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Molecular profiling and genetic diversity of mecA-mediated methicillin-resistant *staphylococcus aureus* isolates from healthcare workers in a major hospital

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ABSTRACT

Objectives: To investigate the genetic diversity and molecular characteristics of methicillin-resistant *staphylococcus aureus* (MRSA) isolates from healthcare workers in a Jordanian hospital, focusing on the role of the *mecA* gene in antibiotic resistance.

Methods: A total of 25 *staphylococcus aureus* isolates were collected from healthcare workers' skin, nasal passages, and environmental sources. Biochemical tests identified MRSA isolates, and DNA extraction was followed by polymerase chain reaction amplification of the 16SrRNA gene for genetic analysis. The sequences were analyzed using phylogenetic methods, sequence demarcation tool (SDT) analysis, and median-joining network analysis to determine genetic diversity and evolutionary relationships.

Results: Out of the 25 *staphylococcus aureus* isolates, 10 were identified as methicillin-resistant. Phylogenetic analysis revealed distinct clustering patterns, indicating genetic heterogeneity. SDT analysis highlighted the genetic complexity of the MRSA strains. The median-joining network identified haplotype 1 as the predominant haplotype, underscoring significant genetic diversity. The mecA gene was detected in all MRSA isolates, emphasizing its critical role in methicillin resistance.

Conclusion: The study demonstrates significant genetic diversity among MRSA isolates from healthcare workers in a Jordanian hospital, with the mecA gene playing a vital role in antibiotic resistance. These findings underscore the importance of molecular diagnostics in MRSA detection and the need for targeted infection control strategies to manage antibiotic resistance effectively in healthcare settings.

Keywords: methicillin-resistant *staphylococcus aureus*, mecA gene, antibiotic resistance, healthcare workers, Jordanian hospital, 16S rRNA gene

INTRODUCTION

The escalating threat posed by methicillin-resistant *staphylococcus aureus* (MRSA) to human health is a stark reality, driven by the insidious infections they cause and their increasing resistance to multiple antibiotics [1, 2]. This resistance, predominantly stemming from the overuse of antibiotics in healthcare settings, has rendered these bacteria formidable adversaries [3]. *Staphylococcus aureus*, often commensal to humans, can transition into opportunistic pathogens, and the distribution and isolation of MRSA isolates reveal a complex interplay of factors that contribute to their prevalence [4, 5]. Simultaneously, the resistance mechanism conferred by the mecA gene provides resistance against a range of beta-lactam antibiotics, such as carbapenems, cephalosporins, cephamycins, and monobactams [6, 7]. The mecA gene, originating from staphylococcal species, showcases its adaptability and efficient dissemination among different staphylococcal species through the mobile genetic element SCCmec [8, 9].

MRSA isolates exhibit a wide distribution across various healthcare settings, including hospitals, clinics, and long-term care facilities [10]. The nature of close and continuous contact among patients, healthcare workers, and environmental surfaces provides an ideal environment for MRSA transmission. In particular, the isolation of MRSA from skin and nasal swabs is crucial for understanding colonization patterns and potential sources of transmission [11, 12]. Skin isolates underscore the role of direct contact and fomites in the spread of MRSA, while nasal isolates highlight the bacterium's ability to colonize and persist in the nasopharynx, serving as a reservoir for subsequent infections [13].

The challenges associated with MRSA extend beyond its antibiotic resistance. The ability of MRSA to persist on environmental surfaces for extended periods contributes to its resilience and makes infection control measures more

intricate. Moreover, the bacterium's ability to cause both community-acquired and hospital-acquired infections adds complexity to surveillance and prevention strategies [14, 15]. The emergence of community-associated MRSA strains has introduced a new dimension, emphasizing the need for a holistic approach to combat these infections [16].

Skin isolates are often indicative of localized infections or colonization, emphasizing the importance of proper hygiene and infection control practices to prevent the spread of MRSA on surfaces and among individuals in close proximity. Environmental isolates, recovered from various hospital surfaces, underscore the challenges in maintaining a clean and MRSA-free healthcare environment [17]. Nasal isolates play a critical role in understanding the reservoir of MRSA, as the nasal passages serve as a primary colonization site [18]. Investigating nasal isolates helps identify asymptomatic carriers, allowing for targeted interventions to reduce the risk of subsequent infections. The detection and analysis of MRSA isolates from different anatomical sites contribute to a comprehensive understanding of its epidemiology, transmission dynamics, and the development of effective prevention strategies [19, 20].

In summary, the escalating threat of MRSA necessitates a thorough exploration of its distribution, isolation patterns, and the challenges posed by its antibiotic resistance. Understanding the dynamics of MRSA in diverse contexts, including the skin, environment, and nasal passages, is pivotal for developing targeted interventions and implementing robust infection control measures to mitigate the impact of this resilient pathogen on human health.

METHODS

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 this study, a total of 175 bacterial isolates were obtained, with 25 isolates identified as *staphylococcus aureus* through a series of biochemical tests. 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. Following isolation, the bacterial strains undergo preservation before being subjected to molecular studies aimed at analyzing certain genetic structures.

DNA Extraction for *Staphylococcus Aureus* Isolates

Our study commenced by isolating *staphylococcus aureus* 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.

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

No	IC	AN	Source	IY	Location
1	IHE1A	OQ568766	Environment	2022	Jordan
2	IHE2A	OQ568767	Environment	2022	Jordan
3	IHN1A	OQ568768	Human nasal	2022	Jordan
4	IHN2A	OQ568769	Human nasal	2022	Jordan
5	IHN3A	OQ568770	Human nasal	2022	Jordan
6	IHN4A	OQ568771	Human nasal	2022	Jordan
7	IHN5A	OQ568772	Human nasal	2022	Jordan
8	IHN6A	OQ568773	Human nasal	2022	Jordan
9	IHN7A	OQ568774	Human nasal	2022	Jordan
10	IHN8A	OQ568775	Human nasal	2022	Jordan
11	IHN9A	OQ568776	Human nasal	2022	Jordan
12	IHN10A	OQ568777	Human nasal	2022	Jordan
13	IHN11A	OQ568778	Human nasal	2022	Jordan
14	IHN12A	OQ568779	Human nasal	2022	Jordan
15	IHN13A	OQ568780	Human nasal	2022	Jordan
16	IHN14A	OQ568781	Human nasal	2022	Jordan
17	IHN15A	OQ568782	Human nasal	2022	Jordan
18	IHN16A	OQ568783	Human nasal	2022	Jordan
19	IHS1A	OQ568784	Human skin	2022	Jordan
20	IHS2A	OQ568785	Human skin	2022	Jordan
21	IHS3A	OQ568786	Human skin	2022	Jordan
22	IHS4A	OQ568787	Human skin	2022	Jordan
23	IHS5A	OQ568788	Human skin	2022	Jordan
24	IHS6A	OQ568789	Human skin	2022	Jordan
25	IHS7A	OQ568790	Human skin	2022	Jordan

Note. IC: ısolation code; AN: Accession number; & IY: Isolation year

Polymerase Chain Reaction Amplification for 16SrRNA Identification

After DNA extraction, polymerase chain reaction (PCR) was utilized to amplify the 16SrRNA 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. These primers were designed to target highly conserved regions of the 16SrRNA gene, facilitating precise amplification of the intended gene. PCR was conducted using LILIF 2× master mix, Korea in a 50 µL reaction under meticulously adjusted conditions. The annealing temperature was set at 58°C, and the extension temperature at 74 °C.

Sequence Editing, National Center for Biotechnology Information Submission, and Bioinformatic Analysis

The resultant PCR products were sequenced, and the obtained sequences underwent meticulous editing using the EditBio program to remove any artifacts or low-quality regions [21]. 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). 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 MRSA 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**.

Phylogenetic Tree Construction with MEGA11

MEGA11 software was employed for constructing phylogenetic trees based on the edited 16SrRNA sequences [22]. This facilitated the visualization of evolutionary

relationships among MRSA isolates. Parameters such as tree-building methods and bootstrap values were carefully considered to ensure robustness of the phylogenetic analysis.

PCR Amplification of mecA Gene for Methicillin Resistance Testing

To assess methicillin resistance, PCR amplification targeted the mecA gene, a key determinant of resistance in *staphylococcus aureus*. 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 mecA gene, ensuring accurate detection of methicillin resistance in the MRSA isolates.

Haplotype Analysis

A previous phylogenetic tree, constructed using 16SrRNA sequences, was utilized to explore haplotype diversity among the *staphylococcus aureus* and MRSA 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 performed using DnaSP6 and PopArt programs [23, 24] . These software tools allowed for the examination of haplotype frequencies, nucleotide diversity, and the visualization of haplotype networks, enhancing our understanding of the *staphylococcus aureus* and MRSA population dynamics and evolutionary patterns.

Identity and Similarity Percentage Calculation

Specialized bioinformatics programs were employed to calculate identity and similarity percentages among the *staphylococcus aureus* and MRSA 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 [25] and TBtools-I software [26], was utilized to facilitate this analysis, providing robust metrics for assessing genetic identity and similarity among the isolates.

RESULTS

One hundred and seventy-five bacterial isolates were obtained from healthcare workers at a targeted hospital in Jordan. Among these, 25 isolates were identified via biochemical tests as staphylococci and were determined to be methicillin-resistant (data not shown).

Among the 25 *staphylococcus aureus* isolates, 7 (28%) originated from skin swabs, 16 (64%) from nasal swabs, and 2 (8%) from environmental swabs. Subsequently, DNA extraction was performed on these specific samples. Variations in coloration and growth rates among these isolates suggested potential genetic diversity, prompting consideration for future investigation. Notably, the 25 isolates exhibited varying resistance patterns to a group of antibiotics (data not shown).

Molecular identification of the staphylococcal bacterial isolates involved successful PCR amplification of nucleic acids extracted from 25 isolates using primers specific to the 16S rRNA gene. Sequences obtained ranged between 1,040 and 1,150 base pairs in length. The entirety of the resulting

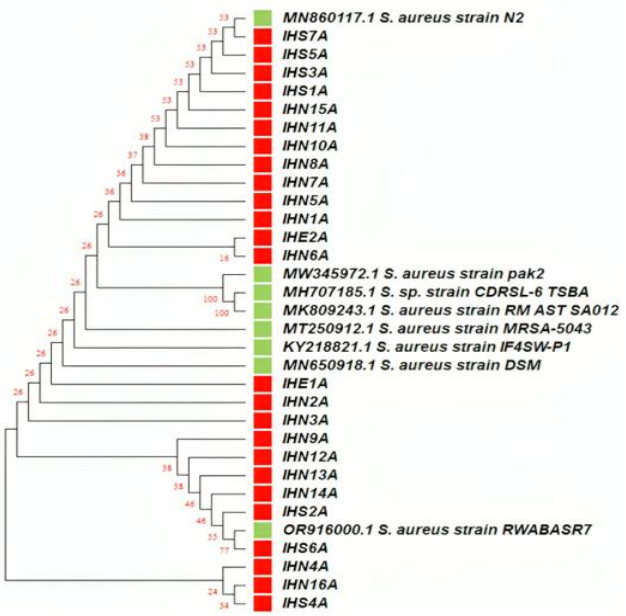

Figure 1. The evolutionary history was inferred by using the maximum likelihood method and Tamura-Nei model [27] (the tree with the highest log likelihood (-1,691.75) is shown, initial tree(s) for the heuristic search were obtained automatically by applying the maximum parsimony method, this analysis involved 33 nucleotide sequences, all positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 956 positions in the final dataset, evolutionary analyses were conducted in MEGA11 [22], and the red box signifies that this isolate is part of the samples investigated in this study, whereas the green box indicates isolates sourced from GenBank) (Source: Authors' own elaboration)

amplicons, deemed of high quality, was utilized for analysis. These sequences have been deposited in GenBank and are presented in **Table 1** alongside their corresponding accession numbers.

A phylogenetic tree was assembled a dataset consisting of 33 distinct genotypes of *staphylococcus aureus*, which incorporated the addition of 25 new isolate sequences.

Analysis of nucleotide sequences from the 16S rRNA gene via BlastN analysis against globally available *staphylococcus aureus* sequences revealed similarities with *staphylococcus aureus* isolates obtained from diverse hosts, isolation sites, and clinical samples, with similarity ranging between 98% and 99.80%. Consequently, to construct a phylogenetic tree, 16S rRNA genes retrieved from various samples and countries were sourced from GenBank and incorporated into the phylogenetic analysis. Notably, *staphylococcus aureus* reference sequences (with GenBank accession numbers: MN860117.1, KY218821.1, MN650918.1, MT250912.1, MW345972.1, OR916000.1, MK809243.1, and MH707185.1) exhibited similarity within this range with the isolates under investigation. Sequences analysis of the amplified region revealed that these 25 genotypes were distributed alongside closely selected sequences sourced from GenBank (**Figure 1**). Observing the initial configuration of the evolutionary tree, we identified the distribution of our isolates into three distinct groups, alongside strains sourced from GenBank (**Figure 1**). The first group comprised thirteen isolates, inclusive of four from skin, one from the environment, and eight from nasal sources. The second group encompassed

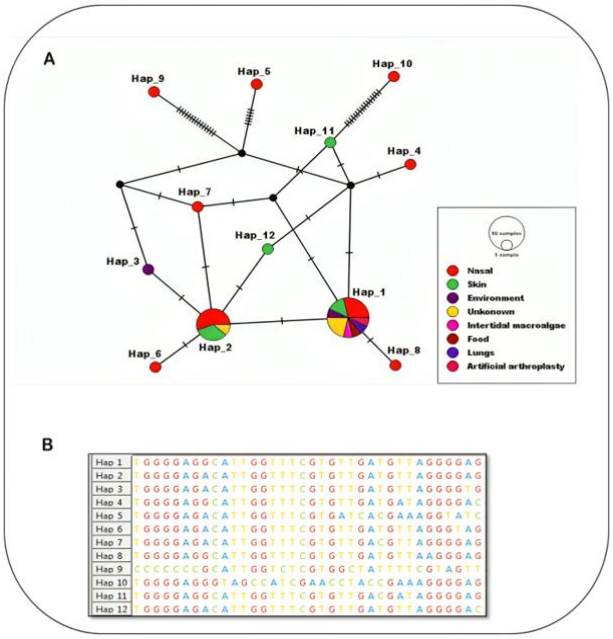

Figure 2. (A) This analysis illustrates the mutation count between each pair of haplotypes, indicated by the number of dashed lines between them and ech mutation represents a variation in nucleotides at specific positions within the sequence & (B) The analysis reveals the distinct positions of targeted sequences needed to derive the haplotypes, based on the discrepancies among the sequences and each mutation signifies a variation in nucleotides at specific positions within the sequence (Source: Authors' own elaboration)

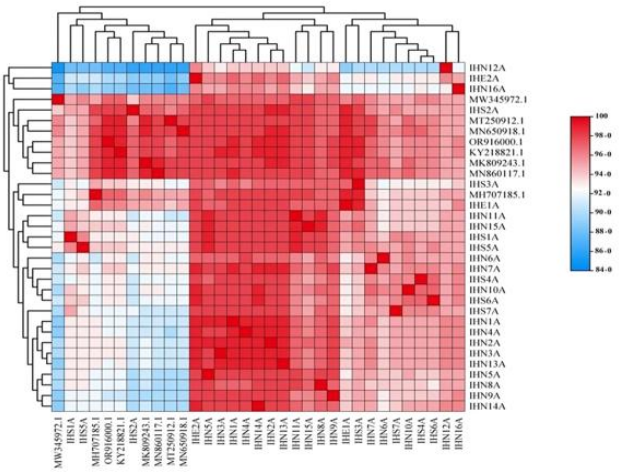

Figure 3. Color-coded pairwise identity matrix generated from 33 *staphylococcus aureus* isolates sequences (each colored cell represents a percentage identity score between two sequences (one indicated horizontally to the left and the other vertically at the bottom, a colored key indicates the correspondence between pairwise identities and the colours displayed in the matrix, and per cent nucleotide identity based heat map created using TBtools-I [26]

eight isolates, comprising one from skin, one from the environment, and six from nasal sources. The third group comprised four isolates, with two from skin and the remainder from nasal sources. The branching diagram depicting *staphylococcus aureus* strains based on the 16SrRNA gene is depicted in **Figure 1**.

In part A in **Figure 2**, PopArt focuses on constructing median-joining networks, which are particularly useful for studying intraspecific genetic variation, such as haplotype data. From this analysis, it's evident that haplotype 1 is the most prevalent haplotype in the dataset, appearing more frequently than any other haplotype, with a frequency of 14. The provided data in part A in **Figure 2** represents genetic sequences categorized into 12 haplotypes. The first haplotype, labeled haplotype 1, consists of 14 sequences identified by their accession numbers, including MH707185.1, OR916000.1, MK809243.1, MW345972.1, MT250912.1, KY218821.1, and MN650918.1, along with alleles such as IHE1A, IHN2A, IHN3A, IHN13A, IHN14A, IHS2A, and IHS6A. Similarly, the subsequent haplotypes, from haplotype 2 to haplotype 12, contain varying numbers of sequences, each associated with specific alleles. Notably, haplotype 2 comprises 9 sequences with the accession number MN860117.1 and alleles including IHN1A, IHN5A, IHN10A, IHN11A, IHN15A, IHS1A, IHS3A, and IHS5A. The remaining haplotypes, haplotype 3 through haplotype 12, each contain a single sequence, with unique alleles designated as IHE2A, IHN4A, IHN6A, IHN7A, IHN8A, IHN9A, IHN12A, IHN16A, IHS4A, and IHS7A, respectively. This categorization provides insight into the genetic diversity present within the studied population. The data in part A in **Figure 2** comprises a matrix delineating haplotype frequencies across various categories, including nasal, skin, environment, unknown, intertidal macroalgae, food, lungs, and artificial arthroplasty. Notably, haplotype 1 emerges as the most prevalent haplotype, with a frequency of 14, surpassing all others. This dominance is further underscored by its presence in intertidal macroalgae, food, lungs, and artificial arthroplasty categories, with a frequency of 1 in each. Detailed sequencing information is provided for haplotype 1, elucidating specific genetic variations associated with this predominant haplotype. Moreover, additional genetic diversity metrics reveal a nucleotide diversity (π) of 0.100642, with 36 segregating sites and 6 parsimony-informative sites. Tajima's D statistic yields a value of -2.12969, indicating a deviation from neutral evolution. These findings underscore the significance of haplotype distribution and genetic diversity in the studied population, warranting further investigation into potential evolutionary dynamics and selective pressures.

The provided data originates from DNA sequence analysis using DnaSP Ver. 6.12.03. It involves haplotype data from 33 sequences related to *staphylococcus aureus* and associated isolates, with a total of 1,100 sites analyzed. The analysis focused on a selected region spanning sites 1 to 1,100, with invariable sites removed. Twelve distinct haplotypes were identified, each labeled from haplotype 1 to haplotype 12. Among these haplotypes, haplotype 1 is the most frequent, observed in 14 sequences, while the remaining haplotypes are each found only once. The sequences associated with each haplotype are listed (part B in **Figure 2**), indicating the genetic diversity within the dataset. Additionally, the DNA sequences corresponding to the haplotypes are provided, with variations in nucleotide positions represented (part B in **Figure 2**). The analysis is conducted within the framework of diploid genomes, focusing on autosome regions. The dataset comprises 36 characters, representing nucleotide positions in the analyzed sequences (part B in **Figure 2**).

In the **Figure 3**, the analysis of nucleotide sequences utilizing the SDT, version 1.2, revealed notable variations in similarity and identity among the isolates under investigation,

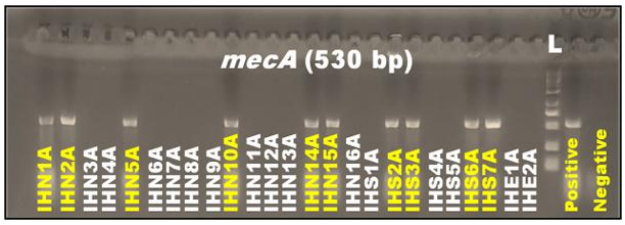

Figure 4. Amplification of the mecA gene was exclusively detected in ten *staphylococcus aureus* isolates highlighted in yellow, signifying their resistance to methicillin (the gel electrophoresis image depicts the [L] lane, representing the ladder [DNA marker] with a size of 1 Kbp and the presence of negative and positive controls is indicated in the gel image) (Source: Authors' own elaboration)

in comparison to selected isolates sourced from the NCBI database.

Notably, certain isolates exhibited a striking degree of similarity with others, occasionally reaching 100% identity. Moreover, specific strains sourced from the NCBI database demonstrated a significant level of identity with certain sequences from our isolates. These findings underscore the heterogeneous nature of the isolates utilized in this study, with clear distinctions and variations observed among them. For more investigation and to obtain greater reliability, we plan to conduct an analysis to elucidate the identity and similarity between our isolates and, on the other hand, between our isolates and the selected isolates from NCBI. The construction of a phylogenetic tree (**Figure 1**) further emphasized the discernible differences and diversities inherent within the dataset. Overall, the comprehensive analysis facilitated by SDT 1.2 elucidates the complex genetic landscape of the investigated isolates, providing valuable insights into their evolutionary relationships and genetic diversity.

During our investigation into the mecA gene, **Figure 4** illustrates the gel electrophoresis results, unveiling a consistent and significant discovery across all 25 *staphylococcus aureus* isolates the presence of the mecA gene within their genetic composition. The clear visualization of PCR amplification products corresponding to the mecA gene confirms its widespread prevalence among these MRSA strains. This revelation solidifies the genetic foundation of methicillin resistance observed in the samples and underscores the crucial role of the mecA gene in transmitting resistance to beta-lactam antibiotics. Additionally, within the same context, out of the 25 isolates examined, only ten displayed positive outcomes for PCR amplification of the mecA gene. Specifically, these isolates include samples IHN1A, IHN2A, IHN5A, IHN10A, IHN14A, IHN15A, IHS2A, IHS3A, IHS6A, and IHS7A.

DISCUSSION

In recent decades, the emergence of bacterial resistance to antibiotics has posed a dilemma for the global healthcare system, and this problem continues to escalate [28, 29]. This increase in bacterial resistance is attributed to misuse and overuse of antibiotics, as well as the spread of these antibiotics in biological environments, leading to increased pressure on bacteria and the development of numerous bacterial mechanisms to resist antibiotics [30, 31] . On the other hand, the emergence of antibiotic-resistant bacterial strains within healthcare facilities and hospitals has added pressure on healthcare workers, necessitating the implementation of mechanisms to limit and reduce this spread within standardized frameworks and tools adopted by specialists and workers within these healthcare environments [32, 33]. One of the most important mechanisms to reduce this spread of antibiotic-resistant bacterial isolates is monitoring, surveillance, and access to antibiotic-resistant bacterial isolates within healthcare facilities and among their workers [34-36].

In our study, we accessed a total of 175 bacterial isolates from a teaching hospital in Jordan, among which we obtained 25 *staphylococcus aureus* isolates. These isolates were molecularly diagnosed using 16S rRNA gene amplification, which is considered the most accurate method for bacterial species identification. Within our study, we examined the distribution of isolates from various sources on an evolutionary tree, alongside bacterial strains sourced from GenBank. We utilized the 16S rRNA gene to assess genetic disparities and distinguish between isolates from the same source. Notably, we observed convergence in evolutionary distances among isolates from different sources, indicating distinct genetic profiles. This finding underscores that isolates obtained from skin, nose, or environmental sources may exhibit varying evolutionary distances and genetic differences. Additionally, it suggests the widespread presence of certain staphylococci isolates across multiple locations. Molecular comparisons of bacterial isolates deposited in GenBank with those isolated from different locations and times serve as a mechanism for monitoring genetic changes at the isolate level of the same species. This surveillance helps understand the mechanisms of resistant gene transmission and the stability of the genetic structures used to evaluate bacterial isolates. The appearance of twelve haplotype patterns during our study of a group of bacterial isolates, consisting of twenty-five *staphylococcus aureus* isolates, in addition to some isolates used in evolutionary tree analysis, is relatively important. The genetic changes observed at the gene level give an impression of the ability of bacterial genomes to acquire or lose certain genetic traits.

The provided results in **Figure 3** depict a comparison of identity percentages between various sequences, organized in a triangular matrix format. Each cell in the matrix represents the percentage identity between two sequences, with the diagonal cells showing 100% identity as a sequence is compared to itself. The matrix is triangular due to the redundancy in comparing pairs of sequences, with only one side being filled with values. At the bottom of the matrix, statistical summary measures such as the minimum (95.08%), maximum (100%), mean (98.68%), and standard deviation (1.16%) are provided, offering insights into the variability and distribution of identity percentages across all pairs of sequences. Some isolates showed high identity, reaching up to 100%, with the isolates obtained from NCBI, indicating strong similarity between these sequences. Conversely, some isolates displayed lower identity levels with both the rest of the compared isolates from NCBI and between our isolates. This variance in identity percentages suggests differing levels of genetic relatedness and divergence among the sequences. This data is commonly utilized in bioinformatics and molecular biology for tasks like sequence alignment, phylogenetic analysis, and identifying homologous sequences. Interpreting these percentages can help gauge the degree of similarity

between sequences, crucial for understanding evolutionary relationships or functional conservation [37].

During this study, we targeted the mecA gene to determine whether the isolates in this study have the ability to resist methicillin, indicating that these isolates possess this resistance gene. The presence of the mecA gene means that the bacteria may be resistant to methicillin and, consequently, may be resistant to a wide range of antibiotics that rely on the same resistance mechanism [38, 39]. This suggests that the presence of the mecA gene may be associated with the presence of a group of antibiotic-resistant genes in different bacterial isolates, which may be carried on plasmids or through mobile elements between bacterial genomes, indicating the possibility of transmission between different bacterial species through horizontal gene transfer [40, 41]. In our investigation of the mecA gene, **Figure 4** provides a visual representation of the gel electrophoresis results, demonstrating a consistent and significant finding across all 25 *staphylococcus aureus* samples. Specifically, the results reveal the presence of the mecA gene within the genetic composition of some isolates and not in the rest. The distinct visualization of PCR amplification products corresponding to the mecA gene confirms its widespread prevalence among these MRSA strains. This detection of the mecA gene serves to strengthen the genetic basis of methicillin resistance observed in the samples, emphasizing the crucial role of the mecA gene in conferring resistance to beta-lactam antibiotics. Furthermore, within the same investigative context, it is noteworthy that out of the 25 isolates examined, only ten exhibited positive outcomes for PCR amplification of the mecA gene. These isolates, namely samples IHN1A, IHN2A, IHN5A, IHN10A, IHN14A, IHN15A, IHS2A, IHS3A, IHS6A, and IHS7A, stand out as exceptions within the broader pattern of mecA gene prevalence observed among the MRSA samples.

In the evolutionary tree analysis, a total of twelve individual patterns were obtained, with the first individual pattern being the most common among bacterial isolates and those used from GenBank. Access to a relatively large number of individual patterns within a group of bacterial isolates from the same geographic area is of utmost importance; it contributes to a deeper understanding of the genetic diversity and biological characteristics of bacteria and can be used as a tool for comparison with other strains, providing insight into the spread of resistant genes and resistance patterns. It also helps guide treatment and makes optimal clinical decisions to combat bacterial resistance and develop effective strategies to control it. Therefore, we reiterate the call for monitoring the escalating problem of bacterial resistance to antibiotics, which poses a significant challenge to the global healthcare system, necessitating precise monitoring and access to antibiotic-resistant bacterial isolates within healthcare facilities to identify individual patterns and effectively guide treatment and prevention.

The insights drawn from the analysis depicted in **Figure 5** of *staphylococcus aureus* isolates in this study unveil intriguing trends regarding their dispersion across various swab types and their resistance to methicillin. Appreciating the prevalence and distribution of *staphylococcus aureus*, especially the methicillin-resistant variants, holds significant importance for deploying efficient infection control protocols and devising treatment approaches. Accordingly, the distribution of *staphylococcus aureus* isolates across different types of swabs indicates varying colonization patterns. The majority of isolates were obtained from nasal swabs (64%), followed by skin swabs (28%), and a smaller proportion from environmental swabs (8%). This suggests that the nasal cavity serves as a significant reservoir for *staphylococcus aureus* colonization, which is consistent with previous research highlighting the nose as a primary site of colonization for this bacterium. Additionally, the presence of *staphylococcus aureus* in environmental swabs underscores the potential for environmental contamination and transmission, although it is observed at a lower frequency compared to nasal and skin colonization. Moreover, the analysis of methicillin resistance among the *staphylococcus aureus* isolates provides insights into the prevalence of antibiotic resistance within different colonization sites. MRSA is a major concern in healthcare settings due to its resistance to multiple antibiotics, posing challenges for treatment and infection control. In this study, the majority of MRSA isolates were found in nasal swabs (60%), followed by skin swabs (40%), while no MRSA was detected in environmental swabs. This distribution suggests that nasal colonization may play a significant role in the dissemination of MRSA, potentially serving as a reservoir for transmission within healthcare facilities and communities. The absence of MRSA in environmental swabs could indicate a lower likelihood of environmental contamination with methicillin-resistant strains in the studied settings. Furthermore, comparing the proportions of MRSA among *staphylococcus aureus* isolates from different swab types reveals important epidemiological trends. This emphasizes the importance of targeted surveillance and infection control measures focusing on nasal colonization to reduce the spread of MRSA.

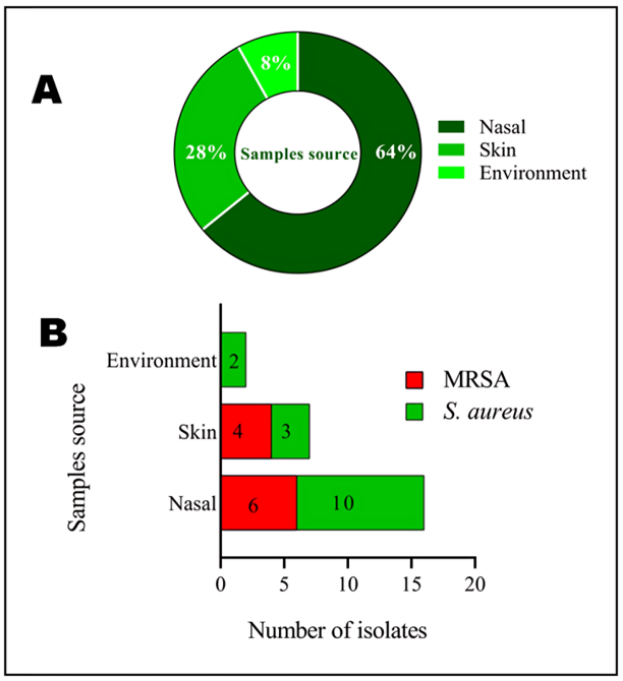

Figure 5. Representation of the distribution frequency of *staphylococcus aureus* isolates from various sources and the presence of MRSA isolates among the total isolates: (A) Pie chart illustrates the total number of isolation sources and the distribution of isolates among these sources & (B) Bar chart displaying the total number of isolates of MRSA and *staphylococcus aureus* from selected sources (Source: Authors' own elaboration)

Overall, these results highlight the complex dynamics of *staphylococcus aureus* colonization and methicillin resistance,

with nasal colonization playing a significant role in both the prevalence and dissemination of MRSA. Targeted interventions aimed at reducing nasal colonization and controlling the spread of MRSA is warranted to mitigate the impact of *staphylococcus aureus* infections in healthcare and community settings. Additionally, continued surveillance and research are essential for understanding the evolving epidemiology of *staphylococcus aureus* and informing effective prevention and treatment strategies.

CONCLUSION

The rising problem of antibiotic resistance poses a significant threat to global healthcare, driven by antibiotic misuse and environmental spread. Our study on *staphylococcus aureus* isolates from a Jordanian hospital highlights the need for effective monitoring and intervention. Using 16S rRNA gene amplification, we identified genetic diversity and methicillin resistance, particularly the mecA gene, in several isolates. The analysis revealed that nasal colonization is a major reservoir for MRSA, emphasizing the need for targeted infection control measures. These findings call for focused efforts to reduce nasal, skin, and other sources colonization and continuous surveillance to manage antibiotic resistance effectively and protect public health.

Author contributions: SA: conceptualization, methodology, project administration, data analysis, writing–original draft preparation, and supervision; **SMA:** writing–original draft preparation; **SMD:** revision of the manuscript and final approval; **NS:** conceptualization, methodology; **EHA:** Bacterial isolates collection and isolation. All authors have agreed with the results and conclusions.

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AI statement: The authors stated that no generative AI or AI-based tools were used in any part of the study, including data analysis, writing, or editing.

Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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