Infection and Drug Resistance

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ORIGINAL RESEARCH

Investigation of Virulence Genes of the Predominant Bacteria Associated with Renal Stones and their Correlation with Postoperative Septic Complications

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Purpose: Nephrolithiasis is a worldwide disease, and 4.7% of the patients may develop postoperative sepsis. Characterization of virulence genes of bacteria associated with renal stones is still lacking in the literature. The study aimed to investigate the virulence genes of the predominant stone bacterial isolate and their association with postoperative septic complications in patients treated with percutaneous nephrolithotomy (PCNL).

Methods: Stone and midstream urine samples were collected from 200 nephrolithiasis patients who underwent PCNL. Microbiological examination and virulence profile were studied for the common bacteria isolated from the stones.

Results: Microbiological analysis revealed that *Staphylococcus aureus* was the predominant organism in stone samples (42.8%), while *Escherichia coli* (56.6%) was the dominant pathogen in midstream urine. Eight patients (4%) developed septic complications; stone culture was positive for *S. aureus* in seven and *E. coli* in one patient, while all but one had negative midstream urine. The patient with positive midstream urine culture had also *S. aureus* infection. Detection of virulence genes in *S. aureus* isolated from stones showed a high positivity of the hemolysine gene *hla* (93.3%) and adhesion gene *fnbA* (73.3%), whereas enterotoxin genes (*sec* and *sea*) were negative in all *S. aureus* stone cultures. Moreover, the adhesion genes (*fnbB* and *can*), hemolysine gene (*hlb*), panton-valentine leukocidin (*pvl*) gene and the enterotoxin gene (*seb*) were significantly higher in septic patients compared to the non-septic ones ($p< 0.05$). Interestingly, there was a significant relation between the existence of virulence genes and the resistance of antibiotics ($p < 0.05$).

Conclusion: There has been a notable shift toward gram-positive organisms (*S. aureus*) in the stone culture. Moreover, *S. aureus* virulence genes were significantly attributed to the resistance of some antibiotics and postoperative septic complications, suggesting that the stone culture could be more informative than urine culture, especially in predicting the risk of postoperative sepsis.

Keywords: *S. aureus*, nephrolithiasis, antibiotic resistance, percutaneous nephrolithotomy

Introduction

Urolithiasis is a common worldwide disease. The prevalence depends on the geographical location and ranged between 5% and 20%. Moreover, during the last 20 years, the prevalence of urolithiasis has interestingly increased, especially in developing countries. The rate of stone recurrence is quite high; it can be up to 50–70% over 10 years of follow-up.

Urolithiasis is considered as a significant health problem with high socioeconomic costs. Several models might expect dramatic rises in urolithiasis in the future.[1]

Percutaneous nephrolithotomy (PCNL) is the treatment of choice for large renal stones beyond the scope of indications of shock wave lithotripsy. Numerous studies have concluded that about 30% of the patients undergoing PCNL developed perioperative complications, and 10% to 30% of the patients had postoperative complications.[2]

Received: 5 April 2022
Accepted: 30 June 2022
Published: 9 July 2022

Although fever is usually related to an immunological reaction to the operation, 4.7% of the patients may develop a postoperative septic complications.[3]

Historically, the association between bacteria and kidney stones was traditionally restricted to the relationship between urease-splitting bacterial species and magnesium-ammonium-phosphate calculi (struvite stones). However, infection stones represented just 4% of the stones, the majority of stones are calcium-based (calcium oxalate and calcium phosphate) and uric acid stones.[4] Kidney stones have been found to be commonly associated with bacteria. Frequently, kidney stone patients have concomitant urinary tract infections (UTI), regardless its chemical composition. Following stone removal, resolution of bacteriuria is highly expected.[5]

Techniques for precluding postoperative infectious complications are nowadays based on the data provided by midstream urine culture. However, there are many reports of septic complications in patients who received a dose of antibiotic based on urine culture.[6] The American Urological Association (AUA) advises antibiotic prophylaxis after PCNL. Nevertheless, several reports have noted that even with using a prophylaxic dose of antibiotics, septic complications occur in around 8–10% of the patients.[6,7] Some authors' data reported that the bacterial agents isolated from midstream urine samples were entirely different from the agents isolated from the stones of patients who suffered from infectious complications after PCNL[6,8]. Moreover, recent studies tried to change the old belief that gram-negative species commonly represent the kidney stone's micro-flora rather than gram-positive species.[4,9]

The pathogenicity of bacterial agents and their ability to cause infectious complications is basically determined by the acquisition of virulence characteristics encoded by certain genes, which enable pathogens to colonize over host mucosal surfaces and invade the typically sterile urinary tract.[10]

Virulence factors such as adhesion and biofilm have been extensively studied by scientists on bacteria isolated from UTI.[11] Few investigations have been performed to study the prevalence of virulence genes of bacteria associated with stones. Studies of the virulence genes of bacteria isolated from stone samples are scanty and are commonly restricted to gram-negative bacteria.[12,13]

Therefore, the present study is proposed to investigate the precision of midstream urine culture and stone culture in predicting patients at high risk for postoperative septic complications. Moreover, we aim to study the virulence features of the most common stone bacterial isolates and investigate their significance in developing septic complications.

Patients and Methods

Patients

This prospective study included 200 consecutive patients with kidney stones scheduled for PCNL in a tertiary referral center, admitted to the hospital between December 2019 and October 2021. Midstream urine culture was obtained from all patients prior to PCNL. Those who showed positive culture received a full course of antibiotics based on culture sensitivity. All patients who underwent PCNL had negative urine culture and received a perioperative prophylactic single dose of broad spectrum antibiotics. Stones were managed by mechanical fragmentation using ultrasound or pneumatic lithoclast. Stone fragments were collected under complete aseptic condition. Post-operative follow-up was carried out, and the primary endpoint was the development of septic complications; in terms of systemic inflammatory response syndrome (SIRS). SIRS was defined as tachypnea (>20 breaths per min) or PCO_2 < 32 mmHg, fever or hypothermia, leukopenia (<4000 cells/mm^3), leukocytosis (>12,000 cells/mm^3), tachycardia (>90 beats per min) or more than 10% of immature neutrophils.[14] Sepsis was considered SIRS with the suspicion or confirmation of a microorganism-caused infection.[15] Patients with indwelling urinary catheters and urinary stents were excluded from the study.

Methods

Stone fragments were obtained under complete aseptic condition and subjected to microbiological analysis including bacterial identification, antimicrobial susceptibility testing and virulence profile of the most common bacterial isolates. Moreover, the stone fragments were subjected to chemical analysis using Fourier-transform infrared spectroscopy (FTIR).

Analysis of Chemical Composition

A portion of the removed stone was utilized for chemical composition analysis using Perkin Elmer FTIR (FTIR 2000, Perkin-Elmer Co., USA) according to the instructions of the manufacturer. Briefly, the stone fragments were washed with distilled water to remove any residuals of blood and tissue and allowed to be dry. Then, the stones were ground manually by mortar, and the FT-IR spectral analysis was performed with the attenuated total reflectance (ATR) technique.[16]

Microbiological Processing of the Samples and Bacterial Identification

The remaining part of the stones was subjected to bacteriological examination according to Tavichakorntrakool et al[17] method. The stone fragments were immediately washed in sterile phosphate buffer saline (PBS) and then crushed with a sterile grinding mortar. The crushed fragments of the stones were cultured in a brain heart infusion broth medium and then incubated at 37°C for 18–24 h aerobically, and then the stone homogenate and urine samples were cultured on cysteine lactose electrolyte deficient (CLED) agar and blood agar using a standard loop technique and then incubated at 37°C for 18–24 h. As previously described, sterile PBS was used as a negative control for assessing the quality assurance and testing for the contamination.[18] Bacterial colonies were identified by Gram stain and a standard biochemical reaction for full identification of Gram-positive and Gram-negative bacteria. VITEK2 system version 08.01 (BioMérieux, France) was used to confirm the bacterial identification. Bacterial isolates were then preserved for additional molecular testing in nutrient broth media, supplemented with glycerol (30%) at −80°C.[19]

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility pattern was tested by the Kirby–Bauer disc diffusion method using Mueller–Hinton agar (MHA). The following antibiotic discs (Oxoid, UK) were used: vancomycin (VA, 30 µg), cefoxitin (Fox, 30 µg), amoxicillin/clavulanic acid (AMC,20/10 µg), nitrofurantoin (F, 300 µg), norfloxacin (NOR, 10 µg), cefaclor (CEC, 30 µg), gentamicin (CN, 10 µg), cephalothin (CE, 30 µg), ampicillin–sulbactam (SAM, 20 µg) and levofloxacin (LEV, 5 µg). Disc diffusion procedure was conducted based on the guidelines of the clinical and laboratory standard institute (CLSI 2018).

DNA Extraction

Bacterial isolates were cultured in Luria–Bertani (LB) broth at 37 °C for 18 h prior to the DNA extraction. Bacterial cells were collected from 1.5 ml LB broth, then suspended in 100 µl PrepMan™ Ultra Sample Preparation Reagent (Applied Biosystems®) and incubated at 100 °C for 10 min. The cell debris was collected into a pellet by centrifugation, and the supernatant was collected into a fresh tube. The DNA extraction was performed according to the manufacturer's instructions. Finally, DNA was checked using 1.5% agarose gel electrophoresis.

Polymerase Chain Reaction (PCR) for Detection Virulence Genes

Multiple PCR assays were carried out to detect the following 9 *S. aureus* virulence genes, including the Panton-Valentine leukocidin (*pvl*) gene, the enterotoxin a (*sea*), b (*seb*), and c (*sec*) genes, the hemolysin alpha (*hla*) and beta (*hlb*) genes, and the adhesion factor genes including collagen-binding protein (*can*), fibronectin-binding proteins A (*fnbA*) and B (*fnbB*) genes. The primers are listed in Table S1. The PCR mixture with a final volume of 25 µL contained 1 µL of DNA template, 2 µL of a reverse and forward primers (10 µM), 12.5 µL of Maxima Hot Start PCR master mix (2X) (Thermo Scientific®), and nuclease-free water (9.5 µL). A negative control sample for each gene was prepared by replacing the DNA sample with nuclease-free water. The conditions of PCR were performed as follows: the step of initial denaturation (95°C for 10 min), followed by 35 cycles of denaturation stage for 30s at 94°C, 45s for annealing stage; the corresponding annealing temperatures are listed in Table S1, extension stage for 45s at 72°C, and final extension stage at 72°C for 10 min. And, 1.5% agarose gel electrophoresis was used to visualize all PCR products.

Statistical Analysis

Continuous variables were presented as mean ± standard deviation (SD) when normally distributed and use median and range when non-normally distributed, whereas categorical data were presented as frequency (number and percentage). Comparison between the two types of samples was carried out by Fisher's exact test. When the *P* value was <0.05, the results were considered statistically significant. The SPSS 19 for Windows (SPSS Inc., Chicago, IL, USA) was used.

Results

Patients' Demographics

Table 1 provides demographic characteristics of patients enrolled in the study. Of all patients, bacterial culture was positive for 70 (35%) and 60 (30%) for stone and midstream urine samples, respectively. Eight patients (4%) developed

Table 1 Patient Demographics

Variable	
Patient's characteristics	
Age, years (Mean ± SD)	49.3±12.3
Gender, no. pts (%)	
- Male	77 (38.5)
- Female	123 (61.5)
Comorbidities, no. pts (%)	
- Hypertension	58 (29)
- Diabetes	34 (17)
Septic patients, no. pts (%)	8 (4)
Stones' characteristic	
- Stone density (Mean ± SD), HU	737.2±349.8
- Stone size (Median, range), mm^2	42.6 (2.8 −1680)
Operation time (minutes) (Mean ± SD)	88.43±24.34
Bleeding, no. pts (%)	
- Yes	11 (5.5)
- No	189 (94.5)
Tracts, no. pts (%)	
- Single	180 (90)
- Multiple	20 (10)
Method of disintegration, no. pts (%)	
- Ultrasound	152 (76)
- Pneumatic	27 (13.5)
- Combined	21 (10.5)
Positive bacterial culture, no. pts (%)	
- Stone samples	70 (35)
- Midstream urine samples	60 (30)
Stone infection in relation to chemical composition, no. pts (%)	
- Calcium-based stones	33/77 (42.8)
- Calcium oxalate	27/60 (45)
- Calcium phosphate	5/9 (55.5)
- Calcium oxalate/calcium phosphate	1/8 (12.5)

(*Continued*)

Table 1 (Continued).

Variable	
Non-calcium-based stones	35/110 (31.8)
- Uric acid	28/94 (29.7)
- Cystine	3/10 (30)
- Magnesium ammonium phosphate (Struvite)	4/6 (66.6)
Mixed stones	2/13 (15. 3)
- Calcium oxalate/Uric acid	2/13 (15. 3)

Abbreviations: no. pts, Number of patient; HU, Hounsfield units; SD, standard deviation; mm^2, square millimeter.

postoperative septic complications. For all patients, the mean operative time was 88.43±24.34 minutes. For septic patients, it was 98.12±14.03 minutes, and for non-septic was 87.93±24.62 minutes. Bleeding occurred in 11 (5.5%) patients; 3 of them had postoperative septic complications. The tracts were single for 180 (90%) patients and multiple for 20 (10%) patients. Three septic patients had multiple tracts, and five had a single tract. Stone disintegration was performed using the ultrasound disintegration for 152 patients, of whom 7 were septic. Pneumatic disintegration was used for 27 patients; 1 was septic, and combined disintegration was utilized for 21 patients.

Stone Chemical Composition

Calcium-based stones were found in 77 (38.5%), while the remaining 110 (55%) had non-calcium-based stones and only 13 (6.5%) had combined ones. Calcium oxalate and uric acid stones were the most common types of calcium-based and non-calcium-based stones, respectively. Table 1 shows the frequency of positive stone culture in all types of stones.

Microbiological Study

The bacterial spectra of samples collected from the stone and midstream urine are shown in Table 2. The highest percentage of bacterial isolates detected in the stone samples was *Staphylococcus aureus* (42.8%), and for midstream urine was *Escherichia coli* (56.6%). The similarities between the type of organism detected in stone samples and those from the midstream urine are shown in Table 2. Compared to 70 positive stone cultures, midstream urine samples showed positive culture with the same organism in 26 (37.1%), positive culture with different organism in 12 (17.1%) and sterile

Table 2 Comparison Between Positive Bacterial Stone Culture and Midstream Urine Culture

Type of Bacteria	Positive Midstream Urine Culture, n (%)	Positive Stone Culture, n (%)	Isolates from Midstream Urine Culture Versus Stone Culture		
			Similar	Different	Sterile
E. coli	34 (56.6)	10 (14.2)	8	0	2
S. aureus	12 (20)	30 (42.8)	12	6	12
K. pneumonia	8 (13.3)	2 (2.8)	2	0	0
E. faecalis	4 (6.6)	10 (14.2)	4	2	4
P. aeruginosa	2 (3.3)	10 (14.2)	0	2	8
S. epidermidis	0	8 (11.4)	0	2	6
Total, n (%)	60 (100)	70 (100)	26 (37.1)	12 (17.1)	32 (45.7)

Note: Values are expressed as n (%).
Abbreviation: n=Number.

culture in the remaining 32 (45.7%) samples. It was observed that there was no statistical significance between the type of bacterial stone culture and their chemical composition (Table 3).

Of the eight patients who developed septic complication, all had positive stone culture and only one had positive midstream urine culture. Stone culture was positive for *S. aureus* in 7 and *E. coli* in one patient. The only patient who had positive midstream urine culture had also *S. aureus*.

Because of the findings that *S. aureus* was the most predominant isolate detected in all stone samples and also associated with postoperative infectious complications, its virulence genes and antibiotic sensitivity were investigated.

Virulence Genes Profile

The distribution of the virulence genes was studied among the 30 *S. aureus* samples, as shown in Table 4. For hemolysin genes, *hla* was positive in 28 (93.3%), while *hlb* was positive in 17 (56.7%). *pvl,* a Panton-Valentine leukocidin gene, was positive in 17 (56.7%) specimens. Regarding adhesion genes, *fnbA* was positive in 22 (73.3%), *fnbB* in 7 (23.3%) and *can* in 7 (23.7%). On the other hand, among enterotoxin genes, only *seb* gene was positive in 5 (16.7%). An example of PCR results is shown in Figures S1–S4 for detection of the adhesion genes (*fnbA, fnbB* and *can*), hemolysine genes (*hla* and *hlb), pvl* gene, and enterotoxin gene (*seb*), respectively.

Virulence Genes Among Septic and Non-Septic Patients

Comparison of the distribution of the virulence genes among septic (n=7) and non-septic (n= 23) patients with positive *S. aureus* showed that *hla* gene and *fnbA* gene had no statistical significance, while *fnbB, can, seb, pvl* and *hlb* genes showed high statistical significance (Table 4).

Antimicrobial Susceptibility

S. aureus strains were resistant for cephalotin (66.6%), norfloxacin (56.6%), cefoxitin (36.6%), amoxicillin/clavulanic and cefaclor (30%), and levofloxacin and gentamicin (26.6%). On the other hand, they showed high sensitivity for both nitrofurantoin and ampicillin–sulbactam (83.3%) and vancomycin (76.6%) (Figure S5).

The presence of *hlb* gene was associated with resistance to amoxicillin/clavulanic acid, norfloxacin, cefaclor and ampicillin–sulbactam (P = 0.002, 0.02, 0.001 and ≤0.001, respectively). *pvl* existence was associated with the resistance of amoxicillin/clavulanic acid, cefaclor, cephalotin and ampicillin–sulbactam (P= ≤0.001, 0.002, ≤0.001 and ≤0.001,

Table 3 The Distribution of the Types of Bacterial Stone Culture in Relation to Its Chemical Composition

	Stone Culture n= 70						
Chemical Composition	***E. coli* n=10**	***S. aureus* n=30**	***K. pneumonia* n=2**	***E. faecalis* n=10**	***P. aeruginosa* n=10**	***S. epidermidis* n=8**	***P****
Calcium-based stone (n=33)	4	18	1	3	4	3	0.32
- Calcium oxalate (n=27)	3	14	1	2	4	3	
- Calcium phosphate (n=5)	1	3	0	1	0	0	
- Calcium oxalate/calcium phosphate (n=1)	0	1	0	0	0	0	
Non-calcium-based stone (n= 35)	6	11	0	7	6	5	
- Uric acid (n=28)	4	9	0	7	5	3	
- Cystine (n=3)	1	1	0	0	1	0	
- Magnesium ammonium phosphate (Struvite) (n=4)	1	1	0	0	0	2	
Mixed stone (n=2)	0	1	1	0	0	0	
- Calcium oxalate/Uric acid (n=2)	0	1	1	0	0	0	

Note: *Fisher's exact test.
Abbreviation: n, Number.

Table 4 Distribution of Virulence Genes Among *S. aureus* Strains and Among Septic and Non-Septic Patients

Distribution of Virulence Genes (n=30)									
	Hemolysine Genes		**Panton-Valentine Leukocidin Gene**	**Adhesion Genes**			**Enterotoxin Genes**		
	hla	***hlb***	***pvl***	***fnbA***	***fnbB***	***can***	***seb***	***sea***	***sec***
Positive, n (%)	28 (93.3)	17 (56.7)	17 (56.7)	22 (73.3)	7 (23.3)	7 (23.3)	5 (16.7)	0	0
Negative, n (%)	2 (6.7)	13 (43.3)	13 (43.3)	8 (26.6)	23 (76.7)	23 (76.7)	25 (83.3)	30 (100)	30 (100)
Distribution of virulence genes among *S. aureus* strains associated with Septic and non- Septic patients									
Septic patients (n=7)	7 (25)	7 (41.1)	7 (41.1)	7 (31.8)	7 (100)	7 (100)	5 (100)	0	0
Non-septic patients (n = 23)	21 (75)	10 (58.8)	10 (58.8)	15 (68.1)	0	0	0	0	0
P value	1.0	0.01*	0.01*	0.1	≤0.001*	≤0.001*	≤0.001*	-	-

Notes: Values are expressed as n (%). *p* values were obtained from Fisher's exact test. *Statistically significant.
Abbreviations: *hla*, Hemolysine a; *hlb*, Hemolysin b; *seb*, staphylococcal enterotoxin b; *sea*, staphylococcal enterotoxin a; *sec*, staphylococcal enterotoxin c; *pvl*, Pantone-Valentine leukocidin; *can*, collagen binding protein; *fnbA*, fibronectin binding proteins A; *fnbB*, fibronectin binding proteins B; n, number.

respectively). A part of amoxicillin/clavulanic acid and cefaclor resistance showed a significant increase with the existence of *fnbA* (P = 0.01 and 0.01, respectively). The *seb* gene showed a significant association with the resistance to cefoxitin, amoxicillin/clavulanic acid, cefaclor and ampicillin–sulbactam (P= 0.008, 0.001, 0.001 and 0.003, respectively). On the other hand, *fnbB* and *can* show a significant association with the resistance to cefoxitin, amoxicillin/clavulanic acid, norfloxacin, cefaclor, ampicillin–sulbactam and levofloxacin (P = 0.004, ≤0.001, 0.01, 0.003, ≤0.001 and 0.04, respectively) (Table 5).

Discussion

Postoperative complications are observed in patients with renal stones treated by PCNL in up to 4.7% due to bacterial infection.[3] Some studies reported that midstream urine culture is not helpful for the prediction of septic complications, therefore, suggested the use of stone culture.[3,6,20] Recent studies showed a trend towards a change in stone micro-flora from gram-negative to gram-positive bacteria.[4,9] Nevertheless, characterization of bacterial stone species and analysis of their virulence nature is still lacking in the literature, especially for cases who developed bacterial septic complications.

Previous studies reported that the prevalence of bacterial infection in urine and stone cultures are not always similar.[3,21] This fact was confirmed by the present study that showed that the urinary cultures were different from that of stone culture. This study indicated a 35% and 30% prevalence of stone and midstream urine infections, respectively. These results are nearly similar to the studies of Mariappan and Loong[22] and Shah et al,[23] who showed a prevalence of stone infection in 34.3% and 36.4%, respectively. Nevertheless, different frequencies of infection from midstream urine (22%) were reported by Wang et al.[24]

In the past, it was known that gram-negative pathogens were the most common organism associated with renal stone infection. However, many recent reports stated that change of the offending bacteria associated with stones become with more gram-positive organism. Recent reports documented that *Staphylococcus* spp., either *epidermidis* and/or *aureus*, are incriminated in stone infection. One of these reports stated that gram-positive organisms (*Staphylococcus* spp.) were the dominant pathogen in preoperative urine and stone cultures.[9] Other studies that used microbiome analysis revealed that the *Staphylococcus* spp. was the dominant genera in the stone and urine of kidney stone patients.[4,25] Also, recent studies concluded that renal stones were significantly associated with *S. aureus* infection as it can colonize the stones.[6,26,27] These findings again were confirmed in this study, as *S. aureus* was found to be the most predominant organism in stone

Table 5 Relation of Antibiotic Resistance to Virulence Genes of *S. aureus*

Genes / Antibiotic	*hla*		*hlb*		*pvl*		*fnbA*		*fnbB*		*can*		*seb*	
	hla+ **n=28** **n (%)**	***hla-*** **n=2** **n(%)**	***hlb+*** **n=17** **n (%)**	***hlb-*** **n=13** **n (%)**	***pvl+*** **n=17** **n (%)**	***pvl-*** **n=13** **n (%)**	***fnbA+*** **n=22** **n (%)**	***fnbA-*** **n=8** **n (%)**	***fnbB+*** **n=7** **n (%)**	***fnbB-*** **n=23** **n (%)**	***can+*** **n=7** **n (%)**	***can-*** **n=23** **n (%)**	***seb+*** **n=5** **n (%)**	***seb-*** **n=25** **n (%)**
Vancomycin n=7	7 (25)	0 (0)	4 (23.5)	3 (23)	4 (23.5)	3 (23)	7 (31.8)	0 (0)	0 (0)	7 (30.4)	0 (0)	7 (30.4)	0 (0)	7 (28)
P value	1.0		0.5		0.5		0.1		0.02*		0.02*		0.5	
Cefoxitin n=11	9 (32.1)	2 (100)	9 (52.9)	2 (15.3)	7 (41.1)	4 (30.7)	9 (40.9)	2 (25)	5 (71.4)	6 (26)	5 (71.4)	6 (26)	5 (100)	6 (24)
P value	0.2		0.1		0.8		0.3		0.004*		0.004*		0.008*	
Amoxicillin/Clavalanic Acid n=9	9 (32.1)	0 (0)	9 (52.9)	0 (0)	9 (52.9)	0 (0)	9 (40.9)	0 (0)	7 (100)	2 (8.6)	7 (100)	2 (8.6)	5 (100)	4 (16)
P value	1.0		0.002*		≤0.001*		0.01*		≤0.001*		≤0.001*		0.001*	
Nitrofurantoin n=5	5 (17.8)	0 (0)	2 (11.7)	3 (23)	2 (11.7)	3 (23)	5 (22.7)	0 (0)	0 (0)	5 (21.7)	0 (0)	5 (21.7)	0 (0)	5 (20)
P value	1.0		0.6		0.6		0.2		0.3		0.3		0.5	
Norfloxacin n=17	15 (53.5)	2 (100)	13 (76.4)	4 (30.7)	11 (64.7)	6 (46.1)	13 (59)	4 (50)	7 (100)	10 (43.4)	7 (100)	10 (43.4)	5 (100)	12 (48)
P value	0.4		0.02*		0.4		0.6		0.01*		0.01*		0.052	
Cefaclor n=9	9 (32.1)	0 (0)	9 (52.9)	0 (0)	7 (41.1)	2 (15.3)	9 (40.9)	0 (0)	5 (71.4)	4 (17.3)	5 (71.4)	4 (17.3)	5 (100)	4 (16)
P value	0.6		0.001*		0.002		0.01*		0.003*		0.003*		0.001*	
Gentamicin n=8	8 (28.5)	0 (0)	5 (29.4)	3 (23)	5 (29.4)	3 (23)	8 (36.3)	0 (0)	3 (42.8)	5 (21.7)	3 (42.8)	5 (21.7)	3 (60)	5 (20)
P value	1.0		0.5		0.3		0.07		0.6		0.6		0.2	
Cephalotin n=20	20 (71.4)	0 (0)	13 (76.4)	7 (53.8)	17 (100)	3 (23)	16 (72.7)	4 (50)	7 (100)	13 (56.5)	7 (100)	13 (56.5)	5 (100)	15 (60)
P value	0.1		0.2		≤0.001*		0.3		0.06		0.06		0.1	

Ampicillin–sulbactam n=5	5 (17.8)	0 (0)	5 (29.4)	0 (0)	5 (29.4)	0 (0)	5 (22.7)	0 (0)	3 (42.8)	2 (8.6)	3 (42.8)	2 (8.6)	3 (60)	2 (8)
P value	1.0		≤0.001*		≤0.001*		0.4		≤0.001*		≤0.001*		0.003*	
Levofloxacin n=8	8 (28.5)	0 (0)	6 (35.2)	2 (15.3)	6 (35.2)	2 (15.3)	6 (27.2)	2 (25)	2 (28.5)	6 (26)	2 (28.5)	6 (26)	2 (40)	6 (24)
P value	1.0		0.1		0.1		1.0		0.04*		0.04*		0.7	

Notes: Values are expressed as n (%). *p* values were obtained from Fisher's exact test. *Statistically significant.

Abbreviations: *hla*, hemolysine a; *hlb*, hemolysine b; *seb*, staphylococcal enterotoxin b; *pvl*, Pantone-Valentine leukocidin; *can*, collagen binding protein; *fnbA*, fibronectin binding proteins A; *fnbB*, fibronectin binding proteins B; n, number; (+), presence of gene; (-), absence of gene.

https://doi.org/10.2147/IDR.S368852

isolates (30/70). On the other hand, *E. coli* was the predominant organism isolated from the midstream urine samples in our study. These findings also are proved by Eswara et a[20] and Zeng et al.[3]

Several studies reported that the emergence of *S. aureus* is attributed to the presence of foreign bodies like stones. This information clarifies the high rate of *S. aureus* infection in stone samples.[26,28,29] Moreover, recent in vitro studies demonstrated that *Staphylococcus* spp. has crystal aggregation potential.[30] Furthermore, it is clear that the stone disease microbiology has significantly changed over the last decade, with gram-negative organisms giving way to gram-positive organisms.[9]

Shoshany et al[31] and Zeng et al [3] found that the incidence of postoperative septic complication was highly associated with positive stone culture. Similar to the previous studies, our results showed that 4% (8/200) of patients developed postoperative septic complications, seven patients their stones were associated with *S. aureus* infection, and one with *E. coli*. One of these samples was associated with positive *S. aureus* stone and midstream urine culture. Walton-Diaz et al[6] recorded that 5.7% (7/122) of percutaneous nephrolithotomy patients had fever or sepsis, in one patient *S. aureus* was the causative agent, as *S. aureus* was present in the renal stone culture and not detected in the midstream urine or urine sample from pelvis.

The ability of *S. aureus* to cause infection is probably due to the expression of a wide range of virulence factors, including adhesions and toxins. Several studies highlighted the role of Staphylococcal toxins and adhesion genes that could be critical in the physiopathology of UTI.[32,33] So, the present study focused on detecting the main *S. aureus* virulence genes previously described in the case of UTIs, which included hemolysin (*hla* and *hlb)*, Panton-Valentine leukocidin (*pvl*), adhesion (*can, fnbA*, and *fnbB*), and enterotoxin *(seb, sec* and *sea*) genes.[32,33] Based on this, we studied the pathogenic potentiality of *S. aureus* isolated from stones concerning those genes.

Hemolysin genes produced by *S. aureus* are a crucial virulent factor. They have cytotoxic action responsible for lysing red blood cells and culminating in worsening of clinical conditions[34]. *S. aureus* has been shown to produce alpha, beta, gamma, and delta toxins, although different strains may vary in level of their expression. Among these toxins, alpha and beta toxin were detected by the most pathogenic *S. aureus* strains and considered a significant virulence factor. In the present study, *hla*- and *hlb*-positive isolates represented 93.3% and 56.7%, respectively; these results are more or less similar to results obtained by Li et al[35] as they reported that 99% and 52% of *S. aureus* isolated from blood samples harboring *hla* and *hlb* genes, respectively. On the other hand, Ando et al[36] stated that *hla* and *hlb* were detected in 81% and 76% of *S. aureus* isolated from UTI, respectively.

The *pvl* is a leukotoxin-forming pore produced by *pvl* gene. It can attack and destroy host leukocytes via plasma membrane perforation along with intracellular organelle membranes.[19] In the last decades, in vitro studies pointed the potential role of *pvl* in inducing cell lysis and programmed cells death.[37] In the current study, we observed that the *pvl* gene was detected in 56.7% of isolated *S. aureus*. Sultan and Nabiel[19] reported that the *pvl* gene was detected in 23% of *S. aureus* isolated from urine samples.

The first stage in the occurring of *S. aureus* infection is adherence to host tissues. There are many types of adhesions, the so-called "microbial surface components recognizing adhesive matrix molecules" or MSCRAMM. Attachment of *S. aureus* to the human body occurs by the use of microbial surface components such as *can, fnbA*, and *fnbB*. The reported observation in this issue is variable. In the present study 73.3% of the strains harboring the *fnbA* gene, *can* and *fnbB* genes were detected in 23.3% of the isolates. These results are inconsistent with the results obtained for *S. aureus* isolated from urine samples.[36] While, in the study performed by Baba-Moussa et al,[33] the *fnbA* represents 17% in *S. aureus* isolated from UTI. Shahmoradi et al[11] reported that *fnbA* present in 70% of *S. aureus* isolated from different clinical samples (blood, tracheal, aspirate, and urine).

Some *S. aureus* strains express pyrogenic exotoxins, such as staphylococcal enterotoxins.[1] Regarding the findings of PCR in this study, it was clear that 16.7% of the isolates had *seb* gene; this result is near to the result obtained by Sabouni et al,[38] as they reported that 11.5% of *S. aureus* isolated from urine were positive for *seb* gene. The *sec* and *sea* genes were not detected among *S. aureus* isolates these results are in agreement with the results obtained by Sabouni et al.[38]

In the current study, all *S. aureus* associated with septic patients developed most of the studied virulence genes and showed significance with the *hlb, pvl, fnbB, can* and *seb* genes. These results could explain the occurring of postoperative

sepsis. The expression of these virulence genes could help in the persistence of infection. So, monitoring these strains and their virulence genes is of great importance.

S. aureus isolates had demonstrated higher resistance rates to norfloxacin and cephalothin. On the other hand, they showed a high sensitivity rate for nitrofurantoin, vancomycin, and ampicillin–sulbactam. The previous result is in agreement with Shah et al,[23] as they concluded that *S. aureus* isolated from stones was sensitive for nitrofurantoin and vancomycin.

Bacterial infection and antimicrobial susceptibility are related to the local antibacterial spectrum. The most prevalent uro-pathogens' resistance rate considerably differed across nations. Higher resistance rates to fluoroquinolones, amoxicillin/clavulanate, fosfomycin, and aminoglycosides were observed in North Macedonia. The cephalosporins, piperacillin/tazobactam, carbapenems, and nitrofurantoin recorded a high resistance rate in Spain, while Trimethoprim-sulfamethoxazole showed high resistance in Turkey.[39] A Chinese study reported that *Staphylococcus* spp. showed resistance to cephalosporins, penicillins, fluoroquinolones, and trimethoprim-sulfamethoxazole.[40] In a Russian study, *Staphylococcus* spp. were resistant to bacitracin, ampicillin, cefepime, cephalexin, nitrofural, levofloxacin, streptomycin, ofloxacin, sulfanilamide, and tetracycline.[27]

The antibiotic resistance was compared with virulence genes, and the study showed that the resistance of some antibiotics was associated with the existence of virulence genes. This could be because virulence genes help bacteria to establish chronic infection and enhance their antibiotic resistance.

Conclusions

Our data confirmed that the microorganism profile of the stone had been changed from gram-negative pathogen to gram-positive (*S. aureus*), which showed a high prevalence of virulence genes, especially in patients who developed post-operative septic complications. Furthermore, the existence of these genes was significantly related to antibiotic resistance. Therefore, the stone culture could be more reliable than the urine culture in predicting patients at high risk for postoperative infectious complications.

We recommend taking a small piece of the stone for culture during PCNL. This simple procedure may save the lives of a considerable number of patients at risk of developing septic complications through tailoring of postoperative antibiotic therapy.

Nevertheless, our study is not free of limitations, of these: the small number of studied patients with *S. aureus* and the small number of investigated septic patients. Still, the incidence of sepsis due to infection does not excessed 4.7%. So, to consolidate this data, we encourage to perform multi-center studies. Moreover, the use of antibiotics prior to PCNL may have an impact on changing the type of stone bacterial flora.

In this study, we used PCR technology to detect virulence genes, the most popular and well-established method for identifying pathogenic bacteria. It is considered a powerful technology for the detection of virulence genes, it is not only sensitive and specific but it also allows quick screening for bacterial toxins. Nevertheless, it does not reflect the actual behaviour of virulence factors. Meanwhile, based on the PCR results, further research is warranted to assess the role of these virulence factors by analyzing their corresponding proteins and judging their actual biological behaviour. Therefore, we recommend further research to avoid such shortcomings to consolidate our initial observations.

Data Availability

The original contributions presented in the study are included in the article and Supplementary Materials.

Ethical Consideration

Prior to specimen collection, the research was accepted by the Faculty of Medicine – Mansoura University institutional review board and the local ethical committee (ID: RP.20.12.90). Informed consent was taken from all patients, and all of them were treated according to the principles of declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

Funding

This work was supported by Science, Technology and Innovation Funding Authority (STDF) in Egypt for the financial support of this study [Grant No 43356].

Disclosure

The authors declare no conflicts of interest in relation to this work.

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