37	100	15	100	52	100

Table 2 **Distribution of the 52 patients according to history of previous urinary stone disease**

Previous history	Males No.	Males %	Females No.	Females %	Total No.	Total %
Present	6	16	3	20	9	17
Absent	31	84	12	80	43	83
Total	37	100	15	100	52	100

Qualitative chemical analysis of urinary stones revealed the presence of mixed calcium oxalate, calcium phosphate and magnesium phosphate (S3) in 10 (19%) patients; mixed uric acid, calcium phosphate and magnesium phosphate (S6) in 8 (15%) patients; mixed calcium phosphate and magnesium ammonium phosphate (S9) in 8 (15%) patients; mixed calcium oxalate, calcium magnesium phosphate and calcium carbonate (S2) in 7 (13%) patients; pure calcium oxalate (S1) in 5 (10%) patients; and pure uric acid (S5) in 4 (8%) patients (Table 3).

The serum uric acid was higher than normal in 7 patients who had calculi where uric acid was one of the components (S5, S6 and S7). The serum calcium level was also raised in 3 patients with calculi containing calcium as one of the components (S1 and S4). Serum phosphorus was elevated in 2 patients with calculi containing phosphorus as one of the components (S8 and S9), and elevated serum levels of both uric acid and phosphorus were found in 3 patients with calculi containing uric acid and phosphorus (S6 and S7) (Table 3).

Nineteen (37%) patients, 12 females and 7 males, had an associated UTI, 14 (73%) of them symptomatic. Of these 19 patients, 15 (79%) had pyuria, 11 (58%)

Table 3 **Normal and abnormal serum level of calcium, phosphorus and uric acid in relation to various chemical types of urinary stones**

Urinary stone type	Increased serum level of:						Normal	Total
	Uric acid	Calcium	Phosphorus	Calcium + phosphorus	Calcium + uric acid	Phosphorus + uric acid		
S1	0	1	0	0	0	0	4	5
S2	0	0	0	0	0	0	7	7
S3	0	0	0	0	0	0	10	10
S4	0	2	0	0	0	0	1	3
S5	4	0	0	0	0	0	0	4
S6	2	0	0	0	0	2	4	8
S7	1	0	0	0	0	1	0	2
S8	0	0	1	0	0	0	2	3
S9	0	0	1	0	0	0	7	8
S10	0	0	0	1	0	0	1	2
Total (%)	7 (13)	3 (6)	2 (4)	1 (2)	0 (0)	3 (6)	36 (69)	52 (100)

S1 = calcium oxalate. S2 = calcium oxalate + calcium phosphate + magnesium phosphate + calcium carbonate. S3 = calcium oxalate + calcium phosphate + magnesium phosphate. S4 = calcium oxalate + uric acid. S5 = pure uric acid. S6 = uric acid + calcium phosphate + magnesium phosphate. S7 = uric acid + ammonium urate + ammonium phosphate. S8 = calcium phosphate. S9 = calcium phosphate + magnesium phosphate + ammonium phosphate. S10 = calcium phosphate + magnesium phosphate + calcium carbonate.

had epithelial cells in the urine and 13 (68%) had proteinuria. The most common microorganisms isolated from 52 pre-operative urine specimens were *E. coli* and *P. mirabilis* with 5 isolates each. The same type of microorganisms were found in both pre-operative and intra-operative urine specimens except in 5 patients where the intra-operative urine was sterile. In contrast, the bacterial species isolated from the calculi of the patients were dominated by *P. mirabilis* and *Staphylococcus* spp., with 5 isolates each. Of the 6 *Staphylococcus* species isolated (1 from pre-operative urine and 5 from calculi), 1 was identified as *S. saprophyticus* (from pre-operative urine), 3 were *S. aureus* and 2 were *S. epidermidis* (all from calculi) (Table 4).

Of the 19 urinary calculi associated with a UTI, 12 bacterial isolates were identical with those isolated from the pre-operative urine specimens, while 6 isolates (1 *Pseudomonas aeruginosa* and 5 *Staphylococcus* spp.) were isolated from calculi but not from urine specimens.

Urease-producing microorganisms (i.e. *P. mirabilis, Enterobacter aerogenes* and *K. pneumoniae*) were detected in 8 (42%) of 19 pre-operative urine specimens and 7 (50%) of 14 intra-operative urine specimens. They could therefore be responsible for UTI in these patients. Furthermore, 58% of the 19 urinary calculi specimens were positive for urease-producing microorganisms (5 *P. mirabilis*, 2 *S. aureus* and 1 *S. epidermidis*, 2 *K. pneumoniae* and 1 *Enterobacter aerogenes*).

Interestingly, *E. coli* was found to be associated with stone types S1, S2 and S3, in which calcium oxalate is one of the com-

Table 4 **Bacterial species isolated from pre-operative urine, intra-operative urine and renal calculi of the 52 patients**

Bacterial species isolated	Urinary isolates				Calculi isolates	
	Pre-operative urine		Intra-operative urine			
	No.	%	No.	%	No.	%
Proteus mirabilis	5	9.6	4	7.7	5	9.6
Escherichia coli	5	9.6	4	7.7	3	5.7
Enterobacter aerogenes	1	1.9	1	1.9	1	1.9
Klebsiella pneumoniae	2	3.8	2	3.8	2	3.8
Citrobacter freundii	1	1.9	1	1.9	0	0.0
Pseudomonas aeruginosa	1	1.9	1	1.9	2	3.8
Staphylococcus spp[a]	1	1.9	1	1.9	5	9.6
Streptococcus faecalis	1	1.9	0	0.0	0	0.0
Mixed growth	2	3.8	0	0.0	1	1.9
No growth	33	63.4	38	73.0	33	63.4
Total	52	10.0	52	100.0	52	100.0

[a]Staphylococcus *spp.* = S. aureus *and* S. epidermidis *(from calculi) and* S. saprophyticus *(from urine).*

ponents in addition to phosphate. However, *P. mirabilis* isolates were associated with stone types S2 and S9, where phosphate is the major component. Types S5 and S6, in which uric acid is the main component, were not associated with microbial growth (Table 5).

There were differences between urinary isolates and calculi isolates in the antimicrobial susceptibility patterns. The calculi isolates were less sensitive than urinary isolates to most of the antibiotics tested (Table 6).

Discussion

In our study, male patients were affected with urolithiasis more often than female patients, with a male to female ratio of 2:1. This result is in agreement with other studies, which show that urinary tract stone disease affects men more frequently than women; the reported ratio of male to female stone disease is 3:1 [*6–8*]. From our study, it also appears that renal stone disease develops more frequently in the age group of 30–49 years. This result is in agreement with other studies from Saudi Arabia, which show urolithiasis occurs more frequently in the third decade of life [*1,9–11*].

Sixteen patients had abnormal serum levels of uric acid, calcium or phosphorus. Hyperuricaemia was observed in some patients whose calculi contained uric acid as one of their components. These types of stones (i.e. S5, S6 and S7) together constituted 27% of the total. Only one patient (7%) with uric acid-containing stones had an infection of the urinary tract; the remaining (93%) presented without UTI. This could indicate that metabolic disorders are greater risk factors than urinary tract infection. However, the small number of patients involved should be borne in mind.

Table 5 **Frequency of occurrence of renal stones in relation to urinary tract infection in study patients**

Urinary stone type	Frequency of occurrence of renal stones in relation to urinary infections [No. (%)]										Total
	Prot.	Esch.	Ent.	Kleb.	Cit.	Pseud.	Staph.	Strep.	MG	NG	
S1		1 (20%)		1 (20%)						3 (60%)	5
S2	2 (29%)	1 (14%)			1 (14%)					3 (43%)	7
S3		2 (20%)						1 (10%)		7 (70%)	10
S4							1 (33%)			2 (67%)	3
S5										4 (100%)	4
S6										8 (100%)	8
S7		1 (50%)								1 (50%)	2
S8			1 (33%)	1 (33%)						1 (33%)	3
S9	3 (37%)					1 (13%)				4 (50%)	8
S10									2 (100%)	0 (0%)	2
Total	5 (10%)	5 (10%)	1 (2%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)	2 (4%)	33 (63%)	52

S1 = calcium oxalate. S2 = calcium oxalate + calcium phosphate + magnesium phosphate + calcium carbonate. S3 = calcium oxalate + calcium phosphate + magnesium phosphate. S4 = calcium oxalate + uric acid. S5 = pure uric acid. S6 = uric acid + calcium phosphate + magnesium phosphate. S7 = uric acid + ammonium urate + ammonium phosphate. S8 = calcium phosphate. S9 = calcium phosphate + magnesium phosphate + ammonium phosphate. S10 = calcium phosphate + magnesium phosphate + calcium carbonate.

Prot. = Proteus mirabilis, *Esch.* = Escherichia coli, *Ent.* = Enterobacter aerogenes,
Kleb. = Klebsiella pneumoniae, *Cit.* = Citrobacter freundii, *Pseud.* = Pseudomonas aeruginosa
Staph. = Staphylococcus *spp.*, *Strep.* = Streptococcus faecalis, *MG = Mixed growth, NG = No growth.*

Gad et al. have shown that UTI was considered a high risk factor in Libyan patients with uric acid stones associated with UTI, rather than metabolic disorders [*12*]. Hypercalcaemia was observed in 6% of our patients. This may be due to some predisposing condition, such as hyperparathyroidism, which causes an increase in calcium turnover in bone and is considered to be an important cause of hypercalciuria. These patients with hypercalcaemia had calculi of types S1 and S4 and no UTI. Hypercalcaemia may therefore predispose to their formation.

It is important to elucidate whether a UTI associated with calculi is related to the stone formation. It is understood that in the presence of accelerated ureolysis, the alkalinity and concentrations of ammonia, bicarbonate and carbonate in the urine are sufficient for crystallization of struvite and carbonate apatite [*13*]. Holmgren reported that of 796 consecutive patients at a stone clinic over a 10-year period, 52 (7%) had recurrent or chronic UTI that was considered to be of pathogenic importance in the stone formation [*14*]. Our study shows that 37% of patients had UTI associated with urinary calculi. *E. coli* and *P. mirabilis* were the most commonly isolated microorganisms.

Urease-producing microorganisms were detected in 42% of 19 patients with UTI, and they may therefore play a role in

Table 6 **Susceptibility of Gram-negative bacteria isolated from urine specimens and calculi to antimicrobial agents**

Antibiotic	Sensitivity (%) Urinary isolates ($n = 28$)	Calculi isolates ($n = 13$)
Ampicillin (10 mg)	30	12
Augmentin (30mg)	68	48
Carbenicillin (100 mg)	43	24
Cephalexin (30mg)	24	20
Cefamandole (30 mg)	75	72
Cefotaxime (30 mg)	100	100
Ceftazidime (30 mg)	100	100
Chloramphenicol (10 mg)	50	18
Gentamicin (10 mg)	92	75
Oxytetracycline (30 mg)	50	27
Norfloxacin (10 mg)	100	100
Co-trimoxazole (25 mg)	47	30
Nalidixic acid (30 mg)	76	56
Nitrofurantoin (30 mg)	66	30
Colistin sulfate	100	100

stone formation in these patients. The enzyme urease hydrolyses urea to ammonium and carbon dioxide, which leads to the crystallization of magnesium ammonium phosphate and carbonate apatite. In our study, 60% of *P. mirabilis* isolates were associated with S9 type stones, which are struvite mixed with calcium phosphate, while 40% were associated with S2 type stones, which are struvite mixed with calcium carbonate, calcium phosphate and calcium oxalate. This could be explained if urease-positive *P. mirabilis* isolates led to the formation of struvite. These struvite stones may be formed first following UTI by *P. mirabilis*. Subsequently, other types of salts, such as calcium phosphate, may be precipitated on them as in S9 type stones, or may be formed secondary to metabolic stones such as calcium oxalate stones after infection with *P. mirabilis*, as in S2 type stones [*3*]. *E. coli* isolates were associated with 20% of S1 type stones (calcium oxalate), 14% of S2 type stones (calcium oxalate, calcium phosphate and magnesium ammonium phosphate) and 20% of S3 type stones (calcium oxalate mixed with calcium phosphate). *E. coli* is a non-urease-producing microorganism and may have led to secondary infection in these cases [*3*].

We observed that calculi isolates were more resistant than urinary isolates to most of the antimicrobial agents tested. This 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 [*15*]. If the antibiotic therapy is stopped while the infected stone fragment is still present, the original infection will recur [*16*].

In conclusion, urine culture of patients with infections associated with urolithiasis can identify the infecting organisms associated with stone formation and the organisms residing within urinary calculi in more than 50% of patients. Bacteria present within the calculi may not be detected by urine culture, and may act as foci of persistent infection of the urinary tract. As observed clinically, when these stones are treated by any method that leads to their disruption, the bacteria will be released and may cause bacteraemia or endotoxaemia. To prevent such complications, prophylactic antibiotic therapy should be given to the patients in the pre-operative period. As the calculi isolates were more resistant than urinary isolates to most antibacterial agents tested, broad spectrum antibiotic therapy should be used prophylactically before the operation. Finally, chemical analysis of the urinary stones, to elucidate any factors predisposing to stone formation, will help in the management and prevention of stone recurrence.

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