Original Article

Multi-locus Sequence Typing of *Staphylococcus aureus* isolated from different Clinical Isolates

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ABSTRACT

OBJECTIVE: To evaluate the prevalence and genetic diversity of *Staphylococcus aureus* (*S.aureus*) isolates from different clinical samples using Multilocus Sequence Typing (MLST), with special attention to identification of clonal lineages and their associated resistance and virulence genes.

METHODOLOGY: A cross-sectional observational study consisted of 301 clinical specimens, including throat swabs, urine samples, wound swabs, and burn swabs, collected from patients presenting with varying illnesses and attending the Al-Dewaniyah Teaching Hospital, Al-Dewaniyah province, Iraq, from January to March 2025. Isolation of *S. aureus* was performed using Mannitol Salt Agar, and the VITEK 2 system was used for corroborative identification. Three to four isolates underwent MLST. Patients with an age range between 18 and 60 years who presented with various illnesses and signs of pyogenic infections were included. Patients with other infections or chronic diseases, and those under 18 years old, were excluded.

RESULTS: Out of 301 samples, 56 (18.60%) tested positive for *S. aureus*. The highest prevalence was found in burn (21.88%) and throat (21.05%), followed by urine (16.51%) and wound (16.92%). Statistical analysis showed no significant association between sample type and *S. aureus* prevalence (p = 0.82). MLST identified distinct lineages: ST5/CC5 (throat, HA-MRSA), ST8/CC8 (urine, USA300 CA-MRSA), ST30/CC30 (wound, Southwest Pacific clone), and ST45/CC45 (burn, European CA-MRSA). These results demonstrate significant genetic heterogeneity, with both HA- and CA-associated strains present.

CONCLUSION: The study highlights the genetic diversity and presence of *S. aureus*, including high-risk Methicillin-Resistant S. *aureus* (MRSA) clones such as ST5 and ST8, in various clinical specimens.

KEYWORDS: *Staphylococcus aureus,* Genetic diversity, Multi Locus Sequence Typing (MLST), Clonal lineages.

INTRODUCTION

A common and opportunistic pathogenic organism that constitutes a major global healthcare concern is Staphylococcus aureus *(S. aureus)*[1,2]. It is causing a lot of interest because it may quickly develop resistance to a variety of antibacterial medications, making it an important contributory factor of several medical diseases, such as endocarditis, pneumonia, bacterimia, as well as skin and inflammation of the soft tissues[3,4]. The difficulty of treating *staphylococcal* infections has been worsened by the emergence and spread of methicillin-resistant S. aureus (MRSA) in communities and hospital environments[5,6]. Establishing efficient infection prevention plans and directing the appropriate use of treatment measures requires a comprehensive understanding of S. aureus's genetic makeup and geographic distribution of S. Aureus[7,8]. A powerful technique for examining the genetic epidemiology of Staphylococcus aureus is Multilocus sequence typing (MLST), which allows identification of different clonal lineages and the resistant and virulent characteristics associated with S. aureus. MLST relies on the analysis of sequence variations in seven housekeeping genes, providing a standardized and reproducible approach to characterize the genetic relatedness among S. aureus isolates[9]. This technique has been instrumental in elucidating the global distribution and transmission dynamics of various S. aureus clonal complexes (CCs) and sequence types (STs), including pandemic MRSA. A lineage, such as C.C. ,5C.C. ,8and C.C. .30[10]Numerous studies are being conducted on the frequency and dispersion of S. aureus across various healthcare settings, including hospitals, long-term care facilities, and general settings[11]. The simultaneous appearance of hospital-associated M.R.S.A (HA-MRSA) as well as community-associated MRSA. A (CA-MRSA) strain has been shown in several studies, underscoring its pathogenicity and complicating epidemiological data[12]. HA-MRSA lineages, such as ST5 and ST239, are often associated with multidrug resistance and increased virulence, posing significant challenges in healthcare settings. Conversely, CA-MRSA strains, exemplified by ST8 (USA300) and ST30 (Southwest Pacific Clone), have emerged as community-based clones capable of causing severe infections in otherwise healthy individuals[13]. The genetic diversity of *Staphylococcus aureus* has also been linked to its ability to colonize and infect various anatomical sites,

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such as the skin, respiratory tract, and urinary tract[14]. Certain clonal lineages have been observed to predominate in specific clinical settings, suggesting adaptations to diverse host environments and niches[15].

Furthermore, ST. 239 strains were frequently detected among bloodstream and respiratory tract infections, whereas ST. 30 isolates have been linked to infectious diseases of the skin and soft tissues[16]. The rationale of present study due to the emergence of novel and highly transmissible *S aureus* lineages, such as the European CA-MARS clone (ST45), through further highlighted the need for continuous genomics surveillance as well as implementation of effective controlling infection measurement and the safety of humanity is seriously threatened due to such quickly changing strains because they can spread quickly across the population as well as hospitals. The present study is the first local study to use Multilocus Sequence Typing to analyze genetic variation in S aureus clinical isolates from various sources, providing insights into molecular resistance and public health implications.

METHODOLOGY

A cross-sectional observational study was designed, 301 specimens tested for *Staphylococcus aureus* isolation have been obtained from patients who presented varying illnesses and attending the Al-dewaniyah Teaching Hospital, Al-dewaniyah province, Iraq, from January to March 2025, specimens including burn-wound swabs (*n*=32), wound swabs (*n*=65), urine samples (*n*=109), as well as throat swabs (*n*=95), after being aseptically collected. Isolation of *S. aureus* was done using Mannitol Salt Agar, Gram staining, and the VITEK 2 system for corroborative identification. The inclusion criteria involved patients with an age range between 18 and 60 years who presented with various illnesses and signs of pyogenic infections. Exclusion criteria included patients with other infections (not pyogenic infections) and chronic disease who were taking chemotherapy, as well as patients under 18 years old. Every specimen was brought to the microbiological lab for analysis. The inclusion criteria involved patients with an age range between 18 and 60 years who presented with various illnesses and signs of pyogenic infections. Exclusion criteria included patients with other infections (not pyogenic infections) and chronic disease who were taking chemotherapy, in addition to patients under 18 years old.

Bacterial Isolation & Identification:

A selected, as well as differentiated, medium for the isolation of *S. aureus*, Mannitol Salt Agar, has been inoculated with the medical specimens. The dishes that were infected were incubated for 24 to 48 hours at 37°C. The VITEK 2-automate system (bioMérieux -France)

Multilocus Sequence Typing (MLST):

To identify the sequencing of *S. aureus isolates and clonal complexes, MLST was conducted for 4 typical isolates, with 1 isolate per specimen* (burn, wound, urine, and throat swab). This limitation of numbers is due to the difficulty of sending samples abroad and the high cost of analyzing them.

DNA-Extraction:

Utilizing the Presto-Mini-Kit (Geneaid, Taiwan), genomic DNA was extracted from the *S. aureus* isolates in accordance with guidelines provided by the company.

Polymerase chain reaction(PCR) Amplifications & Sequencing:

Utilizing the primer pairs given in **Table I**, the 7 housekeeping genes utilized for MLST (***arc.C, aro. E, glp. F, gm.k, pt. a, tp. i, and yqi.L***) were amplified through PCR. Initial denaturation at 94°C for 5 minutes, 30 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, as well as extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes.

Sequence Analysis and Allele Assignment:

Allele numbers for each of the 7 housekeeping genes were assigned by analyzing and comparing the acquired sequences with the current MLST database (https://publicist.org/s aureus/).

The confirmation genetic identification of MLST type study depends on a phylogenetic-tree analysis of the partial sequencing(Sanger's method) of MLST housekeeping-genes within pathogens *S. aureus*. The evolutionary relationships were calculated using the Maximum Composite Likelihood method in MEGA 6.0 to construct the phylogenetic tree.

Ethical Consideration

The Ethics Studies Commission of Al-Qadisiyah

Table I: Primer-sequences and P.C.R product sizes utilized within MLST of *Staphylococcus aureus*

Gene:	Forward-Primer (5’-3’)	Reverse-Primer (5’-3’)	PCR-Product Size (b.p)
Arc	TTGATTCACCAGCGCGTATTGTC	AGGTATCTGCTTCAATCAGCG	456
AroE	ATCGGAAATCCTATTTCACATTC	GGTGTTGTATTAATAACGATATC	456
glpF	CTAGGAACTGCAATCTTAATCC	TGGTAAAATCGCATGTCCAATTC	465
Gmk	ATCGTTTTATCGGGACCATC	TCATTAACTACAACGTAATCGTA	429
Pta	GTTAAAATCGTATTACCTGAAGG	GACCCTTTTGTTGAAAAGCTTAA	474
Tpi	TCGTTCATTCTGAACGTCGTGAA	TTTGCACCTTCTAACAATTGTAC	402
yqiL	CAGCATACAGGACACCTATTGGC	CGTTGAGGAATCGATACTGGAAC	516

University's Medicine College approved the researchers' methodology. Additionally, everyone who participated, as well as the controllers, verbally gave their understanding.

Statistical Analysis

The Chi-square test was used to assess the relationship between S. aureus frequency and specimen source. S.P.S.S version 26.) 0I.B.M Corp. , Armonk. ,N.Y, U.S.A) was used for the statistical analysis, and a *P* value of less than 0. 05was deemed statistically significant.

RESULTS

Bacterial isolation

Staphylococcus aureus was isolated from 301 clinical specimens using selective media and confirmed by Gram staining and VITEK-2 analysis, including coagulase, catalase, and carbohydrate utilization tests.

Staphylococcus aureus Frequency in Clinical Samples

Staphylococcus aureus was found in 18.60% of clinical specimens (throat, urine, wound, and burn samples). Throat and burn samples had the highest prevalence rates, but no significant association was found between sample source and positivity (**Table II**) and **Figure 2**.

Table II: The Isolation Frequency of *Staphylococcus aureus* from clinical infectious specimens

Source	No. of Tested Samples	No. of Positive Isolates	Prevalence (%)
Throat	95	20	21.05
Urine	109	18	16.51
Wound	65	11	16.92
Burn	32	7	21.88
Total	**301**	**56**	**18.60**

Chi-square test: $\chi^2 = 1.03$, Degrees of freedom df = 3, p-value = 0.82

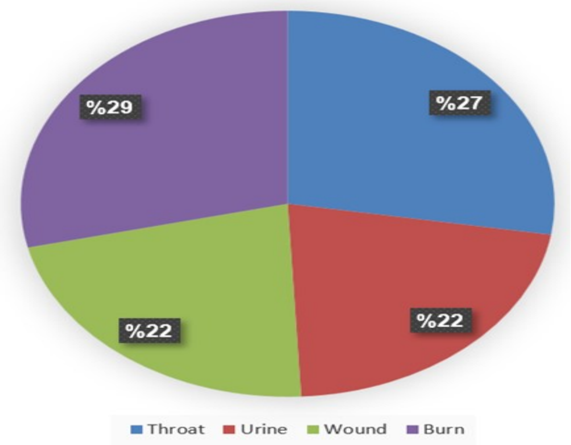

Figure 1: Frequency of *Staphylococcus aureus* from clinical specimens

Multilocus Sequence Typing (MLST)

The MLST analysis of four *Staphylococcus aureus* isolates from different infection sources revealed high genetic diversity, with each strain belonging to a distinct clonal complex and sequence type (**Table II**). MLST analysis identified four distinct MRSA isolates: ST5/CC5 (HA-MRSA), ST8/CC8 (USA300 CA-MRSA), ST30/CC30 (Southwest Pacific clone), and ST45/CC45 (European CA-MRSA). The findings demonstrate the coexistence of HA- and CA-MRSA clones in the clinical environment, with distinct genotypic-phenotypic correlations **Figure 2**.

Table II: Multilocus Sequence Typing of *Staphylococcus aureus* Isolates from Clinical Specimens

Isolate ID	Source	Allelic Profile	ST	CC	Predicted Clonal Lineage
SA-01	Throat swab	3-3-1-1-4-4-3	ST5	CC5	Hospital-associated MRSA
SA-02	Urine	2-2-2-2-3-3-1	ST8	CC8	USA300 (CA-MRSA)
SA-03	Wound	1-4-1-4-3-1-4	ST30	CC30	Southwest Pacific clone
SA-04	Burn	12-12-1-1-4-16-16	ST45	CC45	European CA-MRSA

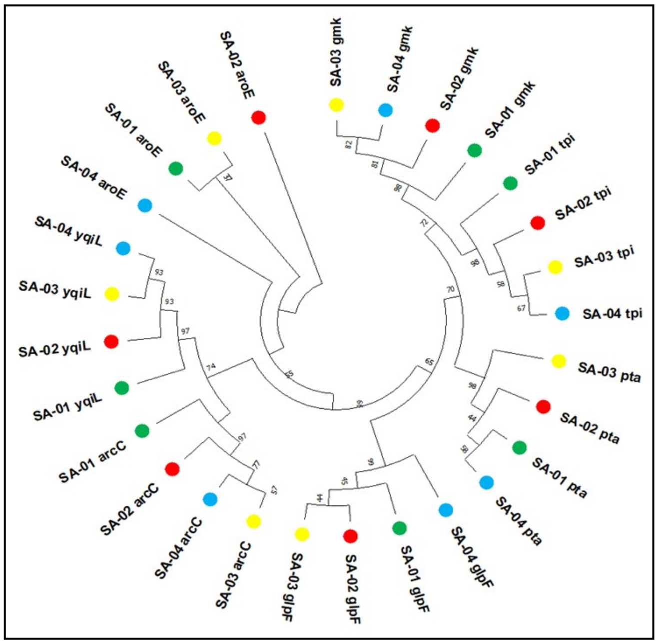

Figure 2: Phylogenetic analysis of partially sequenced MLST housekeeping genes in Staphylococcus aureus isolates revealed genetic differences in some HKG genes, with a total genetic change of 3-0.5%. The local Diarrheagenic. Escherichia isolate showed clear genetic differences in some HKG-genes at total genetic changes (3-0.5%)

DISCUSSION

The results of the current research provide important insights into the molecular mechanisms of resistance in Staphylococcus aureus in a clinical context, emphasizing the genetic diversity and coexistence of strains linked to hospitals and the broader population. The overall prevalence of *S. aureus* across the various sample sources (18.60%) underscores the ubiquitous nature of this opportunistic pathogen and its ability to colonize and infect diverse anatomical sites[15]. The prevalence rates observed in this investigation are consistent with previous reports from other geographical regions, reinforcing the global burden of staphylococcal infections[16]. The statistical analysis revealed no significant association between sample source and *S. aureus* prevalence, suggesting a random distribution of the pathogen across clinical settings. This finding aligns with the observations of, who also reported a lack of significant correlation between infection site and *S.aureus* carriage. The heterogeneous distribution of *S.aureus* across anatomical sites likely reflects the pathogen's remarkable adaptability and its ability to exploit diverse host niches, highlighting the need for comprehensive surveillance and infection control measures targeting all potential reservoirs[17]. The multilocus sequence typing (MLST) analysis of representative S. aureus isolates revealed extensive genetic heterogeneity, with each isolate considered a distinct sequence type (S.T.) and a clonal complex (C.C.). That confirms the result of many previous works focused on the high clonal diversity of *S. aureus*, especially in the context of hospitals[18]. The identification of the ST5/CC5 lineage in the throat swab isolate (SA-01) is striking, as this lineage has been well documented as a highly hospital-associated MRSA (HA-MRSA) strain, with heightened pathogenicity and antimicrobial resistance, and is responsible for many hospital infections[12,14]. The S.T5 clone of *S. aureus* is highly multidrug-resistant, making the management of β-lactam- and macrolide-resistant staphylococcal infections in healthcare settings very difficult. To minimise these infections, strict infection control measures and careful prescribing of antimicrobial agents are imperative [10,12]. The urine isolate (SA-02), classified as ST8/CC8, corresponds to the pandemic USA300 clone, a widely disseminated community-associated MRSA (CA-MRSA) strain known for its enhanced virulence and rapid dissemination[19]**.** The S.T.8 lineage is associated with invasive diseases and serious skin and soft tissue infections in a previously well-defined population, underscoring the need for community awareness and early detection of CA-MRSA infections[20]. The wound isolate (SA-03) belonging to S.T.30/C.C30 is linked to the Southwest Pacific clone, which represents CA-MRSA from many geographical areas, including Asia and Oceania[21]. The S.T30 clone's ability to thrive in many host habitats and adapt to various clinical scenarios, as evidenced by links with skin and soft tissue infections in the current study11,15, also suggests a significant natural affinity of S.T30 for skin and soft tissue infections that was not found in the S.T8 lineages. The burn isolate (SA-04), representing a European CA-MRSA lineage, is ST45/CC45 and was most frequently detected in both community and clinical environments[2,22]. The emergence of this new clone underscores the need for ongoing genomic surveillance, in addition to the practice of effective infection control measures, of curb the spread of highly transmissible strains, as this clone has the potential for rapid spread and adaptation across diverse clinical niches[23]. The co-occurrence of HA-MRSA and CA-MRSA, as noted in this study, underscores the evolving complexity of S. aureus epidemiology and represents a major obstacle to effective control and management of staphylococcal infections. The prevalence of high-risk clones such as ST5 and ST8 strongly underscores the necessity of these approaches. It suggests that infection control measures could be more effective if they are designed and implemented to control the spread of these resistant strains, not only in healthcare settings but also in the community[10].

Further, the results of the current investigation also demonstrate the versatility of S. aureus microbiology across different clinical environments, as distinct clonal lineages were isolated from different specimen sources [14]. This is because certain *S. aureus* genotypes might be more proficient or adapted to colonize and infect certain anatomical regions because of site-specific adaptations or expressions of specialized virulence factors. The molecular mechanisms underlying the seemingly reciprocal associations of *S. aureus* genotypes and their infection potential in diverse clinical scenarios remain unexplored, which is a gap that warrants attention. Comprehensive genomic surveillance is necessary due to the high prevalence of *S. aureus* across all sample sources, as well as the predominance of resistant clones ST5 and ST8, highlighting the need for comprehensive genomic surveillance to guide the development of effective infection control strategies and inform antimicrobial stewardship programs [24,25]. The integration of MLST and other molecular typing techniques into routine diagnostic and epidemiological investigations can provide critical insights into the evolution and transmission dynamics of *S. aureus*, ultimately contributing to the advancement of public health measures[26].

CONCLUSION

The investigation reveals the preliminary descriptive genetic diversity of Staphylococcus aureus clonal lineages in clinical isolates, underscoring the need for genomic surveillance and infection control measures. The findings contribute to the development of more potent therapies against *S. aureus* infections.

Ethical permission: University of Al-Qadisiyah, College of Medicine, Iraq, ERC approval letter No. 62/213.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Abbas FH: Contributed to the literature search, study design, and concept

Aubaid AH: Contributed with questionnaire design, data collection, data analysis, data interpretation, and drafting.

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