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Hospital-Acquired *Staphylococcus aureus* in Thi-Qar Governorate: A Molecular and Epidemiological Perspective

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Abstract— *Staphylococcus aureus* (*S. aureus*) is a pathogenic strain of bacteria, that resists multiple multi-antibiotics used for common bacterial infections. Most *S. aureus* infections occur among people consulting hospitals or other healthcare settings such as nursing homes and dialysis centers. This study aimed to molecularly identify *S. aureus* strains associated with nosocomial infections in hospitals of Thi-Qar. A total of 100 samples were collected from various locations in three hospitals in Thi-Qar Governorate. Polymerase Chain Reaction (PCR) technique was utilized to assay the presence of *S. aureus* via *16S rRNA* gene. The study population consisted of both male and female patients, with a male predominance (82% males vs. 18% females), showing statistically significant differences ($p \leqslant 0.01$). The highest frequency of isolates (65%) was observed in the 21 – 31 age group, which also demonstrated a significant difference compared to other age groups. The PCR amplification of the *16S rRNA* gene was successfully submitted to GenBank and assigned the accession numbers LC866513, LC866514, and LC866515. Phylogenetic analysis using MEGA-10 software revealed distinct molecular relationships between the local *S. aureus* isolates and genetically related strains from various regions worldwide.

The outcomes of this study provide valuable insights that could inform and enhance infection control practices, thereby contributing to the mitigation of disease transmission within healthcare environments.

***Keywords*— *S. aureus* , Nosocomial infections , *16S rRNA* , PCR.**

I. INTRODUCTION

Staphylococcus aureus (S. aureus) is an opportunistic organism that can infect various parts of our bodies. Numerous virulence factors enable bacteria to infiltrate host tissues and successfully elude the immune system [1]. Methicillin-resistant S. aureus (MRSA) is known to be a significant bacterial pathogen on a global scale. With a marked rise in death and morbidity rates, it poses a threat to world health. Multidrug resistance (MDR) has been recognized as one of the biggest risks to human health and is an issue that is becoming worse globally [2]. In general, MRSA can be classified into two main groups:

Group 1: Hospital-associated Staphylococcus aureus (HA-MRSA).

Group 2: Community-associated Staphylococcus aureus (CA-MRSA).

Globally, the majority of MRSA infections are HA-MRSA acquired from healthcare facilities. Recently, a third group has been added, Livestock-associated MRSA (LA-MRSA). After acquiring genetic adaptations, particularly the staphylococcal cassette chromosome mec (SCCmec) element that provides methicillin resistance, the livestock-associated MRSA clonal complex 398 (LA-MRSA CC398) evolved from methicillin-sensitive Staphylococcus aureus (MSSA) strains originally adapted to humans. Livestock colonization by LA-MRSA is known to occur asymptomatically. In pig farms throughout Europe, the clone is common; up to 50% of the pigs are colonized. To the best of the authors' knowledge, LA-MRSA has never been linked to an outbreak, despite reports of its presence in the UK. Uncertainty surrounds the clinical relevance of LA-MRSA in human colonization and infection [3,4]. Multiple virulence determinants, including a range of enzymes and exotoxins, significantly enhance the pathogenic potential of Staphylococcus aureus. These factors facilitate effective host colonization by promoting immune evasion and enabling adaptation to various tissue types and environmental niches. Key virulence elements include hemolysins, leukocidins, proteases, enterotoxins, exfoliative toxins, and immune-modulatory factors, all of which are critically implicated in the pathogenesis and persistence of infection [5-6]. The expression of these virulence factors is tightly regulated throughout the bacterial growth cycle. A well-established regulatory mechanism responsible for this control is the quorum-sensing system, commonly known as the accessory gene regulator (agr) system [7]. Staphylococcus aureus, a Gram-positive bacterium, remains

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one of the leading causative agents of nosocomial infections (NIs), particularly in intensive care units (ICUs). The increasing prevalence of antibiotic-resistant strains in such settings is largely attributed to the indiscriminate sale, overprescription, and misuse of antimicrobial agents, which collectively accelerate the emergence and dissemination of resistance among bacterial pathogens [8-9]. Nosocomial infections (NIs) are commonly transmitted through several pathways, including direct patient-to-patient contact, interactions between patients and healthcare personnel, exposure to surgical and medical equipment, and contact with contaminated environmental surfaces [10-11]. Globally, there is growing concern regarding the need to evaluate and characterize the diversity, density, and complexity of microbial populations present in hospital settings, particularly in high-risk areas such as operating rooms [12]. In intensive care units (ICUs), patients are especially vulnerable, with approximately 80% of healthcare-associated infections (HAIs) attributed to bacteremia, surgical site infections, pneumonia, and urinary tract infections. Immunocompromised individuals are at heightened risk for HAIs,often involving opportunistic pathogens such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida* species [13]. This study aimed to molecularly identify *S. aureus* strains associated with nosocomial infections in hospitals of Thi-Qar.

II. PATIENTS AND METHODS

Samples Collection: A total of 100 different swab samples were enrolled in the present study. Collection included Iraqi patients from the period between December 2024 to March 2025, in three hospitals in Thi-Qar Governorate, Southern Iraq.

Methods: Initially, Samples were collected from nasal swabs of healthcare patients. Isolation and identification of isolates were possible using microbiological techniques. The isolates were identified by culture, microscopic analysis and biochemical tests such as (oxidase test).

Molecular Detection of *Staphylococcus aureus*: Genomic DNA was isolated from the samples of patients using a DNA Extraction kit (Anatolia/Turkey). Each bacterial isolate was then analyzed for the presence of *16S rRNA* gene by conventional PCR method. The gene amplification process was conducted in a thermocycler (Hamburg, Germany), with PCR cycling conditions meticulously set according to the primer specifications and PCR protocol Table (1). Estimation of the DNA concentration and purity concentration and purity of the DNA were carried out by using Nanodrop (BioNeer /Korea), Lot No. FI28506

TABLE(1) : Primer sequences and PCR conditions of *16S rRNA* gene.

gene		Sequence (5'-3')	Tm (C)	Amplicon	PCR Conditions	Reference
16S rRNA	F	5'-GGTCTTGCTGTCACTTATAGATGG-3'	60	164	94C-5 min; 56 C-30s; 72C- 5 min; 35 cycles	[14]
	R	5'-CGGAAGATTCCCTACTGCTG-3'				

Gel electrophoresis: (1X TBE) for all Fifty *S. aureus* isolates was performed at 80 volts for 30 minutes. PCR products were stained with ethidium bromide and then examined at 280 nm under UV light. gSYNC DNA extraction kit/Geneaid (Lot No.)

Sequencing analysis: The targeted gene (*16S rRNA*) was partially sequenced (n=10) for selected *S. aureus* isolates from nosocomial patients, and the PCR results was then compared to standard strains of S. aureus in the NCBI data.

Ethics permission: Thi-Qar Health Directorate has authorized the study via their agreement coded 838/2024.

III. RESULRS AND DISCUSSION

The results of the current study were classified according to age and sex. The age group of (21-31) years, with highly significant differences, contains most of patients those infected with S. aureus with 65%. On the other hand, the lowest patients were noticed in the age group of (>10 years old) with 4 patients ($p \leq 0.01$) (Table 2). According to sex and as shown in (Table 3), the same significant differences were also recorded in male/female with a ratio of 82/18 ($p \leq 0.01$).

Different strains of MRSA can result in a broad range of clinical symptoms, from minor infections to acute infections that can be fatal. *S. aureus* is the second most prevalent cause of nosocomial bacteremia in hospitals and the most common cause of infections acquired in the community [15]. Important information on the frequency and variance of illnesses at various places can be gleaned from the distribution of bacteria in various sample types.

Additionally, the present investigation documented differences in the prevalence and distribution of *S. aureus* by sample sources (urine, sputum, blood and nasal swabs), with a highest rate (69%) of *S. aureus* was isolated from nasal swabs, indicating their important role in pyogenic soft tissue. High prevalence of *S. aureus* in nasal samples compared to other specimens that isolated from pus swabs have been reported in hospitals of Duhok Province, Iraq [16]. Moreover, patients at ages over 21-31 years showed the highest carriage rate (65%) with a higher prevalence among males. This could be attributed to the aggressive inflammatory reaction of their immune system to microbial agents as compared with females who possess high immunity against microbes, hence male patients had a higher mortality rate [17-18].

TABLE (2): distribution of patients according to age group.

Age group (year)	No.	%
<10	4	4
10-20	10	10
21-31*	65	65
32-43	6	6
>44	15	15
Total	100	100

***Significant differences.**

TABLE(3): Distribution of patients according to sex.

Sex	No.	%
Male*	82	82
Female	18	18
Total	100	100

***Significant differences.**

Gene detection: A total of 50/100 was screened for *16S rRNA* (*S. aureus* specific gene), whom showed a 100% positive amplification for the target gene with a size of the products around 164 bp. (Fig. 1). A similar study was conducted in Baghdad City revealed same result. While some studies have shown similar results. A study was conducted in Baghdad on clinical samples showed a 50% positive result for the *16S rRNA* gene. This was proven using conventional polymerase chain reaction at an annealing temperature of 55°C, which is the specific temperature for *S. aureus*, and which is considered an essential feature for distinguishing it from other types of *Staphylococcus aureus* [19-21].

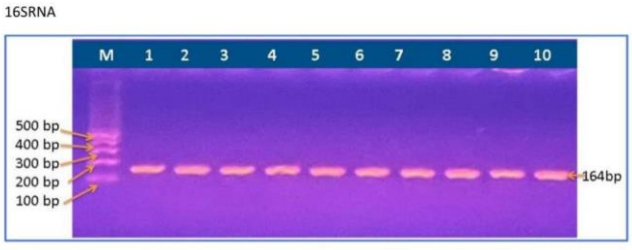

Fig.1: Agarose gel electrophoresis of *16S rRNA* gene. M: (1-1500 bp) ladder; Lanes (1-10) were positive with a product size of approximately 164 bps.

Phylogenetic analysis: The three local *S. aureus* strains for the gene gained the official GenBank accession numbers of LC866513, LC866514 and LC866515. The constructed phylogenetic tree revealed that the local *S. aureus* isolates and similar ones from around the world have distinct molecular relationships. The Phylogenetic tree related to *16S rRNA* gene comprised of two genetic groups;
First Group: Includes *S. aureus* isolates (2,3,4,5,6,9 and 10) local strains which were highly related to each other and constitute a separate cluster.

Second Group: Includes *S. aureus* isolates (1,7 and 8) local strains which were highly related to each other and constitute a separate cluster (Fig.2).

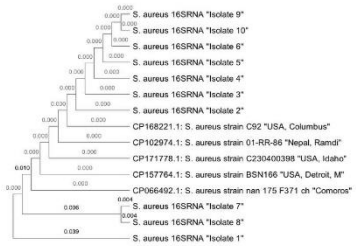

Fig. 2: Neighbor joining phylogenetic tree analysis based on *16S rRNA* gene association of nearby *S. aureus* isolates and related strains from GeneBanK.

IV. CONCLUSIONS

Increasing prevalence of MRSA in nosocomial infection seems to be an important medical phenomenon and prevent, control their prevalence among community and health care facilities around the world need a lot to be done.

AUTHORS CONTRIBUTIONS

All authors had seen and approved the submission of the manuscript with full responsibility, and this research had not been published or under consideration by any other journal.

CONFLICT OF INTEREST

The authors certify that they have no competing interests.

FINANCIAL DISCLOSURE

The authors deny receiving any financial support or grant from any organization.

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