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Molecular Detection of Nuc and Hib Genes of Staphylococcus Aureus Isolated from Burn Patients in Baghdad Province

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Abstract. The current study was conducted to identify pathogenic Staph. Aureus that coexist with Burn Patients and characterize these bacteria based on some virulence genes using bacteriological and molecular techniques such as PCR. The study was carried out on 66 samples isolated from Burn patients in different hospitals in Iraq, Baghdad Province. These samples were cultivated on traditional and specific media agars for the identification of certain pathogenic bacteria. The results the results show that out 66 wound swabs, 55 (88.3%) of them growth Staph. aureus. The highest susceptible antibiotics against Staph. Aureus in this study were Imipenem (IPM), Cefepime (CPM), Ceftriaxone (CRO), and Cefotaxime (CTX) as (76%, 66%, 63%, and 61, respectively), while the highest resistant antibiotics were Cefixime (CFM), Vancomycin (VAN), and Amikacin (AK) as (84%, 75%, and 50%, respectively). In the current study, the molecular detection showed that 55(83.3%) of staph aureu were positive for Nuc gene. In the current study demonstrate that the S. aureus is adequate medium for growth of and production of enterotoxins. The study findings reveal a diverse community of bacterial species present in samples isolated from patients with Burn infections with high virulence, detected by the presence of virulence genes.

Highlights:

1. High Prevalence of Staph. aureus: 88.3% of wound swabs from burn patients tested positive for Staph. aureus, indicating a significant presence in burn infections.
2. Antibiotic Resistance Patterns: The bacteria showed high resistance to Cefixime (84%) and Vancomycin (75%), raising concerns about treatment effectiveness.
3. Virulence Gene Detection: 83.3% of Staph. aureus isolates carried the Nuc gene, highlighting their potential for high virulence and enterotoxin production.

Keywords: Staph. aureus, Burn Infections, Antibiotic Resistance, Virulence Genes, PCR Detection

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Introduction

Staphylococcus aureus represents a significant bacterial pathogen in humans, eliciting a diverse array of clinical presentations [1]. Infections are prevalent in both community-acquired and nosocomial environments, and therapeutic management continues to pose challenges owing to the rise of multidrug-resistant variants, exemplified by methicillin-resistant Staphylococcus aureus (MRSA). [2]

Injury of burn is significant comprehensive of epidermal, resulting in negative control of the systemic and local immune of responses. As we see, wounds of burn turn into a model refinement ground for microorganisms [3]. Methicillin-resistant Staphylococcus aureus (MRSA) is frequently contracted through interactions within hospital environments and various healthcare institutions, resulting in a range of significant infections associated with healthcare settings [4]. MRSA is determined by the availability of weak patients, selective pressure exerted by antimicrobial use, increased potential for transmission from larger numbers of colonized or infected patients (colonization pressure), and the impact of implementation and adherence to prevention efforts [5].

The identification of the mecA gene through the application of polymerase chain reaction (PCR) methodology is presently considered the worldwide standard for the detection of methicillin resistance in Staphylococcus aureus. [6] The phenomenon of methicillin resistance in the Staphylococcus aureus species is attributable to the mecA gene, which is derived from the chromosomal regions of Staphylococcus genomes. [7].

In the current work, was used to discover MARSA producing Staphylococcus. aureus.as Isolation and identification of S. aureus by using conventional and confirmatory techniques.

Materials and Methods

A. Collection and Characterization of Bacterial Isolates

A total of sixty wound swab specimens were subjected to analysis. A total of 66 S.aureaus isolates were obtained from the aforementioned samples collected from both male and female subjects throughout the study interval spanning from December 2024 to March 2025, specifically from Iraqi patients in a selection of

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hospitals located within the city of Baghdad. S. aureaus isolated was identified by using the VITEK 2 compact system and the biochemical test.

B. Resistance to Antibiotics

The disc diffusion method, as described by the Clinical and Laboratory Standards Institute (8.CLSI), was used to assess the antibiotic susceptibility of 55 strains. In short, each A cotton swab was immersed in the bacterial strains suspension after it had been adjusted to a McFarland standard of 0.5.spread evenly on Mueller Hinton agar after the colony had been streaked. The chosen antibiotic discs, which include Amikacin (AK), Cefepime (CPM), Cefixime (CFM), Cefotaxime (CTX), Ceftriaxone (CRO), Gentamicin (CN), Imipenem (IPM), and Vancomycin (VAN), were then added to the plates by sterile forceps after 15 minutes, and the cultures were incubated for 18 to 24 hours at 37°C. After that, measurements and evaluations of the inhibition zone diameters (IZD) were made [8].

C. Genetic Detection

Sequencing with Primers the primer sequencing applied for detecting. The Company of Macrogen provided the primers which were suspended and lyophilized in free nuclease water with volume of 100pmol/l as a stock solution. To make a 10 pmol/µl as primer solution, mixing with 10 µl of standard solution of primer (kept in -20 C) with 90 µl of free nuclease water.

D. PCR Amplification and Sequencing

DNA extract from the bacterial isolates, the ABIOpureTM kit was utilized. A polymerase chain reaction (PCR) analysis using specific primers was carried out in order to look for carbapenemase genes in S. aureaus: Nuc and Hib .The reaction was performed with 20µl volumes containing 10µl GoTaq Green Master Mix (2X), 1µl for each primer (10pmol), 6µl nuclease-free water, and 2µl of template DNA. , DNA was extracted as mentioned by Jasim and Alzubaidy in [9].

E. DNA test with gel electrophoresis

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The Gel Electro was designed according to the manufactured company. The amplified PCR products were detected using gel electrophoresis and observed using a UV trans illuminator documentation system and Ethidium bromide dye. Where one-gram agarose was added to 100 ml 1x TBE buffer.after covering the gel with TBE buffer and closing the tank, the electrophoresis for DNA extract and PCR products ran for an hour at 5 volts per centimeter of the gel. The agarose gel was taken out of the tank during electrophoresis, examined with a UV transilluminator documentation system, and then captured on camera with a digital camera. DNA ladders were always run concurrently with each electrophoretic run to detect the size of the PCR product. Prior to electrophoresis, 3.5µl of extracted DNA was combined with 1.5µl of loading dye (Bromo phenol blue) and loaded into the gel's wells. For the PCR product, each well was loaded with 5µl of the monoplex PCR products [10]. The UV transilluminator documentation system was used to visualize the DNA bands.

F. Statistical Evaluation

Microsoft Excel 2023 and the data were entered and assessed using the Statistical Package for the Social Sciences software, version 26. The results were presented using tables and/or graphs. For statistical analysis of categorical variables, chi-square was employed. The P-value, or level of significance, was set for all statistical analyses at 0.05.

Results and Discussion

The samples for (66) were gathered between October 2024 and April 2022 from the Baghdad Teaching Hospital in the governorate of Baghdad. According to the study's findings, S. aureus had an 83.3% favorable growth rate. The isolates were initially recognized as S. aureus after the specimens were cultivated on blood agar. Using morphological traits on blood agar, Gram stain, biochemical testing, and mannitol salt agar to detect mannitol fermentation—a trait of S. aureus—all isolates were identified. The VITEK 2 system was then used to confirm the diagnosis. As seen in Table 1, the percentage of S. aureus isolates from wounds was 55, or 83.3%. Figure1. The study's findings were consistent with [11].

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Table 1. Distribution Samples Examined the Positive and Negative Samples

Strain	Frequency	Percent
Staph. aureus	55	83.3%
No growth	11	16.67%
Total	66	100.0%

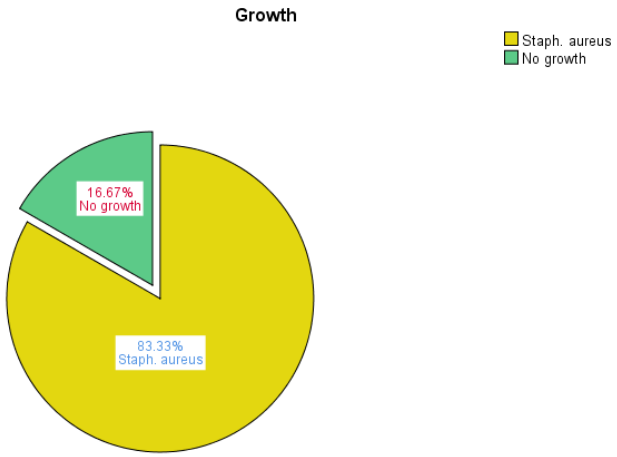

Figure 1. Distribution Samples Examined the Positive and Negative Samples

1. Data Distribution Based On Age & Sex Categories

There were 66 isolates of S. aureaus. Distributed In table 2. Below, the results show the participants' distribution by age & sex categories. Males between the ages of 30 and 60 made up the largest percentage of this study. A p-value of 0.6>0.05 indicated that there was no statistically significant association between sex & age categories in this investigation.

Table 2. Distribution of Data according to Gender and Age Categories

Age Categories		Gender		Total	Stat.
		Male	Female		
10-30yrs	N	12	5	17	r=0.828 P=0.661 (N.S.)
	%	18%	8%	26%	
31-60yrs	N	28	15	43	

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	%	42%	23%	65%	
61-90yrs	N	3	3	6	
	%	5%	5%	9%	
Total	N	43	23	66	
	%	65%	35%	100%	

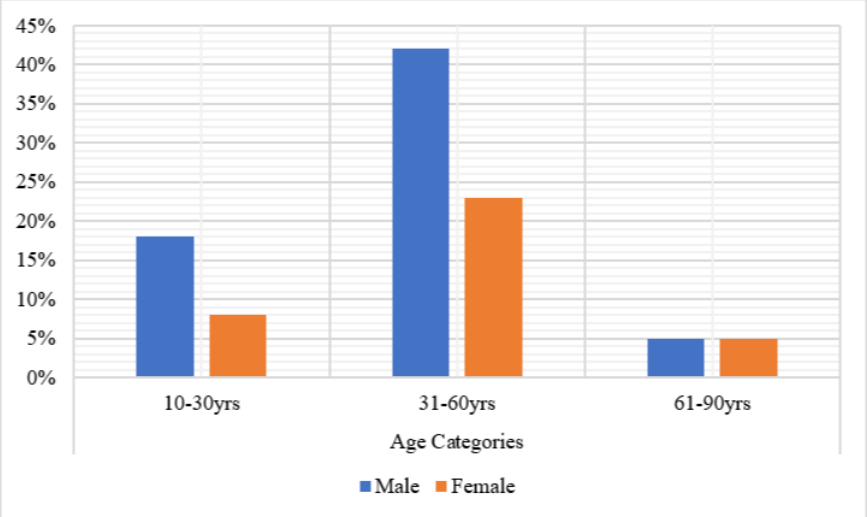

Figure 2. Distribution of participants according to Gender and Age Categories.

2. Resistance of Antibiotics

In the present investigation, the following figure (3 and 4) shows the susbtibility test by agar diffusion method, where the resistance isolates are shown by observing the growth despite the presence of the antibiotics. the highest susceptible antibiotics against Staph. aureus in this study were Imipenem (IPM), Cefepime (CPM), Ceftriaxone (CRO), and Cefotaxime (CTX) as (76%, 66%, 63%, and 61, respectively), while the highest resistant antibiotics were Cefixime (CFM), Vancomycin (VAN), and Amikacin (AK) as (84%, 75%, 50%, respectively) were employed to examine the patterns of antibiotic susceptibility of 38 S. aureus isolates. According to the results of the current study, the antibiotics with the highest antibiotic resistance against the S. aureus isolates were imipenem and cefepime, while the antibiotics with the lowest resistance activity were vancomycin and amikacin. The antibiotics' overall antibiotic-resistant activity against the S. aureus was disclosed by the antibiotic. Due to these prevalent bacterial isolates' strong resistance to widely accessible antibiotics, the

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findings of antimicrobial resistance patterns are extremely concerning; they nearly concur with study by [12].

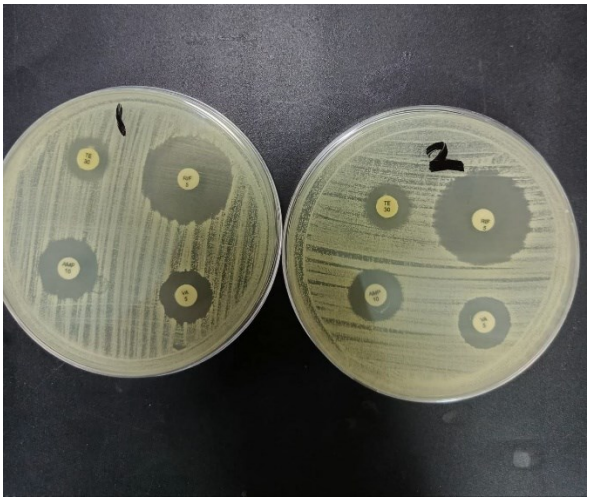
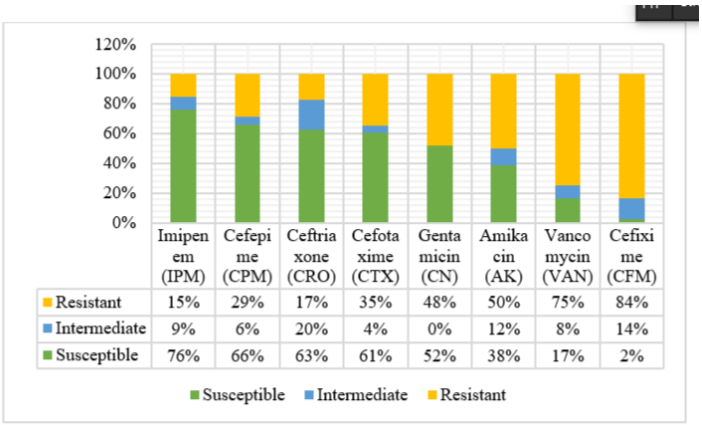


Figure 3. Antibiotic Sensitivity Test on S. aureaus.



	Imipenem (IPM)	Cefepime (CPM)	Ceftriaxone (CRO)	Cefotaxime (CTX)	Gentamicin (CN)	Amikacin (AK)	Vancomycin (VAN)	Cefixime (CFM)
Resistant	15%	29%	17%	35%	48%	50%	75%	84%
Intermediate	9%	6%	20%	4%	0%	12%	8%	14%
Susceptible	76%	66%	63%	61%	52%	38%	17%	2%

Figure 4. Results of AST Against Staph. aureus in This Study

3. Detection of Nuc and Hlb gene.

The 55 S. aureus isolates in the current investigation had an 83.3% nuc gene prevalence, which showed a rather weak positive connection with the burn genes. Similar research was found by [13]. Additionally, the study supports the findings of [14] in Iraq, which show that the PCR approach may be used to more accurately detect the selected isolates at the molecular level by amplifying the nuc gene, which is particular to S. aureus., In 2022, Karimzadeh and Ghassab stated that all S. aureus

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isolates tested positive for the nuc gene. This is in contrast to [15], who stated that 34.2% of S. aureus isolates had the nuc gene.

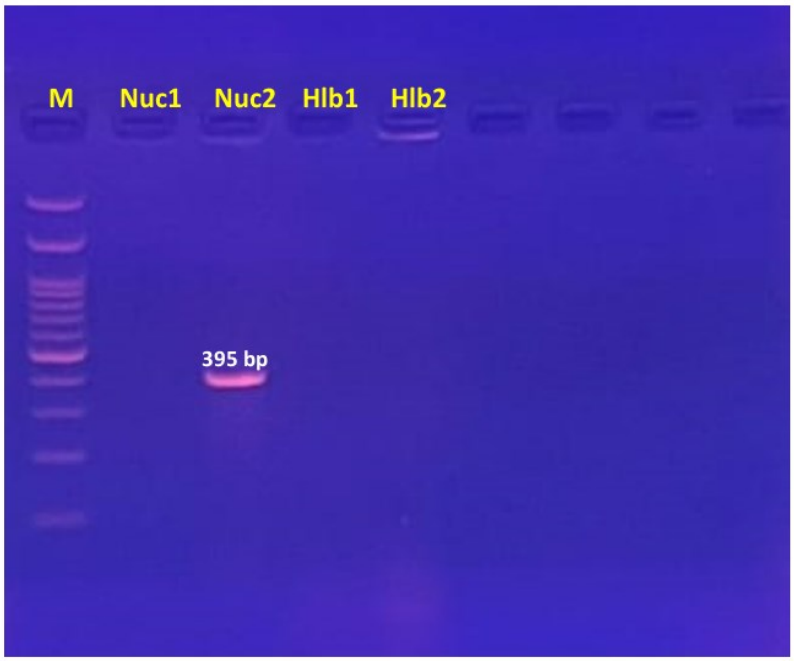

Figure 5. Agarose Gel Electrophoresis of Staphylococcus aureus (1.5% agarose,7v/cm2 for 60 min) for Nuc Gene (395 bp amplicon) represent by Lanes Nuc1,Nuc2, for Hlb Gene (309 bp Amplicon) represent by Lanes Hlb1,Hlb2,lane M represent M100bp DNA Ladder

Conclusions

In this study, Most S. aureaus. isolates showed resistance to MBL production, especially among hospitalized patients, which is alarming. Whereas the spread of the Nuc, can be anticipated in any Middle Eastern nation and might be more common in Iraq. the virulence genes were detected in the identified bacterial species. The study findings reveal a diverse community of bacterial species present in samples isolated from patients with Burn infections with high virulence, detected by the presence of virulence genes.

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