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Original Article

Molecular Detection of *Clumping factor A gene* and Antibiotic Susceptibility Evaluation of *Staphylococcus Aureus* Isolated from Urinary Tract Infections

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Abstract

The present study aimed to isolate and diagnose *Staphylococcus Aureus* (*S. aureus*) from clinical specimens of patients infected with urinary tract infections and evaluate the bacteria's resistance to antimicrobial agents. Additionally, it attempted to study the existence of the *clumping factor A* (*clfA*) gene. This study took place in Najaf province, Iraq, from December 2020 to April 2021 and included 40 clinical specimens taken from urine. In order to make an initial diagnosis of *S. aureus* isolates, microscopic evaluation was used in conjunction with culture and biochemical features. The automatic final diagnostic provided by the VITEK-2 compact system (bioMérieux, France) was utilized, which had a significant advantage. The results showed that 27 (67.5%) isolates gave positive results for *S. aureus*, 2 (5%) isolates for *Streptococcus pyogenes*, 6 (15%) isolates for *Lactobacillus*, and 5 (12.5%) for *Escherichia coli*. Antibiotic sensitivity test was conducted by disk diffusion methods, in which the isolates showed high resistance to ceftriaxone, as well as erythromycin, and they showed sensitivity to vancomycin, gentamicin, amikacin, and ciprofloxacin. The findings led to a concluding remark that all of the *S. aureus* isolates were *clfA* gene positive.

Keywords: *Clumping factor A* gene, *Staphylococcus aureus*, Urinary tract, VITEK

1. Introduction

Every year, 250 million occurrences of urinary tract infections (UTIs) are expected globally (1), which may be caused by gram-positive bacteria. *Staphylococcus aureus* (*S. aureus*), a bacterium that may invade the urinary tract and cause sickness, represents the most popular reason behind UTIs. The *S. aureus* is responsible for 0.5% to 6% of UTIs, but if left untreated, the infection can progress to a serious and potentially life-threatening disease (2).

This public health issue is becoming more widespread throughout the world as the multidrug-resistant *S. aureus* continues to rise in prevalence. Bio-films are thought to be responsible for more than 65% of nosocomial infections and 80% of bacterial infections in healthcare settings worldwide. Bacterial biofilms have been implicated in the progress of recurrent UTIs and antibiotic resistance. "The proximity of cells inside a biofilm structure" might enable the interchange of genetic elements, potentially increasing the transmission of antibiotic-resistant genes (3). Biofilms of *S. aureus* have been found on a variety of surfaces. This bacterium can infiltrate renal tissue and cause UTIs by adhering to the uroepithelium and forming a

biofilm. Due to the tendency of *S. aureus* to build biofilms, which can result in antibiotic resistance, hospitalized patients infected with this bacteria have a high risk of treatment failure (4).

Polysaccharide intracellular adhesion, expressed by the icaADBC operon, controls the formation of *S. aureus* biofilms by facilitating cell-to-cell attachment. Furthermore, surface-associated proteinaceous adhesins may perform an essential function in *S. aureus* adhesion, colonization, and biofilm construction. Several microbial surface components recognizing adhesive matrix molecules, such as clumping factors A and B, can be expressed by the *clumping factor A* (*clfA*) gene (5). The *S. aureus* infections in the urinary system are still a mystery regarding biofilm production and toxicity. Many scientists have recently paid attention to the investigation of genes included in the production of biofilms and their importance in infections resulting from *S. aureus* (6). Given the importance of biofilm-related genes in the production of biofilms and the development of antibiotic resistance, there is currently an increasing need for further research. Consequently, the major goal of the current investigation was to investigate the construction of biofilms and the progress of antimicrobial resistance in *S. aureus* isolates from UTIs.

2. Materials and Methods

A total of 40 clinical specimens were obtained between December 2021 and April 2022 from patients suffering from UTI at Al-Hakim General Hospital in Najaf province, Iraq. The specimens were inoculated onto three distinct types of culture media, including blood agar and mannitol salt agar, which were regarded to be the major enrichment, selective, and differential media, respectively. A single pure isolated colony was obtained after 24 h of incubation at 37˚C, and the isolates were transferred to nutrient agar for preservation, as well as biochemical tests, which validated their identity. A second incubation at 37˚C for another 24 h followed this step in the process. Gram-positive (GP-ID) and gram-negative Identification (GN-ID) cards, as well as an integrated VITEK-2 small device (bioMérieux, France), were used in the most recent coincidence.

2.1. Susceptibility Testing to Antibiotics

This card was composed of antibiotics, including ceftriaxone, erythromycin, trimethoprim-sulfamethoxazole, ceftazidime, ceftriaxone, Augmentin, gentamycin, amikacin, as well as ciprofloxacin, which were administered using the automated VITEK-2 small device (bioMérieux, France) and the Sensitivity card.

2.2. Genotypic Study

2.2.1. DNA Extraction and Molecular Identification

A commercial DNA extraction technique was used to get the genomic DNA samples. The detection of DNA by an ultraviolet (UV) transilluminator was accomplished by the use of gel electrophoresis. The Polymerase chain reaction (PCR) experiment was carried out in order to identify the *clfA* genes in *S. aureus* bacteria. This primer was produced by the Alpha DNA company in Canada, as mentioned in table 1. To verify the presence of amplified products, 0.8% agarose gel electrophoresis was employed to validate the existence of amplified products. The gel was stained with 4 µL of 10 mg/mL ethidium bromide (Sigma, USA) and was run at 70 v for 1.5 h. This process was conducted utilizing a UV light transilluminator and a Gel Documentation System. The measurement of the molecular weights of the amplified products was carried out using a 100bp ladder (Bioneer, Korea) (7). An initial 5-min denaturation phase at 95˚C was followed by 35 amplification cycles of 1 min, each at 95˚C, 30 sec at various temperatures for individual genes, and 50 sec at 72˚C with a final 10-min extension step at 72˚C.

Table 1. Primers utilized in the current investigation

Primer Type	Primer Sequence (5'-3')	Amplicon Size (bp)	Reference
clfA	F- ATTCTGCTGTTAAAGGTGACACAT R- GTGTTGTAATTTGATCATCAGGCG	657	(8)

3. Results and Discussion

3.1. Isolation of Pathogenic Bacteria

A total of 40 clinical specimens were gathered from patients during the trial, which ran from December 2021 to April 2022 using a swab collected from urine. The results showed that 40 (100%) samples contained bacterial growth. A total of 27 isolates of *S. aureus* were found, which grows as *Staphylococcus* species (spp.) on mannitol agar and produces catalase, as well as coagulase. Furthermore, two *Streptococcus pyogenes* isolates were observed, which produce beta hemolysis on blood agar and are sensitive to bacitracin. Additionally, six *Lactobacillus* bacteria isolates were obtained during this investigation. *Lactobacillus* bacteria is a rod-shaped gram-negative, lactose-fermenting that exists as a mucoid lactose fermenter on MacConkey agar (9). As shown in table 2, five *Escherichia coli* bacterium isolates played a part in infection, as five isolates were also identified during this investigation, giving lactose fermenter on MacConkey agar and indole positive (10).

Table 2. Types and numbers of bacteria recovered from a urinary tract infection

Type	Number (total 40)
Staphylococcus aureus	27
Streptococcus pyogenes	2
Lactobacillus	6
Escherichia coli	5

3.2. Bacterial Specimen Identification

Bacterial specimens were initially identified using gram staining, culture tests, morphology, and biochemical assays in the automated VITEK-2 compact system (bioMérieux, France), which included 64 biochemical tests and one negative control. Exactly 40 isolates were identified and validated using the VITEK-2 method (bioMérieux, France), employing four kits (GP-ID cards) for gram-positive bacteria and eight kits (GN-ID cards) for gram-negative bacteria. Some gram-positive bacteria grow on blood agar and some others on mannitol agar, with 27% of those growing on mannitol agar being identified as *Staphylococcus* spp. and exhibiting catalase positivity. They also have bacitracin resistance; bacitracin is an antibiotic that prevents bacteria from synthesizing peptidoglycan, a key component of their cell walls. Bacitracin susceptibility, which varies depending on the bacterium, is useful in identifying gram-positive *cocci* since some are susceptible and some are resistant to bacitracin. However, coagulase-positive as *S. aureus* is identified by Cramton, Gerke (11) because only *S. aureus* produces coagulase enzyme, which reacts directly with plasma fibrinogen and results in rapid cell agglutination. The ability to recognize bacitracin susceptibility is important in the detection of a gram. Tube coagulase recognizes extracellular free coagulase that reacts with a plasma protein called "Coagulase-Reacting Factor" to form a complex that subsequently reacts with fibrinogen to form fibrin. Tube coagulase is used to detect the presence of extracellular free coagulase (the clot) (12). This bacterium has the rare capacity to thrive on mannitol agar, which contains 7.5% sodium chloride and hence, has a high salt concentration. Most microorganisms, except for *S. aureus*, are inhibited by high salt content (13). Pathogenic *S. aureus* grows in microscopic colonies on mannitol salt agar due to the mannitol sugar fermenting, resulting in the generation of acid, which shifts the indicator from pink to yellow (14).

3.3. Antimicrobial Susceptibility

Images of the CoNS strains' resistance profiles to the eight tested antibiotics are shown in table 3. According to Ma, Wang (15), medications were categorized into three groups based on the CoNS strains' resistance levels to these antibiotics as follows: 1) high resistance, including ceftriaxone and erythromycin, 2) moderate resistance, including trimethoprim-sulfamethoxazole, ceftazidime, ceftriaxone, as well as Augmentin, and 3) sensitive gentamycin, amikacin, and ciprofloxacin, as illustrated in table 3. The results of resistance profiles were correlated with the findings of the study by Ma, Wang (15).

Table 3. Sensitivity test to *Staphylococcus aureus*

Type of antibiotic (cell wall)	Sensitivity
Augmentin	M
Vancomycin	S
Ceftazidime	M
Cefotaxime	M
Ceftriaxone	R
Type of antibiotic (DNA synthesis)	**Sensitivity**
Ciprofloxacin	S
Type of antibiotic (Folic acid metabolism)	**Sensitivity**
Trimethoprim	M
Type of antibiotic (Protein synthesis)	**Sensitivity**
Azithromycin	
Erythromycin	R
Gentamycin	S
Amikacin	S

Many nosocomial infections are caused by microbes that are resistant to medicines, which may be transferred readily through the hospital environment and by hospital employees, as is the case with *Methicillin-resistant Staphylococcus aureus* (MRSA). Antimicrobial susceptibility testing can assist physicians in the prescription of suitable medicines, as well as the prevention of the development of drug resistance in patients. The occurrence of *S. aureus* in UTIs might be attributed to factors, such as the increased use of instrumentations, including bladder catheterization, in patients brought to the hospital with primary illness and longer periods of hospitalization. The *S. aureus* was found to be 100% sensitive to vancomycin, and teicoplanin, which was in line with an investigation conducted by Looney, Redmond (16). However, these medications are preserved for life threatening infections by MRSA. Amoxicillin, Amoxy/Clavulanic acid, and Tobramycin showed higher resistance towards the MRSA in the present study, compared to that revealed by a previous study by Looney, Redmond (16).

The findings of the present study were in agreement with previously-published studies by Dibah, Arzanlou (17), Almayali and AL-Kraety (18), AL-MUHANNA, AL-KRAETY (19), as well as Almayali, AL-Kraety (20). The antibiotic resistance pattern revealed that 15.1% of the MRSA isolates displayed resistance to vancomycin, intermediate level of resistance to clindamycin (54.5%), gentamicin (45.5%), as well as tetracycline (48%), and a high level of resistance to ciprofloxacin (72.7%), as well as erythromycin (91%). While vancomycin and gentamicin had the lowest levels of resistance in *Methicillin-Susceptible Staphylococcus aureus* (MSSA) with 5.8% and 17.6%, respectively, tetracycline (47.0%), clindamycin (52.9%), as well as ciprofloxacin (52.9%) had intermediate levels of resistance, and erythromycin had a high degree of resistance (82.55%). This indicates a general increase in resistance patterns in both MRSA and MSSA, which is disconcerting and concerning. The MRSA resistance to kanamycin was assessed to be 77%, whereas the MSSA resistance was projected to be 25%, which is higher than the results reported by Ahaduzzaman, Hassan (21). This discrepancy might signal the emergence of a kanamycin resistance trend.

3.4. Genotyping Detection of Biofilm Formation of *Staphylococcus aureus*

3.4.1. Detection of Clumping factor A gene

It was discovered that 27 (100 %) *S. aureus* isolates tested positive for the *clfA* gene, as shown in figure 1, after the amplification findings of a PCR investigation for the *clfA* (657bp) (1). According to the findings of a study by Yousefi, Pourmand (8), *clfA* genes were discovered in all *S. aureus* isolates, and the PCR identified *clfA* genes in 100% of the isolates. Based on the findings of other investigations, biofilm development may not occur *in vitro* even when genes, such as *clfA*, are present (22). The gene for the biofilm-associated protein (Bap) was not found in the current analysis, although certain investigations have shown that Bap is involved in biofilm formation on a rare occasion. The *S. aureus* isolates have yielded similar results in previous research, which supports these findings. The findings of the present study showed that a range of factors, including environmental circumstances, impact the formation of biofilms. Due to the great sensitivity of *S. aureus* isolates to environmental variables, including the quantity of

glucose or glucosamine available for matrix formation, biofilm development may be inhibited *in vitro* despite the presence of the *icaA* gene (11).

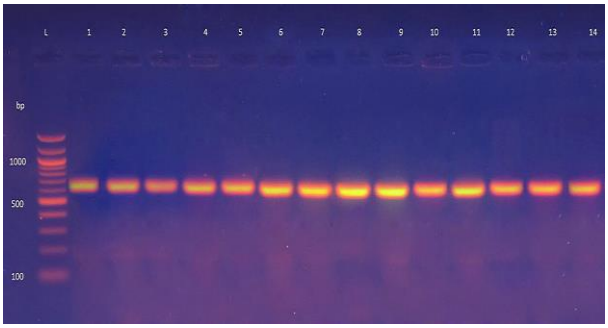

Figure 1. PCR amplicon of *S. aureus clfA* gene. Product size 657bp. Lane (L), DNA marker (100-bp ladder), Lanes (1 to 14) positive results.

The *S. aureus* has been recognized as a major public health problem, especially among UTI patients, which appears highly resistant to ceftriaxone and erythromycin. The phenotypic and genotypic mismatch between biofilm development and the presence or absence of biofilm-associated genes may affect bacteria's genetic origins. The findings of this study imply that biofilm formation is extremely complicated and is not only dependent on the presence of the *icaA* gene in *S. aureus*. While molecular techniques are frequently employed to find genes implicated in biofilm formation, they are not suitable for identifying the real biofilm phenotype *in vitro*.

Authors' Contribution

Study concept and design: A. M. N. A.
Acquisition of data: I. A. A. A.
Analysis and interpretation of data:
Drafting of the manuscript: A. S. Y. A.
Critical revision of the manuscript for important intellectual content: I. A. A. A.
Statistical analysis: S. G. A.
Administrative, technical, and material support: A. M. N. A.

Ethics

The studies were approved by the Human Research Ethics Committee of the University of Alkafeel, Najaf, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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