



Molecular and biochemical detection of bacteria in adult patients with urinary tract infection associate renal stones

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ARTICLE INFO

Article Type:
Original

Article History:
Received: 8 Apr. 2024
Accepted: 9 Jun. 2024
Published online: 24 Jun. 2024

Keywords:
Renal stones
16SrDNA sequencing
Bacteria
Antibiotics

ABSTRACT

Introduction: This study aimed to identify bacterial species isolated from urine and stones, to determine their sensitivity to antibiotics, and also to perform a chemical analysis of stones, focusing on the most prevalent type.

Patients and Methods: One hundred adult patients with renal stones admitted to the surgical department of Al-Zahrawi surgical hospital in Maysan governorate were enrolled, then urine and surgical stone cultures were gathered. Bacterial identification was conducted using *16S rDNA gene* sequencing. Additionally, a qualitative chemical composition analysis of stones was conducted.

Results: A total of 130 bacterial isolates were identified (comprising nine different species), *Escherichia coli* was the predominant bacterium in both urine and stone cultures (41 and 20 isolates respectively), followed by *Klebsiella pneumoniae* (15 and 9, respectively) and *Proteus mirabilis* (11 and 8 respectively). Most isolates showed high sensitivity to imipenem and amikacin (80% and 71.5%, respectively; $P < 0.01$), while sensitivity to tetracycline, sulfamethoxazole/trimethoprim, nitrofurantoin, and ampicillin was notably lower (30.7%, 24.9%, 21.5% and 12.3% respectively). The incidence of renal stones was more frequent in males (64%), particularly among patients aged 31-40 years (60%), with a significant difference at $P \leq 0.05$. Additionally, calcium oxalate was the most common biochemical composition of stones (51%), followed by calcium phosphate and uric acid (19% and 15% respectively), since magnesium-ammonium phosphate, calcium carbonate, and cystine were less common (9%, 4%, and 2%, respectively). There was also a significant relationship between bacterial urinary tract infection and the presence of renal stones.

Conclusion: The most common bacteria isolated from adult patients with renal stones in Maysan governorate were *Escherichia coli*, followed by *Klebsiella pneumoniae* and *Proteus mirabilis*. Calcium oxalate stones were most frequent, followed by uric acid. The infection rate between urinary tract infection and renal stones was 65% and this is considered significant.

Implication for health policy/practice/research/medical education:

In the presented study, we showed that the most common urinary tract infection bacteria associated with renal stones was *Escherichia coli*. In addition, our study indicated that imipenem was the most effective drug against bacterial isolates.

Please cite this paper as: Al-Tulaibawi NAJ, AL-Nussairawi M, AL-Zuhairy NAS. Molecular and biochemical detection of bacteria in adult patients with urinary tract infection associate renal stones. J Renal Inj Prev. 2024; 13(3): e38324. doi: 10.34172/jrip.2024.38324.

Introduction

Renal stone disease is one of the most painful and common urological disorders worldwide. Approximately 10%-15% of the general population is affected by this disease (1,2). The epidemiology of kidney calculus varies by geographical region, gender, age, and stone constituents (3-5). A stone is a solid deposit of dissolved minerals and salts in the urine that develops inside the urinary system. Kidney stones can form when the crystals aggregate and precipitate under the typical crystallization conditions of urine. Chemically, the most prevalent type of stone includes calcium together with oxalate or phosphate (6). Calcium stones comprise about 75% of overall kidney stones (7,8). Urinary bacteria may promote stone formation by increasing crystal attachment, which facilitates inflammation, the development of an organic matrix, and crystal matrix interaction (9). Infection stones are composed of magnesium-ammonium phosphate or carbonate apatite crystals and make up approximately 15% of kidney stone disease (10). Any type of stone can become infected; however, the term "infection stones" exclusively refers to stones that develop when urease-producing bacteria are present (11). Both infection stones and other types of stones, including calcium oxalate, are associated with infections. Finally, uric acid and cystine comprise the remaining kidney stones (12).

Objectives

In the last decade, numerous cases of renal stones and their complications have been reported in the Maysan governorate among individuals of different genders and ages. This study focuses on the molecular detection of bacterial isolates from urine and stones, determining bacterial sensitivity to antibiotics, understanding the chemical composition of stones, and identifying the most prevalent types of stones.

Materials and Methods

Study design

A descriptive study was conducted at the College of Pharmacy, University of Misan, Maysan governorate, Iraq, from May to November 2022. Ethical clearance was obtained from the Misan Health Directorate, Research and Development Department, and College of Pharmacy, Misan University, with consent from the patients. Age, gender, and nutritional type were addressed through a questionnaire.

Sample collection

Isolation and identification of bacteria from urine and stone samples

One hundred mid-stream urine samples were obtained from 100 adult patients (64 males and 36 -females) with renal stones aged between 20 and 65 years, admitted to the surgical department of Al-Zahrawi Surgical Hospital, Maysan governorate, Iraq. The urine samples were

collected in sterile containers and then cultured using semi-quantitative methods before surgical stone removal (13). A plate containing 10^5 colony-forming units per ml was considered significant growth (14). Following surgical stone removal from the same patient, the stone was washed multiple times with sterile distilled water and grinded to powder using a sterilized electrical mortar. Subsequently, the entire stone was divided into two parts, with the first part reserved for culture and the second one for biochemical analysis. Whole powdered stone samples were inoculated into Brain-Heart infusion broth (Oxoid, UK) and incubated aerobically at 37°C for 8 hours. A 100 microliter sample of each (urine and broth) was inoculated onto MacConkey agar and blood agar (Oxoid, UK) and then incubated under aerobic conditions overnight at 37 °C (15). The grown colonies were identified using 16S rDNA analysis.

Detection of bacterial genomic DNA

The bacterial genomic DNA was extracted using a bacterial genomic miniprep kit (Sigma, Aldrich, Hungary). The picked bacterial colonies were washed with phosphate-buffered saline (PBS). The procedure described by Lee and colleagues (16) involved dissolving 0.25g of agarose powder in 25 ml of 1X TBE buffer, gently mixing, heating to almost boiling, and adding 0.5 µL of ethidium bromide dye. The mixture was then poured into a casting tray with a comb inserted to create the proper special wells for adding the DNA, and it was left to solidify. Subsequently, the seal and combs were carefully taken out of the tray, and the gel was carefully placed inside the electrophoresis chamber that had been saturated in diluted TBE. Next, 5 µL of extracted DNA on parafilm paper was combined with 3 µL of bromophenol blue dye, put into agarose wells using a micropipette, and electrophoresed using a ban electric current from a power supply set at 110 mA 70 V for 25 minutes. A gel imaging instrument containing a UV light transmitter was employed to identify the bands that had migrated.

Polymerase chain reaction amplification of 16S rDNA gene

Two universal primers were conducted to amplify the 16S rDNA gene, 27-forward 5'AGAGTTTGATCCTGGCTCAG-3' and 1492-reverse 5'GGTTACCTTGTTACGACTT-3' (17). A mixture was used to amplify the *16S rDNA gene* in an Eppendorf tube (50 µL) (Promega, USA) consisting of 35 µL nuclease-free water, 11 µL Mastermix, 10 pmol primer (1 µL) for each bacterium sample, and 2 µL DNA template. The polymerase chain reaction (PCR) protocol was as follows: 92 °C for two minutes; 35 cycles of 94 °C denaturation for 30 seconds; 53 °C annealing for 45 seconds; 72 °C extension for 1.5 minutes; and lastly, 72 °C for five minutes. The 1500 bp bands were observed by adding 4 µL of the PCR product to a 2% agarose gel with 0.5 µL ethidium

bromide, electrophoresing the gel with 4 μ L of a 1 kb DNA ladder (Sigma Aldrich, Hungary), and capturing photos with a digital camera.

16S rDNA gene purification and sequencing

The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) and then sequenced by an automated DNA sequencer following the procedure of Macrogen Company (South Korea).

Antibiotic sensitivity testing

Antibiotic sensitivity patterns for bacterial isolates were determined using the standard disc diffusion method on Muller-Hinton plates (LAB, UK) according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2016). The antibiotics tested included; amikacin 30 μ g, ampicillin 30 μ g, ceftriaxone 30 μ g, cefixime 5 μ g, ciprofloxacin 30 μ g, sulfamethoxazole/trimethoprim 25 μ g, gentamicin 10 μ g, imipenem 10 μ g, nitrofurantoin 300 μ g, and tetracycline 30 μ g (Biolab Zrt. Hungary). The inhibition zones were measured in millimeters using an ordinary ruler.

Chemical analysis of stones

The chemical constituents of the stones were analyzed using a stone analysis set (Biolabo, France) to screen for calcium, oxalate, carbonate, phosphate, magnesium, ammonium, uric acid, and cystine. This analysis was performed using the powder obtained from the second part of the whole stone (15).

Statistical analysis

Statistical Package for Social Science Software (Version 19) was utilized to analyze all the data using the chi-square test and one-way ANOVA analysis. A P value of 0.05 or less was considered statistically significant.

Results

Genomic bacterial DNA confirmation

The genomic DNA of bacterial isolates was confirmed by agarose gel electrophoresis. A total of one hundred thirty DNA bacterial isolates were observed in the gel imaging instrument (Figure 1).

Identification by 16S rDNA gene sequencing

As shown in Figure 2, the 16S rDNA gene from 130 bacterial isolates was visible on an agarose gel at the appropriate size (1500 bp) according to the DNA marker. Although two hundred samples were cultured, only one hundred thirty bacterial isolates were obtained. *Escherichia coli* was the predominant organism in both urine and stone culture (41 and 20 isolates, respectively), followed by *Klebsiella pneumoniae* (15 and 9, respectively), and *Proteus mirabilis* (11 and 8, respectively). Additionally, *Enterococcus faecalis* accounted for 5 and 6 isolates, respectively, whereas *Staphylococcus haemolyticus*

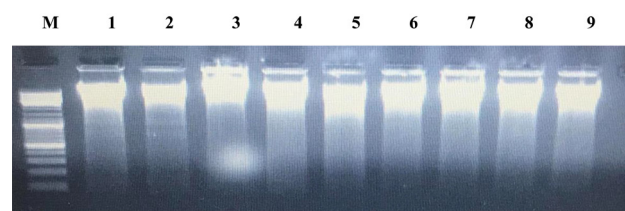


Figure 1. DNA bands of the bacterial isolates.

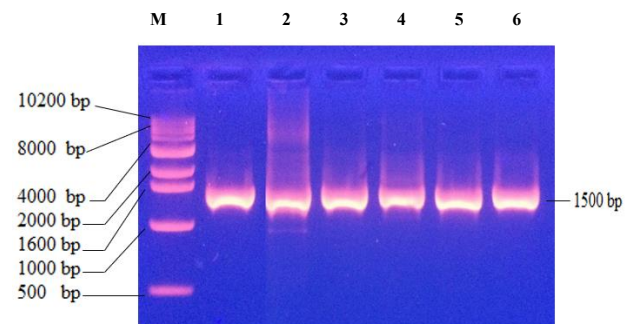


Figure 2. PCR products confirmation for the 16S rDNA genes of the bacterial isolates (M= Marker, 1-6=16S rDNA genes) between urine and whole stone culture.

accounted for 2 and 3 isolates, respectively. Furthermore, *Pseudomonas aeruginosa* and *Enterobacter cloacae* were isolated only from urine (5 and 3, respectively), while *Staphylococcus epidermidis* and *Staphylococcus lugdunensis* were isolated only from whole stone (one for each) (Figure 3).

Antibiotic sensitivity patterns

The antibiotic sensitivity profile for bacterial isolates (130) showed high sensitivity to imipenem and amikacin (80% and 71.5%, respectively) with a significant difference at $P < 0.01$. However, sensitivity to cefixime, ciprofloxacin, and gentamicin was 56.9%, 53%, and 51.5%, respectively, without significance. Moreover, there was low sensitivity to tetracycline, sulfamethoxazole/trimethoprim, nitrofurantoin, and ampicillin (30.7%, 24.6%, 21.5%, and 12.3%, respectively; Table 1).

Prevalence of renal stones

Data analysis of this study reported that the prevalence of kidney stones was more frequent in males and the age group (31-40 years) with 64% and 60%, respectively, with a significant difference at $P \leq 0.05$ (Table 2).

Biochemical analysis of stones

A total of 100 stones were analyzed. Calcium oxalate was the most common biochemical type with 51%, followed by calcium phosphate and uric acid (19% and 15%, respectively). Magnesium-ammonium phosphate, calcium carbonate, and cystine were less common (9%, 4%, and 2%, respectively) (Table 3).

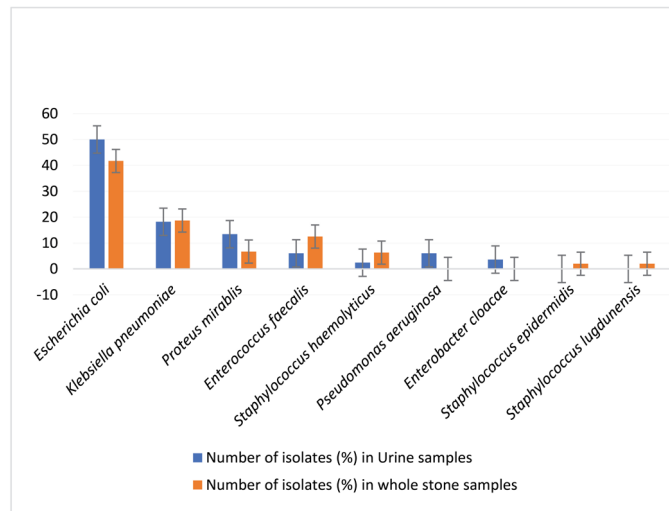


Figure 3. Frequency of bacterial species between urine and whole stone samples.

The relationship between urinary tract bacterial infection and renal stones

Overall, the rate of urinary infection was 65% in both urine and whole stone samples with a significant difference at $P < 0.05$. Among urine samples (n=100), the infection rate was 82 % with a signification at $P < 0.01$. There was also a significant relationship between bacterial urinary tract infection and the presence of renal stones ($P < 0.05$).

Discussion

16S rDNA gene sequencing has become a popular, accurate, and quick technique for identifying bacteria (18). The bacteriology of urine and stone culture mainly

includes gram-negative bacterial infections with fewer infections by gram-positive bacteria. Among the 130 isolates identified, *E. coli* was the predominant organism in both urine and stone culture, followed by *K. pneumoniae* and *P. mirabilis*. Chutipongtanate and colleagues (19) demonstrated that enteric bacteria such as *E. coli* and *K. pneumoniae* increase the level of calcium oxalate crystals compared to the control. Moreover, some uropathogens can lyse citrate, which lowers the urine citrate level leading to supersaturation of urine and crystal formation (20). Additionally, urea-splitting bacteria like *Proteus*, *Klebsiella*, and *Staphylococcus* play a significant role in elevating urinary pH and promoting precipitation and

Table 1. Antibiotic sensitivity profile of bacterial species isolated from urine and stone samples in adult patients with urinary tract infection

Bacterial species	n	Antibiotic sensitivity, n (%)									
		IMP	AK	CFX	CIP	CN	CTR	TE	SXT	F	AMP
<i>Escherichia coli</i>	61	52 (85.2)	49 (80.3)	34 (55.7)	33 (54.1)	40 (65.5)	34 (55.7)	24 (39.3)	26 (42.6)	20 (32.7)	12 (19.7)
<i>Klebsiella pneumoniae</i>	24	18 (75)	15 (62.5)	16 (66.7)	15 (62.5)	13 (54.2)	13 (54.2)	10 (41.6)	4 (16.6)	6 (25)	4 (16.6)
<i>Proteus mirabilis</i>	19	13 (68.4)	11 (57.8)	10 (52.6)	9 (47.4)	5 (26.3)	5 (26.3)	2 (10.5)	2 (10.5)	1 (5.2)	0 (0)
<i>Enterococcus faecalis</i>	11	9 (81.8)	8 (72.7)	6 (54.5)	4 (36.4)	3 (27.2)	1 (9.1)	1 (9.1)	0 (0)	0 (0)	0 (0)
<i>Staphylococcus haemolyticus</i>	5	5 (100)	3 (60)	3 (60)	3 (60)	2 (40)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Pseudomonas aeruginosa</i>	5	3 (60)	3 (60)	1 (20)	1 (20)	1 (20)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Enterobacter cloacae</i>	3	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)	0 (0)	0 (0)
<i>Staphylococcus epidermidis</i>	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
<i>Staphylococcus lugdunensis</i>	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)
Total	130	104 (80) ^a	93 (71.5) ^b	74 (56.9)	69 (53)	67 (51.5)	60 (46.1)	40 (30.7)	32 (24.6)	28 (21.5)	16 (12.3)

IMP: Imipenem; AK: Amikacin; CFX: Cefixime; CN: Gentamicin; CIP: Ciprofloxacin; CTR: Ceftriaxone; TE: Tetracycline; SXT: Sulfamethoxazole/Trimethoprim; F: Nitrofurantoin; AMP: Ampicillin; n: Number of bacterial isolates.

^a $P < 0.01$, ^b $P \leq 0.05$.

Table 2. Frequency of renal stones among adult patients according to gender and age distribution

Gender	Age group (y)					
	Male (%)	Female (%)	(20-30)	(31-40)	(41-50)	(51-65)
(64%)*	(36%)	(11%)	(60%)*	(22%)	(7%)	
(100%)		(100%)				

* $P \leq 0.05$.**Table 3.** Chemical composition of stones collected from renal stone patients

Chemical composition	Number of stones
Calcium oxalate	51
Calcium phosphate	19
Uric acid	15
Magnesium-ammonium phosphate	9
Calcium carbonate	4
Cystine	2
Total number	100

aggregation of struvite crystals (21,22). Alternatively, the antibiotic sensitivity profile for bacterial isolates showed high sensitivity to imipenem and amikacin, while there was low sensitivity to nitrofurantoin and ampicillin, consistent with some studies (23,24). Imipenem is a broad-spectrum carbapenem that acts as an antibacterial through the inhibition of cell wall synthesis of numerous gram-positive and gram-negative uropathogens, including staphylococci, enterococci, *E. coli*, *Klebsiella*, *Enterobacter*, and *Pseudomonas* (25). On the other hand, amikacin is a semisynthetic broad-spectrum aminoglycoside antibiotic with high activity against multi-drug-resistant gram-negative bacteria and is widely used for the treatment of urinary tract infections.

The present study showed that the incidence of renal stones was more frequent in males and the age group (31-40 years of age), which agreed with previous studies (26,27). Males are more prone than females to consume more meat and excessive amounts of coffee. Additionally, estrogen appears to prevent the development of stones by controlling the synthesis of 1,25-dihydroxyvitamin D, whereas testosterone can stimulate the formation of stones (28). Furthermore, middle-aged people are more likely to develop kidney stones than others since they perform more rigorously than others (29), and this can lead to a decrease in fluid intake across with dehydration. Otherwise, calcium oxalate was a common type of stone, followed by calcium phosphate and uric acid, in agreement with those reported by others (30-32). Intake of a diet with high animal protein, rice, and oxalate content elevates the acidity of urine, favoring calcium oxalate stone formation (33,34). Although a low urinary reaction is crucial for the development of uric acid renal calculi, a defect in renal acid excretions also leads to hypocitraturia, a significant risk factor for calcium stones (35). On the other hand, the

sharp edges of calcium stones damage the epithelial urinary tract and encourage bacterial growth leading to infection (36). Moreover, calcium stones are often established in the urinary tract, providing a surface for bacteria to create a biofilm (37). These factors significantly contribute to the rise in bacterial colonization and urinary tract invasion.

Conclusion

The most frequent bacteria isolated from adult patients with renal stones in Maysan governorate were *E. coli*, followed by *K. pneumoniae* and *P. mirabilis*. Although the growth of urine bacterial species was more than that of the whole stone culture, no significant difference between the bacterial species was obtained. Calcium stones were more common, followed by uric acid. Our study showed a significant relationship between bacterial urinary tract infection and the presence of renal stones.

Limitations of the study

The only limitation of our study is using the traditional method for identifying the types of stones and their chemical compositions due to the absence of a specific instrument that measures the total compositions and their percentage.

Acknowledgments

We would like to thank all staff from the College of Pharmacy, University of Misan, for supporting this work. We are also grateful to all staff of the surgical department at Al-Zahrawi Surgical Hospital for assistance in the collection of samples.

Authors' contribution

Conceptualization: Nooralden Abdulkarem Jasim Al-Tulaibawi, Mohammed AL-Nussairawi.

Data curation: Noor AL-Huda Salah Al-Zuhairy.

Formal analysis: Noor AL-Huda Salah Al-Zuhairy, Mohammed AL-Nussairawi.

Funding acquisition: Personal budget.

Investigation: Nooralden Abdulkarem Jasim Al-Tulaibawi.

Methodology: Mohammed AL-Nussairawi, Nooralden Abdulkarem Jasim Al-Tulaibawi.

Project administration: Nooralden Abdulkarem Jasim Al-Tulaibawi.

Resources: Mohammed AL-Nussairawi.

Software: Noor AL-Huda Salah Al-Zuhairy.

Supervision: Mohammed AL-Nussairawi.

Validation: Nooralden Abdulkarem Jasim Al-Tulaibawi.

Visualization: Mohammed AL-Nussairawi.

Writing—original draft: Mohammed AL-Nussairawi and Nooralden Abdulkarem Jasim Al-Tulaibawi.

Writing—review & editing: All authors.

Conflicts of interest

The authors declared no competing interests.

Ethical issues

The current study adhered to the principles outlined in the Declaration of Helsinki. This research protocol was approved

by the ethical review boards of the College of Pharmacy at Misan University and the Misan Health Directorate (Ethical code # EA314). Written informed consent was obtained from all participants prior to their enrollment in the investigation. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

Personal funding from the authors.

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