Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.158

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 is a major bacterial pathogen of humans that causes a wide range of clinical manifestations[1]. Infections are common in both community-acquired and hospital settings, and treatment remains difficult due to the emergence of multidrug-resistant strains such as methicillin-resistant Staphylococcus aureus (MRSA). [2]

Injury of burn is a major comprehensive public health crisis. It disrupts the barrier of epidermal, leading to negative regulation of both local and systemic immune of responses. As we see, wounds of burn become an ideal refinement ground for microbes[3]. Methicillin-resistant Staphylococcus aureus (MRSA) is usually acquired during exposure to hospitals and other healthcare facilities and causes a variety of serious healthcare-associated infections [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 detection of the mecA gene by using PCR technique is currently regarded the global method for identifying methicillin resistance in S. aureus. [6], Methicillin resistance is generated in the Staphylococcus cassette chromosomal genomic zone by the mecA gene, which originates in Staphylococcus chromosomes. [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 Identification of Bacterial Strains

Sixty sex samples of, wound swab, were analyzed. A total of 66 S.aureaus were isolated from those samples by males and females during the study period from (December 2024-march 2025) from Iraqi patients in some selected hospitals in Baghdad city. S. aureaus isolated was identified by using the biochemical test, VITEK 2 compact system.

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B. Resistance to Antibiotics

The antibiotic susceptibility of 55 strains was evaluated by using disc diffusion method. Briefly, suspension of each Bacterial strain was adjusted to a McFarland standard of 0.5, and a cotton swab was soaked in adjusted suspension and spread evenly on After streaking the colony on Mueller-Hinton agar, the selected antibiotic discs, which include Amikacin (AK), Cefepime (CPM), Cefixime (CFM), Cefotaxime (CTX), Ceftriaxone (CRO), Gentamicin (CN), Imipenem (IPM), & Vancomycin (VAN), were added after 15 minutes on the plates by sterile forceps, then, cultures incubated at 37°C overnight (18- 24 hours). The inhibition zone diameters (IZD) were measured and evaluated. Disk diffusion method as mentioned by Clinical and Laboratory Standards Institute [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. Gel Electro-Phoresis and DNA Test

The Gel Electro was designed according to the manufactured company. Gel electrophoresis was used for the detection of amplified PCR products, which visualized with the aid of Ethidium bromide dye and UV transilluminator documentation system. Where one-gram agarose was added to 100 ml 1x TBE

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buffer. TBE buffer was added to cover the gel, then tank was closed, and electrophoresis runs for an hour at 5 volt/cm2 of the gel for DNA extract and PCR products. After electrophoresis, the agarose gel removed from the tank and visualized by UV transilluminator documentation system and then photographed using digital camera. Before electrophoresis, 3.5µl of extracted DNA was mixed with 1.5µl of loading dye (Bromo phenol blue) and loaded into the wells of the gel, while for PCR product, each well was loaded with 5µl of the monoplex PCR products [10], DNA ladders were always run concurrently with each electrophoretic run to detect product size of PCR. DNA bands were visualized by UV transilluminator documentation system.

F. Statistical Analysis

The data were entered and analyzed by using Microsoft Excel 2023 and Statistical Package for the Social Sciences software version 26. The results were presented as tables and/or graphs. Chi-square was used for statistical analysis of categorical variables. In all statistical analysis, level of significance (P-value) was set at 0.05.

Results and Discussion

The samples for (66) were collected from Baghdad Teaching Hospital in the province of Baghdad during the period from October 20224 until April 2022. The result of the study showed that the positive growth for S. aureus was 83.3%. The specimens were cultured on blood agar at first; isolates were primary identified as S. aureus. All isolates were diagnosed by morphological charecters on blood agar, Gram stain, biochemical test, mannitol salt agar to detect mannitol fermentation which consider a specific feature for S. aureus and at last confirmed with VITEK 2 system. The percentage of S. aureus isolates were distributed as 55(83.3%) isolatesm from wounds as show in table 1. Figure 1. The outcomes of the study was agree with [11].

Table 1. Distribution Samples Examined the Positive and Negative Samples

Strain	Frequency	Percent
Staph. aureus	55	83.3%

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No growth	11	16.67%
Total	66	100.0%

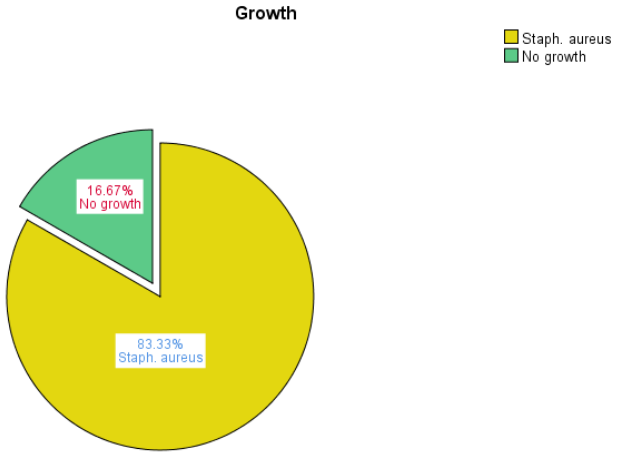

Figure 1. Distribution Samples Examined the Positive and Negative Samples

1. Distribution of data according to Gender and Age Categories

There were 66 isolates of S. aureaus. Distributed In table 2. Below, the results show the distribution of participants according Gender and Age Categories. In this study, the highest percentage were males those aged between 30- 60 decades. There was no statistically significant correlation between age categories and gender in this study, with a p-value of 0.6>0.05.

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

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	%	65%	35%	100%	

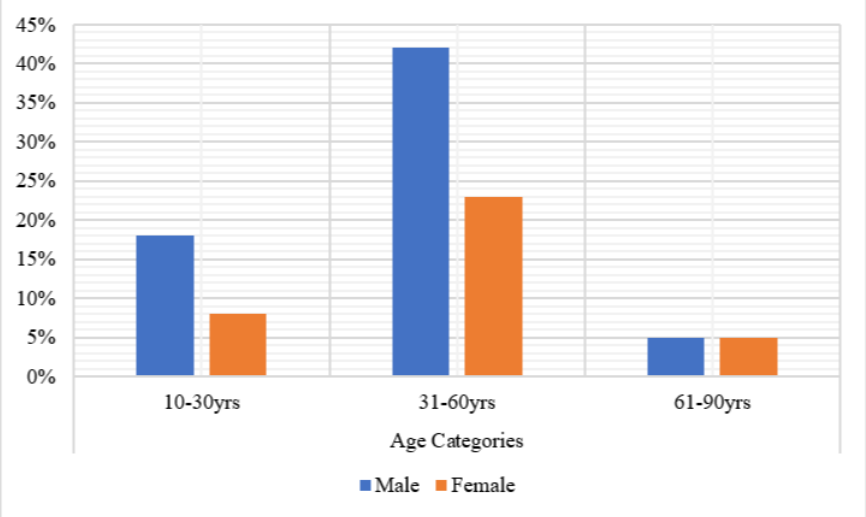

Figure 2. Distribution of participants according to Gender and Age Categories.

2. Antibiotic Resistance

In the current research study, 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 used to check the antibiotic susceptibility patterns of 38 S. aureus isolates, In the current research findings, the highest antibiotic resistance against the S. aureus isolates was exhibited by the Imipenem and Cefepime and the lowest resistance activity was exhibited by Vancomycin and Amikacin the total antibiotic-resistant activity against the S. aureus was revealed by the antibiotic. The results of antimicrobial resistance patterns are of great concern due to these predominant bacterial isolates which are highly resistant to commonly available antimicrobial, the result almost agree with research by [12].

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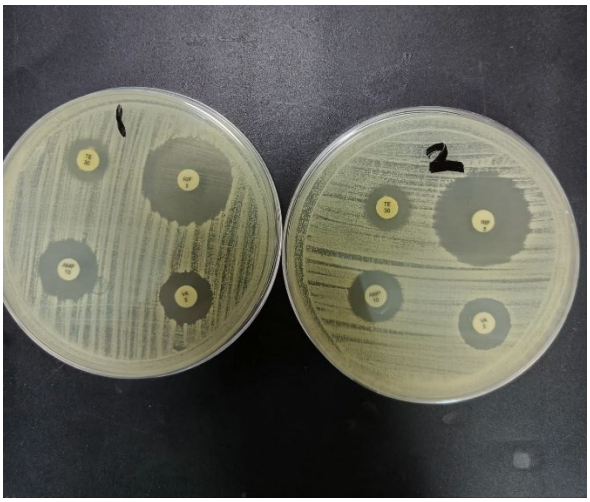
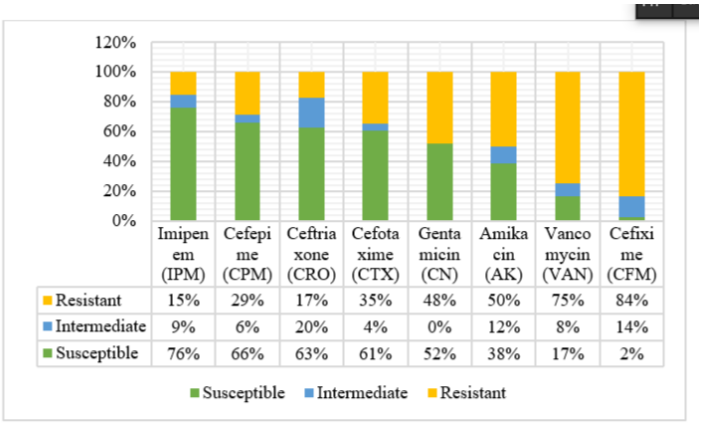


Figure 3. Antibiotic Sensitivity Test on S. aureaus.



	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 current study, the prevalence of nuc gene was 83.3% in the 55 S. aureus isolates, which had a relatively low positive correlation with the burn genes. Similar study were obtained by [13]. Also the study is in agreement with findings of [14] in Iraq who demonstrate that the molecular detection of the selected isolates up to the species level by amplifying the nuc gene, which is specific to (S.aureus) for more accuracy using PCR technique., Karimzadeh and Ghassab in 2022 reported in their article that 100% of S. aureus isolates were positive for nuc gene and disagree with [15] who reported that 34.2% of S. aureus have nuc gene.

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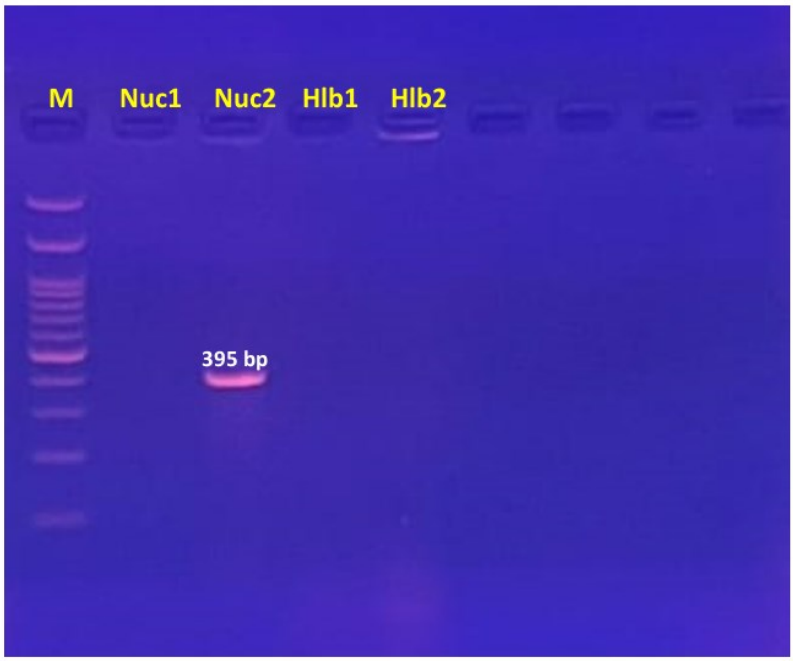

Figure 5. Agarose Gel Electrophoresis of Staphylococcus aureus (1.5% agarose,7v/cm2 for 60 min) for Nuc Gene (395 bp amplicon) represent by Lanes Nuc1,Nuc2, for Hlb Gene (309 bp Amplicon) represent by Lanes Hlb1,Hlb2,lane M represent M100bp DNA Ladder

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, may be more widespread in Iraq and can be expected in any country in the Middle East. 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.

Acknowledgments

The authors would like to thank and appreciate the Medical city in Baghdad.

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