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Investigating the Presence of *eap* and spa Genes of S*taphylococcus aureus* and their Relation to Antibiotic Resistance

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Abstract

Staphylococcus aureus is an extracellular and intracellular microbe that can infect different mammalian cells like epithelial, endothelial, and osteoblasts. It is a source of recurrent infection and has persistently caused a variety of human diseases, like skin infections and systemic diseases such as osteomyelitis, endocarditis, pneumonia, bacteremia, sepsis, and toxic shock syndrome. Diagnosed (30) isolates of *S. aureus have been* taken from urine. A clinical sample has been done according to the culture on mannitol salt agar and biochemical tests, then identification through amplification of the *16srRNA* gene by conventional PCR, detection of *spa and eap* genes by conventional PCR found all isolated to have a *spa* gene with a percentage of 100% but *an eap* gene found with a percentage of 53.3%, and detection of their relation with antibiotic resistance found 76.70% of this isolates resistance to different types of antibiotic. Many factors contributed to the rapid development of antimicrobial resistance, including cell membrane disruption, environmental factors, and DNA damage. All of these factors contributed to the rapid development of antibiotic resistance. *Staphylococcus aureus is resistant* to a lot of commonly used antibiotics, such as Beta-lactams, Tetracycline, Aminoglycosides, Fluoroquinolones, Macrolides, and Chloramphenicol.

Keywords :*S. aureus*, *16srRNAgene*, *eap* gene, *spa* genes, antibiotic resistance.

1. Introduction

Staphylococcus aureus is an extracellular and intracellular microbe that can infect different mammalian cells like epithelial, endothelial, and osteoblasts. It is a source of recurrent infection and a persistent cause of a variety of diseases in humans, like skin infections and systemic diseases such as osteomyelitis, endocarditis, pneumonia, bacteremia, sepsis, and toxic shock syndrome [1, 2]. Many factors helped to develop antimicrobial resistance rapidly, including cell membrane disruption, environmental factors and DNA damage, all of which fastened the development of antibiotic resistance. *S. aureus* is resistant to a lot of commonly used antibiotics, such as beta-lactamases, tetracycline, aminoglycosides, fluoroquinolones, macrolides, and chloramphenicol [3]. *S. aureus* has different virulence factors that enable the microorganism to be affected. This causes a wide range of infections because this factor helps the pathogen in attachment to host cells, tissue invasion, and weakness of the host immune system. *S. aureus*

occurs in 20–50% of humans.The pathogenic capacity is combined with the effects of extracellular factors, toxins, and the invasive properties of the strain [4-6].

2. Materials and Methods

2.1. Sample collection:

150 clinical samples were collected during the period (February to May 2022) using sterile swaps taken from tonsils and sterile cups for the collected urine samples. All the samples were taken from people returning to Tuz General Hospital in the Salah al-Din Governorate and from patients broadcasting in hospitals.

2.2. Bacterial identification:

All clinical samples cultured on the selective media (Mannitol salt agar) that differentiate *S. aureus* isolates through mannitol fermentation [7, 8], then taking smears of isolates and staining them with Gram's stain investigated through an oil immersion microscope lens, the cells appear under light microscopes. Gram-positive cocci clustered mostly in grape-like irregular clusters, circular clusters, single, paired, or short chains because *S. aureus* bacteria divided into three planes during binary fission [9, 10]. Biochemical tests include The catalase test was distinguished by transporting some pure colonies of bacterial isolates taken from mannitol salt agar media to a clean glass slide by a sterilized loop, then adding a few drops of 3% H_2O_2 to it. The production of air bubbles is referred to as a positive result that evidences enzyme production [11, 12]. The coagulase test was evaluated to distinguish free plasma coagulase enzymes that lead to coagulation products from interactions between blood and *S. aureu*s bacteria, which indicates a positive result. While the clotting did not emerge at room temperature, that refers to a negative result [13].

2.2.1. Confirmed this method of identification through detection *16srRNA* by PCR (Polymerase chain reaction) includes two steps.

2.2.1.1. Extraction and determine *S. aureus* DNA:

Genomic DNA extraction according to Onasanya *et al. 2*003 and Geneaid Biotech kit instructions, with some modifications, agarose gel electrophoresis that was stained by adding ethidium bromide was used for determining DNA extraction presence and integrity. The optimum DNA concentration was detected for the PCR, and all the samples were conserved at -20 °C until use.

The genomic DNA samples were mixed with the loading buffer (DNA: loading 7/2 v/v) and then loaded inside the wells of the gel [15].

2.2.1.2. *16srRNA* gene amplification:

The *16srRNA* gene commonly used for identification, classification, and quantitation of microbes that codes for the small subunit of the ribosome found in prokaryotes such as bacteria and archaea has several factors that make it the perfect target to complete phylogeny studies. [16] used a specific primer [17] in the PCR program according to the TRANS protocol detected 16srRNA.

Table 1. PCR reaction components for detection 16srRNA according to Trans protocol

Primer	Type of reaction	Master mix volume	Volume of F-primer (10pmol/*m*l)	Volume of R-primer (10pmol/*m*l	Nuclease free water	DNA	Final volume
16s rRNA	uniPCR	10	1	1	6 *m*l	2 *m*l	20 *m*l

Table 2. PCR program to amplify 16srRNA

Steps	ºC	Min-Sec	Cycle	Product band
Initial denaturation	95 ºC	4min	1	
Denaturation	95 ºC	4 min	30	
Annealing	53 ºC	45 sec		257
Extension	72 ºC	45 sec		
Final extension	72 ºC	7 min	1	

2.3. Antimicrobial resistance test

Detection of S. *aureus* and determination of the antimicrobial resistance profile of each isolate Antimicrobial resistance testing was carried out by the Kirby Bauer disc diffusion method according to the Clinical Laboratory Standards Institute on Muller Hinton agar [18, 19]. The antibiotics used to determine antibiotic resistance include amoxicillin (AMC 30), chloramphenicol (C10), erythromycin (E10), tetracycline (TE10), penicillin (P10), and vancomycin (VA30), all obtained from the Bioanalyse company. The ranges of antibiotic zone diameter (mm) that are suggested for *S. aureus* according to research in reference [20]

2.4. Detection of *S. aureus* virulence factors *spa* and *eap* genes

Detection of *S. aureus spa and eap* genes that were encoding for regulating virulence factor was carried out by the amplification of the extraction DNA of *S. aureus* with the target gene by using PCR, which included using various specific sets of primers encoding for each target gene mixed with the template (DNA sample) and master mix reagents (PCR buffer, MgCl2, Taq polymerase, and dNTPs). The end constituent was deionized water. The mixture was then mixed and centrifuged for 3 seconds to collect the drops from the walls to ensure a final volume of 25 ml. The mixture was transferred to a thermal cycler to start the reaction according to the steps of the specific program [21].

Table 3. PCR reaction components according to (Trans protocol)

Primer	Type of reaction	Master mix volume	Volume of F-primer (10pmol/*m*l)	Volume of R-primer (10pmol/*m*l	Nucleas e free water	DNA	Final volume
Spa gene	uniPCR	10	1	1	6 *m*l	2 *m*l	20 *m*l
Eap gene	uniPCR	10	1	1	6 *m*l	2 *m*l	20 *m*l

Table 4 explains the PCR program to amplify the *spa* gene using a specific primer [22].

Table 4. PCR program to amplify *spa* gene

Steps	ºC	Min-Sec	Cycle	Product band
Initial denaturation	95 ºC	4min	1	
Denaturation	95 ºC	30 sec	30	
Annealing	50 ºC	45sec		variable
Extension	72 ºC	45 sec		
Final extension	72 ºC	7min	1	

Table 5 explains the PCR program to amplify the *eap* gene using a specific primer [23].

Table 5. PCR program to amplify *eap* gene

Steps	°C	Min-Sec	Cycle	Product band
Initial denaturation	95 °C	4min	1	
Denaturation	95 °C	30 sec	30	230
Annealing	50 °C	45sec		
Extension	72 °C	45 sec		
Final extension	72 °C	7min	1	

3. Results

The number of samples taken from people returning to Tuz General Hospital were125, collected from urinary tract (100) samples with a percentage of 66.6 percent, and from tonsils (25) samples with a percentage of 16.6%. Also collected from patient broadcasts in hospitals were 24 samples with a percentage (16%) from tonsils and one sample with a percentage (0.6%) from the urinary tract, as shown in **Table 6.**

Table 6. the sample source and patient type

Patient type	Sample source			
	Urinary tract	Percentage	tonsil	percentage
Patient return to hospital	100	66.6%	25	16.6%
Patient broadcasts in hospitals	1	0.6%	24	16%

3.1. Bacteria identification

Staphylococcus aureus isolates identification include Mannitol salt agar culture, biochemical tests, and confirmed primary identification by detection of 16srRNA by PCR (polymerase chain reaction).
Only from all samples identified 30 isolates with a percentage of 29.7% of *S. aureus* bacteria this percentage is shown in **Table 7.** This result found S. *aureus* bacteria infect the urinary tract more than the upper respiratory tract.

Table 7. positive culture result and negative result

Bacterial source	Positive result	percentage	Negative result	percentage
Urinary tract	30	29.7%	71	70.2%
tonsil	0	0	49	100%

3.1.1. Biochemical test

All *S. aureus* bacteria mannitol fermenter appear with golden yellow on mannitol salt agar plates, Gram-positive cocci, catalase positive, coagulase positive, and oxidase, motility negative and more strain hemolysis it is lysis the red blood cell.

3.1.2 molecular detection of *Staphylococcus aureus* includes two steps, the first one is extraction *S. aureus* DNA

Extracted DNA and purified from all *S. aureus* using the manufacturer's DNA purification kit (DNA extraction kit) used agarose gel electrophoresis and stained by adding ethidium bromide for determined DNA extraction presence and integrity. The optimum DNA concentration was detected for the PCR. **Figure 1** shows DNA extraction.

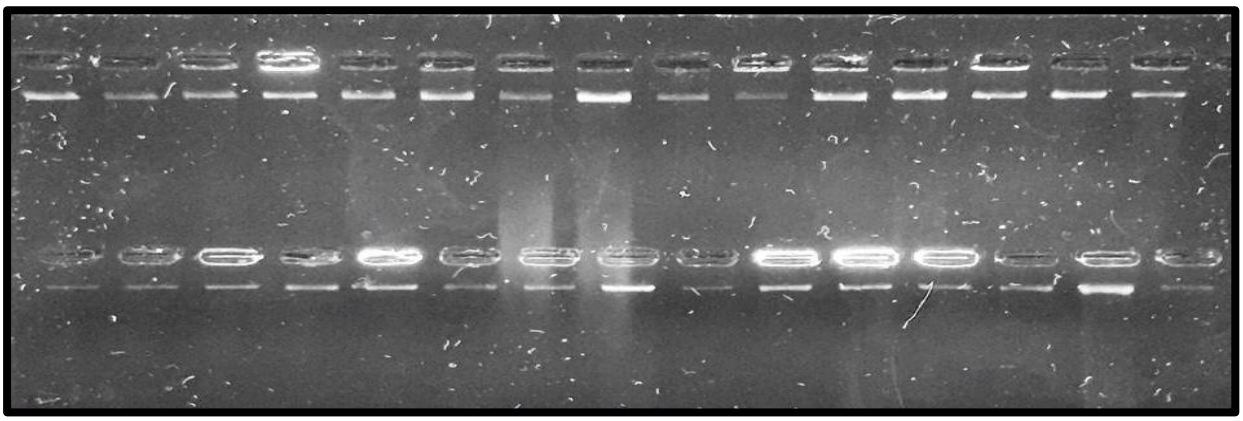

Figure 1. showed *S. aureus* DNA extraction visualized by 1 % agarose gel electrophoresis that stained with ethidium bromide by 1xTBE and used UV transilumiator at 350nm.

The second step used the PCR technique to amplify the 16srRNA gene was applied on the 30 isolates taken from the urinary tract after primary identification, and all isolates were *S. aureus* bacteria. **Figure 2** clarify 16SrRNA amplification.

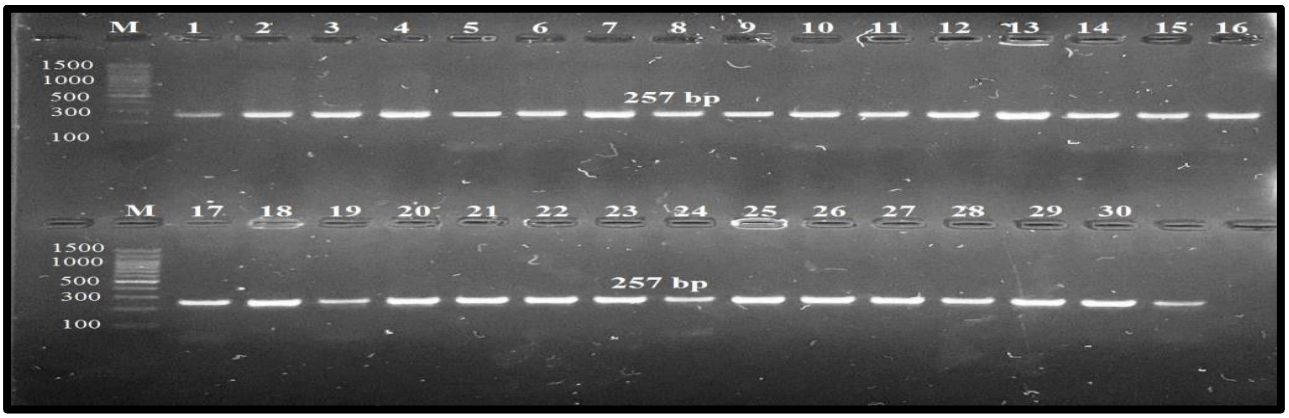

Figure 2. PCR amplification of 16srRNAgene (257bp) of *S. aureus*, visualized by 1 % agarose electrophoresis that using TBE to stained by ethidium bromide, for 30 min at 90 Volt. , Lane M: Marker Lane (1-30): Urinary tract sample from people return to Hospital.

3.2. Antimicrobial resistance test by disk diffusion method

Antimicrobial resistance testing was performed using the disk diffusion method on 30 *S. aureus* isolates against six commonly used antibiotics (P, TE, AMC, C, and VA). All *S. aureus* isolates showed varying resistance percentages to the different antibiotics used in the study, as shown in **Figure 3**.

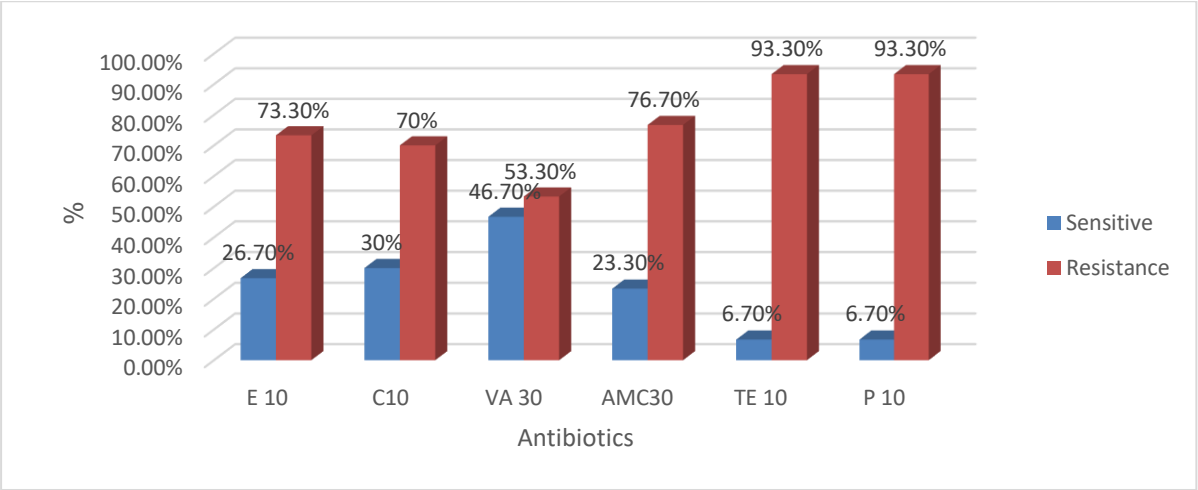

Figure3. result of antimicrobial resistance test by disk diffusion method

3.3. PCR reaction result for detected some *S. aureus* genes

Used conventional PCR to investigate about 2 genes (the *eap gene* and the *spa gene)*. This gene is responsible for the virulence factor in 30 isolates of *S. aureus* bacteria. All isolates showed a *spa* gene with a 100% percentage. **Figure 4** shows the *spa* gene in all isolates.

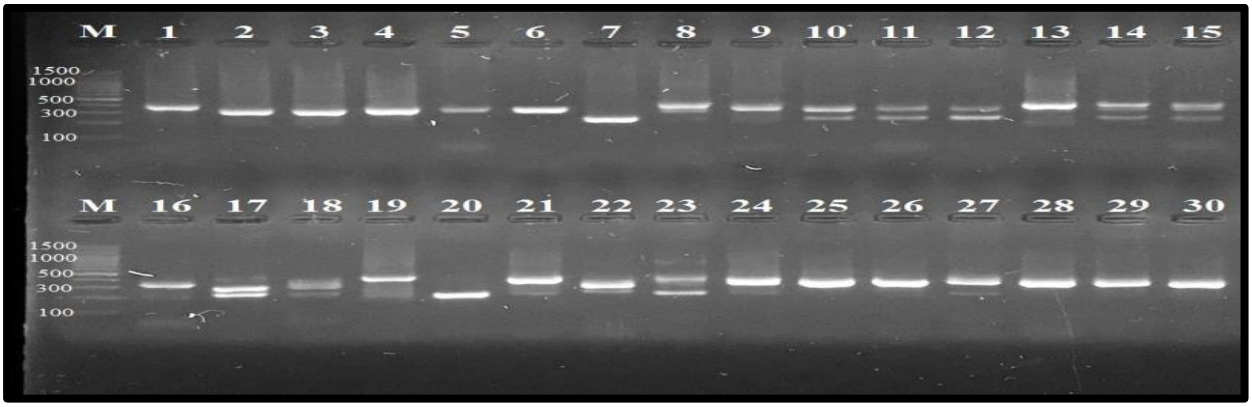

Figure 4. PCR amplification of the *spa* gene of *S. aureus*, visualized by 1% agarose electrophoresis, using TBE to stain with ethidium bromide for 30 min at 90 volts Lane M: 1500 Marker.

The *eap* gene detected in this study with percentage of 53.3% from all stains, that show in **figure 5.**

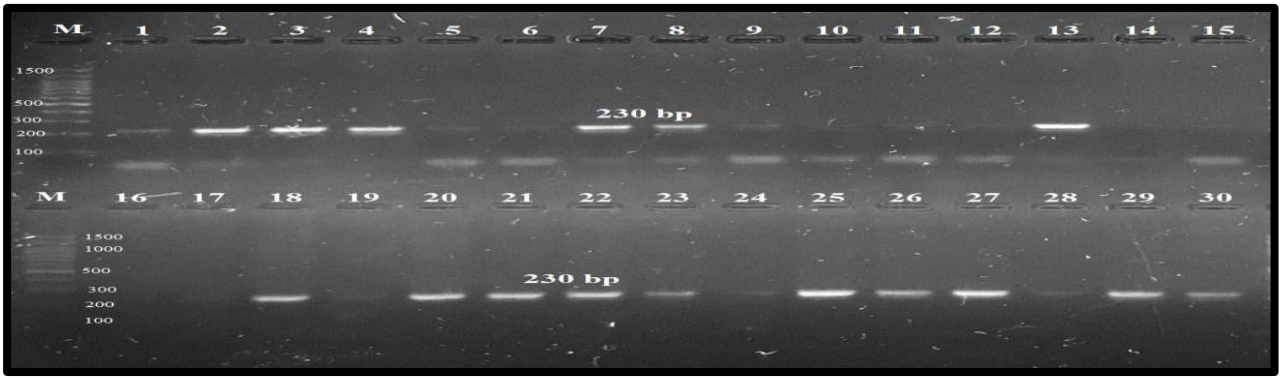

figure 5. PCR amplification of *eap* gene (230bp) of *S. aureus*, visualized by 1 % agarose electrophoresis that stained with ethidium bromide by using TBE, for 30 min at 90 Volt. Lane M: 1500 Marker

3.4. Antibiotic resistance relation with *spa* and *eap* virulence genes

Antimicrobial therapy plays an important role in controlling *S. aureus* toxicity and infectivity that cause *S. aureus* harm is stronger this is related to the virulence genes but has become less effective due to widespread drug resistance, the resistance returns to bacterial capsular polysaccharide and some virulence gene like *spa* gene and *eap* gene [32, 33]. In this study, it was shown that *S. aureus* has a high ability to resist some types of antibiotics, such as erythromycin, chloramphenicol, vancomycin, amoxicillin, and penicillin, with percentages of 73.3%, 70.0%, 53.3%, 76.7%, 93.3%, and 93.3, respectively. Because of the *S. aureus* virulence gene *spa*, it was found with a 100% considered molecule that binds to Fcγ of human and animal immunoglobulin (Ig), a defense mechanism that hinders the capacity of antibodies with specific binding activities for the *S. aureus* surface to enable Fc receptor-mediated opsonophagocytosis and bacterial killing that allows *S. aureus* to avoid innate and adaptive immune responses [34].

While the eap gene found with a percentage of 53.3% is considered a protein, it has two structural homologues, *EapH1* and *EapH2*, which impair the adherence of neutrophils to non-stimulated and TNF-alpha-stimulated endothelial cells, may prevent the release of neutrophil serine proteases, inhibit extracellular killing of bacteria, cause trans-endothelial migration of neutrophils, and reduce inflammation responses because it directly interferes with neutrophil serine protease activity. All these genes of *S. aureus* bacteria contribute to bacterial escape through immunity defenses and antibiotic resistance [35, 36]. **Table 8** shows all the results.

Table (8) *S. aureus spa* and *eap* virulence genes relation with antibiotic resistance

Resistance Antibiotic	R	%	*Spa* gene	%	*eap* gene	%
erythromycin 10	22	73.3	30	100%	16	53.3%
Chloramphenicol 10	21	70.0	30	100%	16	53.3%
vancomycin 30	16	53.3	30	100%	16	53.3%
Amoxicillin30	23	76.7	30	100%	16	53.3%
Tetracycline 10	28	93.3	30	100%	16	53.3%
Penicillin 10	28	93.3	30	100%	16	53.3%

4. Discussion

The first step in diagnosis, all samples cultured on Mannitol salt agar medium for 24 hours at 37ºC are selective and differential growth mediums that contain a high concentration (~7.5%–10%) of NaCl, Mannitol sugar, and phenol red as an indicator for differentiation between two groups of *staphylococci* [24, 25]. *S. aureus* growth on the mannitol salt agar and changed the culture color from pink to yellow, this bacteria was isolated from the urinary tract of people returning to Tuz General Hospital and found S. *aureus* bacteria infect the urinary tract more than the upper respiratory tract. The second step of diagnosis used the PCR technique to amplify the 16srRNA gene, which helped to identify the bacterial genome, it was a highly developed and effective tool that helped to recognize specific bacterial strains, the increasing number of multidrug-resistant (MDR) *staphylococcal* infections leads to investigating basic questions about the genetic variations because antibiotic resistance evolved within the bacterial population. Therefore, in recent years, 16srRNA gene amplification has become more common as a genetic marker to confirm *S. aureus* phylogeny and taxonomy identification, which is increasingly prevalent in the clinical environment [17, 26].The most common resistance mechanisms include active efflux of the antibiotic, enzymatic inactivation of the drug, and mutations in the rRNA. For example,

penicillin resistance returns to antibiotic action, plasma membrane, penicillin-less bacterial permeable to the antibiotic, and modification of the receptor site of the bacterial protein [27, 28]. Through genetic study found *spa* acts as a genetic marker to distinguish *S. aureus* strains and has an important role in *S. aureus* resistance to different types of antibiotics, especially erythromycin [29]. *The eap* gene which is involved in invasion of host tissues, [30] showed in their study *eap* gene was with a percentage 100%, but the [31] study showed the *S. aureus* adhesion gene (*eap*) responsible for antibiotic resistance that led to evolution and development has become hyper virulent and spread in hospitals and communities. Through the relation between antibiotic resistance with *spa* , *eap* virulence genes ,found all these genes of *S. aureus* contribute about bacterial escape through immunity defenses and antibiotic resistance [35, 36]

5. Conclusion:

The urinary tract system was more infected with *S. aureus* bacteria compared with the pharynx. The return patient to the hospital was more infected with *S. aureus* bacteria than patients in the hospital. Through antibiotic resistance tests, *S. aureus* bacteria found more resistance to three antibiotics TE, P, and AMC. *The spa* gene is found in all strains of *S. aureus* bacteria, but *the eap* gene is not found in all strains, and these genes have an important role in antibiotic resistance.

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Conflict of Interest

No, conflict of interest

Funding

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Ethical Clearance

The samples taken from people return to Tuz general Hospital collected from urinary tract and tonsils, also taken samples from patient broadcasts in hospitals .

References

1. Xiao, X; Li; Y; Li; L; Xiong, Y.Q. Identification of Methicillin-resistant *Staphylococcus aureus* (MRSA) genetic factors involved in human endothelial cells damage, an important phenotype correlated with persistent endovascular infection. *Antibiotics*. **2022**, *11(3)*, 316. https://doi.org/10.3390%2Fantibiotics11030316.
2. Cheung, G.Y.C; Bae, J.S.; Otto, M. Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*. **2021**, *12(1)*, 547-569, https://doi.org/10.1080%2F21505594.2021.1878688.
3. Rasheed, N.A.; Hussein, N. R. *Staphylococcus aureus*: An Overview of Discovery, Characteristics, Epidemiology, Virulence Factors and Antimicrobial Sensitivity. *European Journal of Molecular & Clinical Medicine*. **2021**, *8(3)*.
4. Riedel, S; Morse, A.S.; Mietzner, T.; Miller, S. *Jawetz, Melnick & Adelbergs' Medical Microbiology*, **2019** .28th Edition.
5. Kadhim, B.A; Essa, M.A; Sabbah, M.R. Prevalence of enterotoxin type A in *Staphylococcus aureus* isolated from different sources. *Iraqi Journal of Agriculture Sciences*. **2022**, *53(6)*, 1325-1330. DOI: https://doi.org/10.1590%2FS1517-83822013000200008.

6. Abdrabaa, M.K.; Aburesha, R.A. Gene expression evaluation of intracellular adhesion and regulatory genes among biofilm producing MRSA isolates. *Iraqi Journal of Science*. **2023**, *1(64),* 75-83. DOI: https://doi.org/10.24996/ijs.2023.64.1.8.
7. Obaid, W.A.; Abdulwahhab, A.S. Impacts of starvation stress on biofilm formation and expression of virulence genes in mono-and mixed –species cultures of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Biochemical & Cellular Archives.* **2021**, *21(1)* 685-693.
8. Najem, M.J.; Lafta, I.J. Characterization of Mannitol fermenter and salt Tolerant *Staphylococci* from breast tumor biopsies of Iraqi women. *Baghdad Science Journal*. **2020**, *17(2),* 415-425. DOI: https://bsj.uobaghdad.edu.iq/index.php/BSJ/article/view/3661.
9. Al-Hasnawi, E.A. Isolation of *Staphylococcus aureus* from ear swab in Iraq children as a causative agent of otitis externa. *Journal of Medicine- Baghdad*. **2017**, *59(3),* 258-261, DOI: https://doi.org/10.32007/jfacmedbagdad.593100.
10. Chotigarpa, R.; Lampang, K.; Pikulkaew, S.; Okonogi, S.; Ajariyakhajorn, K.; Mektrirat, R. Inhibitory effects and killing kinetics of lactic acid rice gel against pathogenic bacteria causing bovine mastitis. *Scientia pharmaceutica Multidisciplinary Digital Publishing Institute*. **2018**, *86(3),* 1-10. DOI: https://doi.org/10.3390/scipharm86030029.
11. Omran, A.S.; Hussein, A.N. Molecular investigation of *mec* gene Among coagulase negative *Staphylococcus* isolated from different cases. *Journal of Physics: Conference Series.* **2019**, *1234(1),* 12074, DOI: https://doi.org/10.1088/1742-6596/1234/1/012074.
12. Mahdi, E.A.; Al-Shaibani, A.; Zbar, N.S. The impact of glucse and chloride on the biofilm formation of *Pseudomonas aeruginosa*& *Staphylococus aureus*. *Ibn AL-Haitham Journal For Pure and Applied Sciences*. **2020**, *33(4)*, 1-9. https://doi.org/10.30526/33.4.2525.
13. Rakotovao-Ravahatra, Z.D.; Randriatsarafara, F.M.; Milasoanjara, R.N.; Ranaivosoa, M.K., Rakotovao, A.L.; Rasamindrakotroka, A. Assessment of the Coagulase Test in the Identification of *Staphylococcus aureus* Strains. *Journal of Biotechnology and Biomedicine*. **2019**, *2(3),* 105-111.
14. Onasanya, A.; Mignouna, H.D.; Thottappilly, G. Genetic fingerprinting and phylogenetic diversity of *Staphylococcus aureus* isolates from Nigeria. *African Journal of Biotechnology*. **2003**, *2(8),* 246-250. DOI: https://doi.org/10.24996/ijs.2021.62.1.6.
15. Al-Noaami, R.J. *Detection the genotype estimation of allele frequencies for ABO groups an Rh factor in population of Selah Al-Dine and Kirkuk and Neneva provinces*. PhD. Thesis, Tikrit University, **2014**.
16. Jassim, S.A.; Kandala, N.J. Molecular detection of enterotoxin genes of multiresistant *Staphylococcus aureus* isolates from Different sources of food. *Iraqi journal of Science*, **2021**, *62(1),* 61-74. https://doi.org/10.24996/ijs.2021.62.1.6.
17. Gumaa, M.A.; Idris, A.B.; Bilal, N.E.; Hassan, M.A. First insights into molecular basis identifcation of 16 s ribosomal RNA gene of *Staphylococcus aureus* isolated from Sudan. *BMC Research Notes*. **2021**, *14(1)*, 240. DOI: https://doi.org/10.1186/s13104-021-05569-w.
18. Clinical and Laboratory Standaards Institute (CLSI). *Performance Standard for Antimicrobial Susceptibility testing*. 32nd ed, West Valley Road, Pennsylvania, USA, **2023**.
19. Sastry, A.S.; Bhat, S.; Janagond, A.B.; Deepashree, R.; Ray, P.; Sistla, S. *Essentials of Medical Microbiology*. 3rd ed , Jaypee Brothers Medical Publishers The Health Sciences Publisher New Delhi London, **2021**.
20. Clinical and Laboratory Standards Institute (CLSI). *Performance Standard for Antimicrobial Susceptibility testing*. 30th ed, West Valley Road, Pennsylvania, USA, **2021**.
21. Fadhil, H.; Mohammed, J. Molecular and Classical identification of *Staphylococcus aureus* , isolated from Iraqi Patients with recurrent tonsillitis. *Medico-legal Update*. **2022**, *22(1).* https://doi.org/10.37506/mlu.v22i1.3176.

22. Alwash, S.J.H. *Molecular analysis of toxin associated genes in clinical and non-clinical methicillin-resistant Staphylococcus aureus isolates*. PhD. thesis, Baghdad university, College of Science, Biology, **2021**.
23. Hussain, M.; Eiff, C.V.; Sinha, B.; Joost, I.; Herrmann, M.; Peters, G.; Becker, K . *Eap* gene as novel target for specific identification of *Staphylococcus aureus*. *Clinical Microbiology*, **2008**, 470-476. DOI: https://doi.org/10.1128/jcm.01425-07.
24. Burke, K.C. Introduction of microbiology 22.2 Selective and differential media-MacConkey, *EMB, MSA*. Libre Texts, **2020**.
25. Aryal, S. Mannitol salt agar for the isolation of Staphylococcus aureus. *Microbiology info.* **2022**, 10.
26. Jaddoa, N.T.M.; Al-Mathkhury, H.J.F. Biofilm shows independency form hemolysin genes arsenal in methicillin resistant *Staphylococcus aureus*. *Iraqi Journal of Science*. **2018**, *59(4)*, 2184-2194. DOI: https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/591.
27. Guo , Y.; Song, G.; Sun, M.; Wang, J.; Wang, Y. Prevalence and Therapies of Antibiotic- Resistance in *Staphylococcus areus*. *Frontiers in Cellular and infection Microbiology*. **2020**, *10*, 107. DOI: https://doi.org/10.3389%2Ffcimb.2020.00107.
28. Parthasarathy, A.K; Chougale, R.A. *Insights Into Drug Resistance in Staphylococcus aureus, Antibiotic Resistant Staphylococcus aureus*, **2021**. 23rd https://doi.org/10.5772/intechopen.100057.
29. Naorem, R.S; Urban, P; Goswami, G; Fekete, C. Characterization of methicillin-resistant *Staphylococcus aureus* through genomics approach. *Biotech*, **2020**, *10(9), 401*. DOI: https://doi.org/10.1007/s13205-020-02387-y.
30. Afzal, M.; Vijay, A.K.; Stapleton, F.; Willcox, M. Virulence genes of *staphylococcus aureus* associated with keratitis, conjunctivitis, and contact lens–associated inflammation. *Transl Vis Science Technology*. **2022**, *11(7)*, 5. https://doi.org/10.1167/tvst.11.7.5
31. Khan, S.; Marasa, B.S; Sung, K.; Nawaz, M. Genotypic Characterization of Clinical Isolates of *Staphylococcus aureus* from Pakistan. *Pathogens*. **2021**, *10*, 918. https://doi.org/10.3390/pathogens10080918.
32. Zhang, Z.; Chen, Y.; Li, X.; Wang, X.; Lia, H. Detection of Antibiotic Resistance, Virulence Gene, and Drug Resistance Gene of *Staphylococcus aureus* Isolates from Bovine Mastitis. *Microbiology Spectrum*. **2022**, *10(4)*.DOI: https://doi.org/10.1128/spectrum.00471-22.
33. Alwash, S.J; Aburesha, R.A. Detection of toxin-associated genes in seven *spa*-types of *Staphylococcus aureus* in Iraq. *Indian Journal of forensic medicine & Toxicology*. **2021**, *15(3)*,DOI: https://doi.org/10.37506/ijfmt.v14i4.11540.
34. Gonzalez, C.D.; Ledo, C.; Giai, C; Garófalo, A.; Gómez, M. The *Sbi* Protein Contributes to *Staphylococcus aureus* Inflammatory Response during Systemic Infection. *PLOS One*. **2015,** *10(6)*. DOI: https://doi.org/10.1371/journal.pone.0131879.
35. Eisenbeis , J.; Saffarzadeh, M.; Peisker, H.; Jung, P.; Thewes, N.; Preissner, K.T.; Herrmann, M.; Molle, V.; Geisbrecht, B.V.; Jacobs, K.; Bischoff, M. The *Staphylococcus aureus* Extracellular Adherence Protein *Eap* Is a DNA Binding Protein Capable of Blocking Neutrophil Extracellular Trap Formation. *Front. Cell. Infect. Microbiol*, **2018**, *8*, 235. DOI: https://doi.org/10.3389/fcimb.2018.00235.
36. Aburesha, R.A; Hussein, H; Khalaf, Z.Z; Mohamed, B.J. Extraction of methicillin resistant *Staphylococcus aureus* (Mrsa) golden pigment and study it antioxidant activity on macrophage killing pathway. *ISOR Journal of Pharmacy and biological Sciences(ISOR-JPBS)*. **2018**, *13(4)*, 40-43. DOI: https://doi.org/10.9790/3008-1304054043.