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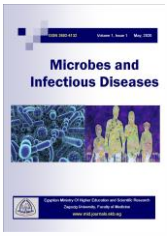

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Original article

Molecular profile of *Staphylococcus aureus* related with UTIs pregnant women in Al-Nasiriyah City, Iraq

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ABSTRACT

Background: *Staphylococcus aureus* (*S. aureus*) is a facultative anaerobic, Gram-positive (GP), catalase-positive, non-motile bacterium that infects humans and animals, causing illness in a wide range of individuals as an opportunistic organism in various parts of the body, including urinary tract. The purpose of the current study was to clarify the potential impact of bacterial infection on pregnant patients at Bint Al-Huda Teaching Hospital in Thi-Qar Governorate during the period from August 2023 to January 2024. **Methods**: All patients provided 40 blood samples, which were then used to perform a conventional Polymerase Chain Reaction (PCR) test utilizing the universal gene. Seven *S. aureus* PCR products were selected and subjected to partial DNA sequencing for the 16S rRNA gene to follow up their possible relationship among what was recorded globally in GenBank. **Results**: The results revealed that 7/40(17.5%) isolates were *S. aureus* according to 16S rRNA were recorded globally in GenBank under the official accession numbers of PP396125, PP396121, PP396120, PP396122, PP396124, PP396126, and PP396123. The phylogenetic tree that was constructed by MEGA-10 software showed that there were different molecular relationships among the local *S. aureus* isolated from urinary tract infections (UTIs) pregnant women with analogous ones around the world. **Conclusion**: Pregnant women's UTIs remain one of the most common socio-health issues that require extensive diagnosis and care. When it comes to accurately diagnosing related illnesses, molecular approaches to bacterial identification in the blood stream may be helpful with faster and with high accuracy.

Introduction

Staphylococcus aureus (S. aureus) is a facultative anaerobe, Gram-positive (GP), catalase-positive, non-motile bacterium that infects humans and animals, causing illness in both immunocompetent and immunocompromised individuals. It inhabits the epidermis, nasal cavities, and natural biological niches. Under a microscope, it resembles grapes and can infect people and create biofilms. Exotoxins, including enterotoxins, hemolysin, leukocidin and toxic shock syndrome type-1 (TSST-1), cause various illnesses in vulnerable hosts [1].

The human pathogen *S. aureus* is an opportunistic organism that can infect various parts of the body. Numerous virulence factors, which enable the bacteria to infiltrate host tissues and successfully elude the immune system [2].

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For individuals suffering from *S. aureus* bacteremia, the source of the infection is a key indicator of mortality. Some causes are considered low-risk, such as catheter-related infections, while others, such as endocarditis, are considered high-risk [3]. Significant side effects of *S. aureus* sepsis include infectious endocarditis. Superantigens have a role in infectious endocarditis, and fatal sepsis is attributed to TSST-1 [4]. These variations in risk might be linked to the bacterial inoculum associated with each source or to the potential for timely source management. Furthermore, individuals with a bloodstream infection caused by *S. aureus* have been shown to have worse outcomes when they are methicillin-resistant *S. aureus* (MRSA). Despite notable national variations, the mean proportion of MRSA in the European Union in 2018 was 16.4%, with 24% in Spain [5].

One of the most common bacterial diseases that afflict people is urinary tract infections (UTIs), which are more common in women. UTIs can have serious repercussions for both the mother and the fetus and are more common in pregnant women. Numerous bacteria may cause UTIs, and while untreated UTIs can have major repercussions, it is noteworthy that drug-resistant UTIs are common in maternity and pediatric hospitals. Pregnancy-related morphological and physiological modifications to the genitourinary tract raise the risk of UTIs [6,7]. Infections brought on by several bacterial pathogens, like S. aureus, that are spread by hospitals and the general public [8]. The UTIs are categorized as either asymptomatic bacteriuria (ASB) or symptomatic UTIs. The presence of considerable bacteria (i.e., more than 105 bacteria per milliliter of urine) without any signs of UTIs is known as ASB [9]. There is a correlation between UTIs and a higher risk of stillbirth and neonatal sepsis. Treatment is therefore crucial for both the mother and the child [10]. There is a wide range of clinical presentations, from simple UTIs (cystitis or pyelonephritis in young, healthy women with no problems in their urinary tract) to complex UTIs that affect vulnerable people (e.g., elderly patients, patients with neurogenic bladders, patients undergoing urinary diversion, and catheter-related UTIs). These complicated UTIs are frequently associated with morbidity in these populations, carrying a high risk of developing urosepsis, acute or chronic renal failure, and even mortality. Recurrent urinary tract infections (rUTIs) are characterized as either simple or complex UTIs that recur three times a year or twice in the preceding six months. Given their shorter urethral length than males and their close proximity to the external urethral meatus and the anus, females are among the most susceptible to developing ASB and UTIs. Additional risk factors for rUTI in females encompass the utilization of spermicides, increasing sexual activity frequency, family history, declining estrogen levels, elevated post-void residual urine, incontinence, and pelvic prolapse [11].

The most sophisticated molecular technique now available for the identification of a variety of illnesses is thought to be polymerase chain reaction (PCR) [12]. The 16S rRNA gene sequencing yields trustworthy results. Because of its highly variable portions that vary considerably throughout bacterial species, leading to discrete sequences for each kind, and its relatively stable portions that do not change over time, this gene is considered one of the key classification criteria. This appears to be the rationale behind the importance of this gene for the diagnosis [13].

The present study aimed to detect bacterial infections in the blood stream of UTI pregnant women via the 16S rRNA gene.

Patients and methods

Collection of Samples

A 40 blood samples were collected from all participants who consulted Bint Al-Huda Teaching Hospital in Thi-Qar Governorate during the period between August 2023 and January 2024. The following patient data were collected: name, age, months of pregnancy, presence of immunological or chronic disorders, number of births and abortions, use of antibiotics, socioeconomic position, and educational attainment. All patient blood samples (5 ml) were collected from pregnant women who had positive urine cultures and brought to the laboratory. Then, it was frozen at -20 ºC until used subsequently for the 16S rRNA gene assay.

Molecular detection of S. aureus

With a DNA Extraction Kit (Qiagen, Germany), genomic DNA was isolated from the blood samples of UTIs pregnant women. Using specialized primer pairs for each gene, a typical PCR approach was employed to determine the presence of the universal gene, 16S rRNA, in all bacterial isolates (Table 1). The amplified genes were placed in a thermal cycler (Hamburg, Germany), and the primer-specific conditions were changed to set the proper cycling program

parameters. The process of thermal cycling is described in (Table 2).

Gel electrophoresis

For the 16S rRNA gene in all isolates of *S. aureus* was performed at 80 volts for 30 minutes. PCR products were stained with ethidium bromide and seen at 280 nm under UV light as shown in (Figure 1).

Sequencing analysis

The 16S rRNA gene was partially sequenced for seven selected *S. aureus* isolates from pregnant individuals who had UTIs, and the PCR results was then compared to standard strains of *S. aureus* in the NCBI. The sample sequences designated as: (PP396125, PP396121, PP396120, PP396122, PP396124, PP396126, and PP396123). Using MEGA-10 program, a phylogenetic tree for the sequenced genes was constructed [14].

Statistical analysis

A chi-square test was employed, with ($p \leq 0.01$) being considered statistically significant. The graphs were made using Microsoft® Excel, 2016.

Ethics permission

Thi-Qar Health Directorate has authorized the study via their agreement coded 265/2023.

Results

A total of 35 pregnant patients enrolled in the present study, who were aged from 17 to 40, with an age mean ±SD (27.44±6.66). Also, the study included Five apparently healthy pregnant women (mean ± SD (27.11±5.59)) who were considered as controls. And were screened by 16S rRNA as a universal gene; 14 (35%) samples showed positive bacterial culture (Table 3). The results revealed a similar distribution for GP and Gram-negative (GN), with 7 isolates (50%) for both ($p > 0.01$).

As shown in (Figure 2), *S. aureus*, with a high significant differences, was the most common isolate among UTIs pregnant women, with a recovery of 7 isolates (50%). According to the current study findings, the percentages of GN bacteria were 7(50%), distributed to *Escherichia* sp. 4 (28.57%), *Pseudomonas* sp., *Klebsiella* sp., and *Salmonella* sp. identified in a single isolate for each (7.14%) ($p \leq 0.005$).

16S rRNA detection

To verify the identity of *S. aureus* and other bacterial species among the confirmed isolates, the 16S rRNA gene was amplified in all 14 isolates using a conventional PCR approach. For the 16S rRNA gene, the size of the products was around 1500 bp on the ladder (3000 bp) **(**Figure 1).

Phylogenetic analysis

The official GenBank accession numbers of PP396125, PP396121, PP396120, PP396122, PP396124, PP396126, and PP396123 were assigned to the local seven selected *S. aureus* strains. The constructed phylogenetic tree revealed that the local *S. aureus* isolates and similar ones from around the world have distinct molecular relationships (Figure 3).

Phylogenetic tree was constructed based on 16S rRNA gene sequences of the seven local isolates of *S. aureus* with eight isolates sequences derived from GenBank database. Phylogenetic tree comprised of three genetic groups;

First Group: Includes PP396121, PP396120, and PP396122 *S. aureus* local strains which were highly related to each other and constitute a cluster with other local *S. aureus* PP396125 which are closer to MW453041.1 strains isolated in Pakistan.

Second Group: Includes PP396124 and PP396126 *S. aureus* local strains which were genetically closer to OP889692.1 and JF513981.1 strains isolated in Egypt and China, respectively.

Third Group: Includes a single PP396123 *S. aureus* local strains which were genetically closer to LC752325.1, OQ996944.1, PP504268.1, MN519401.1, MT355444.1 strains isolated in Japan, China, India, India, and Pakistan.

Table 1. Primers sequence.

Primer		5-Sequence-3	Reference
Universal gene (16S rRNA)	27 F	AGAGTTTGATCMTGGCTCAG	[15]
	1492 R	TACGGYTACCTTGTTACGACTT	

Table 2. PCR condition for universal gene.

Steps	Temperature (°C)	Time (min)	No. of cycle	Reference
Taq polymerase activation	95	5	1	[16]
Denaturation	94	1	35	
Annealing	55-60	0.5		
Elongation	72	1		
Final elongation	72	7	1	

Table 3. bacterial types that were isolated from patient blood.

Bacterial type	No.%	*P*-value
GP	7 (50%)	N.S
GN	7 (50%)	N.S
Total	14 (100%)	
N.S: Non-significant.		

Figure 1. Agarose gel electrophoresis of 16S rRNA gene. M:3000 bp ladder; Lanes (1, 5, 6, 8, 9, 13 and 14) were positive *S. aureus* with a product size of approximately 1500 bp.

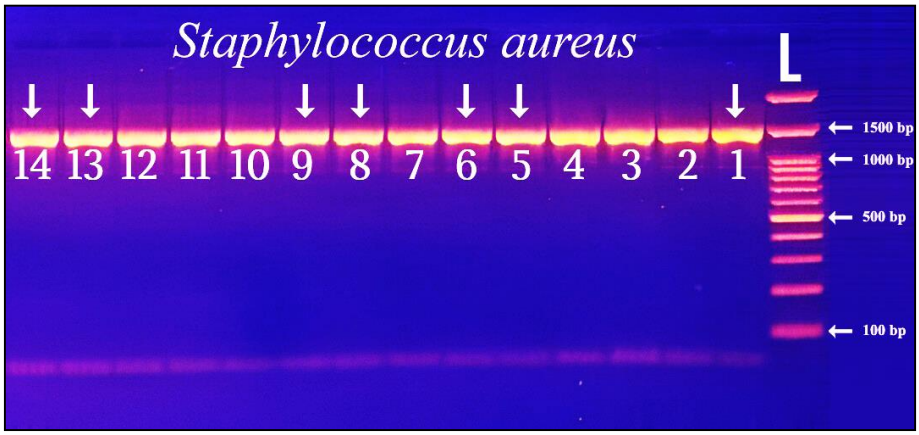


Figure 2. The percentages of bacteria that were directly extracted from blood using PCR.

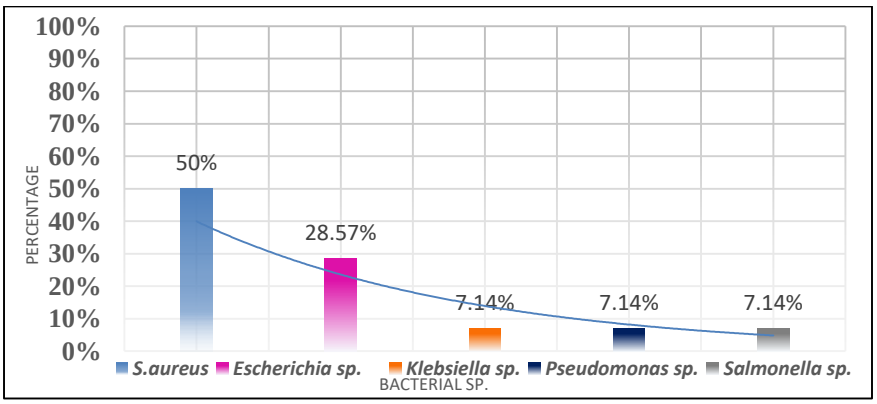
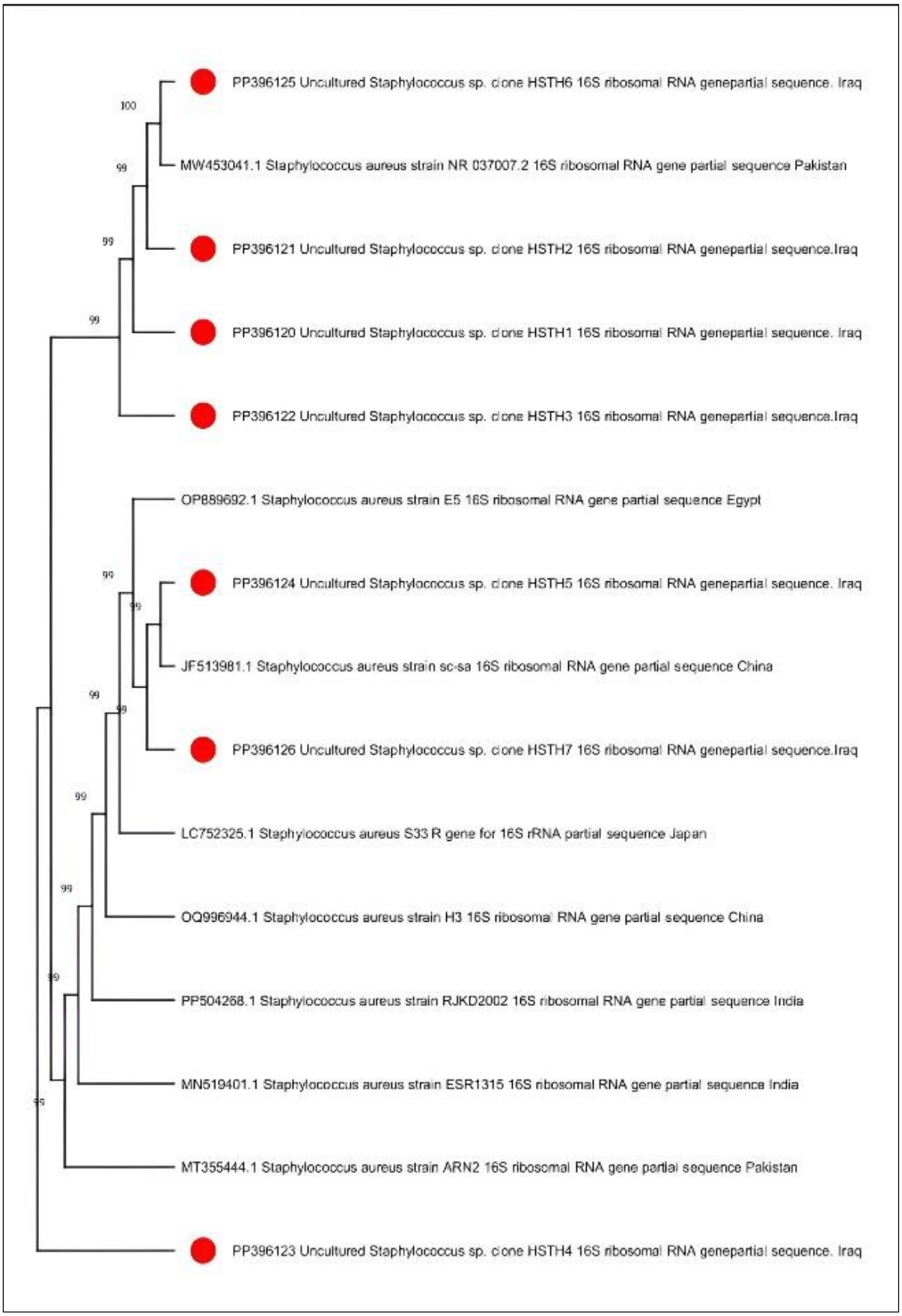


Figure 3. Neighbor joining phylogenetic tree analysis based on 16S rRNA gene association of nearby *S. aureus* isolates and related strains from GeneBanK.



Discussion

Blood-stream infections (BSIs) caused by *S. aureus* are thought to be secondary to *S. aureus* bacteriuria [17]. The main cause of *S. aureus* bacteriuria is contamination or an initial infection related to urinary catheter usage [18]. According to a recent Canadian study, only 7% of individuals who have *S. aureus* bacteriuria also go on to acquire *S.aureus* BSIs (SA-BSIs). [19]. About 50% of infections are caused by UTIs, which can lead to bacteremia and hospitalization, that raises morbidity and mortality. Furthermore, urosepsis, a systemic infection, may transpire as a consequence of microorganisms that cause UTIs to spread from the urinary tract to the bloodstream [20].

The following risk factors for SA-BSIs: urinary tract blockage, advanced age, hospitalization, urinary tract catheterization, urologic surgical operations, and cancer [21].

With 16S rRNA gene sequencing, bacteria that are difficult to identify using automated and manual approaches may be properly identified. It can also be applied to describe species that have not yet been described. 16S rRNA sequencing importance for precisely identifying microorganisms and comprehending their diversity. The advantages of the 16S rRNA sequencing technology have grown in significance, and it would be beneficial to include it in clinical practice in developing nations due to its affordability, accessibility, and ease of use [22].

A similar Japanese study used blood analysis by 16S rRNA gene amplicons as a novel technique for identifying pathogenic bacteria in patients with UTIs. The study demonstrated the benefits and drawbacks of using 16S rRNA testing on blood samples against traditional culture testing on urine and blood samples. It was shown that the next three points are clinically significant when using blood sample testing. First off, the findings suggest that 16S rRNA testing on urine samples is not only a viable replacement for urine culture testing but also helpful in identifying other potentially harmful, unculturable bacteria. Second, in situations of severe urosepsis, blood sample testing by 16S rRNA may be helpful in identifying pathogenic bacteria that are spreading widely. Finaly, it seems likely that non-severe, non-sepsis cases reflect the potential of 16S rRNA for detecting false-positive bacteria due to contaminants or noise, as a result of low abundance of infecting bacteria in the blood [23].

Testing a blood sample by 16S rRNA is the most significant and influential pathogenic microorganism in terms of clinical impact and may thus be identified. For blood culture testing in cases of febrile UTIs, this criterion also applies [24].

according to another study of ICU patients with suspected sepsis. Moreover, blood testing by 16S rRNA demonstrated much greater diagnostic sensitivity than blood culture [25].

The GP bacteria usually constitute the most isolated type among pregnant patients with UTIs in Iraq, while *S. aureus* takes the lead. This outcome was clearly in agreement with the present study findings [26,27,28,29,30].

One of the most sophisticated development techniques in molecular biology nowadays is the sequencing process. This allows for the quick detection of genetic relationships and mutations between bacterial isolates [31,32].

Conclusions

Pregnant women UTIs remain one of the most common socio-health issues that require extensive diagnosis and care. When it comes to accurately diagnosing related illnesses, molecular approaches to bacterial identification in the blood stream may be helpful with faster and with high accuracy.

Author contributions

All authors had seen and approved the submission of the manuscript with full responsibility, and this research had not been published or under consideration by any other journal.

Conflict of interest

The authors certify that they have no competing interests.

Financial disclosure

The authors deny receiving any financial support or grant from any organization.

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