INVESTIGATION OF RESISTANCE AND VIRULENCE GENES OF STAPHYLOCOCCUS AUREUS ISOLATED FROM PATIENTS UNDERGOING HEMODIALYSIS

Mohammed Khalid mohammed Aladeeb¹, Halah Abdulkhaleq awadh²

¹Department of Medical Laboratory Techniques, Technical Institute/mosul, Northern Technical University, Nineveh Governorate, Iraq
²Department of biology, College of Science, University of Tikrit, Salah al-Din Governorate, Iraq

¹mohammedkhalidmohammed87@gmail.com
²d.hala2010@yahoo.com

ABSTRACTS

Eight isolates of Staph.aureus were subjected to a PCR technique to investigate of 6 genes in Staph.aureus (6 isolates from blood samples and 2 isolates from urine), including the nuc gene, which was used as a diagnostic gene for Staphylococcus aureus isolates. The results showed that the percentage of isolates carrying the nuc gene was 87.5%. All isolates of Staphylococcus aureus possess genes (mecA, blaz, spa) that encode for (methicillin resistance, beta-lactamase production, virulence factor protein A), respectively.

While 87.5% of Staphylococcus aureus isolates possess two genes (ermA and coa) that encode for (resistance to the antibiotic erythromycin, coagulase enzyme) respectively.

Keywords: Staph.aureus; mecA; blaz; spa; ermA; coa

I. INTRODUCTION:

Hemodialysis is one of the types of dialysis, this process is done by delivering the blood of kidney failure patients to the hemodialysis machine in order to remove waste products and toxins (creatinine, urea and uric acid) and excess fluids from the blood, then return the filtered blood to the patient's body (Krans, 2019). Hemodialysis patients suffer from a weak immune response and therefore more at risk of bacterial infection. Urinary tract infection is one of the most common types of acquired infection, and urinary tract infection often appears without symptoms in patients with kidney failure. (Shankar et al., 2021; Arjumand et al., 2021). Inflammation of the bloodstream that leads to bacteremia is one of the main problems of hemodialysis patients. The risk of bacterial infection for dialysis patients is usually related to the hemodialysis procedure itself, and specifically the means and methods of access between the patient’s blood and the dialysis device, which is the main cause of their bacteremia (Alhazmi et al., 2019).

Hemodialysis patients are frequently exposed to Staphylococcus aureus infection due to their prolonged presence in dialysis centers or hospitals, Especially when using a central venous catheter, which increases the risk of bacteremia compared to an arteriovenous fistula (Scheuch et al., 2019) Staphylococcus aureus is considered highly pathogenic due to its virulence factors such as the speed of penetration, multiplication and spread of these bacteria in the tissues of the host and their production of many extracellular substances (toxins and enzymes), and these factors are genetically controlled (Carroll et al., 2016) Staphylococcus aureus is inherently resistant to many antibiotics due to its acquisition of many resistance genes, and the transferability of these resistance genes between bacterial strains such as resistance to the anti-vancomycin that is used as an alternative therapy to anti-beta-lactam type, Methicillin-resistant Staphylococcus aureus (MRSA) is a problem Global health, as it is responsible for most cases of Staphylococcus aureus bacteremia compared to methicillin-susceptible Staphylococcus aureus, as well as the emergence of Erythromycin resistant Staphylococcus aureus (ERSA) strains (Tille, 2017; Hassoun et al., 2017; Taddesse et al., 2014)
II. MATERIALS AND METHODS

collection of samples

Samples were collected from renal failure patients undergoing dialysis at Ibn Sina Hospital in Mosul for the period from 1 October, 2020 to 1 March, 2021, where 200 samples (100 urine samples and 100 blood samples) from 100 cases of hemodialysis patients for different age groups and for both sexes

Diagnostic methods:

First. depending on The phenotypic and biochemical tests that Include: gram stain, Catalase test, Oxidase test, Coagulase test, Mannitol fermentation test on Mannitol Salt Agar, Triple iron sugar agar TSI, Urease test (Granato et al., 2019; Wanger et al., 2017)

Second. using (VITEK 2 system) device from Biomerieux the company of French, The VITEK test was conducted in the laboratory of Dr. Muhammad Dawaj in Mosul, Iraq

DNA Extraction

The process of extracting the genetic DNA of Staphylococcus aureus bacteria samples was carried out, in which the ready-made kit was used to extract the DNA prepared by (Geneaid) company, and the extraction method was followed according to the protocol attached by the manufacturer of the kit

Electrophoresis and Polymerase Chain Reaction PCR Assay: Detection of Resistance and Virulence Genes

Electrophoresis was used to separate DNA molecules of different sizes and electrophoresis was performed based on (Sambrook and Russell, 2001)

The primers were prepared in the form of a dried powder (Lyophilized) from the manufacturer (Scientific Researcher.Co.Ltd) with different concentrations of picomoles.

Table.1 Sequences of primers used to target genetic determinants responsible for resistance and virulence in Staphylococcus aureus

<table>
<thead>
<tr>
<th>gene</th>
<th>Primer sequence(5'-3')</th>
<th>Size Bp</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>nuc</td>
<td>F GCGATTGATGGTGATACGGTT</td>
<td>270</td>
<td>Brakstad et al, 1992</td>
</tr>
<tr>
<td></td>
<td>R AGCCAAAGCCTTGACGGA.ACTAAAGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mecA</td>
<td>F AAAATCGATGGTAAAGGTTGCG</td>
<td>532</td>
<td>Strommenger et al, 2003</td>
</tr>
<tr>
<td></td>
<td>R AGTTCTGCAGTACCGGATTTGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BlaZ</td>
<td>F ACTTCACACCTGCTGTTTCC</td>
<td>240</td>
<td>Martineau et al, 2000</td>
</tr>
<tr>
<td></td>
<td>R TAGGTTCAAGATGGCCCTTAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>coa</td>
<td>F CGAGACCAAGATTCAACAAG</td>
<td>730</td>
<td>Aslantas et al, 2007</td>
</tr>
<tr>
<td></td>
<td>R AAAGAAAACCTCACTACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spa</td>
<td>F CAA GCA CCA AAA GAG GAA</td>
<td>320</td>
<td>Frenay et al, 1996</td>
</tr>
<tr>
<td></td>
<td>R CAC CAG GTT TAA CGA CAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ermA</td>
<td>F TATCTTATCGTTGAGAGGAT</td>
<td>139</td>
<td>Martineau et al, 2000</td>
</tr>
<tr>
<td></td>
<td>R CTACACTTGCTAGATG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The main reaction mixture was prepared for each PCR reaction by mixing the DNA sample and the special primer for each gene with the components of the Premix inside a 0.2 ml Eppendorf tube supplied by the American company promega, as the reaction volume was fixed to 20 μl with distilled water. After that, the reaction tubes were transferred to the Thermocycler for the purpose of conducting the replication reaction using the special program for each reaction, then the samples are loaded into the pits of the previously prepared agarose gel at a concentration of 1% with the addition of Ladder DNA (supplied by Biolaps Company) in one of the pits, and the samples are transferred Samples are transferred through an electrophoresis device for (60-70) minutes, after which the gel is photographed using a UV-Transillumination device.

III. RESULTS AND DISCUSSION

The results showed that among the 8 isolates diagnosed for Staphylococcus aureus (6 isolates from blood samples and 2 isolates from urine), 7 isolates were carrying the nuc gene with a percentage of 87.5% at a molecular size of 270 bp as shown in Figure (1), that the molecular identification of the nuc gene responsible for TNase production is specific to Staph bacteria. aureus as a diagnostic gene (Thiele, 1990).
Our results were in agreement with the results of the researcher Khaleel et al., (2018), where the proportion of the nuc gene was 83.33% among their isolates, and the results also converged with the researcher Salih et al., (2015) when these genes were isolated by 100%, while our results did not agree with the researcher Mkize et al., (2017) when they proved that the percentage of Staph. aureus carrying the nuc gene was 54%.

In some cases, it is not possible to determine the presence of the nuc gene in some strains of Staph. aureus, due to the difference in the nucleotide sequence between the genes of the nucleus resulting from some mutations, so if a negative result for the nuc gene appears, this does not mean the absence of Staph aureus bacteria among the clinical isolates (Sahebnasagh et al., 2014).
The results also showed that the percentage of Staph.aureus carrying the \textit{mecA} gene was 100% at a molecular size of 532 bp as shown in the picture (2). The results of the current study were in agreement with the study of researchers Ibrahim and Al-Mathkhury, (2018), as their results showed 80% of the \textit{Staph.aureus} is a carrier of the \textit{mecA} gene, and our results also converged with that of the researcher Asante et al., (2019), where the percentage of \textit{Staph.aureus} carrying the \textit{mecA} gene was 74%, while the results of the researcher Motamedi et al., (2015) disagreed, as they stated that the proportion of \textit{Staph.aureus} contains the \textit{mecA} gene. \textit{mecA} was 30%.

Phenotypic resistance to methicillin in non-protective isolates of the \textit{mecA} gene may not be due to their ability to produce PBP2a, which is encoded by the \textit{mecA} gene, which are motile genetic elements of different size and genetic composition among MRSA strains. Resistance may be due to other factors such as beta-lactamase production (Monecke et al., 2018; Ciftci et al., 2009) or the change in the pBPs of beta-lactam antagonists, in addition to the presence of determinants located on the chromosome that lead to methicillin resistance, such as the genes (\textit{femB} and \textit{femA}) that encode the protein involved in the formation of the cross-bridges of glycine to the peptidoglycan layer and thus affect the expression levels of methicillin resistance in addition. Therefore, methicillin resistance may be due to the presence of the \textit{mecB} and \textit{mecC} genes (Becker et al., 2018; Towner et al., 1998).

The results of the study showed that 8 isolates were carriers of the \textit{blaZ} gene, which is responsible for encoding the enzyme $\beta$-lactamase or penicillinase enzyme, with a percentage of 100% at a molecular size of 240 bp. All of their isolates were carriers of the \textit{blaZ} gene with a percentage of 100%, while the study of the researcher Asante et al., (2019) differed, which found that the percentage of isolates possessing this gene was 33%.

Regarding the \textit{ermA} gene, it came at a rate of 87.5% and at a molecular size of bp139 as shown in Figure (3), and this result was in agreement with the results of the researcher Abdulamir, (2012), as its results showed that 81.8% of \textit{Staph.aureus} bacteria carry the \textit{ermA} gene. While these results differed, they did not agree with the results of the researcher Gan et al., (2021), as their isolates showed a percentage of 11.44 %, and Timsina et al., (2020) stated that the percentage of \textit{ermA} was 15.6% and this also did not agree with our results.

The reason behind the phenotypic resistance to erythromycin in aureus may be due not only to the \textit{ermA} gene, but also to other types of genes encoded by some \textit{Staph aureus} that are responsible for resistance to erythromycin, such as \textit{ermB} and \textit{ermC} (Talebi et al., 2019).
The current results showed that the percentage of *Staph. aureus* carrying the *coa* gene was 87.5% and at a molecular size of 730 bp, as shown in Figure (4). This result was in agreement with the results of the researcher Khaleel, *et al.*, (2018), where their isolates of *Staph. aureus* carrying the *coa* gene showed a rate of 85.7%, and our results were also close to the results of the researchers Abdul-Kareem and Husain, (2015), as their results showed the presence of the *coa* gene. 100% in *Staph. aureus* isolates from wounds and burns, while our results differed and did not agree with the results of Sharma *et al.* (2017), as their isolates showed 41.1%.

![Electrophoresis of the PCR reaction products to determine the presence of the *coa* gene](image-url)

**Figure (4)** Electrophoresis of the PCR reaction products to determine the presence of the *coa* gene

Regarding the *spa* gene, it came 100% at a molecular size of 320 bp, as shown in Figure (5). This percentage was in agreement with the results of the researcher Parth *et al.*, (2016) in India when they investigated the *spa* gene in *Staph. aureus* isolates and it was 92.45%. The results also agreed with the study of researcher Bhati, *et al.*, (2016) when 15 of their isolates showed that they carried the *spa* gene 93.7%. While the results of the researcher Li *et al.*, (2019) in China showed that the *spa* gene was 34.8% among their isolates, and in Egypt the researcher Kamel (2020) mentioned that the percentage of the *spa* gene among *Staph. aureus* isolates was 6.9% and this percentage did not agree with our results.
Figure (5) Electrophoresis of the PCR reaction products to determine the presence of the Spa gene

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