

The Main Bacterial Isolates Associated with Urolithiasis in Thiqr Province

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Abstract:

Urinary tract stones are big problem in both developed and developing countries word wide distribution, in late decades prevalence and incidence of urolithiasis were increased. Thirty urinary calculi were collected from Al-Hussein Teaching and Al-Habobi hospitals, during a period from 1/10/2020 to 1/2/2021 from both sex. Results explained that from 30 urinary stones were 15 (50%) were contain bacterial isolates (infection stones) and the main an important bacterial isolates were *E.coli*, *Staphylococci caprae*, *Ochrobacterum anthropi*, *Staphylococcus epidermidis*, *Micrococcus* spp, *Staphylococcus xylosus*, *Pseudomonas aeruginosa*, and *Chromobacterium violaceum* in 33.3%, 20%, 13.3%, 6.6%, 6.6%, 6.6% and 6.6% respectively. Antimicrobial susceptibility test show most isolates were resistance to Amikicin, ceftraxone, vancomycin and Gentamycine, while it was sensitive to Imipenem, Amoxicillin- Clavulanic acid and Ciproflaxacin. On the other hand, chemical analysis refer that 73.3% were uric acid stones and 26.6% were calcium oxalate stones.

Key words: urinary tract stones, *E.coli*, *Pseudomonas aeruginosa*.

Introduction:

Urinary tract stones (UTS) remain a public health problem around the word, however about 15 to 20% of population suffered from urolithiasis (1). There are several risk factors which responsible for stones formation represented by environmental, metabolic, dietary, racial, sex, obstructive uropathology and urinary tract infection (2). The prevalence and incidence of urolithiasis have changed in different countries over the years (3). Urolithiasis is one of the most word wide in prevalence, incidence and composition of calculi, consequently it changes in last several decades, with prevalence ranging from 7% to 13% in North America, while 5% to 9% in Europe, on the other hand, 1% to 5% in Asia (4). In different countries of Asia, the prevalence and incidence may be dropped or ascended, that due to climate effect, scocioeconomic status, geographic variation and genetic variation (5).

Urolithiasis are polycrystalline, which are often associated with crystalluria is the excretion of different crystals in urine, which is a marker of transient supersaturation of urine present both in normal physiology and also in pathological conditions (6). Crystals of size less than 5 mm pass easily through the urinary tract, while crystals of size more than 5 mm precipitate, leading to the stone formation (7).

Urinary stone disease (USD) is an expanding problem in United States that 185000 hospitalization patients suffered from USD and about 2.1 billion dollars expended annually for management (8,9). The supersaturation of urine with crystal which is a risk factor, alone it is insufficient for stone formation, however supersaturation of urine and bacterial isolates should be recognized as source of stones formation, many drugs used for reduce the supersaturation of urine but the stone formation problem still, that prevalence in United States adults and children has recently increased by 40% and 23% respectively (10,11).

Study occurs in Taiwan, referred that urinary tract infection, history was the most common associated condition in children with newly diagnosed urinary stone disease, overall, 34% of children diagnosed with USD had a history of urinary tract (12). Bacteria can be isolated from the stones themselves, study from 1973 to several decades in both Asia and North America have referred that bacteria can be isolated from approximately 15-70% of stones following clinical culture (13,14). *Escherichia coli* and *Pseudomonas* spp. were the most common bacteria isolated from calcium oxalate stones, followed by the urease-splitting bacteria which is isolated from struvite stones (15). Molecular study such as 16S rRNA gene sequencing refer that multiple bacteria were isolated from different urinary stones, these isolates include *Pseudomonas*, *Gardnerella*, *Lactobacillus* and *Enterobacteriaceae* especially *E. coli* (15).

Infection stones account for 15 to 20% of urinary stones, it may occur as primary event or infection may induce further stone formation, however, infection stones commonly manifest as renal or bladder calculi but infrequently as ureteral calculi (16). There several mechanisms which described the role of bacteria in stone formation, one of them refer, that some bacteria selectively aggregate to certain crystal types but not others and that bacteria are associated with an increased number of crystal-crystal agglomeration (16). Another way that bacteria could contribute to

urinary stone disease is bacterial production of citrate lyase, which could decrease the urine citrate level, that lead to supersaturated urine and crystal formation(17).

Aim of study: Isolation and identification of main bacterial isolates associated with urinary tract stones in Thiqr province.

Materials and methods:

- 1- Sample collection: thirty urinary tract stones were aseptically collected from Al-Hussein Teaching and Al-Habobi Hospitals during a period from 1/10/2020 to 1/2/2021, directly transported by cleaning sterile plastic container to advance microbiology laboratory, medicine college, thiqr university.
- 2- Cleaning and washing with physiological saline then crushing of stones by mechanic mortar.
- 3- The crushing stones were divided into two parts, part for bacteriological examination, the other for chemical analysis test.
- 4- Part of bacteriological examination, direct culturing within brain- heart infusion broth and incubated at 37°C for 24 hours.
- 5- The previous culturing samples were reinoculated on blood agar and macConkey agar.
- 6- After obtained pure cultures, primary diagnosis will be done by Gram stain.
- 7- Final diagnosis, which occurs by biochemical reaction and API system.
- 8- Antimicrobial susceptibility test will be done for stones bacterial isolates.

Results:

1- Numbers of infection stones

During a period 1/10/2020 to 1/2/2021, there were 30 urinary tract stones were collected from Al-Hussein teaching and Al-Habobi hospitals. There are 50% of collected stones were considered as infection stones.

2- Bacterial isolates which isolated from infection stones

The main bacterial isolates were isolated and identified were *Escherichia coli* (33.33%), *Staphylococcus caprae* (20%), *Ochobacterum anthropi* (13.33%), *Staphylococcus epidermidis*, *Staphylococcus xylosus*, *Micrococcus* spp, *Chromobacterium violaceum* and *Pseudomonas aeruginosa* were (6.66%) for each one as show in table 1.

Table (1): Numbers and percentages of bacterial isolates from infection stones

Bacterial isolates	Number	Percentage(%)
<i>E.coli</i>	5	33.33
<i>Staph. caprae</i>	3	20
<i>Ochobacter anthropi</i>	2	13.33
<i>Staph. epidermidis</i>	1	6.66
<i>Staph. xylosus</i>	1	6.66
<i>Microcococcus</i>	1	6.66
<i>Chromo. Violaceum</i>	1	6.66
<i>Pseud. aeruginosa</i>	1	6.66
Total	15	100

3: Antibiotic susceptibility test of Gram positive isolates.

Most Gram- positive bacterial isolates which isolated from urinary tract stones were resistant to variety of antibiotics disk will be used in current study, table (2) refer that isolates were resistance to Ceftraxone and Amikicin, while were sensitive to Amoxicillin- Clavulanic acid, Imipenem, Vancomycin, Amoxicillin and Ciprofloxacin.

Table**(2): Susceptibility test for Gram- positive isolated from urolithiasis.**

Bac. Isolates	Imi 10 mcg	Amox 20 mcg	Am.cla 30 mcg	Amiki 10mcg	Vanc 30mcg	Gent 10mcg	Cipro 10mcg	Ceftr 10 mcg
<i>Stap.caprae</i>	S	S	S	R	S	S	S	R
<i>Stap.epidermitis</i>	S	S	S	R	S	S	S	R
<i>Stap.xylosus</i>	S	S	S	R	S	S	S	R
<i>Microcococcus</i>	S	S	R	S	S	S	S	R

4: Antibiotic susceptibility test of Gram – negative isolates.

Escherichia coli, *Chromobacterium violaceum*, and *Pseudomonas aeruginosa* isolates which appear resistance to most antibiotic disk will be used in the present study, except they were sensitive to Imipenem and Ciprofloxacin, while the bacterium *Ochrobactrum anthropi* was sensitive to Amikicin, Amoxicillin-Clavulanic acid, Gentamycin, Vancomycin, and Amoxicillin. On the other hand, it was resistance to Imipenem and Ceftraxone, as show in table (3)

Table (3): Susceptibility test for Gram- negative isolates associated with urolithiasis

Bac. Isolates	Imi 10 mcg	Amox 20 mcg	Am.cla 30 mcg	Amiki 10mcg	Vanc 30mcg	Gent 10mc g	Cipro 10mc g	Ceftr 10 mcg
<i>E.coli</i>	S	R	R	R	R	R	S	R
<i>Ps.aeruginosa</i>	S	R	R	R	R	R	S	R
<i>Chromobacterium violecium</i>	S	R	R	R	R	R	S	R
<i>Ochrobactrum anthropi</i>	R	S	S	S	S	S	S	R

3- Chemical analysis of urinary tract

Urinary tract stones were chemically analyzed in chemical laboratory, science college in Thiagar university. Infra-red (IR) spectroscopy (Vivek and Pradeep, 2014) which be used for obtained the results. Results showed that Uric acid stones were 73.34%, while Calcium oxalate stones were 26.66%, as show in table.

Table (4): Chemical analysis of urinary tract stones

Type of stones	Numbers	Percentage
Uric acid	22	73.34%
Calcium oxalate	8	26.66%
Total	30	100%

Discussion:

Urinary stone disease (USD) is an increasing clinical problem in both children and adults. One in ten individuals will experience a urinary stone, yet the mechanisms responsible for urinary stones remain largely unknown. Bacteria have long been recognized to contribute to urinary stones formation. In present study 50% of stones were contain bacterial isolates (infection stones), and this agreement with Andrew et al., (2017) were explained that bacteria can be isolated from approximately 15-70% of stones following clinical positive culture. On the other hand, the current study which disagreement with Andrian and co-workers (2015), which explained that 24.2% of stones were positive culture, this may be due to number of samples collection, examination, nutrition style and geographic region variation.

The main bacterial isolates from infection stones were *Escherichia coli* (33.33%), *Staphylococcus caprae* (20%), *Ochrobactrum anthropi* (13.33%) and *Pseudomonas aeruginosa* (6.66%), however, these results which

disagreement with Ogata and co-worker, (2003) were explained that *E. coli* and *Pseudomonas* species were the most bacterial isolates from stones culture, this may be due to previous urinary tract infections, geographical variation, stones chemical composition and life style.

Results of antimicrobial susceptibility test, were referred that most bacterial isolates which resistance to different generation of antibiotics, that appeared resistance to Ceftraxone (100%) and Amoxicillin in (75%) and these results which similar with Andrian and co-workers were explained that bacterial isolates were resistance to the second generation Cephalosporin.

Chemical analysis of urinary tract stones showed that, only two types stones, which are uric acid stones, which represented 73.34% , while calcium oxalate stones formed 26.66%. these results were unlike from other researchers, Andrian and his team (2015) were explained that calcium oxalate is found in greater than 60% of urinary stones and the reasons may be diet style, genetic variation and number of stones which be examined

From the current study we, conclude that bacterial stone infection has an important role in formation of urolithiasis and most of these bacterial isolates were resistance to antimicrobial agents, on the other hand, the present study explain, most urinary tract stones were uric acid stones (chemical composition) in Thiqr province, south of Iraq

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