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Molecular Typing of Clinical, Colonizing and Environmental *Staphylococcus aureus* Isolates Using Multilocus Sequence Typing (MLST): A Global Comparative Analysis

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Abstract

Introduction: *Staphylococcus aureus* (*S. aureus*) is a facultative anaerobic bacterium that colonizes the nasal cavity, skin and other mucosal surfaces of humans. It causes a wide spectrum of infections, ranging from localized skin and soft tissue infections to severe systemic conditions such as bacteremia, endocarditis and osteomyelitis. The pathogen is a major contributor to hospital-acquired infections and the emergence of Methicillin-Resistant *S. aureus* (MRSA) poses significant therapeutic and epidemiological challenges.

Methods: This retrospective analytical study analysed 100 archived *S. aureus* isolates from clinical, colonizing and environmental sources in a hospital setting.

Multilocus Sequence Typing (MLST) was performed on 55 of these isolates. Fragments of seven housekeeping genes were amplified following the protocol outlined on the PUBMLST website. The resulting amplicons were sequenced and Sequence Types (STs) were assigned by comparing allele profiles with the PUBMLST database. Novel allelic combinations were designated as new STs.

Results: The identified STs included ST22, ST5, ST364, ST1910, ST5939, ST5529, ST7442, ST672 and ST9162. Forty-four novel STs were submitted to the PUBMLST site and were designated new STs. Most of these isolates belonged to Clonal Complex 5 (CC5). A comparative analysis was conducted against Indian and global *S. aureus* isolates available in the PUBMLST database to assess phylogenetic relationships and geographic distribution.

Conclusion: Understanding the molecular epidemiology and evolutionary dynamics of *S. aureus* through MLST provides valuable insights into strain diversity and transmission patterns. These findings underscore the importance of robust infection-control and targeted surveillance strategies to mitigate the spread of MRSA in healthcare settings.

Keywords: *Staphylococcus aureus*; MRSA; MLST; Burst Analysis; Clonal Complex

Introduction

Staphylococcus aureus (*S. aureus*) is a colonizer of the nasal cavity, skin and mucosal surfaces, with 20-30% of healthy individuals being persistent carriers [1,2]. While colonization is often asymptomatic, carriers act as reservoirs and infections can occur when bacteria enter through breaks in the skin or mucous membranes. *S. aureus* causes a wide spectrum of diseases, including skin and soft tissue infections, bacteremia, endocarditis and osteomyelitis and remains a major contributor to hospital-acquired infections [3].

The emergence of Methicillin-resistant *S. aureus* (MRSA) in hospitals poses challenges for treatment [4]. Hospital-associated MRSA (HA-MRSA) strains are linked to severe infections in healthcare settings, while community-associated MRSA (CA-MRSA) strains circulate outside hospitals, often differing genetically and clinically from HA-MRSA. Understanding genetic and phenotypic differences between these strains is essential for focused treatment and preventative plans [5].

Nasal carriage of *S. aureus* occurs in approximately 20-30% of healthy individuals, who are considered persistent carriers [6]. Colonizers serve as reservoirs for the bacterium, potentially leading to infection. Several factors, including host genetics, immune response and environmental conditions, influence the colonization [7]. Colonizing strains may be methicillin-sensitive or resistant, influencing transmission dynamics in both communities and hospitals. Molecular characterization of colonizing strains helps identify potential reservoirs and supports the development of effective decolonization strategies to prevent infections [8]. Host genetics, immune responses and environmental factors all shape colonization patterns.

Environmental reservoirs further complicate control. *S. aureus* can persist on hospital surfaces, medical equipment and the hands of healthcare workers, facilitating nosocomial spread. In community settings, gyms, schools and public transport serve as sources of transmission [9]. The ability of *S. aureus* to persist in the environment presents a significant public health challenge, underscoring the importance of sustained surveillance and preventive measures [10].

Molecular characterization and typing of *S. aureus* isolates are essential for epidemiology, outbreak investigation and infection control. Typing methods reveal resistance profiles, genetic diversity and clonal relationships [11]. It enables outbreak investigations by linking isolates from patients, environments and carriers, thereby supporting targeted interventions. PCR-based methods, such as spa typing and Multi-Locus Sequence Typing (MLST), aid in tracking the clonal spread of bacteria with similar genetic makeups [12,13]. The role of mobile genetic elements in MRSA evolution is particularly significant. These facilitate horizontal gene transfer, allowing rapid acquisition of resistance traits. For example, Rolo, et al., investigated a hospital outbreak in Portugal using MLST and found that a single MRSA clone, enriched with mobile genetic elements carrying resistance genes, was responsible. This highlighted the adaptability of *S. aureus* and the importance of molecular epidemiology in outbreak monitoring [14].

By comparing isolates from hospitals, communities and environmental sources, researchers can trace transmission pathways and identify reservoirs. Such insights guide decolonization strategies, shape infection control policies and support the design of preventive measures. Ultimately, integrating molecular typing with surveillance programs strengthens our ability to contain MRSA spread and mitigate its public health impact. Hence, in the present study, *S. aureus* strains of different origins were typed using MLST and compared with the world isolates.

Methodology

This retrospective analytical study utilized *Staphylococcus aureus* isolates stored in the departmental repository at the Vallabhbhai Patel Chest Institute, University of Delhi. Isolates were obtained from diverse sources: Clinical samples (sputum, bronchial aspirate, blood, endotracheal tips, Foley's catheter tips, pleural fluid) from patients in OPD, Emergency, HDU and ICU wards. Those isolates obtained from clinical samples showing significant counts only were included to rule out colonizers.

Surveillance swabs from healthcare workers and patients (nose and hand). Isolates from nasal swabs and hand swabs from patients and health care workers were considered colonizers irrespective of the infection/colonization status of the patient Environmental surfaces (bed railings, switchboards, side tables, stethoscopes) within Vishwanathan Chest Hospital, which caters to patients from across northern India.

A total of 100 *S. aureus* isolates representing clinical, colonizing and environmental categories were included. This study was approved by the Institutional Human Ethics Committee (Ref: VPCI/DIR/IHEC/2023/249) and adhered to the ICMR National Ethical Guidelines and the Declaration of Helsinki. Data confidentiality was strictly maintained and no conflicts of interest were declared.

All isolates were preserved in 16% glycerol broth at −80°C. They were revived and reconfirmed using conventional biochemical tests and MALDI-TOF mass spectrometry before molecular characterization.

DNA Isolation
Genomic DNA was extracted using HIMedia's Genomic DNA Purification Kit (Cat. No. MB505) following the manufacturer's protocol. Briefly, 1.5 ml of the overnight broth culture was centrifuged at 13,000 rpm for 2 minutes. The pellet was resuspended in 200 µl lysozyme solution and incubated at 37°C for 30 minutes. Proteinase K (20 µl) and RNase A (20 µl) were added, followed by 200 µl lysis solution, vortexing and incubation at 55°C for 10 minutes. Ethanol (200 µl, 95–100%) was added and the lysate transferred to a HiElute Miniprep Spin Column. After sequential washes, DNA was eluted with 200 µl elution buffer and stored at −20°C.

Multilocus Sequence Typing (MLST)
Fragments of seven housekeeping genes (arcC, aroE, glpF, gmk, pta, tpi, yqiL) were amplified using primers and protocols from the PUBMLST database (https://pubmlst.org/organisms/staphylococcus-aureus). PCR reactions (50 µl) contained 1U Taq polymerase, 1× PCR buffer with $MgCl_2$, 40 pM primers, 0.2 mM dNTPs and 2 µl DNA template. Amplified fragments were sequenced by Bio Serve Biotechnologies (India) Pvt Ltd, Hyderabad. Allele sequences were uploaded to the *S. aureus* MLST database (https://pubmlst.org/organisms/*staphylococcus-aureus*) to determine allele types and Sequence Types (STs). Clonal Complexes (CCs) were assigned using eBURST analysis (http://pubmlst.org/analysis) with a definition of 6 out of 7 matching alleles and classified into Single-Locus Variants (SLVs) and Double-Locus Variants (DLVs). Isolates were compared with global entries by country and source type to assess genetic relatedness and epidemiological patterns.

Results
A total of 100 *Staphylococcus aureus* isolates from the departmental repository were revived and analyzed. These comprised 50 clinical isolates from patients, 40 colonizing isolates from patients and healthcare workers and 10 environmental isolates from hospital beds, bed railings and side tables. All isolates were confirmed as *S. aureus* using MALDI-TOF MS. Fifty isolates were from male patients, 40 from female patients and 10 from hospital environmental surfaces. Forty-six isolates were Methicillin-resistant and 54 were methicillin-sensitive. The details are provided in Table 1,2.

Multilocus Sequence Typing (MLST)
Study Isolates
MLST was performed on 55 isolates from diverse sources: sputum (24), bronchoalveolar lavage (1), hand (8), nose (10), pleural fluid (1), environmental medical surfaces (9), urine (1) and blood (1). Sequence types (STs) identified included ST22 (4), ST5 (1), ST364 (1), ST1910 (1), ST5939 (1), ST5529 (1), ST7442 (1), ST672 (1) and ST9162 (1) (Table 3). Importantly, 44 novel STs were submitted to the PUBMLST database, underscoring regional diversity and the emergence of new clones.

ST22 and ST5 were exclusively identified among clinical isolates. Among 40 colonizing isolates from asymptomatic carriers (23 nasal, 17 hand swabs), MLST was performed on 18, yielding ST364, ST1910 and ST7442, with the remainder assigned to novel STs. Most isolates are clustered within clonal complexes CC5 and CC22 (Table 2, Fig. 1). BURST analysis categorized the dataset into four groups and 24 singletons. Group 1 was the largest, comprising 18 isolates across 16 STs, all affiliated with CC5. Group 2 included 9 isolates representing 6 STs, of which 4 were ST22, corresponding to CC22 (Fig. S1).

Indian Isolates
From the PUBMLST database (accessed 21 February 2025), 286 Indian isolates were retrieved, of which 177 were from humans. These originated from diverse clinical conditions: pharyngitis (33), wound infections (25), invasive infections (33), skin infections (13), bacteremia (6), pneumonia (5), among others. Twenty isolates were from asymptomatic carriers, including 18 from the present study. Of the 177 human isolates, 91 were MRSA.

BURST analysis of the 177 human *S. aureus* isolates revealed 12 clonal groups and 33 singletons (Fig. S2). Group V was the largest, comprising 45 isolates across 20 STs, with ST22 as the primary founder (9 ST22 isolates, 13 SLVs, 4 DLVs). Group 1 was the second largest, with 39 isolates across 10 STs, led by ST772 (28 ST772 isolates, 6 SLVs, 2 DLVs). Group 3 comprised 16 isolates and 13 STs, with ST239 as the founder.

ST772 was the most frequent (28 isolates), first reported in India (2003) from pus and later detected in Bangladesh, the Netherlands and Norway. Despite its prevalence, ST772 was absent in the present study. Other notable STs included ST2371 (n=13, CC2), associated with recurrent furunculosis in Mysuru; ST22 (n=9), reported from MRSA blood isolates in Lucknow (2019); ST672 (n=12), first seen in 2003, with one isolate identified in the present study; ST1037 (n=7, CC22), reported from nasal carriers in Algeria and later eye infections in India; ST1713 (n=4), observed in UK and Indian pharyngitis cases; and ST30 (n=3), linked to invasive infections and abscesses.

Among 14 carrier isolates, 12 originated from the present study. The remaining two were ST1298 (2007) and ST772 (2009), both from nasal swabs (Fig. 2).

World Isolates

As of 20 February 2025, the PUBMLST database contained 43,233 global *Staphylococcus aureus* isolates, of which 11,326 were from humans. Most originated from Europe (4,811), followed by Asia (2,455), North America (1,266), Africa (1,095) and South America (769). Disease status was unspecified in 44.3%. Among isolates with clinical context, carriers were most common (1,734; 15.3%), followed by invasive infections (1,387; 12.2%), bacteremia (1,160; 10.2%), skin infections (420; 3.7%) and wound infections (394; 3.5%). Carrier samples were primarily nasal (1,193), with additional sources including skin (74), feces (26) and throat (18).

Grape Tree analysis revealed ST5 as the predominant global type (620 isolates), followed by ST8 (423), ST30 (313), ST22 (274), ST15 (205) and ST45 (201) (Fig. 3).

Common Global STs (Fig. 3)

ST5: Most prevalent globally (618 isolates), initially identified from carriers (1997) and later from blood samples. These were methicillin-sensitive and widely distributed across regions. Only two ST5 isolates from India are recorded in the database—one from sputum (present study) and one from pus, both of which were MRSA. Several additional sequence types from the present study, including ST364, ST1910, ST9412, ST5939, ST9416 and ST9417, also belonged to clonal complex 5.

ST8: Second most common (423), first identified in 1997 from a CA-MSSA blood infection in Oxford, found in both carriers and patients.

ST30: Third most common (313), reported in England (1997) and later in Canada and other countries (2002–2014); only three Indian isolates.

ST22 (CC22): Fourth most common (266), first detected in Ireland (1993), later in blood isolates (1997).

ST15: Fifth most common (205), first isolated in Oxford (1997) as MSSA, associated with invasive infections and bacteremia.

STs from Carrier Isolates

Among 1,734 global carrier isolates, the largest numbers were reported from the UK (275), Germany (265), USA (141), Switzerland (137), China (121) and India (20). Europe contributed the majority (893), followed by Africa (287), Asia (283), North America (146) and South America (101).

ST22 (n=99) was the most prevalent among carriers, followed by ST30 (92), ST45 (79), ST5 (68) and ST15 (58). Overall, 89% were MSSA, including 55% of Indian carrier isolates.

In the present study, among 40 carrier isolates, 15 were MRSA and 25 MSSA. MLST was performed on 18, revealing 15 novel STs. Three were SLVs of ST5 (ST9412, ST1910, ST364), one was an SLV of ST22 (ST7442) and one was a DLV of ST22 (ST9413). Although ST5 and ST22 were not directly identified among carrier isolates, most belonged to their clonal variants.

Methicillin resistance	Clinical (n=50)	Colonizer (n=40)	Environmental (n=10)	Total (n=100)
MRSA	29	15	2	46
MSSA	21	25	8	54

Table 1: Methicillin resistance in Clinical, colonizing and Environmental isolates of *S. aureus*.

Sl.No	Lab ID	ST Type	Source Category	Methicillin R/S	Details (First isolation, Year, Country, etc)
1	609/23	22 (4)	Clinical	MRSA	Ireland, 1993, CC 22, MRSA
2	3210/23				
3	1475/22				
4	4849/22				
5	496/23	5			England, 1997, carrier and Invasive, MSSA, CC5
6	2212/22	9411*			SLV of ST6014, 2020, UK
7	2877/22	9445*			New
8	2211/22	9418*			SLV of ST4313, England, 2017
9	980/22	9440*			New
10	3317/23	9441*			New
11	3397/22	9442*			SLV of 9162, CC2
12	4626/22	9451*			From ST12
13	3120/23	9462*			New
14	384/23	9464*			CC22, DLV of 2101, Spain, 2009
15	2236/21	9469*			CC22, DLV of ST636, single isolate from Algeria, 2004, wound swab
16	392/23	9466*			CC5, DLV of ST462,
17	2186/22	9416*		MSSA	CC5, SLV of 5529
18	3580/21	672			India 2003, 5 isolates, MRSA, Later isolates from Iran, Egypt, Haiti and Australia
19	3735/21	8914*			CC5 descendant of ST5
20	1910/22	9444*			CC5, SLV of ST5529, China, Human carrier
21	3657/22	9458*			New
22	2351/21	9459*			New
23	2260/21	9460*			From ST 361
24	1050/21	9461*			New
25	4593/22	9450*			CC5, DLV of ST6743, China, Human carrier, 2017
26	4861/23	9452*			CC5, SLV of ST5529, China, Carrier, Human faeces
27	2302/22	9470*			DLV of ST5638
28	2196/21	9468*			DLV of ST7077, Single isolate from Jordan, 2019, Environment
29	C8/23	9414*	Colonizer	MRSA	New
30	1014/23	9415*			New
31	C21/23	7442			Single isolate, Jordan 2018 carrier CC22
32	1116/24	9438*			CC5, DLV of ST5638, Ethiopia, 2018, Human
33	C26/23	9447*			New
34	1216/14	9456*			New
35	2918/23	9467*			DLV of ST9444 (All study isolates
36	3414/23	364	Colonizer	MSSA	CC5, two isolates, one each from Japan, type unknown and the present study
37	3950/23	1910			CC5, Human lung aspirate, Poland, 2005, MSSA
38	C3/23	9412*			CC5, SLV of ST5
39	C16/24	9413*			CC22, DLV of ST2892, UK, Blood, 2002
40	3289/23	9453*			CC5, SLV of 5529
41	C6/23	9444*			CC5, SLV of ST5529, China, Human carrier
42	C12/24	9446*			CC5, SLV of ST4184, China, Food
43	C4/23	9457*			SLV of ST672,

Sl.No	Lab ID	ST Type	Source Category	Methicillin R/S	Details (First isolation, Year, Country, etc)
44	1037/24	9449*			Originated from ST672
45	3487/23	9463*			From ST7371, Spain, 2015, Carrier isolate
46	4129/23	9465*			CC5, SLV of ST9454
47	En8/23	9439*	Env	MRSA	New
48	En25/23	9162			Single isolate, Netherlands, 2024 CC22
49	En3/23	5939		MSSA	CC5, single isolate, UK, 2019
50	En1/23	9417*			CC5, DLV of ST4005, UK, 2016 Human
51	En7/23	9443*			CC5, DLV of ST950, China, 2006
52	En2/23	9454*			CC5, Origin from ST 860, Norway, human carrier, 2005
53	En20/23	9455*			CC5, DLV of ST9454
54	En4/23	9444*			CC5, SLV of ST5529, China, Human carrier
55	En6/23	9448*			DLV of 6262, China, one carrier isolated from an animal.
Env- Environmental, MRSA- Methicillin-resistant Staph. aureus, MSSA- Methicillin-sensitive Staph. aureus, *-New STs in the study, SLV- Single locus variant, DLV-Double locus variants, CC- Clonal complex					

Table 2: Sequence types of *S. aureus* isolates used in this study (n = 55).

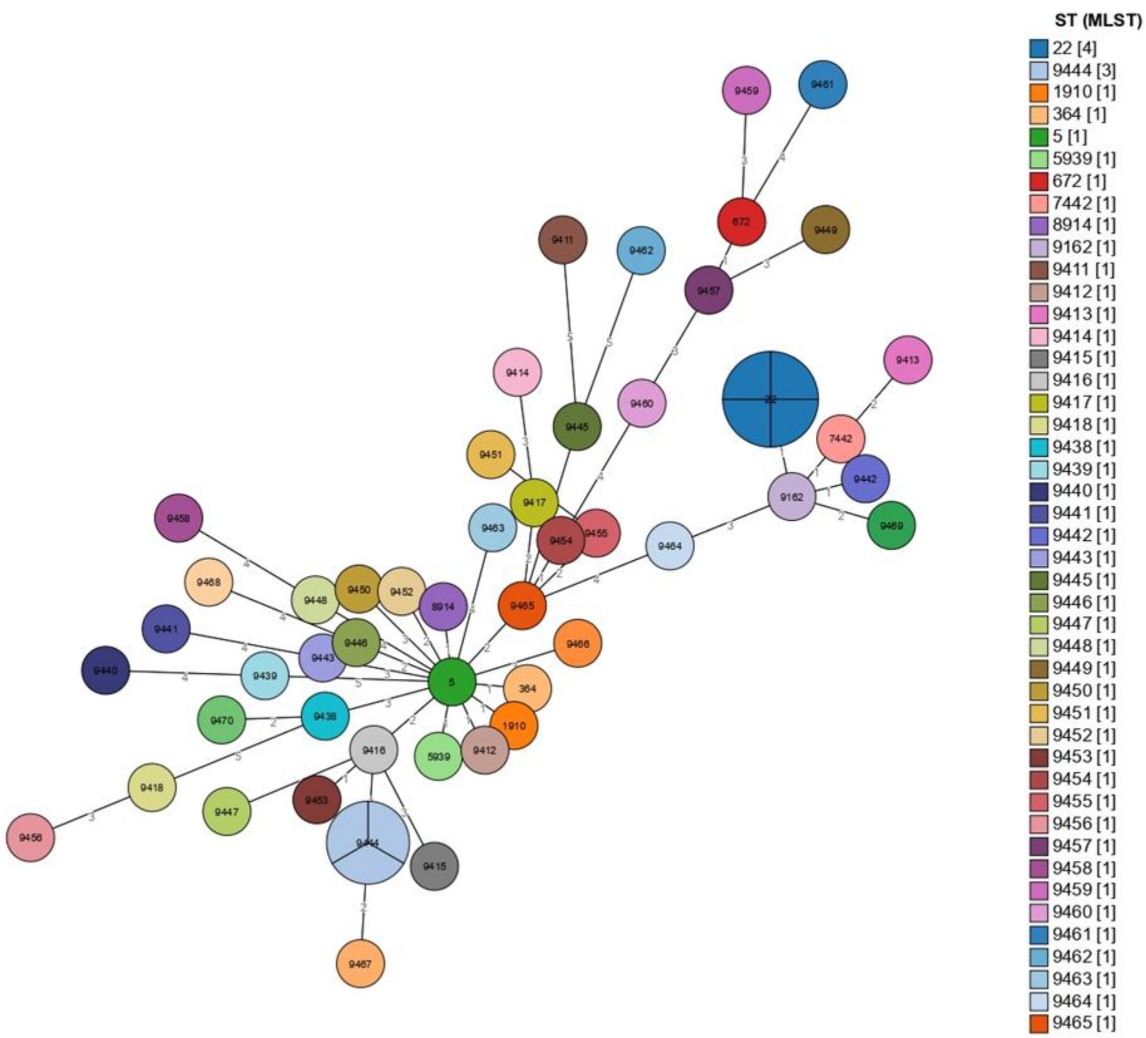

Figure 1: Minimal Spanning Tree (MS Tree) of MLST data of Study *S. aureus* isolates (STs are arranged in order of Prevalence).

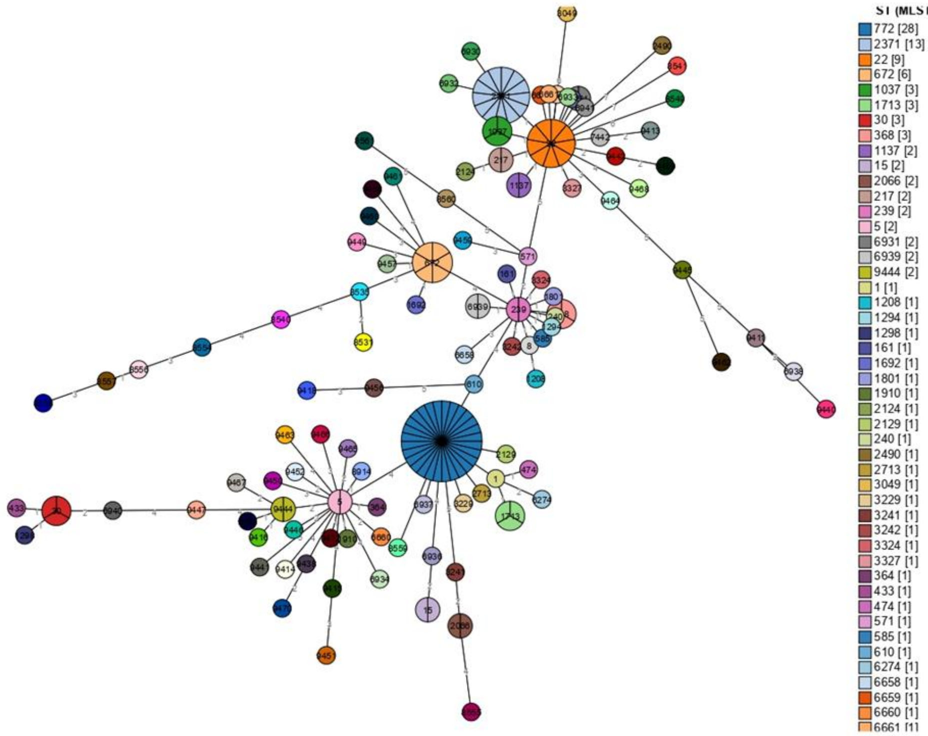

Figure 2: Minimal Spanning Tree (MS Tree) of MLST data on Indian isolates of *S. aureus* showing the major clones and sequence types (STs are arranged in order of Prevalence).

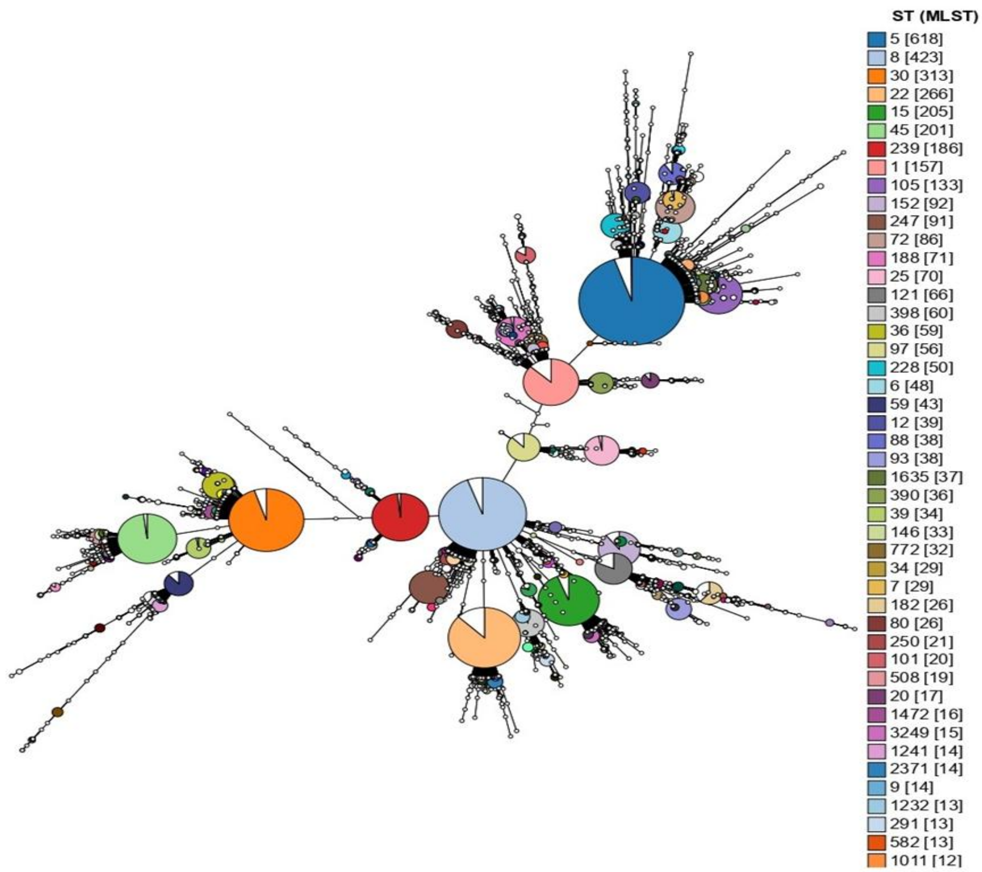

Figure 3: Minimal Spanning Tree (MS Tree) of MLST data showing population snapshot of the world *S. aureus* isolates showing the major clones and sequence types (STs are arranged in order of Prevalence.).

Discussion

Multilocus Sequence Typing (MLST) is a highly discriminatory and widely adopted method for molecular characterization of *S. aureus*, particularly MRSA. It provides detailed insights into genetic relatedness, clonal diversity and evolutionary relationships, facilitating global surveillance of MRSA epidemiology and resistance trends [15]. In contrast to PFGE, MLST provides standardized and reproducible results across laboratories, thereby enabling reliable inter-study comparisons. Globally, ST5 (CC5) is the most prevalent lineage, associated with hospital-acquired MRSA infections in the Western Hemisphere [16]. It has diversified into four distinct clades: Basal (comprising ST5, ST100, ST1125, ST1184, ST1186 and ST2625), CC5-I, CC5-II and CC5-IIb. Asian ST5 isolates, studied by Chen, et al., were grouped into five clades with partial overlap with Western clades, but showed higher carriage of enterotoxin genes (tst, sec, sel) [17]. These genes were often located on blaZ plasmids [18]. The ST5 New York/Japan clone represents a globally distributed lineage, with genetic similarities between American clade I and Asian clade III, while Asian clade II diverged significantly. ST5-MSSA from China, genetically distinct from its MRSA counterpart, was classified under clade IVA, which is globally distributed. Clade IV strains exhibited higher Extracellular Polymeric Substance (EPS) production and gene content than clades II and III. Genomic analyses suggest that Mobile Genetic Elements (MGEs) contributed to clade diversification and the separation of virulence and resistance genes may have delayed the emergence of fully virulent, resistant strains [17].

Several isolates in our study belonged to CC5, including novel STs with varied resistance profiles and sources, indicating ongoing clonal evolution. However, CC5 was less prevalent among other Indian isolates.

ST22 (CC22) has emerged as a globally dominant MRSA clone, replacing earlier lineages across Europe, Asia, Africa, Oceania and the Middle East [19-21]. It is associated with severe infections due to virulence factors such as PVL (lukS/F) and TSST-1. ST22 strains are grouped into clades I–III [22,23], with subclade IIc recently introduced into China. Notably, the ST22-SCCmec IVa clone harboring both pvl and tst genes exhibited enhanced virulence *in-vitro* and *in-vivo*. Similar hypervirulent ST22 variants have been reported in Japan, including the Fukuoka clones I–III, which evolved through sequential acquisition and chromosomal rearrangements [24]. In our study, four ST22 MRSA isolates were identified from clinical samples. Two harbored PVL and Hla and all carried SPA and FnbA (data not shown). While SCCmec typing was not performed in this study, the presence of virulence genes suggests these may represent hypervirulent ST22 strains. ST22 MRSA IV clones have previously been reported in Bengaluru from both hospital and community settings [25]. Most of our isolates represented novel Sequence Types (STs), which limited direct comparisons with existing national or global datasets. These findings highlight the genetic diversity and evolving nature of *S. aureus* in India, particularly within CC5 and CC22 lineages. Ongoing molecular surveillance and genomic analysis are essential for monitoring emerging clones, assessing their virulence potential and informing effective infection control strategies.

Methicillin resistance was detected across all sample categories, with the highest prevalence observed among clinical isolates. This trend may be attributed to the fact that most clinical samples were from admitted patients, where resistant strains circulate due to selective pressure from antibiotic use and close contact within healthcare environments. Healthcare workers may also acquire and transmit these strains.

Limitations of the Study

This was a retrospective study utilizing isolates from our repository. Consequently, comprehensive clinical information, including patient details, treatment history and outcome data, was not available.

Conclusion

This study highlights the genetic diversity of *S. aureus* in India, with novel STs contributing to clonal evolution. Routine MLST is essential for tracking local and global epidemiology. Strengthening infection control and regulating antibiotic use are critical to curbing MRSA transmission. Future studies incorporating whole-genome sequencing and SCCmec typing could further elucidate the evolutionary dynamics of emerging clones.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Data Availability Statement
The study data are available from the authors upon request. Allele types have been deposited in the *Staphylococcus aureus* MLST sequence database (https://pubmlst.org/organisms/*staphylococcus-aureus*) for sequence type (ST) determination. Comparative MLST data, including global and Indian datasets, are accessible through the MLST website (https://pubmlst.org/organisms/staphylococcus-aureus).

Ethical Statement
The Institutional Human Ethics Committee of Vallabhbhai Patel Chest Institute, University of Delhi, Delhi (Ref: VPCI/DIR/IHEC/2023/ 249) approved the project. The study adhered to the Indian Council of Medical Research's National Ethical Guidelines for Biomedical and Health Research Involving Human Participants, in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. Data confidentiality has been strictly maintained and the authors declare no conflicts of interest.

Informed Consent Statement
Informed consent was obtained from all participants included in the study.

Authors' Contributions
M.S. Conceptualization, global data review, Final manuscript writing
A.N. Experiments, data tabulation, draft writing

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Supplementary Files

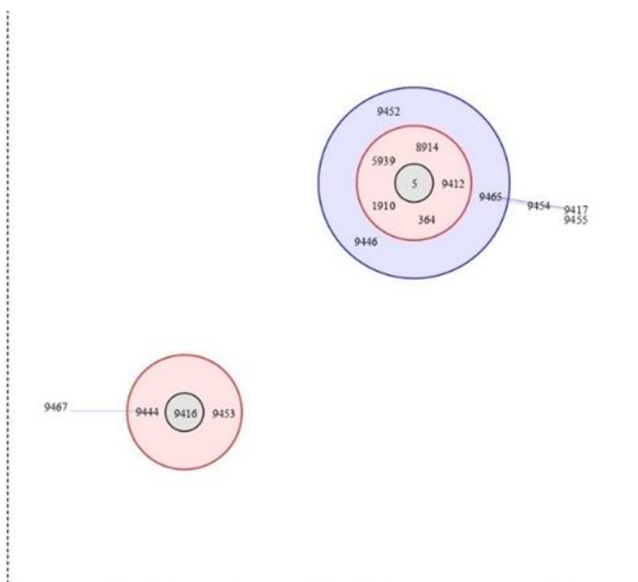
BURST analysis

Groups:

Group definition: 5 or more matches
Groups with central ST will be displayed as an image.

group: 1

ST	Frequency	SLV	DLV	SAT
5*	1	5	6	4
364	1	4	2	9
1910	1	4	2	9
5939	1	4	2	9
8914	1	1	9	5
9412	1	4	2	9
9416	1	2	4	9
9417	1	0	1	14
9444	3	2	4	9
9446	1	0	3	12
9452	1	0	4	11
9453	1	2	4	9
9454	1	1	1	13
9455	1	0	2	13
9465	1	1	9	5
9467	1	0	1	14

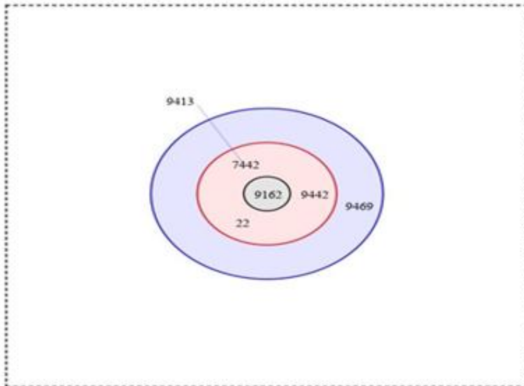


SVG file (right click to save)

group: 2

ST	Frequency	SLV	DLV	SAT
22	4	2	1	2
7442	1	2	2	1
9162*	1	3	1	1
9413	1	0	1	4
9442	1	1	3	1
9469	1	0	2	3

Figure S1: eBURST Analysis of study isolates.



SVG file (right click to save)

group: 3

ST	Frequency	SLV	DLV	SAT
672	1	1		
9457	1	1		

group: 4

ST	Frequency	SLV	DLV	SAT
9438	1	0	1	
9470	1	0	1	

Singletons:

ST	Frequency
9411	1
9414	1
9415	1
9418	1
9439	1
9440	1
9441	1
9443	1
9445	1
9447	1
9448	1
9449	1
9450	1
9451	1
9456	1
9458	1
9459	1
9460	1
9461	1
9462	1
9463	1
9464	1
9466	1
9468	1

Figure S2: eBURST Analysis of Indian isolates

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