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Detection of some virulence genes of *Staphylococcus aureus* that caused Infective Endocarditis

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ABSTRACT: Infective Endocarditis (IE), first documented over 350 years ago, is condition characterized by infection of the endocardial membrane of the heart. The clinical presentations of irreversible endocarditis (IE) can affect all organ systems, with the cardiac manifestations including valve vegetation, abscess, periannular extension of infection, and myopericarditis. 230 Blood sample were collected from Infective Endocarditis patients, blood culture used for bacterial growth and identification of bacterial species were completed by microscopic examination, culture characteristics, and biochemical tests, and the use of the Vitek 2 diagnostic system for final identification of the isolated bacteria. Antibiotic sensitivity test for *S. aureus* were performed by disk diffusion method , *mecA* and *sarA* genes were detected by PCR. 51 positive culture for bacterial growth and 18 were *S. aureus* bacteria, most of Staph aureus were resistant to ampicillin (100%), amoxiclav (83.3%)and pencillin(94.4%) while they were highly sensitive to Azithromycin, Cefotaxime, Ceftriaxone, Gentamicin, Imipenem, Levofloxacin, Amikacin, and Trimethoprim, which reached 72.22%, 72.22%, 83.33%, 88.89%, 83.33%, and 88.8% 9, 83.33%, 83.33%, 72.22%, and 88.89%, respectively. *mecA* gene were found in (42,8%)of *S. aureus* isolates while *sarA* gene were found in (64.2%). *S. aureus* have many virulence factors genes that contributed in the infective Endocarditis infection and they were resistant to many antibiotics due to these genes.

Keywords: *S. aureus*, Endocarditis, PCR, Abscess

1. INTRODUCTION

Infective endocarditis (IE), first documented over 350 years ago, is the infection of the endocardial periphery of the heart. Inflammatory encephalopathy can present with clinical symptoms affecting all organ systems. The cardiac symptoms particularly include valve vegetation, abscess, periannular extension of infection, and myopericarditis. Although echocardiography is essential for diagnosing ischemic attack IE, other imaging modalities are becoming more important in the diagnosis and treatment of IE (1). IE affects both natural and artificial valves, as well as any intracardiac devices operating within the heart. In rare cases, it also affects nonfunctional embryonic remains that are located in the right atrium (RA). Bacterial or, less frequently, fungal species generate this by seeding any of these structures (2). Despite improvements in diagnostic capabilities and

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treatment choices, ischemic embolism remains a rising health concern with a one-year death rate of 30%, which has stayed constant for many decades. *Staphylococcus aureus* is the main bacterium responsible for Infectious IE, accounting for 60-90% of cases. The percentage of methicillin-resistant *Staphylococcus aureus* strains and polymicrobial infections is gradually rising(3). *Staphylococcus aureus* possesses many genes (1441), including the *sarA* gene. This gene is expressed in most *S. aureus* isolates, especially those that possess multiple virulence factors and are resistant to antibiotics, especially the species isolated from hospitals (4). Also it has the mec gene is responsible for the resistance of *S. aureus* bacteria to antibiotics, especially methicillin, which is a type of penicillin, and is called MRSA (methicillin-resistant *Staphylococcus aureus*). This gene encodes a protein that targets beta-lactam antibiotics known as penicillin-binding protein. (PBP2a) (5). Endothelium damage can result in either direct infection by pathogenic microorganisms or the formation of an uninfected platelet-fibrin thrombus that provides a reservoir for temporary bacteremia, unless *S. aureus* is capable of infecting the whole endothelium(6). These organisms infiltrate the circulatory system through the skin, mucosal surfaces, or previously infected areas and attach to nonbacterial blood clots as a result of damage to the valves or turbulent blood flow. Without defensive mechanisms from the host, this bacterium is permitted to multiply, producing tiny colonies and being released into the bloodstream. Left-sided infection is far more prevalent than right-sided illness, with the exception of among those who use intravenous drugs. (7). The study aimed to evaluate the bacterial causes of infective endocarditis and determined the virulence genes of *S. aureus* that played role in infection.

2. MATERIALS AND METHODS

2.1 Study design

This study included 230 patients suffering from heart disease and bacteremia (115 males and 115 females), whose ages ranged between 20-85 years and were admitted to the resuscitation unit, cardiac catheterization unit, internal medicine unit, and dialysis unit in Kirkuk Teaching Hospital and Azadi Teaching Hospital, in addition to 30 groups as a negative control in the time period from October 2023 to March 2024.

230 blood samples were collected from patients with heart disease and bacteremia who suffered from fever, chills, shortness of breath, high blood pressure, and tachycardia. Ten ml of blood was collected by drawing blood from the patient's vein using a vacutainer syringe. Ten ml of blood was injected into two 5 ml blood culture bottles sterilely (5 ml for aerobic culture and the other for anaerobic culture) and the bacteria causing the bloodstream infection were isolated. Take blood

samples directly into two aerobic and anaerobic blood culture bottles containing Brain Heart Infusion Broth and the anticoagulant Sodium-Polyanethole Sulfonate SPS.

2.2 Laboratory Diagnosis

2.2.1 Primary culture on brain heart infusion

Blood culture bottles were checked daily (for up to 7 days) for obvious signs of bacterial growth such as turbidity of the medium, colonies growing on the surface of red cells (cotton balls), hemolysis, gas bubbles, and clots.

2.2.2 Subculture

Suspected secondary cultures from blood cultures were done directly on blood agar, MacConkey agar, and chocolate agar on days 1, 3, and 7. All isolates were incubated aerobically and anaerobically at a temperature of (35-37°C) for 24 hours, then suspicious colonies were selected for final microscopic examination, culture characteristics, and biochemical tests, and the use of the Vitek 2 diagnostic system for final identification of the isolated bacteria.

2.3 Antibiotic sensitivity

S. aureus sensitivity to the antibiotics were detected by disk diffusion method on mullar henton agar and confirmed by Vitek2 compact system.

2.4 Molecular diagnosis

2.4.1 DNA Extraction

The kit was used to extract genomic DNA from Bacteria was DNA Presto™Mini.

2.4.2 Examination of the purity of the extracted DNA

The extracted DNA was detected by using a Nanodrop spectrophotometer, which is a special device for detecting and measuring the concentration of nucleic acid. DNA is detected by determining the concentration of nucleic acid (ng\ml) (DNA) and measuring the purity of the nucleic acid by reading Absorbance at a wavelength between 260/280 nm.

2.4.3 Preparation of polymerase chain reaction components

The polymerase chain reaction mixture was prepared using the PCR PreMix kit prepared by the Korean company Bioneer, according to the company’s instructions.

Table 1: Components of Monoplex PCR master mix and their volumes

Component	20 µl reaction volume
Template DNA	Variable (3 µl)
Forword primer (10 pmole,ul)	1.0 µl
Reverse primer (10pmole, ul)	1.0 µl
Nuclease Free Water	15 µl
Total volume	20 µl

Table 2: Conditions used in the PCR thermocycler condition

Steps	Temperature	Time	Cycles
Pre-denaturation	95 ºC	5min	1 cycle
Denaturation	95 ºC	30 sec	30cycles
Annealing	58 ºC	30sec	30cycles
Extension	72 ºC	30sec	30cycles
Final extension	72 ºC	5min	1 cycle

2.4.5 Agarose gel electrophoresis

This method was used to separate DNA molecules of different sizes, and electrophoresis was performed as stated in Mohammed *et al*., 2014.

2.4.6 Primers used for gene detection

Table 3: Primers used to detect genes

Primers	Sequence of necliotides 5'→3'	Gene size target bp
mecA	F: GCGTTGATTTGGGTAGTATG	317
mecA	R: GCACGTTAAAGTGTCTATAC	
sarA	F: CTTCTACACCTCCATATCAC	589
sarA	R: GGCCAATTCCACATTGTTTC	

3. RESULTS AND DISCUSSION

The result of present study showed 51(22.1%) were positive for cultural growth while 179(177.8%) were negative, as showed in table (4).

Table 4: Results of blood cultures for endocarditis patients and the control group

Result	IE patients	Controls
Positive	51 (22.1%)	0
Negative	179 (177.8%)	30 (100%)
Total	230 (100%)	30 (100%)

The result of bacterial culture revealed 34 (14.78%) were gram positive bacteria while 17 (7.39%) of isolates were gram negative bacteria, isolation results showed that out of 51 positive samples, (18) samples were *S. aureus* 7.83%, (5) samples were *S. epidermidis* 2.17%, and (3) samples were *Micrococcus luteus* 1.30% and the rest of the isolates are as shown in Table (5), where the isolates were identified based on the phenotypic characteristics of the colonies growing on the culture media, biochemical tests, and molecular tests.

Table 5: Types of bacterial isolates that cause endocardial infections and bacteremia

Isolates	**No.**	**Precentage(%)**
Gram positive	34	14.78
S. aureus	18	7.83
S. epidermidis	5	2.17
S. haemolyticus	2	0.87
S. hominis	2	0.87
E. faecalis	2	0.87
Micrococcus luteus	3	1.30
Micrococcus lylae	2	0.87
Gram negative	17	7.39
E. coli	5	2.17
K. pneumoniae	5	2.17
P. aeruginosa	7	3.04
Total	51	100

S. aureus was identified based on their phenotypic characteristics after growing them on blood agar medium under aerobic conditions. The results showed large, smooth, raised, low-convex, opaque colonies. Most of the colonies were dyed in a creamy yellow color, as in Figure (1), surrounded by clear areas. (Areas of beta hemolysis). As for its growth on mannitol salt agar medium, it produces small colonies and the medium turns yellow because the bacteria grow in high concentrations of

salinity and have the ability to ferment mannitol sugar and produce acid, which works to change the color of the medium's reagent from red to yellow.

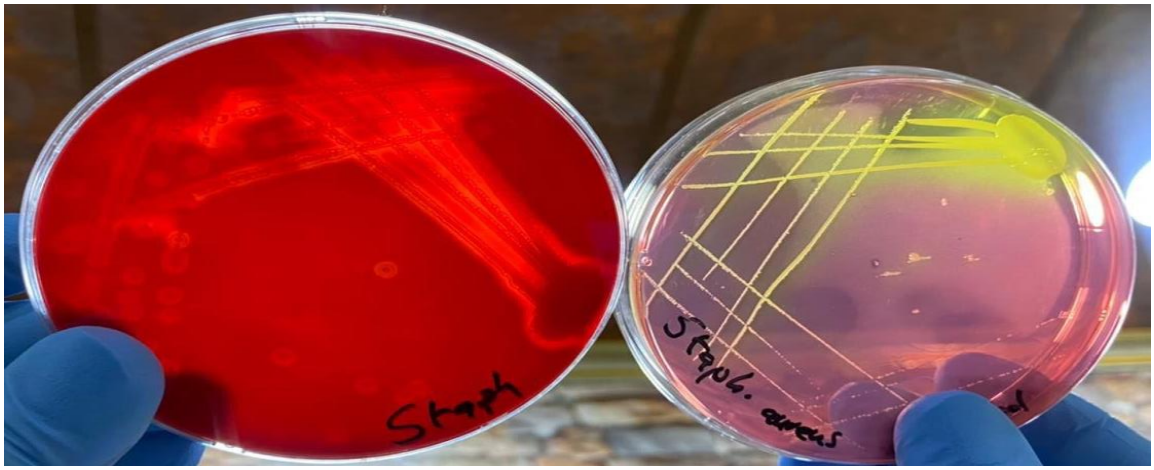

Figure (1): *S. aureus* isolate on Blood agar and mannitol agar

The results showed that the bacteria *S. aureus* was positive for the catalase test, the urease enzyme production test, the methyl red test, the Coagulase test, the Voges Proskauer test, the nitrate reduction test, and the gelatinase dilution test and the test results were negative for the motility test, Indole test, H_2S production, oxidase test and citrate consumption test, Table (6).

Table 6: Biochemical tests for *S. aureus*

Biochemical tests	Indole	Methyle red	oxidase	urease	catalase	motility
S. aureus	-	+	-	+	+	-
	Citrate utilization	H_2S	Gelatinase	Vogas proskaur	Nitrate reduction	Coagulase
	-	-	+	+	+	+

The results of antibiotic sensitivity shown in Figure (2) showed that most of the *S. aureus* bacteria were highly resistant to the antibiotics Ampicillin, Amoxicalv, Penicillin G, Pipracillin, Cefixim, which reached 100%, 83.33, 61.11%. 94.44%, 66.67%, respectively, while most isolates showed high sensitivity to the antibiotics Azithromycin, Cefotaxime, Ceftriaxone, Gentamicin, Imipenem, Levofloxacin, Amikacin, and Trimethoprim, which reached 72.22%, 72.22%, 83.33%, 88.89%, 83.33%, and 88.8% 9, 83.33%, 83.33%, 72.22%, and 88.89%, respectively, while the isolates gave an average sensitivity to Ciprofloxacin, which amounted to 55.56%.

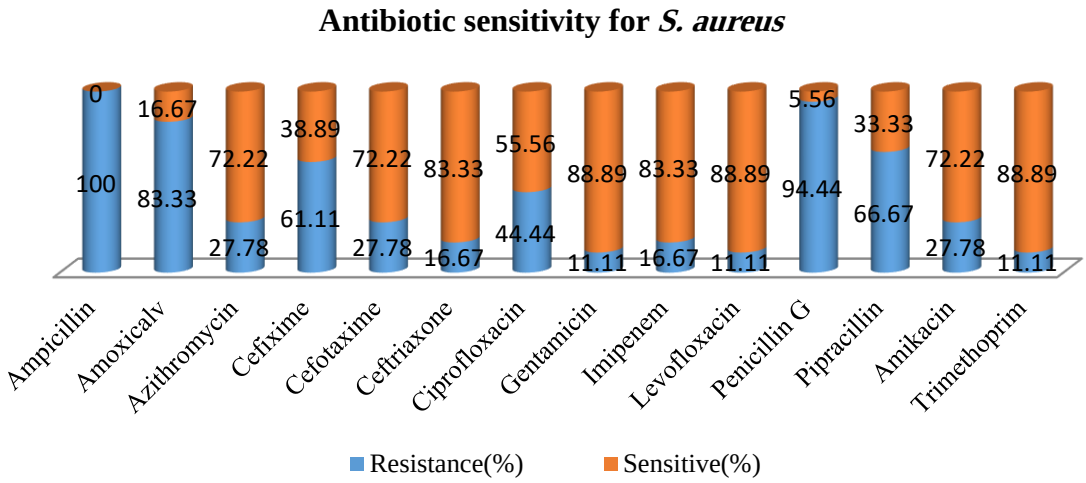

Figure 2: Antibiotic sensitivity for *S. aureus*

Detection of the *mecA* gene

The results of study showed that the detection rate of the *mecA* gene in *S. aureus* bacteria using PCR technology. The results were positive for (6) isolates, at a rate of 42.85% out of (14) isolates, while (8) isolates, at a rate of 57.14%, had a negative result, as shown in Figure (3).

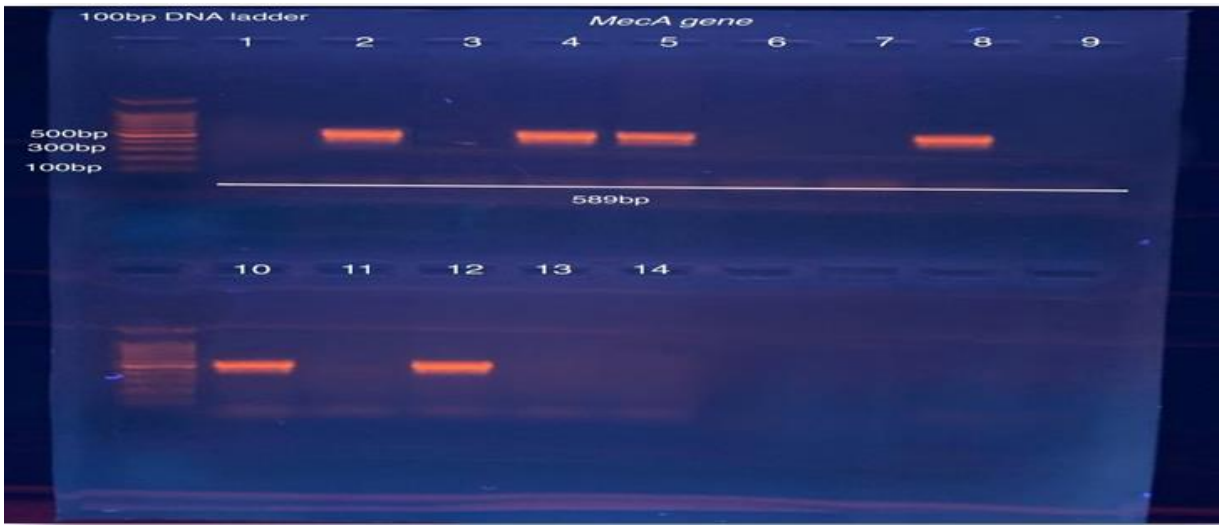

Figure 3: Showed the product of the PCR reaction with a size of 589 bp for the genetic detection of the *mecA* gene of *S. aureus* bacteria, which was migrated in an agarose gel at a concentration of (1)% at a voltage difference of (70) volts for (60) minutes

The results study showed that the detection rate of the s*arA* gene in *S, aureus* bacteria using PCR technology. The results were positive for (9) isolates, at a rate of 64.28% out of (14) isolates, while (5) isolates, at a rate of 35.71%, had a negative result, as shown in Figure (4).

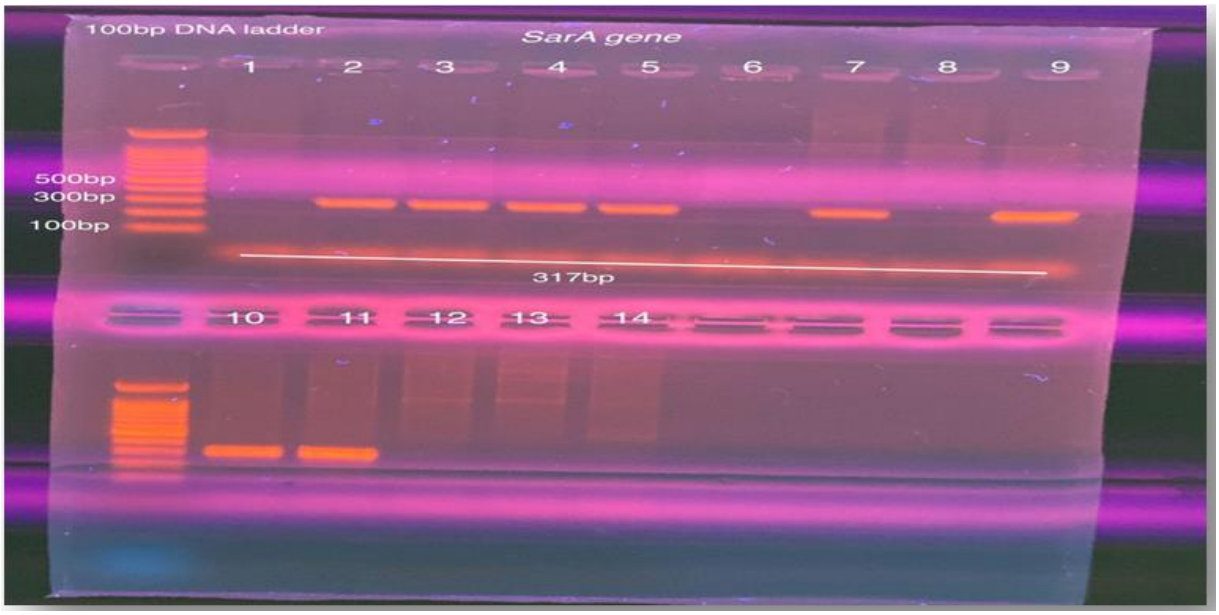

Figure 4: The product of the PCR reaction with a size of 317 bp for the genetic detection of the *sarA* gene of *S. aureus* bacteria, which was migrated in an agarose gel at a concentration of 1% at a voltage of 70 volts for 60 minutes.

Infective endocarditis is an uncommon yet lethal etiology of sepsis, resulting in an overall death rate of 20 to 25% in most studies (8). The results showed that the most common causative agent of infectious endocarditis due to Gram-positive aerobic bacteria was mainly *Staphylococcus aureus* bacteria, as this bacterium was found to be associated with an increased mortality rate, followed by *S. epidermidis*, which is the most common species causing this disease, as well as *E. faecalis*, *Micrococcus luteus*, *S. haemolyticus*, and less common microbial causes of IE for aerobic Gram-negative bacteria were *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. This study agreed with a study (9). *S. aureus*, the primary cause of infective endocarditis, acts as a genuine modulator of immunothrombosis and flourishes in the fibrin-rich milieu of an endocarditis vegetation. Given its pivotal function in infective endocarditis, the coagulation system remains a compelling treatment focus for this lethal condition. Nevertheless, there is a highly intricate equilibrium in operation, and the administration of antithrombotic medications in patients with endocarditis is more typically associated with a significant risk of bleeding(10). *S. aureus* isolates exhibited the greatest resistance to penicillin (100%) and tetracycline (87.50%), which is consistent with the findings of the current investigation (11). An investigation conducted in Kirkuk city by Mohammed SA revealed that the Staphylococci isolates were Methicillin-resistant *Staphylococcus aureus* (MRSA), which exhibited resistance to all other resident agents such as ampicillin, azithromycin, erythromycin, gentamicin, and ciprofloxacin. No evidence of vancomycin resistance was detected. This phenomenon might be attributed to the compounding effects of the widespread use of antibiotics and the overall deficiency in health literacy within the population (12, 13). In a separate investigation conducted in Kirkuk city,

it was observed that 27.3% of the isolates exhibited resistance to oxacillin. This was followed by 24.3% resistance to penicillin G, 15.2% resistance to amoxicillin, 12.1% resistance to erythromycin, and 6.1% resistance to tetracycline. Additionally, 4.1% of the isolates showed resistance to each of the three clindamycins. In the investigation conducted by Rafif Khairullah et al.,(14) three isolates (30%) tested positive for the *mecA* gene in *Staphylococcus aureus* using PCR testing. This finding closely aligns with the current study result(7). A study by Dhungel et al.(15) revealed that out of 524 specimens, 27.5% (144/524) had bacterial growth. The leading bacteria among the 144 culture positive isolates was S. aureus, accounting for 27.1% (39 out of 144). Within the group of 39 S. aureus isolates, all of them exhibited resistance to penicillin, followed by erythromycin (94.9%), gentamicin (94.9%), and cefoxitin (87.2%). The *mecA* gene was present in 82.1% of the 39 S. aureus strains, out of which 87.2% were MRSA.

sarA is the initial member of the s*arA* protein family identified as responsible for crucial functions in the control of virulence genes. A thorough examination of the recently published S. aureus genomes has identified a minimum of 10 more s*arA* homologs, out of which six have undergone partial characterisation. Additionally, members of the s*arA* protein family exhibit sequence homology with the MarR protein or homologs found in Gram-negative bacteria (16).

4. CONCLUSION

S. aureus have many virulence factors genes that contributed in the infective Endocarditis infection and they were resistant to many antibiotics due to these genes.

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