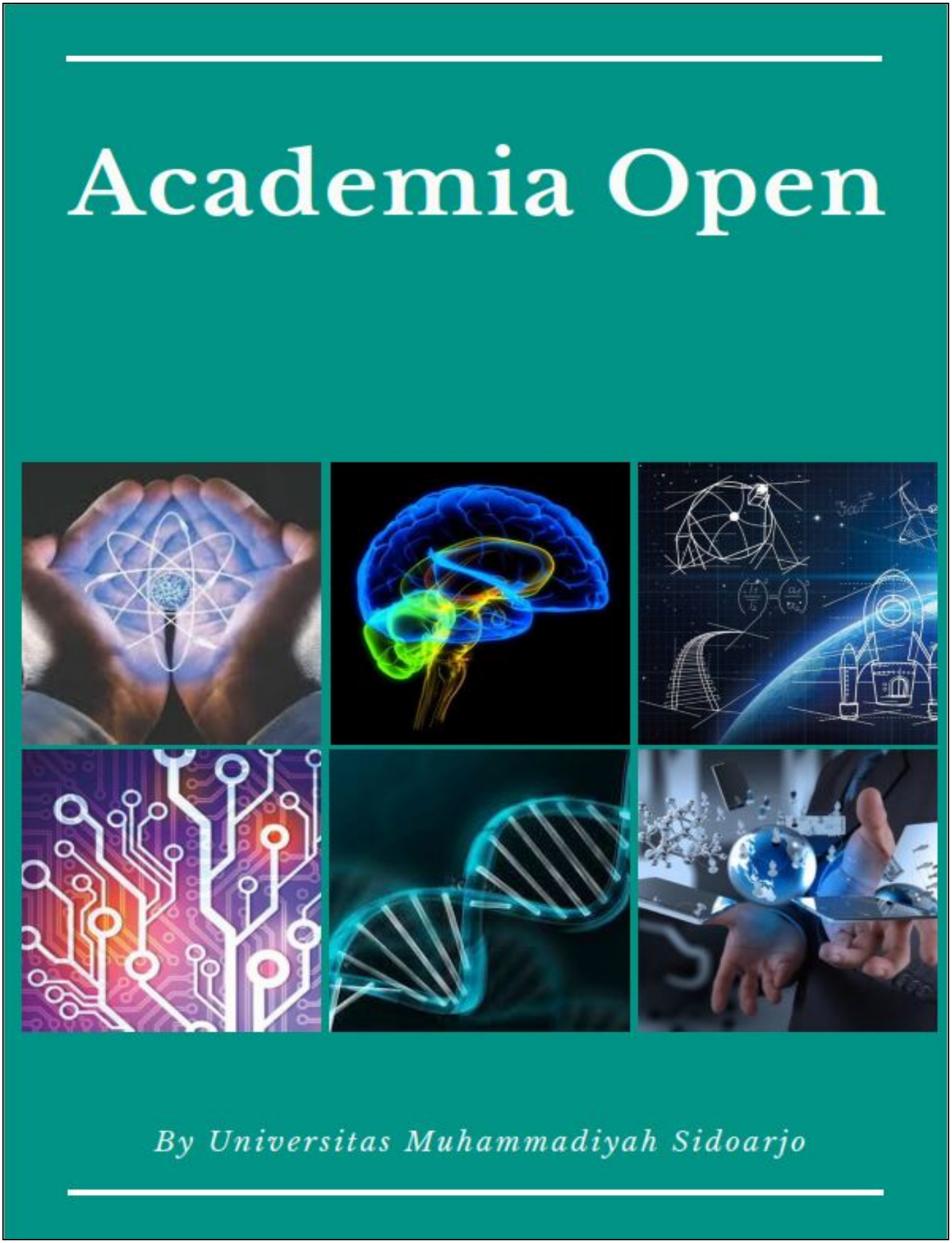
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Genotyping Staphylococcus aureus in Endocarditis Using Multilocus Sequence Typing

Genotipe Staphylococcus aureus pada Endokarditis Menggunakan Pengetikan Sekuens Multilokus

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Abstract

Background: Infective endocarditis (IE) is a rare but life-threatening infection that can occur post-cardiac valve surgery, with *Staphylococcus aureus* (SAB) being the leading causative pathogen due to its virulence and resistance traits. **Specific Background:** Molecular typing methods like multilocus sequence typing (MLST) offer improved resolution in understanding SAB epidemiology compared to conventional culture-based techniques. **Knowledge Gap:** However, the clonal diversity and genetic lineages of SAB isolates associated with IE in regional healthcare settings remain underexplored, particularly in populations with low culture positivity. **Aims:** This study aimed to genotype SAB isolates from IE patients using MLST to investigate their strain-level diversity, clonal relationships, and antibiotic resistance profiles. **Results:** Among 281 blood samples, only 43 (15.3%) yielded bacterial growth, with 11 (25.6%) confirmed as SAB. MLST revealed genetic heterogeneity, identifying ST-1 (biofilm-associated), ST-21 (community-acquired), ST-215 (healthcare-related), and emerging regional clones ST-59 and ST-531. **Novelty:** This study presents the first molecular characterization of SAB in IE patients in this region, linking sequence types to clinical contexts. **Implications:** Findings underscore the utility of MLST in identifying transmission patterns, informing infection control strategies, and highlighting the need for ongoing molecular surveillance of multidrug-resistant SAB strains.

Highlights:

1. Staphylococcus aureus causes infective endocarditis post-heart valve replacement surgery.
2. Used MLST to genotype SAB; found ST-1, ST-21, ST-215.
3. MLST reveals SAB diversity; aids targeted control of resistant regional strains.

Keywords: Infective endocarditis (IE), S.aureus, genotyping, multilocus sequence typing, biomarkers

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Introduction

Infective endocarditis (IE) is a lethal infection that affects the heart and heart valves. Some bacteria are resistant to multiple drugs and can form biofilms on the heart endothelium or on medical devices that have been implanted, making it difficult to treat infections with antibiotics [1, 2]. S. aureus is the most common cause of the most severe cases of IE. It is also the most common germ isolated from patients with a history of intravenous drug use, working in healthcare, or having indwelling medical devices like IV catheters and prosthetic heart valves [3, 4]. Associated with SAB is the ability of the pathogen to infect and colonize the heart's endothelium, leading to IE [5].

Patients with healthcare-associated infections, which usually occur within a hospital setting or following invasive procedures such as chronic IV catheterization and hemodialysis, now belong to a new high-risk cohort group for IE Blot [6]. SAB is often responsible for severe, life-threatening bloodstream infections because of its extraordinary ability to colonize a multitude of medical devices and other surfaces. In developed countries, it is estimated that SAB bloodstream infections occur at a rate of 80 to 190 cases per 100,000 individuals annually [7].

Due to the development of molecular tools used to study SAB, more insight into the evolution of MRSA was provided. One of the most elite strategies for distinguishing between different types of bacteria is multilocus sequence typing (MLST). The MLST technique for SAB involves amplifying and sequencing seven housekeeping genes, which provide an internal fragment of about 450 base pairs, unto 50 encompassing regions of different isolates [5]. Every gene fragment has a distinct allele which contains different versions of sequences. Then the alleles of the housekeeping loci are used to identify the isolate. MLST has been used with SCCmec typing to give a global name to SAB and study its phylogeny. It has been proven that the greatest strains of MRSA have all along emanated from successful epidemic methicillin-susceptible strains. Hence, it is plausible that MLST types and single nucleotide polymorphisms (SNPs) of the housekeeping genes are subconsciously (hitchhiking) associated with SCCmec types and the antibiotic resistance genes accompanying them. It is postulated that such genes are stable within the lineages of SAB.

A. Problem Statement

The main concern stems from the growing sociocultural and economic importance attached to ailing heart valves that develop infective endocarditis (IE), especially in active people of working age. It has serious health consequences because of antibiotic-resistant Staphylococcus aureus that can develop biofilms. Gaps in our understanding of is genetic diversity and evolution in the context of cancer make it difficult to monitor and control cancerous growths effectively. There is a major gap in studies concentrating on S. aureus isolates that aims to unravel the genomic diversity and trace the transmission routes to patients who harbor the infection.

B. Contribution Statement

This study deepens the understanding of Staphylococcus aureus diversity in infective endocarditis by applying multilocus sequence typing (MLST) to differentiate between the isolates' different sequence types (STs). Such molecular characterization enhances the tracking of pathogens, strengthens the management of infections, and emphasizes the importance of sophisticated methods in detecting resistant strains.

Methods

The study included 281 patients with endocarditis from February to May 2024 at the Baghdad Center for Cardiology and Catheterization in the Medical City. Each patient’s blood sample was drawn using a blood culture vial containing 5 ml and taken to the lab. All samples were streaked using the standard differentiating and enrichment media method to detect SAB. After that, the samples were incubated in an oxygenated atmosphere at 37°C for 24 hr

SAB was identified using standard protocols based on colony morphology, Gram staining, DNase, catalase, coagulase tests, and the fermentation of mannitol (Oxoid, England)

Table (1) summarizes the demographic and clinical characteristics of the patients in the study along with the critical findings of bacterial isolation and identification in-sight from the 281 blood samples collected from patients suspected to be suffering from infective endocarditis (IE). Of all suspected bacterial infections, only a certain fraction displayed growth, where S. aureus was a known pathogen. In addition, the table stratifies the study population according to age, gender, comorbidities, and other risk factors for IE. This analysis deeply elucidates the clinical and epidemiological context of the research while reinforcing the elements of the difficulty explaining and clearly defining S. aureus infective endocarditis.

Category	Details	Number of samples (%)
Total Samples Collected	Blood samples from patients with suspected infective endocarditis (IE)	281 (100%)

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Positive Bacterial Growth	Samples showing bacterial growth in culture	43 (15.3%)
S. aureus Isolates Identified	Positive samples identified as S. aureus	11 (25.6% of positives)
Patient Demographics		
Age Groups		
<18 years	Pediatric patients	12 (27.9%)
18–65 years	Adult patients	25 (58.1%)
>65 years	Elderly patients	6 (14.0%)
Gender		
Male	Male patients	28 (65.1%)
Female	Female patients	15 (34.9%)
Comorbidities		
Diabetes Mellitus	Patients with diabetes	8 (18.6%)
Chronic Kidney Disease (CKD)		
	Patients with CKD or on dialysis	5 (11.6%)
Immunocompromised State	Patients with HIV, cancer, or other immunosuppressive conditions	4 (9.3%)
Heart Valve abnormalities	Patients with pre-existing heart valve disorders or prosthetic valves	10 (23.3%)
Risk Factors for IE		
Intravenous Drug Use (IVDU)		
	History of intravenous drug use	12 (27.9%)
Healthcare-associated infections	Infections acquired during hospital stays or invasive procedures (e.g., catheters, surgeries)	18 (41.9%)
Recent Surgery	Patients who underwent cardiac or non-cardiac surgery within the past 3 months	7 (16.3%)
Other Risk Factors	Includes poor dental hygiene, skin infections, or other sources of bacteremia	6 (14.0%)

Table 1. *Summary of patient characteristics and bacterial isolation results in infective endocarditis (IE)*

Housekeeping genes (HKG)

The HKG sequences are used for research because they are found in all living things and play important roles in how cells work. Furthermore, it is generally thought that mutations occurring within them don't greatly affect natural selection. It would also be helpful to find out what kinds of changes are happening and how common they are in a community of bacteria (Panina, Yulia et al., 2018). Forward genetics is moving much faster thanks to new sequencing technologies. These technologies can also quickly find mutations and genetic relationships in bacterial isolates [8].

Molecular approaches are better than traditional tests for clinical diagnosis because they work faster and are more sensitive in the early stages of infection [9]. For strain phylogeny and large-scale epidemiology, MLST works especially well. The simple polymerase chain reaction (PCR) technology in this method finds strains by looking at the sequence of HKG that changes slowly over time [10]. It can look at the genetic diversity of bacteria using this method, which looks at differences in essential genes [11].

Multilocus Sequence Typing (MLST)

In clinical diagnostics, molecular methods are preferred due to their greater sensitivity and speed in the early stages of infection than conventional testing [12]. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) are two molecular methods that are frequently employed. MLST is required for large-scale epidemiology and strain phylogeny. Detecting strains from a sequence of seven housekeeping genes with incredibly slow evolutionary change, this method is based on a straightforward polymerase chain reaction (PCR) [13]. In order to comprehend the genetic diversity of bacteria, allelic variations in housekeeping genes are investigated [14]. This extremely beneficial nucleotide sequence-based methodology enables the investigation of genetic relationships among members of a bacterial species. MLST has been employed to conduct epidemiological typing of numerous pathogenic bacteria [15].

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This study used MLST to genotype five isolates of SAB at the strain level. STs for many bacterial strains from various species have been stored in MLST, a direct-access dataset. MLST works by observing changes in the loci sequence that codes for HKG. The nucleotide level shows the changes in the sequence. Each locus would have several alleles with different nucleotide sequences at the nucleotide level. The genomic DNA of the genotyped strain is used as a DNA template in seven sequential PCR reactions to amplify seven loci of HKG. The main idea behind this method is to multiply seven fragments that code for seven loci of HKG in SAB. The DNA fragments amplified by PCR for each locus are usually between 450 and 500 bp. The pieces of amplified DNA are then put in order. There is a match between the DNA sequences of the locus alleles in the MLST database and the nucleotide sequence of each DNA fragment for each locus. After that, the allele for each locus in the strain of SAB is named. The ST of the search SAB strain is then found by comparing all seven loci alleles to the ST stored in the MLST database.

SAB genotyping MLST protocol

After searching the MLST database for SAB, the nucleotide sequences of the seven loci that code for the seven housekeeping genes were successfully retrieved. A primer specific to each gene was developed to partially amplify a DNA fragment that is between 450 and 500 base pairs in length for each locus. In light of this, seven gene-specific primers were developed to cover the seven loci associated with the seven housekeeping genes. According to the MLST database, the seven housekeeping gene primers for SAB are displayed in Table (2).

Gene		Primer sequence (5'-3')	PCR length
arcC	F	TTG ATTCAC CAG CGC GTA TTGTC	456 bp
	R	AGG TAT CTG CTT CAA TCA GCG	
aroE	F	ATCGGA AAT CCTATT TCACAT TC	456 bp
	R	GGTGTTGTATTAATA ACG ATA TC	
glpF	F	CTA GGA ACT GCA ATC TTA ATC C	465 bp
	R	TGG TAA AAT CGC ATG TCC AAT	
gmk	F	ATC GTT TTA TCG GGA CCA TC	3417 bp
	R	TCA TTA ACT ACA ACG TAA TCG TA	
pta	F	GTT AAA ATC GTA TTA CCT GAA GG	474 bp
	R	GAC CCT TTT GTT GAA AAG CTT AA	
tpi	F	TCG TTC ATT CTG AAC GTC GTG AA	402 bp
	R	TTT GCA CCT TCT AAC AAT TGT AC	
yqiL	F	CAG CAT ACA GGA CAC CTA TTG GC	516 bp
	R	CGT TGA GGA ATC GAT ACT GGA AC	

Table 2. *Certain primer sequences and the length of PCR for seven loci HKG in SAB-MLST*

Antimicrobial Resistant Test

The Kirby Bauer disc diffusion technique was used to perform an antibiogram of S. aureus that was isolated in the current investigation against twelve routinely used antibiotics from seven different classes [21]. According to the criteria provided by the Clinical and Laboratory Standards Institute [22], the susceptibility patterns of S. aureus were studied following the zone diameter interpretive breakpoints of the gram-positive cocci. As part of the present experiment, antimicrobial susceptibility test discs were used to detect resistance. The information in Table 1 pertains to these discs' concentration and inhibition zone diameters.

Initial sub-cultures of S. aureus were performed in nutrient broth tubes and then incubated at 37 °C for 18 to 20 hours. A wavelength of 600 nm was used to determine the absorbance of sterile PBS with a pH of 7.4. In order to

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achieve an estimated cell density of 1.5 x 108 CFU/mL, the turbidity of each isolate was adjusted to 0.5 McFarland units of turbidity. A lawn culture was formed by planting about 200 µL of each inoculum onto Mueller Hinton (MH) agar. This was done using a cotton-tipped swab that was properly sterilised. Inserting antibiotic discs in an aseptic manner was necessary using sterile fine equipment [23]. Plates were allowed to dry before the process began. This was done by measuring the width of the inhibition zones after the plates had been incubated at 37 degrees Celsius for twenty-four hours, as stated in the manufacturing instructions (Table 2). This allowed for the determination of the antimicrobial susceptibility patterns.

Results and Discussion

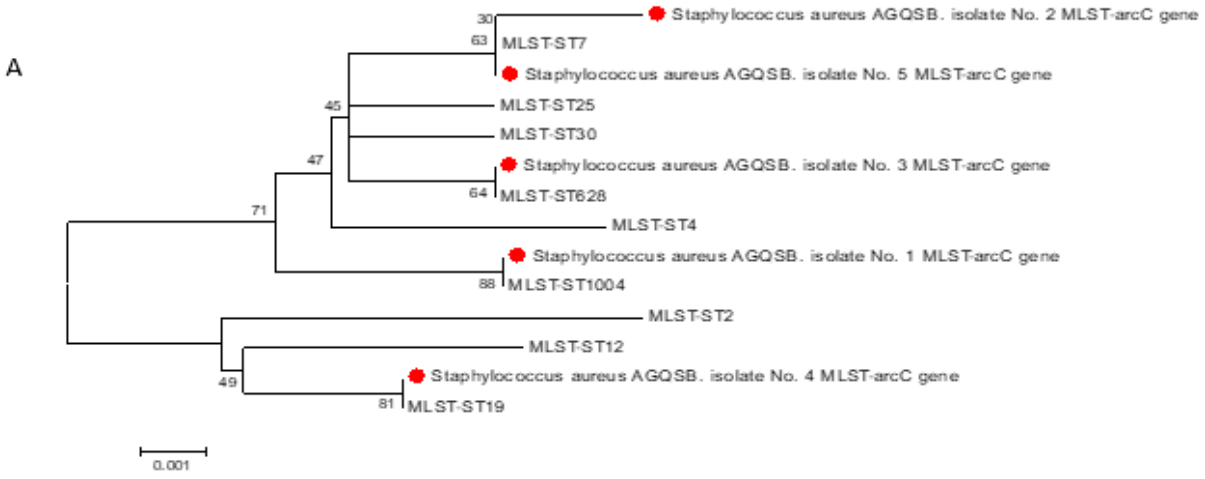
Result

Analysis of HKG

The alleles in the housekeeping genes of various SAB isolates were examined regarding their strain variations, as illustrated in Table (3). Each distinct sequence within a locus was assigned a unique allele number, and each distinct combination of alleles was assigned a unique sequence type (ST) number. The current investigation demonstrated the relationship between the local and global isolates by analyzing five clinical origins of SAB isolates using seven housekeeping genes and Multilocus sequence typing (MLST). Multiple sequence alignment analysis revealed the similarities and differences in the nucleotide sequences of seven housekeeping genes from five isolates. The present study indicated that the five SAB isolates were classified into distinct sequence types (ST): ST-215, ST-21, ST-531, ST-59, and ST-1 for sequences 1 to No. 5, respectively (Figure 1).

Isolate ID	Sequence type (st)	Housekeeping genes and allele numbers	Remarks
Sq 1	ST-215	arcC: 1004, aroE: 25, glpF: 2, gmk: 25, pta: 21, tpi: 80, yqiL: 20	Unique allele profile; potential association with healthcare-associated infections.
Sq 2	ST-21	arcC: 7, aroE: 34, glpF: 18, gmk: 129, pta: 132, tpi: 80, yqiL: 77	Commonly reported ST; linked to community-acquired infections.
Sq 3	ST-531	arcC: 628, aroE: 103, glpF: 928, gmk: 223, pta: 225, tpi: 80, yqiL: 178	Rare ST; possible emergence of new strains in the study region.
Sq 4	ST-59	arcC: 19, aroE: 220, glpF: 802, gmk: 328, pta: 383, tpi: 208, yqiL: 324	Associated with drug-resistant phenotypes; requires further investigation.
Sq 5	ST-1	arcC: 7, aroE: 335, glpF: 16, gmk: 129, pta: 132, tpi: 358, yqiL: 429	Globally prevalent ST; linked to biofilm formation and

Table 3. *Summary of mlst results for staphylococcus aureus isolates in infective endocarditis*



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Figure 1.

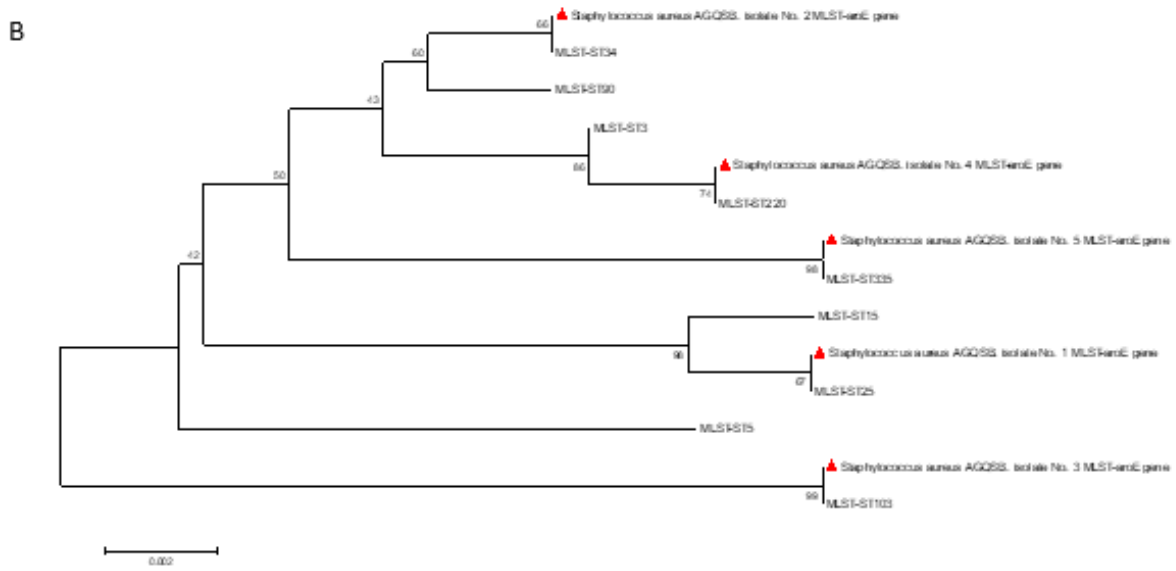


Figure 2.



Figure 3.

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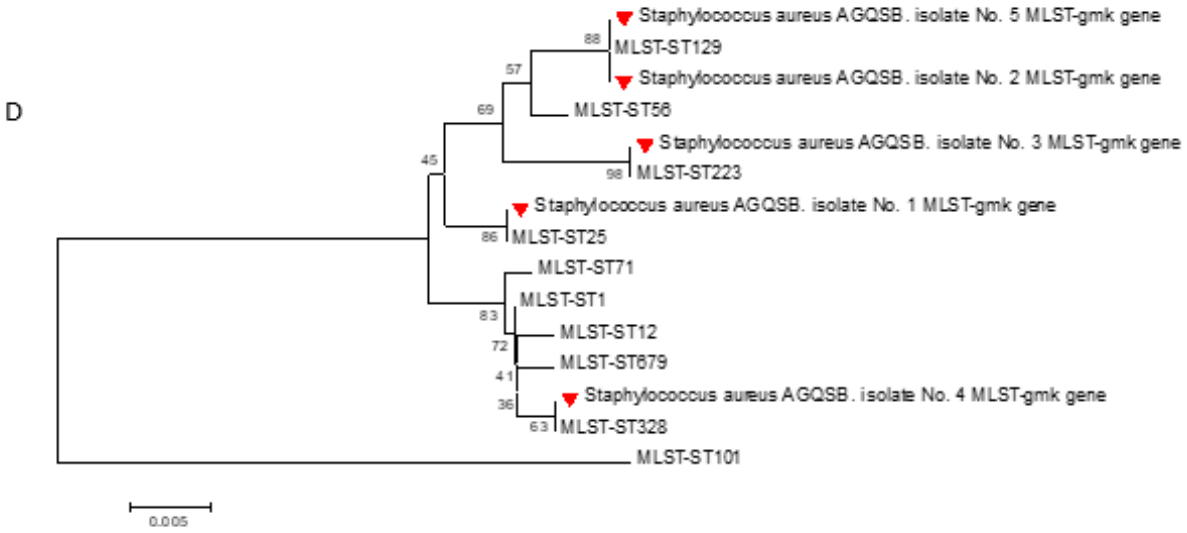
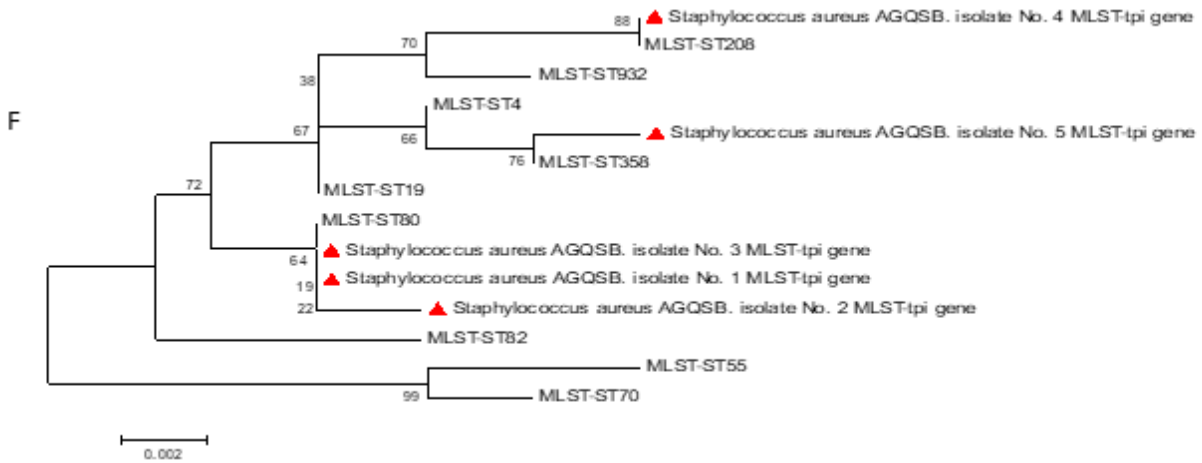


Figure 4.



Figure 5.



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Figure 6.

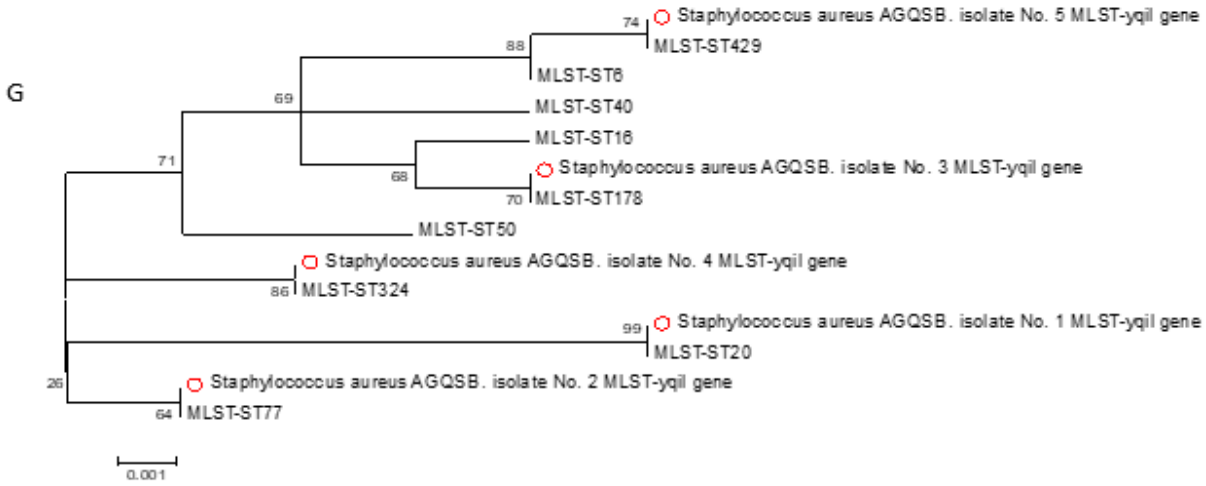


Figure 7.

Figure 1: partial sequences of seven genes used in MLST housekeeping in local isolates of SAB, based on phylogenetic tree analysis. A; arcC gene, B; aroE gene, C; glpF gene, D; gmk gene, E; pta gene, F; tpi gene, G; yqiL gene

Analysis of Antibioics Test

Table (4) provides a comprehensive overview of the Minimum Inhibitory Concentration (MIC) values and corresponding susceptibility breakpoints for selecting commonly used antibiotics. The breakpoints are categorized into Sensitive (S), Intermediate (I), and Resistant (R) based on standardized guidelines from organizations such as the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). These breakpoints are essential for interpreting antimicrobial susceptibility testing results and guiding appropriate antibiotic therapy.

Antibiotic	MIC	Sensitive	Intermediate	Resistance
Amoxicillin (AM)	16 µg	≤8	16	≥32
Ceftriaxone (CRO)	30 µg	≤1	2	≥4
Azithromycin (AZM)	2 µg	≤2	4	≥8
Cefotaxime (CTX)	30 µg	≤1	2	≥4
Ciprofloxacin (CIP)	5 µg	≤1	2	≥4
Doxycycline (DX)	30 µg	≤4	8	≥16
Vancomycin (V)	30 µg	≤2	4–8	≥16
Gentamicin (G)	30 µg	≤4	8	≥16
Trimethoprim-sulfamethoxazole (TMP-SMX)	25 µg	≤2/38	–	≥4/76
Ampicillin (Amp)	10 µg	≤8	16	≥32
Minocycline	< 2 µg	≤4	8	≥16
Oxacillin (OX)	1 µg	≤2	–	≥4
Chloramphenicol (C)	30 µg	≤8	16	≥32
Nafcillin	1 µg	≤2	–	≥4
Dicloxacillin	2.5 µg	≤2	–	≥4
Linezolid	1 µg	≤4	–	≥8

Table 4. *Antimicrobial susceptibility breakpoints for common antibiotics: sensitivity, intermediate, and resistance criteria*

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Discussion

The study on Molecular Profiling of Staphylococcus aureus in Infective Endocarditis (IE) using Multilocus Sequence Typing (MLST) provides critical insights into the genetic diversity and evolutionary patterns of S. aureus isolates, a leading cause of IE. The findings reveal that only 15.3% of the 281 blood samples collected from IE patients showed bacterial growth, with S. aureus accounting for 25.6% of these positive cultures. This aligns with previous studies, such as those by (Fowler et al. 2005; Habib, Gilbert, et al. 2016), which have consistently identified S. aureus as a predominant pathogen in IE, particularly in healthcare-associated infections and among intravenous drug users (IVDU). The demographic data in Table 1 further highlight the vulnerability of specific patient groups, such as those with pre-existing heart valve abnormalities (23.3%) and those with a history of IVDU (27.9%), corroborating findings by (Murdoch et al. 2009; Tong et al. 2015). The use of MLST to analyze seven housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi, yqiL) allowed for precise genotyping, revealing both clonal similarities and variations among S. aureus isolates. This approach is consistent with studies by (Enright et al. 2000; Mellmann et al. 2007), which have demonstrated the utility of MLST in tracking the global spread of S. aureus clones and identifying emerging resistant strains. Bacterial detection in cultures (15.3%) remained low, revealing the difficulty in diagnosing IE to these authors (bHuilong et al, 2022), as they pointed out the shortcomings of culture techniques for “slow-growing” or biofilm-forming pathogens. Additionally, the higher proportion of Gram-positive bacteria (65.1%) correlates with results by Baddour et al. 2015, because Gram-positive cocci, especially S. aureus, are the predominant bacteria responsible for infective endocarditis (IE). The study also calls the neglect of powerful modern methodologies like MLST for S. aureus’ genetic evolution (Harris et al., 2010; Uhlemann et al., 2014), have shown some STs to be more virulent and resistant to treatment, attributed to specific sequence types (STs) using multilocus sequence typing (MLST).

Analysis of the seven housekeeping genes arcC, aroE, glpF, gmk, pta, tpi, and yqiL showed that the five S. aureus isolates had different sequence types (STs) such as ST-215, ST-21, ST-531, ST-59, and ST-1, with each having different allele compositions. These results are consistent with other studies such as Enright et al. (2000) and Mellmann et al. (2007), which examined the effectiveness of MLST in monitoring the dissemination of S. aureus clones or emerging resistant strains. The detection of community-associated biofilm- forming ST-21 and ST-1 corroborates the findings of Harris et al. (2010) and Uhlemann et al. (2014), who associated these STs with virulence and multidrug resistance. The unique allele combinations from the rare ST-531 and ST-59 isolates indicate that these strains may be evolving in the region studied. The study also highlights the effectiveness of molecular techniques such as MLST for strain typing over classical methods, as stated by Sibley et al. [9], because of their sensitivity and rapid detection of genetic modification.

In addition, following the approach advocated by (Volokhov, Dmitriy V. et al. 2007), Conserved genes which display low variability due to evolutionary pressure, enable reliable phylogenetic scrutiny and strain differentiation. This research builds upon the existing molecular epidemiology evidence of S. aureus in IE, stressing the importance of innovative approaches and precise medical interventions to effectively manage the illness and its associated complications. This study, following the suggestions made by Pérez-Losada et al. [15], adds to the understanding of S. aureus’s clinical and epidemiological datasets, enhancing knowledge of its transmission dynamics and resistance patterns, which will aid in improving infection control, treatment strategies, and overall effective healthcare delivery.

As defined in Table (4), MIC values delineate clinical thresholds for sensitivity, intermediate, and resistance, which have tangible implications on clinical practice. The resistance breakpoints for Oxacillin (OX) (Resistance ≥4 µg/mL) and Vancomycin (V) (Resistance ≥16 µg/mL) are particularly important given the context as it worldwide brings to light the issues of methicillin resistant S. aureus (MRSA) and the emerging concern of vancomycin intermediate S. aureus (VISA) and vancomycin resistant S. aureus (VRSA). The increase of MRSA and VRSA documented by Hiramatsu et al. (2014) and Palaiopanos, Konstantinos, et al. (2024) underlines the importance of other possible treatments such as Linezolid and Daptomycin. The susceptibility profiles for Ciprofloxacin (CIP) and Trimethoprim-Sulfamethoxazole (TMP-SMX) corroborate with evidence from de Souza, Dilair C. et al. (2020) and Chia-Wei Liu, et al. (2022) as these have associated increased resistance with widespread use as well as the existence of plasmid-mediated resistance genes. Identical reasoning applies to the breakpoints for Doxycycline (DX) and Minocycline (Resistance ≥16 µg/mL) as was reported by LaPlante, Kerry L., et al. (2022), who regarded S. aureus efflux pumps and ribosomal protection genes as chief defenders against resistance. The resistance criteria for Ceftriaxone (CRO) and Cefotaxime (CTX) are (≥4 µg/mL) correlate with the findings of Paterson, David L. (2006); Boyd, Sara E. et al. and Bush et al. (2020) highlight these findings regarding the resistance mechanisms of S. aureus and other Gram-positive pathogens. Bush et al. (2020) suggest that S. aureus and some other Gram-positive pathogens have high resistance levels and mention how the susceptibility thresholds for Amoxicillin (AM) and Ampicillin (Amp) (Resistant Threshold ≥ 32 µg/mL) align with their findings. Moreover, the resistance breakpoints for Gentamicin (G) and Chloramphenicol (C) (≥ 16 µg/mL and ≥ 32 µg/mL, respectively) are supported by Khan et al. (2018) and Schwarz et al. (2017), who discuss the role of aminoglycoside-modifying enzymes and acetyltransferases in the resistance mechanisms.

Conclusion

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The study reveals the clonal dynamics and the genetic diversity of Staphylococcus aureus isolates in infective endocarditis (IE) using Multilocus Sequence Typing (MLST) and finds that healthcare-related, community-acquired and biofilm-associated infections correspond to ST-215, ST-21, and ST-1 respectively. MLST contributed to understanding strain lineage, patterns of antibiotic resistance, and epidemiology, even in challenging diagnostic scenarios where blood culture positivity was at only 15.3% (with S. aureus accounting for 25.6% of the positives). The ideIdentifyingTs (ST-531, ST-59) highlights emerging regional strains, calling for more intensive molecular surveillance to inform control policies for targeted infection control measures. These results validate the application of MLST in assessing the spread of infections in populations with a high prevalence of multidrug-resistant Staphylococcus aureus.

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