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Genotyping of Methicillin-Resistant *Staphylococcus epidermidis* in Healthcare Workers in a Jordanian Hospital Using Molecular Tools

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Abstract

Methicillin-resistant *Staphylococcus epidermidis* (MRSE) represents a significant challenge in healthcare-associated infections, contributing to increased infection rates, medical complications, and mortality. Originating from human skin, nasal passages, and healthcare environments, MRSE exploits skin breaches and hospital surfaces to spread, complicating treatment due to its antibiotic resistance, particularly conferred by the *mecA* gene. Effective isolation and control of MRSE in healthcare settings require stringent hygiene practices, infection control measures, and continuous education for healthcare workers and patients. Addressing these challenges necessitates a multifaceted approach, including research into new therapeutic strategies and responsible antibiotic usage.

In our study, *mecA* gene analysis was conducted on 24 MRSE isolates using PCR and gel electrophoresis. Results confirmed the prevalence of the *mecA* gene in 95.8% (23/24) of the isolates, highlighting its significant role in methicillin resistance. Additionally, we identified 13 MRSE haplotypes across diverse sources, with Hap_2 being the most prevalent (67%, 26/39 isolates), widely distributed across nasal (53.8%) and skin (34.6%) samples. Phylogenetic analysis of 39 MRSE isolates, including 15 from GenBank, revealed significant genetic diversity. Hap_2 exhibited extensive variation, whereas the isolates designated as IHN (nasal samples) and IHS (skin samples) formed a distinct monophyletic cluster, characterized by high genetic relatedness and a common evolutionary origin. This pattern contrasted with the separate clustering observed for the GenBank strains. These findings emphasize the need for targeted infection control measures, particularly in healthcare settings, to address the prevalence of MRSE and its genetic diversity. Practical applications include the development of screening programs for healthcare workers, improving decontamination protocols, and fostering research into novel therapeutic strategies to mitigate antibiotic resistance.

Keywords: Methicillin-Resistant *Staphylococcus epidermidis* (MRSE), *mecA* gene, Antibiotic resistance, Healthcare workers, Genotyping, Jordanian hospital, *16S rRNA* gene.

1. Introduction

Isolates of methicillin-resistant *Staphylococcus epidermidis* (MRSE) are among the formidable foes in the realm of healthcare-associated infections, contributing significantly to the burden on healthcare systems and public health at large (Mirzaei et al., 2022; Sharma & Kalawat, 2023). These resilient bacteria, resistant to conventional antibiotics like methicillin, present a daunting challenge for treatment, complicating medical interventions and heightening the risk of infection transmission (Sun et al., 2023; Becker et al., 2023). Originating from various sources, including human skin, nasal passages, and the ambient environment within healthcare facilities, MRSE finds ample opportunities to colonize and proliferate (Lee et al., 2023; Guo et al., 2022). Skin breaches, such as wounds and fissures, serve as entry points for these bacteria, while the nasal cavity can harbor them stealthily, devoid of overt symptoms (Liang et al., 2022). It can facilitate the spread of antibiotic-resistant strains to patients, increase the likelihood of hospital-acquired infections, and complicate treatment outcomes. Therefore, identifying and managing MRSE carriers, particularly in healthcare settings, is crucial for infection control and preventing the transmission of resistant bacteria. Furthermore, the hospital environment itself, with its multitude of surfaces and medical apparatus, acts as a reservoir for these resilient microbes, facilitating their spread among patients and healthcare workers (Dai et al., 2023).

The ramifications of MRSE isolates extend beyond individual health concerns to encompass broader public health risks. Increased infection rates, complications in medical treatments, and elevated mortality rates all underscore the urgent need to address this menace (Leung

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et al., 2022; Brown & Smith, 2023). MRSE strains, in particular, serve as a significant reservoir of antibiotic resistance genes, notably the *mecA* gene, which confers methicillin resistance. This genetic trait not only allows MRSE to resist treatment with beta-lactam antibiotics like methicillin, but it can also be transferred to other bacterial species through horizontal gene transfer. Moreover, the emergence of antibiotic resistance compounds the issue, rendering conventional treatment modalities increasingly ineffective (Wang et al., 2023; Yao et al., 2023).

Effectively managing MRSE within healthcare facilities requires a comprehensive approach, combining strict infection control measures, rigorous hygiene practices, and ongoing staff training initiatives. This includes both the careful collection of MRSE isolates for study and the implementation of strategies to limit the bacteria's spread among patients and healthcare workers (Di Russo Case et al., 2023; Fisher et al., 2023). From environmental decontamination to promoting personal hygiene among patients and staff, every aspect of infection prevention must be rigorously implemented and continually reinforced. However, the challenges of isolation extend beyond the realm of technical procedures to encompass human factors and systemic issues within healthcare settings. Time constraints, competing priorities, and the relentless pace of medical practice all contribute to the complexity of ensuring strict adherence to isolation protocols (Hill et al., 2023). Addressing these challenges necessitates a multifaceted approach that goes beyond mere procedural compliance. Continuous education and awareness campaigns targeting both healthcare workers and patients are essential to foster a culture of vigilance and responsibility (Naylor et al., 2023). Additionally, efforts to curb inappropriate antibiotic usage and support research into novel therapeutic approaches are crucial for combating the escalating threat of antibiotic resistance (López-López et al., 2022).

In confronting the menace of methicillin-resistant *S. epidermidis* and its ilk, the imperative for collective action and unwavering commitment to infection control has never been more urgent. Only through concerted efforts, grounded in sound scientific principles and bolstered by a shared sense of responsibility, can we hope to stem the tide of healthcare-associated infections and safeguard public health for generations to come. Our study specifically aims to identify and genotype MRSE isolates from healthcare workers in a Jordanian hospital, focusing on the presence of the *mecA* gene and its impact on antibiotic resistance. We hypothesize that MRSE strains from healthcare workers harboring the *mecA* gene will exhibit higher levels of resistance to methicillin and other antibiotics. The findings could provide crucial insights into the role of healthcare workers in the transmission and evolution of MRSE, offering a pathway for improved infection control measures.

2. Materials and Methods:

2.1. Bacterial Isolation and Identification:

At the selected Jordanian hospital, stringent protocols are implemented to efficiently isolate bacterial groups, particularly when dealing with large healthcare workers. In previous study, a total of 175 bacterial isolates were obtained, with 25 isolates identified as *S. epidermidis* through a series of biochemical tests (Al-Nsour et al., 2024). The isolates from healthcare workers (carriers) and the environment were randomly isolated in roughly equal numbers to ensure a representative selection from both sources. While the exact number of samples from each source varied slightly, this was expected. A substantial number of samples were successfully isolated, identified, genotyped, and characterized. These isolates were sourced from various origins, including the skin and nasal passages of hospital workers, predominantly doctors and nurses, as well as environmental surfaces within the hospital premises. After isolation, the bacterial isolates were preserved and subsequently subjected to molecular analyses focusing on specific genetic structures.

2.2. DNA Extraction for Staphylococcus epidermidis Isolates:

Our study commenced by isolating *S. epidermidis* strains, with a focus on identifying those potentially exhibiting Methicillin-resistant properties. Recognizing the critical significance of employing robust protocols, we prioritized established methods to ensure the extraction of genomic DNA of the highest quality. To achieve this, we utilized a combination of techniques including enzymatic lysis and column-based purification. These methods were meticulously executed to yield pure DNA samples, thereby reducing the risk of contaminants that could compromise subsequent analyses. Importantly, all procedures were conducted in strict adherence to manufacturer instructions to guarantee optimal outcomes (Al-Nsour et al., 2024).

2.3. PCR Amplification for 16S rRNA Identification:

After DNA extraction, Polymerase Chain Reaction (PCR) was utilized to amplify the *16S rRNA* gene. Selecting universal primers like the forward primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3') was crucial to ensure broad applicability across bacterial taxa (Stahl, 1991; Ludwig, 1999). These primers were specifically designed to target highly conserved regions of the *16S rRNA* gene, ensuring accurate amplification of the target gene. PCR amplification was performed using LILIF 2x Master Mix (Korea) in a 50 µL reaction volume. The PCR reaction mixtures were initially incubated at 94°C for 5 minutes for denaturation, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 25 seconds, and elongation at 72°C for 1.20 minutes. A final extension step was performed at 72°C for 5 minutes. The conditions were optimized to ensure efficient amplification of the target gene.

To assess methicillin resistance, PCR amplification targeted the *mecA* gene, a key determinant of resistance in *S. epidermidis*. The primer sequences used for amplification were P2 (5'-ATCGATGGTAAAGGTTGGC-3' and P3 (5'-AGTTCTGCAGTACCGGATTTGC-3' which generated an amplicon of 530 base pairs in size. These primers were carefully designed to specifically target the *mecA* gene, ensuring accurate detection of methicillin resistance in the MRSE isolates. For the amplification of the *mecA* gene, the PCR reaction mixtures were initially incubated at 94°C for 5 minutes for denaturation. This was followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 25 seconds, and elongation at 72°C for 1.20

minutes. A final extension was carried out at 72°C for 5 minutes.

2.4. Sequence Editing, NCBI Submission, and Bioinformatic Analysis:

The purified PCR products were sequenced using a commercial service provided by MACROGEN, Korea, and the obtained sequences underwent meticulous editing using the EditBio program to remove any artifacts or low-quality regions (Hall, 1999). Edited sequences were then subjected to BlastN analysis to confirm their identity and assess their similarity to known sequences (https://www.ncbi.nlm.nih.gov) (Sayers et al., 2020). Subsequently, the edited sequences were submitted to the National Center for Biotechnology Information (NCBI) database, providing a publicly accessible repository for the genetic information of the MRSE isolates. Accession numbers corresponding to each sequence were acquired, associating them with distinct identifiers within the NCBI database. These accession numbers are listed in Table 1.

Table 1. Accession numbers corresponding to each sequence, linked with distinct identifiers in the NCBI database

	Isolate code	Accession number	Source	Isolation Date	Location
1	IHN1B	OQ568718	Human Nasal	2022	Jordan
2	IHN2B	OQ568719	Human Nasal	2022	Jordan
3	IHN3B	OQ568720	Human Nasal	2022	Jordan
4	IHN4B	OQ568721	Human Nasal	2022	Jordan
5	IHN5B	OQ568722	Human Nasal	2022	Jordan
6	IHN6B	OQ568723	Human Nasal	2022	Jordan
7	IHN7B	OQ568724	Human Nasal	2022	Jordan
8	IHN8B	OQ568725	Human Nasal	2022	Jordan
9	IHN9B	OQ568726	Human Nasal	2022	Jordan
10	IHN10B	OQ568727	Human Nasal	2022	Jordan
11	IHN11B	OQ568728	Human Nasal	2022	Jordan
12	IHN12B	OQ568729	Human Nasal	2022	Jordan
13	IHN13B	OQ568730	Human Nasal	2022	Jordan
14	IHN14B	OQ568731	Human Nasal	2022	Jordan
15	IHS1B	OQ568732	Human Skin	2022	Jordan
16	IHS2B	OQ568733	Human Skin	2022	Jordan
17	IHS3B	OQ568734	Human Skin	2022	Jordan
18	IHS4B	OQ568735	Human Skin	2022	Jordan
19	IHS5B	OQ568736	Human Skin	2022	Jordan
20	IHS6B	OQ568737	Human Skin	2022	Jordan
21	IHS7B	OQ568738	Human Skin	2022	Jordan
22	IHS8B	OQ568739	Human Skin	2022	Jordan
23	IHS9B	OQ568740	Human Skin	2022	Jordan
24	IHE1B	OQ568741	Environment	2022	Jordan

2.5. Phylogenetic Tree Construction with MEGA11:

MEGA11 software was employed for constructing phylogenetic trees based on the edited 16S rRNA sequences (Tamura et al., 2021). This facilitated the visualization of evolutionary relationships among MRSE isolates. Parameters such as tree-building methods and bootstrap values were carefully considered to ensure the robustness of the phylogenetic analysis. For the analysis, some *Staphylococcus epidermidis* isolates were retrieved from NCBI and included alongside our experimental data. The evolutionary history of these isolates was inferred using the Maximum Likelihood method and the Tamura-Nei model. The tree with the highest log-likelihood value of -1249.25 is presented. The percentage of trees in which the associated taxa clustered together is indicated next to the branches. Initial trees for the heuristic search were generated automatically using the Maximum Parsimony method. This analysis involved 39 nucleotide sequences, and all positions containing gaps or missing data were excluded (complete deletion option). The final dataset consisted of 676 positions. Evolutionary analyses were performed using MEGA11 software.

2.6. Haplotype Analysis:

A previous phylogenetic tree, constructed using 16S rRNA sequences, was utilized to explore haplotype diversity among the *S. epidermidis* and MRSE isolates. This analysis provided insights into the relatedness and evolutionary history of the isolates, offering a nuanced perspective on their genetic diversity within the population. To further elucidate the genetic variation and population structure, additional analyses were conducted using DnaSP6 (Rozas et al., 2017) and PopArt (Leigh and

Bryant, 2015) programs. In DnaSP6, parameters such as haplotype diversity, nucleotide diversity (π), and the number of polymorphic sites were calculated to assess the genetic variability. For PopArt, haplotype networks were visualized using the Median-Joining method to explore the relationships between different haplotypes. These software tools enabled the examination of haplotype frequencies, nucleotide diversity, and the visualization of haplotype networks, enhancing our understanding of the *S. epidermidis* and MRSE population dynamics and evolutionary patterns.

2.7. Identity and Similarity Percentage Calculation:

Specialized bioinformatics programs were employed to calculate identity and similarity percentages among the *S. epidermidis* and MRSE isolates. This comparative analysis delved into the molecular similarities and differences, contributing to a deeper understanding of the relatedness and divergence within the population at a genomic level. The Sequence Demarcation Tool (SDT), version 1.2, was utilized to facilitate this analysis, providing robust metrics for assessing genetic identity and similarity among the isolates (Muhire et al., 2014); in addition, the TBtools-I software was used to obtain the phylogenetic relatedness between all of isolates (Chen et al., 2023).

3. Results

In this study, we conducted a phylogenetic analysis involving 39 *S. epidermidis* strains. This included one isolate of *S. epidermidis* that does not harbor the *mecA* gene and 23 MRSE isolates from various sources (skin, nasal, and environmental) and 15 additional strains selected from GenBank based on their genetic identity to our isolates. The phylogenetic tree clearly demonstrates the evolutionary relationships between the IHS and IHN isolates (red squares) and various *S.epidermidis* strains from GenBank (green circles). The IHS and IHN isolates form a distinct monophyletic cluster, indicating their close genetic relatedness, with examples such as IHS9B, IHS6B, IHS3B, IHS2B, and IHS1B grouped together with high bootstrap support, suggesting a common or closely related source. Conversely, the GenBank sequences like MN022539.1, LC648284.1, and OU548756.1 also form a monophyletic group, reflecting their tight evolutionary relationship. Despite the clear separation between the IHS/IHN isolates and the GenBank strains, the tree shows they share a distant common ancestor, underscoring a more remote evolutionary connection. This relationship, supported by moderate to high bootstrap values, highlights that while the IHS and IHN isolates are genetically distinct from the GenBank strains, they still belong to the broader phylogenetic context of *S. epidermidis*.

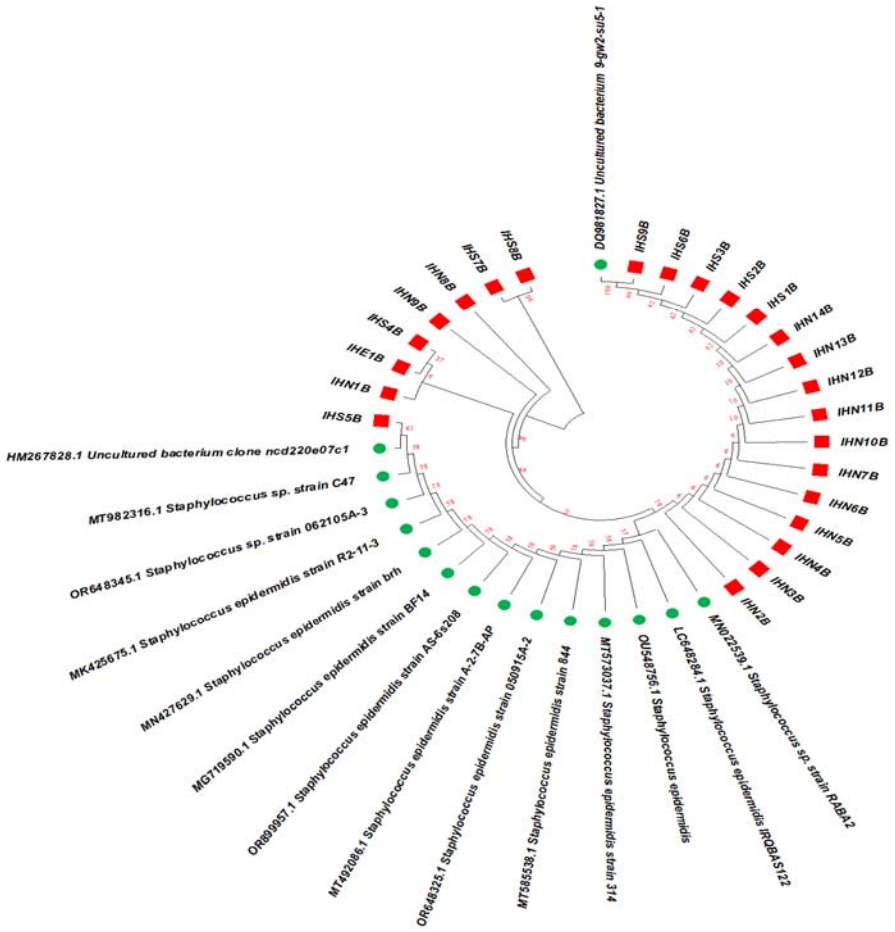

Figure 1. Evolutionary analysis of 16S rRNA sequences by the Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-1249.25) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. This analysis involved 39 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 676 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021). The phylogenetic tree depicts the genetic relationships among *S. epidermidis* isolates. The green circles ● represent strains from the GenBank database, while the red squares ■ indicate isolates from the current study.

The clustering within the IHS and IHN isolates can aid in understanding their diversity and potential functional or pathogenic differences. A monophyletic group (or clade) includes an ancestor and all of its descendants. The cluster of S. epidermidis strains from GenBank (green circles) is a monophyletic group, including sequences such as MN022539.1, LC648284.1, and OU548756.1, all sharing a common ancestor with IHS5B, distinct from other groups in the tree. rue except for IHS5B that is clustered with the GenBank isolates and DQ981827.1 that is clustering with the red squares. Similarly, the IHS and IHN isolates (red squares) form a monophyletic group, which includes sequences such as IHS9B, IHS6B, IHS3B, IHS2B, IHS1B, and their related isolates, all descending from a common ancestor unique to them. However, IHS5B deviates from this pattern, as it clusters with the GenBank isolates, while DQ981827.1 clusters with the red square isolates, indicating some level of genetic diversity within the group. A paraphyletic group includes an ancestor and some, but not all, of its descendants. If we consider the entire group of *S. epidermidis* strains along with the IHS/IHN isolates, excluding certain sequences like DQ981827.1 and HM267828.1, the group could be classified as paraphyletic. This is because it includes their common ancestor and several, but not all, of the descendants. Specifically, HM267828.1 clusters with the other GenBank isolates, while DQ981827.1 is an outlier, sharing a common ancestor with the rest of the group but not fully fitting within it. A polyphyletic group includes members from different ancestral lines and does not include the most recent common ancestor of the group. If we were to group some of the IHS/IHN isolates with certain unrelated GenBank sequences based on some arbitrary similarity, ignoring their actual common ancestors, this would form a polyphyletic group. The phylogenetic tree indicates that IHS9B and DQ981827 are closely related and share a recent common ancestor. The IHS isolates (such as IHS9B, IHS6B) are more closely related to each other, forming a tight cluster. Similarly, the IHN isolates (such as IHN14B,

IHN13B) are more closely related to each other. The GenBank strains of *S. epidermidis* are more closely related to each other than to the IHS/IHN isolates. The overall tree shows that while both the IHS/IHN isolates and the GenBank strains of *S. epidermidis* share a distant common ancestor, they form distinct clades, indicating separate evolutionary paths.

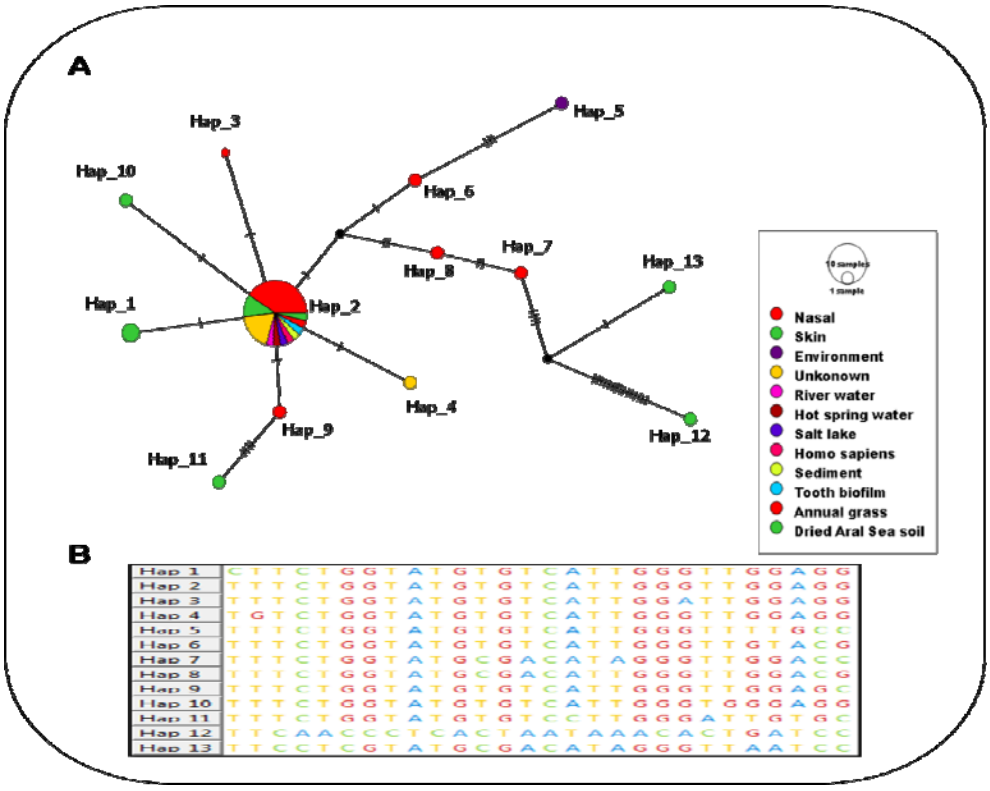

Figure 2 A) This analysis illustrates the mutation count between each pair of haplotypes, indicated by the number of dashed lines between them. Each mutation represents a variation in nucleotides at specific positions within the sequence. **B)** The analysis reveals the distinct positions of targeted sequences needed to derive the haplotypes, based on the discrepancies among the sequences. Each mutation signifies a variation in nucleotides at specific positions within the sequence.

The dataset in figure 2A comprises 13 unique haplotypes (Hap_1 to Hap_13) derived from 39 MRSE isolates, including 15 from NCBI. In figure 2A, the size of the circles represents the number of isolates within each haplotype, with larger circles indicating haplotypes that contain a greater number of isolates. This visual representation helps to illustrate the relative abundance of each haplotype in the dataset. The dominant haplotype, Hap_2, is represented by 26 isolates and encompasses a diverse array of sequences. These sequences include isolates with the following codes: DQ981827.1, OR648345.1, MK425675.1, MN427629.1, MG719590.1, OR899957.1, MT492086.1, OR648325.1, MT573037.1, OU548756.1, LC648284.1, MN022539.1, IHN2B, IHN3B, IHN4B, IHN5B, IHN6B, IHN7B, IHN10B, IHN11B, IHN12B, IHN14B, IHS1B, IHS2B, IHS6B, and IHS9B. These diverse isolates collectively contribute to the extensive variation observed within Hap_2, highlighting the genetic heterogeneity present within the MRSE population, while the rest of haplotypes are singleton haplotypes. By single genotype, we refer to isolates each possessing a unique genetic sequence, with no more than one isolate sharing the same pattern. In contrast, haplotypes represented by more than one isolate, such as Hap_2, indicate genetic clusters where multiple isolates share the same sequence. This distinction highlights the genetic diversity within the MRSE group. The sequence comparison in Figure 2B shows the 16S rRNA sequence exclusively. It demonstrates that all isolates within haplotype 2 share the same sequence, which differs from other haplotypes by one or more nucleotide variations. The haplotypes exhibit notable diversity, as seen in their sequence variations across 28 nucleotide sites. Hap_2 is particularly diverse, represented by sequences with variations at multiple sites. Hap_1, although occurring twice (HM267828.1 and IHS5B), differs from the predominant Hap_2 by a single nucleotide, highlighting its distinct sequence variation. The overall shape of the haplotype distribution suggests a predominant central haplotype (Hap_2) surrounded by several peripheral haplotypes, indicating a core-periphery structure in the MRSE population. These findings underscore the genetic relatedness and variation within the MRSE isolates, providing insights into their evolutionary dynamics and population structure.

Hap_2 emerges as the most prevalent haplotype, with occurrences across multiple sources, including nasal, skin, unknown, river water, hot spring water, salt lake, *Homo sapiens*, sediment, tooth biofilm, annual grass, and dried Aral Sea soil. This suggests a widespread distribution and adaptability of Hap_2 across various environments.

Several other haplotypes, such as Hap_6, Hap_7, Hap_8, and Hap_9, are predominantly isolated from specific sources, indicating potential specialization or niche adaptation. In contrast, haplotypes such as Hap_3, Hap_4, and Hap_5 exhibit a more limited presence, as they tend to originate from a single source, unlike Hap_2, which shows broader distribution across multiple sources. This suggests that these haplotypes may be rare or specifically associated with particular environments, as evidenced by the clustering patterns in Figure 2A. We identified 13 MRSE haplotypes, with Hap_2 emerging as the most prevalent, representing 67% (26/39) of the total isolates. Hap_2 was significantly more common in nasal samples (53.8%, 14/26) compared to skin samples (34.6%, 9/26) ($p < 0.05$, Chi-square test), emphasizing the role of nasal colonization in MRSE dissemination. Other haplotypes, such as Hap_1, Hap_3, Hap_4, and Hap_5, were less common, with 3 or fewer isolates each. Haplotypes Hap_8 to Hap_13 were not detected in either nasal or skin samples. Phylogenetic analysis revealed clustering patterns with bootstrap values >70%, confirming the evolutionary relationships and genetic reliability of the observed haplotype distributions. Overall, the data provides insights into the distribution patterns of MRSE haplotypes across diverse isolation sources, highlighting potential ecological niches and sources of transmission for these bacterial strains.

Continuing with the analysis (Figure 3), each sequence listed, including HM267828.1, DQ981827.1, MT982316.1, OR648345.1, MK425675.1, MN427629.1, MG719590.1, OR899957.1, MT492086.1, OR648325.1, MT585538.1, MT573037.1, OU548756.1, LC648284.1, and MN022539.1, displays varying degrees of identity with other sequences. For instance, HM267828.1 exhibits 100% identity with itself and various percentages of identity with other sequences. Similarly, DQ981827.1 shows 99.89% identity with HM267828.1 and 100% identity with itself, along with varying degrees of identity with other sequences. This pattern persists for the remaining sequences, each demonstrating different levels of identity with others in the dataset. Moving on to the isolates coded with IHN and IHS, each of these isolates, from IHN1B to IHN14B and IHS1B to IHS9B, also demonstrates varied percentages of identity with other sequences, including those with IHN and IHS codes. These identity percentages provide insights into genetic resemblance or divergence among the sequences, aiding in the understanding of evolutionary relationships, genetic variation, and potential functional similarities or differences.

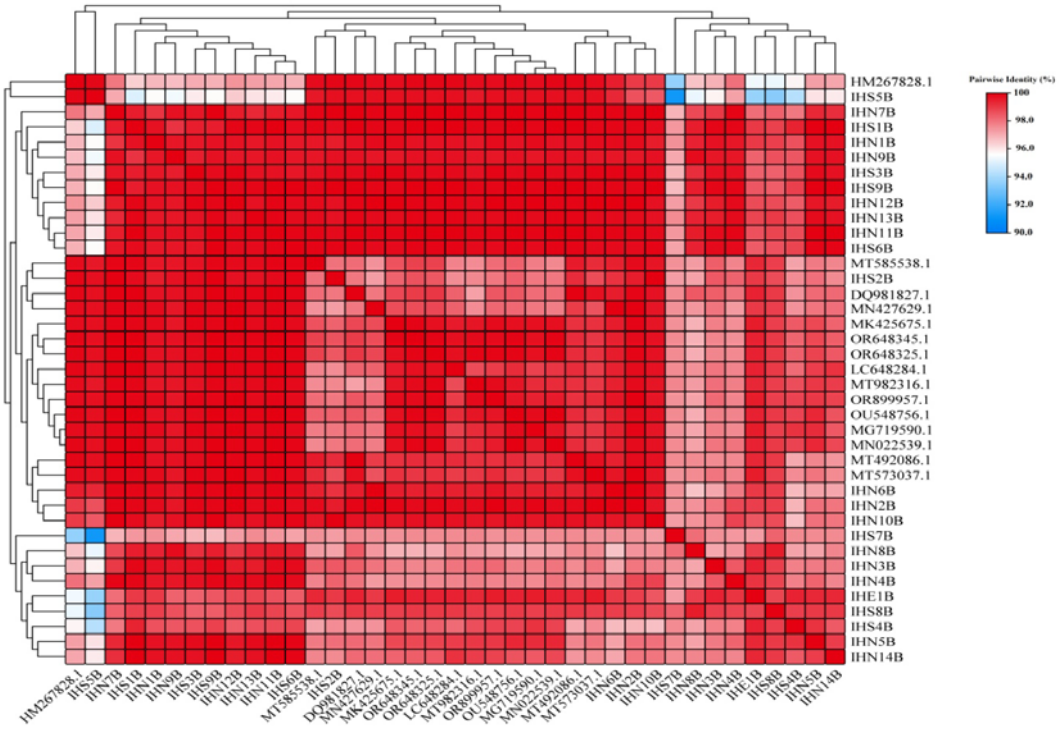

Figure 3 Colour-coded pairwise identity matrix generated from the 16S rRNA sequences of 39 *S. epidermidis* isolates sequences. Each coloured cell represents a percentage identity score between two sequences (one indicated horizontally to the left and the other vertically at the bottom). A coloured key indicates the correspondence between pairwise identities and the colours displayed in the matrix. Per cent nucleotide identity based heat map was created using TBtools-I (Chen et al., 2023).

In our exploration of the *mecA* gene, Figure 4 presents the findings from gel electrophoresis, revealing a noteworthy and consistent revelation across all 25 *S. epidermidis* isolates: the presence of the *mecA* gene within their genetic makeup. The distinct visualization of PCR amplification products corresponding to the *mecA* gene unequivocally confirms its widespread prevalence among these MRSE strains. This significant observation establishes a robust genetic basis for methicillin resistance within the sampled strains, emphasizing the pivotal role of the *mecA* gene in transmitting resistance to beta-lactam antibiotics. Moreover, within the same investigative

framework, among the 24 isolates scrutinized, only one exhibited negative outcomes in PCR amplification targeting the *mecA* gene. Specifically, this isolate is identified as isolate IHN12B, while the remaining 23 isolates demonstrated positive PCR outcomes.

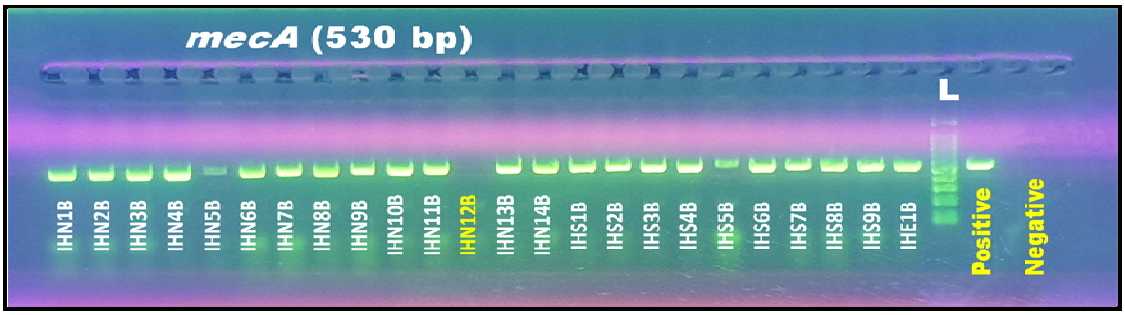

Figure 4. illustrates a compelling finding: amplification of the *mecA* gene was exclusively detected in 23 *S. epidermidis* isolates, signalling their resistance to methicillin. Notably, amidst 24 isolates, only one (**IHN12B**) exhibited a negative result for PCR amplification. The gel electrophoresis image showcases the (L) lane, symbolizing the ladder (DNA marker) with a size of 100 bp. The presence of both negative and positive controls is clearly delineated within the gel image, ensuring the validity and reliability of the experimental results.

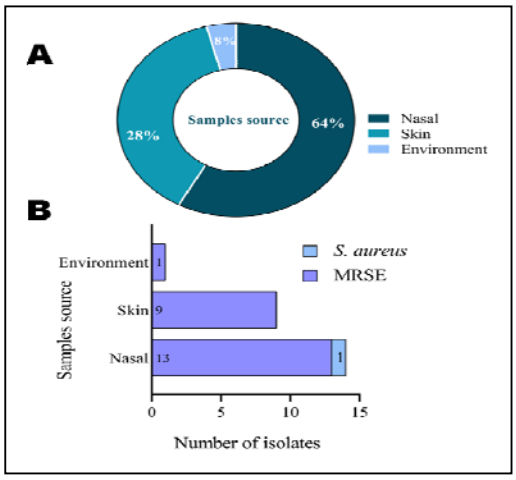

Figure 5. Representation of the distribution frequency of *S.epidermidis* isolates from various sources and the presence of MRSE isolates among the total isolates. **A:** Pie chart illustrates the total number of isolation sources and the distribution of isolates among these sources. **B:** Bar chart displays the total number of isolates of MRSE and *S. epidermidis* from selected sources.

The Pie chart in Figure 5A illustrates the distribution of *S. epidermidis* isolates across different isolation sources. It clearly shows that the nasal cavity is the most significant reservoir for *S. epidermidis*, with 14 isolates, followed by the skin with 9 isolates, and the environment with only 1 isolate. This visualization highlights the predominance of *S. epidermidis* in nasal and skin habitats, reflecting its role as a common commensal organism in these areas. The minimal environmental presence suggests either limited sampling or lower environmental colonization by *S. epidermidis* in the sampled settings. The bar chart data in Figure 5B indicates a significant presence of Methicillin-Resistant *Staphylococcus epidermidis* (MRSE) in skin and nasal passages, with 9 and 13 isolates respectively, and a minimal presence in the environment with just one isolate. In contrast, *S. epidermidis* (that does not contain the *mecA* gene) was only found in one nasal isolate and was absent from skin and environmental samples. This highlights the nasal cavity as a critical reservoir for both MRSE and *S. epidermidis*, although MRSE is more predominant. The findings emphasize the importance of targeted infection control measures, particularly in clinical settings, and suggest the need for increased environmental sampling to better understand the presence of these bacteria. Further research is recommended to explore factors contributing to the higher prevalence of MRSE in the nasal cavity, aiding in the development of effective prevention and treatment strategies.

4. Discussion

The battle against antibiotic-resistant bacteria has intensified and will continue to escalate (O'Neill, 2016). The pace of bacterial resistance to antibiotics is accelerating, making control efforts increasingly challenging (Aslam et al., 2018). Antibiotic-resistant bacteria, especially those exhibiting genetic mechanisms underlying antimicrobial resistance, require ongoing monitoring to address the sources and causes of this increasing resistance (CDC, 2019). In this study, we tracked methicillin-resistant *S. epidermidis* isolates in a teaching hospital in Jordan as part of a surveillance program aimed at monitoring genetic developments in these isolates. We focused on the genetic relatedness and proximity of isolates from various sources, including skin, nose, and the hospital environment, using ribosomal gene analysis. The evolutionary tree in Figure 1 illustrates the relatedness of isolates from different sources and their clustering with those in the gene bank (Kumar et al., 2016). The evolutionary tree revealed three clusters: the first cluster contains IHS8B, IHS7B, IHN8B, IHN9B, IHS4B, IHE1B, and IHN1B; the second cluster includes the GenBank isolates (except for DQ981827.1), along with IHS5B; and the third cluster comprises the remaining isolates. This relatedness indicates a connection between isolates from diverse geographical regions and sources. Our analysis aimed to determine the number of individual haplotypes and their similarities through ribosomal gene sequences. Using the DnaSP software, Hap_2 is the dominant haplotype, found in 26 out of 39 sequences, suggesting it may represent a common ancestral lineage or possess a selective advantage (Rozas et al., 2017). The remaining 12 haplotypes (Hap_1 and Hap_3 to Hap_13) are each found in only one or two sequences, indicating their rarity and possibly reflecting recent mutations, genetic drift, or specific selective pressures (Nei & Kumar,

2000). This distribution points to a diverse genetic population with several rare variants, incorporating sequences from both NCBI and specific isolates from our study. The data in figure 2A provides valuable insights into genetic diversity, evolutionary relationships, and population structure, which can be applied in various fields such as population genetics, conservation biology, and medical research (Hohenlohe et al., 2018). The arrangement and position differences among the haplotypes across all positions provide insights into the evolutionary pressures and genetic drift occurring within bacterial populations. By analyzing these sequence variations, we can infer the evolutionary pathways and selective pressures that have led to the emergence of resistant strains (Didelot & Maiden, 2010). The clustering of haplotypes, as shown in the evolutionary tree, indicates shared evolutionary histories and possible horizontal gene transfer events (Data not shown here), which are common mechanisms for the spread of resistance genes among bacteria (Arnold et al., 2018). In Figure 2B, we noted the different sequences for 13 haplotypes, highlighting the variations in arrangement and the positions of difference among all haplotypes across 1086 positions, which correspond to the length of the sequenced region within the *16S rRNA* gene. The use of software tools to analyze ribosomal gene sequences and identify haplotypes underscores the importance of bioinformatics in modern molecular biology (Gauthier & Singh, 2018). These tools allow for the rapid analysis of large datasets, facilitating the identification of genetic variations that would be challenging to detect through traditional methods. Such comparisons enable us to determine the geographical spread and epidemiological linkages of antibiotic-resistant strains (Benson et al., 2018). If our study isolates show high similarity to strains from different regions or environments deposited in GenBank, it suggests possible transmission routes and sources of infection, aiding in the development of targeted public health interventions (Benson et al., 2018).

The observed genetic relatedness between our isolates and those in GenBank prompted further investigation into the identity and connections between our study isolates and those previously deposited in the Genbank. Using the TBtools-I software, we accessed and analyzed this information, as depicted in Figure 3. The values in this figure demonstrated the identity between bacterial isolate sequences and their closer origins based on ribosomal gene analysis. The investigation into the genetic similarities and connections between study isolates and GenBank sequences, as facilitated by the TBtools-I software and depicted in Figure 3, is a powerful approach in molecular microbiology. It enhances our understanding of the genetic underpinnings of antibiotic resistance, informs clinical practices, and supports public health initiatives. This integrative approach underscores the importance of comparative genomics and bioinformatics in addressing the global challenge of antibiotic resistance (Gauthier & Singh, 2018; Benson et al., 2018). To investigate the presence of the *mecA* gene among our study isolates, we found that all of the isolates, except one (IHN12B), contained the *mecA* gene. The *mecA* gene is a critical determinant of methicillin resistance in *S. epidermidis* and other staphylococcal species. It encodes an alternative penicillin-binding protein (PBP2a) that has a low affinity for beta-lactam antibiotics, including methicillin (Becker et al., 2014). The finding that all but one isolate (IHN12B) possess the *mecA* gene is significant, indicating a high prevalence of methicillin resistance within this bacterial population, which is concerning for treatment options. Moreover, the widespread presence of the *mecA* gene suggests that this resistance mechanism is likely disseminated through horizontal gene transfer or clonal expansion of resistant strains (Howden et al., 2013). Therefore, this highlights the urgent need for effective strategies to combat antibiotic resistance. Significantly, the only exception, IHN12B, may offer insights into potential vulnerabilities in the bacterial population.

5. Conclusion

Methicillin-resistant *S. epidermidis* (MRSE) poses a significant healthcare challenge due to its high infection rates, complications, and resistance, primarily driven by the *mecA* gene. This study confirmed the widespread presence of the *mecA* gene in MRSE isolates and highlighted the genetic diversity and evolutionary dynamics of these bacteria. The nasal cavity and skin were identified as primary reservoirs for MRSE, emphasizing the need for stringent infection control practices. Phylogenetic analyses revealed significant genetic relatedness among isolates, underscoring the necessity for continuous monitoring and research into new therapeutic strategies. The findings underscore the importance of comprehensive infection control measures and the role of bioinformatics in addressing antibiotic resistance, aiding in the development of targeted public health interventions. Our findings highlight the genetic diversity and dominance of Hap_2 among MRSE isolates, underscoring the importance of nasal colonization in the dissemination of MRSE. These results emphasize the need for routine screening programs targeting healthcare workers, particularly nasal carriers, to prevent MRSE transmission.

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