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Prevalence, genotyping, and molecular relatedness of methicillin-resistant *Staphylococcus aureus* isolated from tertiary care hospitals in Jeddah, Saudi Arabia

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Abstract

Methicillin-resistant ***Staphylococcus aureus*** (MRSA) is a major nosocomial pathogen causing severe morbidity and mortality in hospitals globally.Transmission of MRSA occurs within the healthcare sector as a nosocomial infection, primarily facilitated by healthcare workers or patients admitted to medical facilities. The objective of this study was to evaluate the genetic characterization and similarity of MRSA strains isolated from both inpatients and outpatients who visited various healthcare facilities in Jeddah, Saudi Arabia. A total of 200 MRSA strains were isolated from participants between March 2018 and June 2019. The recovered strains were characterized using both phenotypic and genotypic methods. All isolates (n=200) tested positive for the ***S. aureus*** 16S rRNA gene, with 92.5% also testing positive for the mecA gene, while 7.5% were identified as methicillin-susceptible. Furthermore, the typing and subtyping of the staphylococcal cassette chromosome mec (SCCmec) genetic element indicated that 61.6% of the MRSA strains were classified as type III (hospital-acquired), while 32.4% were identified as type IV and 6% remained of an unknown type. Subtyping of SCCmec type IV and the detection of the Panton-Valentine leukocidin (PVL) gene were also conducted. The genetic relatedness among MRSA isolates, assessed through Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR), revealed two primary clusters, with no discernible differentiation between outpatient and inpatient strains. Additionally, Pulsed-Field Gel Electrophoresis (PFGE) fingerprinting of the examined strains identified four major clusters. The first cluster comprised three groups (16 strains), isolated from patients with respiratory and soft tissue infections. The second cluster included two groups (12 strains), all recovered from patients with respiratory, soft tissue, and urinary tract infections (UTIs). The third and fourth clusters each contained one group (6 strains and 5 strains, respectively), all isolated from outpatients. In conclusion, Antimicrobial susceptibility testing showed significant resistance to ceftriaxone, ampicillin, and amoxicillin-clavulanic acid, with vancomycin and gentamicin being the most susceptible. Multiplex PCR identified all positive MRSA strains within hours. Most isolates were SCCmec type III and type IV. The PVL gene was found in all *S. aureus* isolates, especially in type IV and methicillin-sensitive strains, but not in type III. RAPD-PCR analysis revealed distinct profiles for outpatient and inpatient strains.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Genetic variability, Nosocomial infections, Pulsed-Field Gel Electrophoresis.

1. Introduction

Staphylococcus aureus (*S. aureus*) is recognized as one of the most prevalent opportunistic pathogens responsible for a diverse array of human diseases. These include superficial skin and soft tissue infections [1], as well as more severe systemic infections such as bloodstream infections [2, 3], osteoarticular infections, and pneumonia [4, 5]. Furthermore, *S. aureus* is implicated in toxin-mediated diseases, which arise when its toxins are released into the bloodstream or introduced into the gastrointestinal tract via contaminated food sources [6]. Methicillin-resistant *S. aureus* (MRSA) is primarily transmitted as a nosocomial infection within healthcare settings, facilitated by healthcare personnel, through direct contact among hospitalized patients, or via contaminated medical devices and dialysis equipment [7]. In non-healthcare environments, MRSA infections can occur through direct contact with contaminated surfaces, inadequate hygiene and sanitation practices, and overcrowded living conditions [8, 9].

MRSA has emerged as a significant pathogen due to the acquisition of the *mecA* and/or *mecC* genes, which encode a novel type of penicillin-binding protein known as PBP2a [10-12]. This protein enables the bacterium to continue synthesizing its cell wall even in the presence

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of high concentrations of β-lactam antibiotics. The *mecA* and *mecC* genes are located within the staphylococcal cassette chromosome *mec* (*SCCmec*) and are believed to have originated from other species of coagulase-negative staphylococci through horizontal gene transfer [9]. Hospital-associated MRSA (HA-MRSA) is predominantly found in healthcare settings, particularly among patients who have recently been hospitalized [13-15]. The majority of HA-MRSA cases occur in immunocompromised individuals or those at elevated risk, including patients with prolonged hospital stays, those in intensive care units (ICUs), individuals receiving extended antibiotic therapy, patients undergoing surgical procedures, or those in close contact with individuals previously exposed to MRSA [16].

Community-associated MRSA (CA-MRSA) has emerged as a significant public health concern within community populations, particularly among younger individuals [17-19]. A majority of CA-MRSA strains possess the gene responsible for encoding the Panton-Valentine leukocidin (*PVL*) toxin, which is known to induce leukocyte lysis and tissue necrosis [20-22]. The heightened virulence and widespread occurrence of CA-MRSA are largely attributed to the production of the *PVL* toxin. However, the identification of strains lacking the *PVL* gene indicates that additional virulence factors may also play a role in the pathogenicity and transmissibility of this organism [20, 23].

An analysis of 22,793 strains of *S. aureus* published in 2013 revealed a prevalence rate of MRSA of 35.6%. The study documented a prevalence of 6% in Dahran and 95% in Riyadh, with the highest levels of MRSA distribution observed in the Assir and Riyadh regions of Saudi Arabia. The results exhibited variability between the Makkah and Al Gouf regions, with percentages of 40% and 15%, respectively [24]. In a study conducted in hospitals in Jeddah, the incidence of MRSA infections was assessed among medical students and healthcare workers. The findings reveal a significantly elevated incidence of MRSA among healthcare workers, with a prevalence rate of 76%, in contrast to the control group of medical students [25]. These results underscore the necessity for further research aimed at addressing the high prevalence of MRSA infections. Consequently, this study employed SCCmec typing and subtyping, RAPD-PCR, and PFGE techniques to elucidate the genetic relationships among MRSA strains isolated from outpatient and inpatient clinics in Jeddah, Saudi Arabia.

2. Materials and Methods

2.1 Sample collection

A total of 200 clinical isolates of *S. aureus* strains were collected from prominent government hospitals in Jeddah, Kingdom of Saudi Arabia (KSA), between March 2018 and June 2019. The samples were obtained from outpatients diagnosed with UTIs, abscesses, soft tissue infections, and otitis media. In contrast, the strains collected from inpatients were derived from patients experiencing fever, UTIs, and soft tissue infections. According to the definition provided by the Centers for Disease Control and Prevention's Nosocomial Infections Surveillance System (NNIS), the collected samples included encompassed community-associated and HA isolates. We have implemented infection prevention and control measures during specimen handling to minimize contamination risks. This includes the use of personal protective equipment, practicing hand hygiene, and ensuring safe waste disposal. Our technicians are trained in Asepsis and the Aseptic Non-Touch Technique for collecting specimens such as blood cultures and wound swabs. Specimens were collected before patients start antibiotics, with priority given to those at risk of sepsis. Fresh materials are obtained with minimal contamination, such as removing slough from ulcers. This approach ensures that suitable materials are available for analysis and helps to reduce false negatives.

Participants in this study consisted of patients admitted to both outpatient and inpatient clinics at prominent government hospitals in Jeddah, diagnosed with UTIs, abscesses, soft tissue infections, or otitis media. Cases were selected based on the following criteria: the first-time identification of MRSA during a patient's hospitalization under surveillance; the detection of MRSA cases in the emergency department of patients who were subsequently admitted to a hospital under surveillance; and previously identified MRSA-positive patients who had a "For Information" record but lacked a "For Initial" record. The lack of informed consent was a criterion for exclusion.

2.2. Identification of recovered MRSA using standard microbiological techniques

All recovered strains were sub-cultured on mannitol salt agar media (BBL, United Kingdom) and incubated for 24 hours. The isolates that fermented mannitol were subsequently subjected to further examination utilizing Gram staining, catalase testing, DNase testing, and coagulase testing. To accurately identify MRSA strains in pure culture, the VITEK 2 system (BioMerieux SA, France) was employed in conjunction with standard microbiological techniques [26].

2.3. Susceptibility testing

The antibiotic susceptibility of isolated strains was assessed using the disk diffusion method, as outlined previously [26]. A total of eleven antibiotics, relevant to veterinary and/or human medicine, were evaluated: cefoxitin (30 µg), oxacillin (1 µg), vancomycin (30 µg), ceftriaxone (30 µg), ampicillin (10 µg), amoxicillin-clavulanic acid (25 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), erythromycin (15 µg), and chloramphenicol (30 µg) (Oxoid Limited, UK). The antibiogram profile of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates was generated using the VITEK2 automated system, following the manufacturer's instructions. Staphylococcus aureus ATCC 29213 was employed as a quality control reference. Results were interpreted following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [27].

2.4. Molecular typing

2.4.1. Molecular identification of recovered MRSA using multiplex-PCR

The molecular characteristics of MRSA strains isolated from both outpatient and inpatient clinics were analyzed using multiplex-PCR for the simultaneous detection of the 16S rRNA gene of *S. aureus*, the *mecA* gene, and the *PVL* genes. Three sets of primers were employed to amplify specific sequences of these genes [28]. The first primer pair, Staph756F and Staph750R, was designed to amplify a 756 base pair fragment specific to the *16S rRNA* gene of *S. aureus*. The second primer pair, *mecA*; F: GTG GAA

TTG GCC AAT ACA GG and *mecA*; R: TAG GTT CTG CAG TAC CGG AT, was utilized to amplify a 1399 base pair fragment specific to the *mecA* gene. The third primer pair, Luk-PV-1, and Luk-PV-2, was used to amplify a 433 base pair fragment specific to the *lukS/F-PV* genes.

2.4.2. Typing and subtyping of MRSA strains

The characterization of MRSA isolates concerning *SCCmec* genes was conducted utilizing specific and unique primer sets designed to target various types and subtypes of *SCCmec* genes, as detailed in Table 1. The *SCCmec* targets included types I, II, III, and V, as well as subtypes IVa, IVb, IVc, and IVd. Duplex primer sets were employed to detect type III and subtype IVb independently, with the primer set for type III targeting the *mecA* gene and the primer set for subtype IVb targeting the *16S rRNA* of *S. aureus*. The PCR was performed using a Biotech Prime thermocycler (UK) under the following conditions: an initial denaturation step of 1.5 minutes at 95°C, followed by 35 cycles consisting of denaturation for 1 minute at 95°C, annealing for 1 minute at 55°C, and extension for 2 minutes at 72°C. A final extension step was conducted for 1.5 minutes at 72°C. The resulting PCR products were analyzed via electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet transillumination.

2.4.3. Fingerprinting of recovered MRSA utilizing RAPD-PCR

The present study examines the genetic similarity between MRSA isolates obtained from outpatient and inpatient clinics, utilizing RAPD-PCR with short-size primers at a low annealing temperature. The fingerprinting of MRSA via RAPD-PCR was conducted following the methodologies described by Mehndiratta and Bhalla [29], employing primers EP007 (5'-AGC ACG CTG-3') and EP017 (5'-TAC ACC CGT CAA CAT TGA GG-3'). The resulting PCR products were subsequently separated by electrophoresis on a 1.5% agarose gel (Applichem, Germany, GmbH).

2.4.4. PFGE

The isolates were cultivated overnight at 37°C on tryptic soy agar plates. Subsequently, bacterial cells were suspended in 2.5 ml of PIV buffer and vortexed. An aliquot of 500 µl from each suspension was combined with 500 µl of 1.6% low melting point agarose and immediately distributed into the wells of plug molds, where they were allowed to solidify at 4°C for 30 minutes. The resulting plugs were then transferred into tubes containing 1 ml of 1X lysis buffer, which comprised 6 mM Tris-HCl (pH 7.4), 1 M NaCl, 10 mM EDTA (pH 7.5), 0.5% Brij 58, 0.2% deoxycholate, 0.5% sodium lauroyl sarcosine, 0.5 mg/ml lysozyme, and 10 mg/ml RNase A. These tubes were incubated overnight in a water bath at 37°C. Following this incubation, the lysis buffer was replaced with 1 ml of ESP buffer, consisting of 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 100 µg/ml Proteinase K, and 1% SDS (Sigma; St. Louis, MO, USA), and the mixture was incubated overnight in a water bath at 50°C. The bacterial plugs were washed four times with 5 ml of TE buffer for 30 minutes each wash at room temperature. Subsequently, plug slices measuring 3 × 5 mm were placed in a 1.5 ml microcentrifuge tube containing 200 µl of 1X restriction buffer (NE Buffer) along with 50 U of the SMAI restriction enzyme, and incubated overnight at room temperature. The plug slices were then washed for 30 minutes with 0.5X TBE buffer and subsequently inserted into the wells of a 1% agarose gel, which were overlaid with 1% low melting point agarose dissolved in 0.5X TBE. The gel was subjected to electrophoresis after solidification.

2.5. Statistical analysis

All calculations derived from the data collected in our

Table 1. MRSA *SCCmec* genes typing and subtyping primer sequences.

Primer name	Target gene	Primer sequence (5′- 3′)	Size of amplified fragment	Annealing temp.
SCCmec I F	*SCCmec I*	GCTTTAAAGAGTGTCGTTACAGG	613bp	55°C
SCCmec I R		GTTCTCTCATAGTATGACGTCC		
SCCmec II F	*SCCmec II*	CGTTGAAGATGATGAAGCG	398bp	55°C
SCCmec II R		CGAAATCAATGGTTAATGGACC		
SCCmec III F	*SCCmec III*	CCATATTGTGTACGATGCG	280bp	55°C
SCCmec III R		CCTTAGTTGTCGTAACAGATCG		
SCCmecIVa F	*SCCmecIVa*	GCCTTATTCGAAGAAACCG	776bp	55°C
SCCmecIVa R		CTACTCTTCTGAAAAGCGTCG		
SCCmecIVb F	*SCCmecIVb*	TCTGGAATTACTTCAGCTGC	493bp	55°C
SCCmecIVb R		AAACAATATTGCTCTCCCTC		
SCCmecIVc F	*SCCmecIVc*	ACAATATTTGTATTATCGGAGAGC	200bp	55°C
SCCmecIVc R		TTGGTATGAGGTATTGCTGG		
SCCmecIVd F	*SCCmecIVd*	CTCAAAATACGGACCCCAATACA	881bp	55°C
SCCmecIVd R		TGCTCCAGTAATTGCTAAAG		
SCCmec V F	*SCCmec V*	GAACATTGTTACTTAAATGAGCG	325bp	55°C
SCCmec V R		TGAAAGTTGTACCCTTGACACC		
mecA147 F	*mecA*	GTG AAG ATA TAC CAA GTG ATT	147bp	55°C
mecA147 R		ATG CGC TAT AGA TTG AAA GGA T		
Staph756F	*16S rRNA S. aureus*	AACTCTGTTATTAGGGAAGAAC	756 bp	55°C
Staph750R		CCACCTTCCTCCGGTTTGTCACC		

study were conducted utilizing the Statistical Package for the Social Sciences (SPSS) version 20.0.

3. Results

3.1. Prevalence of MRSA isolated from outpatients and inpatients attending various healthcare facilities in Jeddah, Saudi Arabia

A total of 200 MRSA strains were isolated from both inpatients and outpatients, comprising 105 males (52.4%) and 95 females (47.6%), from several prominent hospitals located in Jeddah, Kingdom of Saudi Arabia (KSA). The ages of the participants ranged from less than one year to 84 years; however, the majority fell within the age group of 16 to 56 years, accounting for 43.5% of the total. According to the data from our study, the highest frequency of MRSA strains was obtained from the respiratory tract, with 87 strains (43.5%), followed by skin infections (number and percentage to be provided), wounds (66 strains, 33.5%), urine (15 strains, 7.3%), eye swabs (11 strains, 5.4%), blood (9 strains, 4.7%), ear samples (5 strains, 2.2%), and other tissues (7 strains, 3.4%), as illustrated in Table 1. The isolated MRSA strains were confirmed using the VITEK 2 C15 automated system (BioMerieux®, France), and the results were consistent with those obtained from conventional biochemical tests based on their metabolic characteristics.

3.2. Antimicrobial sensitivity testing

A significant proportion of isolates demonstrated resistance to oxacillin, with over 85% (n=170) exhibiting resistant patterns. Furthermore, approximately 90% (n=180) of the isolates were found to be resistant to cefoxitin. In contrast, all isolates were susceptible to vancomycin. The majority of strains displayed high levels of resistance to ceftriaxone (86%, n=172), ampicillin (84%, n=168), and amoxicillin-clavulanic acid (60%, n=120). Additionally, resistance was observed in 100 strains (50%) to tetracycline, 90 strains (45%) to erythromycin, 80 strains (40%) to ciprofloxacin, and 40 strains (20%) to chloramphenicol. Notably, the lowest resistance rate was recorded for gentamicin, with only 8 strains (4%) exhibiting resistance, as illustrated in Figure 1.

Table 1. The prevalence of MRSA isolated from outpatients and inpatients at various healthcare facilities in Jeddah, Saudi Arabia.

	Variable	No # of strains	Percentage
Sex	Male	105	52.4%
	Female	95	47.6%
Age	≥ 1	17	8.5%
	2- 15	41	20.5%
	16-56	87	43.5%
	57-80	48	24%
	≤ 81	7	3.5%
Sample site	Respiratory tract	87	43.5%
	Skin and wound	66	33.5%
	Urine	15	7.3%
	Blood	9	4.7%
	Eye infection	11	5.4%
	Ear infection	5	2.2%
	Other tissues	7	3.4%

3.3. Molecular detection of MRSA strains using multiplex primers

Three sets of primer pairs were utilized for multiplex-PCR to assess the molecular characteristics of the recovered MRSA. The multiplex primers included a specific derivative of the *16S rRNA* gene for *S. aureus* (756 bp), the *mecA* gene (1319 bp), and the *PVL* gene (433 bp). The results indicated that all isolated strains, identified through standard microbiological techniques, were positive for the presence of the *16S rRNA* specific to *S. aureus*. Furthermore, all phenotypically positive strains for oxacillin and methicillin also tested positive for the *mecA* gene. Additionally, all strains recovered from the outpatient clinic were positive for the *PVL* genes, as illustrated in Figure 2.

3.4. *SCCmec* typing of MRSA isolates

The molecular typing and subtyping of the MRSA isolates are presented in Table 2. A total of 185 MRSA isolates were categorized into different types of *SCCmec*. The

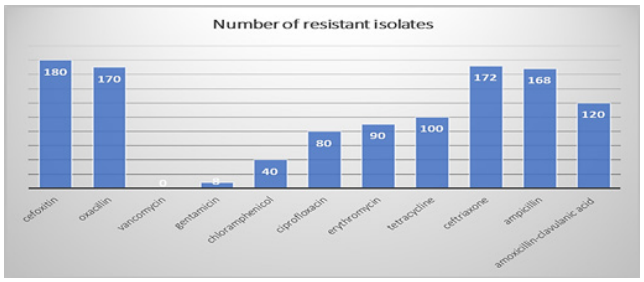

Fig. 1. Antibiotic resistance patterns against isolated S. aureus strains.

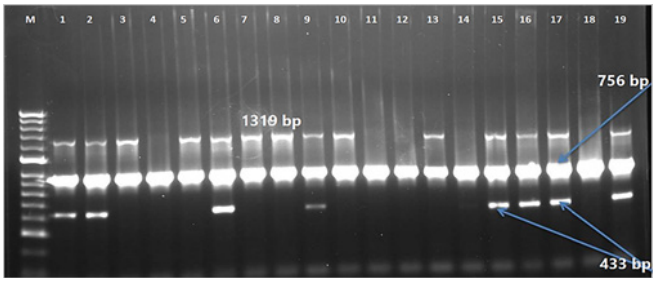

Fig. 2. Multiplex PCR showed amplification of 756 bp fragments of 16S rRNA from S. aureus, 1,319 bp fragments of the mecA gene, and 433 bp fragments of the PVL gene, lane M is 100 base pail ladders.

Table 2. Molecular characteristics and typing of isolated MRSA strains.

No# of mec gene-positive isolates	SCC mec type	Type IV subtyping	PVL positive
0	I	-	-
0	II	-	-
114/185	III	-	-
33/60	IV	IVa	28/33
13/60		IVb	10/13
2/60		IVc	2/2
12/60		IVd	9/12
0	V	-	-
11/185	Non typing		11/11
15/200	MSSA	-	4/15

results showed the predominant *SCCmec* types among the MRSA isolates were type III (61.6%), followed by type IV (32.4%), while the remaining isolates (6%) were reported as non-typeable. Furthermore, the type IV isolates were subtyped into type IVa (33, 55%), type IVb (13, 21.6%), type IVc (2, 3.4%), and type IVd (12, 20%). Of note, none of the MRSA isolates harbored *SCCmec* of type I, II, or V. However, four MSSA strains harbored the *PVL* gene. Furthermore, the *PVL* gene was detected in 28 isolates of type IVa, followed by 10 isolates of type IVb, 9 isolates of type IVd, and 2 isolates of type IVc. Moreover, all of the non-typeable isolates harbored the *PVL* gene.

3.5. Fingerprinting and the similarity of recovered MRSA

3.5.1. Genetic similarity among recovered MRSA utilizing RAPD-PCR

The RAPD-PCR analysis conducted on selected strains from outpatient and inpatient clinics, utilizing EP007 and EP017 primers, revealed distinct RAPD profile fingerprinting patterns. These patterns were characterized by the presence, size, and intensity of the amplified fragments, as illustrated in Figure 3. The amplification reactions produced several bands that were similar in molecular weight (shared bands) across all strains, indicating a high degree of similarity among the strains. However, the intensity of these shared bands varied.

The phylogenetic tree constructed using the similarity index among the strains identified two primary clusters, as illustrated in Figure 4. The first cluster comprises three groups of eight strains, all of which were isolated from both inpatients and outpatients experiencing respiratory and skin infections, without distinguishing between the strains from these two categories. Additionally, the second cluster consists of three groups of ten strains, also derived from inpatients and outpatients suffering from respiratory infections, skin infections, UTIs, and ear infections, again without differentiation between the outpatient and inpatient strains, as depicted in Figure 4.

3.5.2. Fingerprinting of the recovered MRSA through chromosomal DNA analysis utilizing PFGE

The results presented in Figure 5 indicate that 40 distinct chromosomal digestion patterns were identified from the selected isolates of MRSA using the *SmaI* restriction enzyme. This enzyme cleaved the chromosomal DNA of the examined *S. aureus* into 13 to 14 fragments of varying molecular weights. Notably, identical chromosomal restriction patterns were observed among strains isolated from inpatients with respiratory and soft tissue infections, suggesting that these infections were caused by HA strains. Furthermore, the restriction fragment patterns generated by the *SmaI* enzyme revealed several bands of similar molecular weight (referred to as shared bands) across all examined strains, indicating a significant degree of genetic similarity among them, as they were all classified as MRSA. However, the intensity of these shared bands varied, reflecting differences in the concentration of DNA templates present in each sample.

The fingerprinting of the examined strains using pulsed-field gel electrophoresis revealed four major clusters based on the degree of similarity among the strains. The first cluster comprised three groups, totaling 16 strains, all of which were identified as hospital-acquired strains. These strains were isolated from patients suffering from respiratory and soft tissue infections. Notably, some strains within this cluster exhibited identical characteristics, with a similarity index of up to 100%, indicating the presence of the same strain within the hospitals. The second cluster included two groups, consisting of 12 strains, all recovered from patients with respiratory infections, soft tissue infections, and UTIs; some of these strains also demonstrated identical chromosomal patterns. The third and fourth clusters each contained one group, comprising six strains and five strains, respectively, all of which were isolated from outpatient clinics and associated with soft tissue infections, ear infections, and eye infections, as illustrated in Figure 5.

4. Discussion

MRSA is recognized as one of the most significant

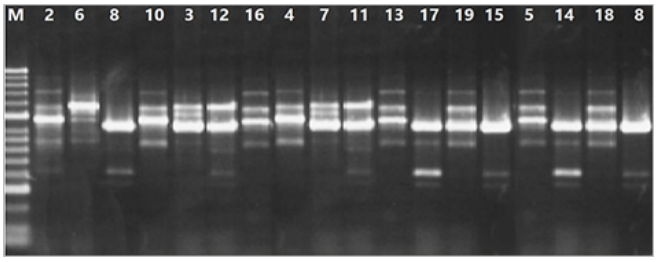

Fig. 3. Agarose gel electrophoresis demonstrates the results of RAPD-PCR for MRSA utilizing the EP007 and EP017 primers. Lane M contains a 100-base pair ladder.

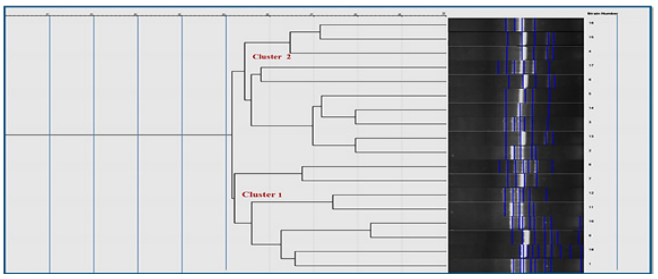

Fig. 4. The phylogenetic tree illustrates the genetic relationships among MRSA utilizing two primers: EP007 and PE017. Two primary clusters have been identified; the first cluster comprises three groups of eight strains, whereas the second cluster consists of three groups of ten strains.

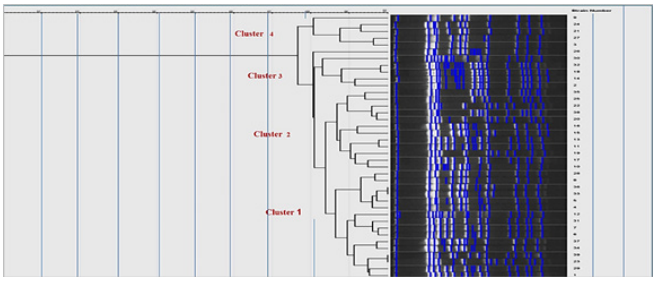

Fig. 5. The dendrogram generated from the examined strains using PFGE reveals the presence of four distinct clusters, which are categorized based on the degree of similarity among the tested strains. The first cluster comprises three groups, totaling 16 strains; the second cluster consists of two groups, encompassing 12 strains; the third cluster is represented by a single group containing 6 strains; and the fourth cluster includes one group with 5 strains.

agents of nosocomial infections globally. The interplay between bacterial infections and antibiotic resistance has resulted in increased morbidity and mortality rates, as well as heightened treatment costs [30-32]. MRSA strains are responsible for both nosocomial and community-acquired infections, which pose challenges in treatment due to their pronounced antibiotic resistance [33-35]. The acquisition of the *mecA* or *mecC* gene leads to altering the results in the modification of PBP2a, genotyping MRSA isolates has PBP2a [36-38]. Consequently, genotyping of essential diagnostic methods emerged as a crucial strain tool for identifying the specific strains involved evaluate the rate of for assessing the rates. In total, 200 strains of *S. aureus* were isolated from both male and female patients, encompassing a broad age range from less than one year to 80 years. Among these strains, 52.4% were derived from male patients, while 47.6% were from female patients. This gender distribution aligns with findings from several studies conducted in Saudi Arabia. For instance, a study by Kashyap et al. [39] reported that 63% of the respondents were male and 37% were female. Another investigation revealed a similar trend, with 73% of the affected individuals being male and 27% female. The highest frequency of isolation was noted in the age group of 16 to 56 years, which accounted for 43.5% of the cases [40]. Previous research has also indicated age-related variations in the frequencies of MRSA isolation [41, 42].

Among the MRSA isolated strains, the highest prevalence was observed in skin and soft tissue infections (40%), followed by respiratory tract infections (31.11%), UTIs (11.11%), bloodstream infections (6.67%), ocular swabs (4.45%), optic infections (3.33%), and infections in other tissues (3.33%). A study by Ahmed et al. [30] at a tertiary hospital in Saudi Arabia found that in 2019, *S. aureus* strains were distributed as follows: 33.3% from pus swabs, 24.5% from blood samples, and 17.24% from UTIs. In 2020, strains from pus swabs increased to 47%, while blood and urine samples accounted for 23% and 12%, respectively. Prior to the COVID-19 pandemic, reports showed varying prevalence rates of MRSA strains in Makkah, Madinah, and Riyadh [43, 44].

The antimicrobial susceptibility testing revealed significant resistance patterns, with high frequencies observed against ceftriaxone (86%), ampicillin (84%), and amoxicillin-clavulanic acid (60%). Intermediate resistance frequencies were identified for tetracycline (50%) and erythromycin (45%), while lower resistance frequencies were noted for ciprofloxacin (40%) and chloramphenicol (20%). Importantly, the highest susceptibility rates were recorded for vancomycin (100%) and gentamicin (96%). The high susceptibility of gentamicin suggests its appropriateness as a treatment option for infections caused by MRSA. Aljeldah et al. [45] conducted an analysis of antibiotic susceptibility data pertaining to MRSA in hospitals located in Northeastern Saudi Arabia. The findings indicated that MRSA isolates demonstrated a higher level of antibiotic resistance. Specifically, all MRSA isolates assessed exhibited resistance to cefoxitin, oxacillin, and penicillin. Notably, none of the hospitals reported the presence of vancomycin-resistant MRSA strains. In 2024, Almutairi and colleagues [46] studied MRSA antibiotic susceptibility at a Saudi maternity and children's hospital. All MRSA isolates were susceptible to vancomycin and linezolid, and 85% (51 out of 60) were sensitive to trimethoprim/sulfamethoxazole.

The multiplex PCR demonstrated the capability to detect all positive MRSA strains within a few hours, achieving 100% sensitivity and specificity. These results corroborate the findings of Moussa et al. [47] and Moussa and Shible [48]. The *mecA* positivity of MRSA strains (n=185) was assessed using specific primers that target various types and subtypes of SCCmec genes, as detailed in Table 2. The majority of MRSA isolates in this investigation were classified as *SCCmec* type III (HA-MRSA, 61.6%), followed by type IV (CA-MRSA, 32.4%), and those of unknown type (6%). These findings are consistent with expectations, given that all isolates were obtained from medical centers. Furthermore, our results align with other studies indicating that *SCCmec* type III strains are the most prevalent in the Middle Eastern region, including countries such as Saudi Arabia [49], Kuwait [50], and Iran [51]. Additionally, the subtyping of *SCCmec* type IV revealed a predominance of subtype IVa (55%), followed by IVb (21.6%), IVd (20%), and IVc (3.4%). The detection of CA-MRSA strains among the tested isolates can be attributed to the collection of samples from both outpatients and inpatients. Notably, none of the isolates were classified as type I, II, or V. The presence of the *PVL* gene was identified in all 200 *S. aureus* isolates. Specifically, the *PVL* gene was detected in *SCCmec* type IV isolates (49/60; 81.6%), non-typeable isolates (11/11; 100%), and methicillin-sensitive *S. aureus* (MSSA) (4/15; 26.6%). However, none of the *SCCmec* type III isolates contained the *PVL* gene. These findings support the hypothesis that the presence of the *PVL* gene is most prevalent among CA-MRSA strains [52]. Bonesso et al. [53] examined CA-MRSA in 127 outpatients with non-outbreak skin infections at a Botucatu, São Paulo, Brazil dermatology clinic. They isolated 66 strains of *S aureus*, accounting for 56.9% of cases. The *mecA* gene was found in seven strains (10.6%), with SCCmec types Ia (three strains), II (three strains), and IV (three strains). The PVL gene was present in 10 MSSA strains (15.1%).

The RAPD-PCR analysis conducted on selected strains from both outpatient and inpatient clinics, utilizing EP007 and EP017 primers, revealed distinct RAPD profiles, as illustrated in Figure 2. The amplification reactions produced several bands of comparable molecular weight (shared bands) across all strains, suggesting a high degree of similarity among them. However, the presence of these shared bands was insufficient to differentiate between outpatient and inpatient strains, primarily due to the limited number of bands generated and the technique's inability to provide a comprehensive representation of the entire genome of the recovered isolates or to accurately assess the degree of genetic similarity among the strains. Additionally, the variability observed in the RAPD profiles upon repeated reactions further underscores the lower specificity of this method in evaluating the genetic relationships among MRSA strains.

Due to its discriminative capabilities in diagnosing MRSA and its applicability in epidemiological studies, PFGE continues to be regarded as the gold standard [54]. The PFGE fingerprinting of isolated strains, based on the degree of similarity among the examined strains, revealed four major clusters, which suggest a common ancestor and a close genetic relationship among these strains. Furthermore, the PFGE fingerprints exhibited sufficient similarity to categorize the strains into four distinct clusters, indica-

ting a significant degree of genetic homogeneity. Notably, the majority of the examined strains displayed identical patterns with a high degree of similarity, particularly those isolated from hospitalized patients with MRSA, which implies the prevalence of hospital-acquired infections. This finding suggests that the transmission of MRSA within hospital settings is more likely to occur through direct contact with infected patients rather than through environmental sources. Environmental cleaning is crucial for reducing MRSA sources and preventing its spread in healthcare settings [53]. MRSA can persist on surfaces after standard cleaning [55]. Enhanced cleaning methods, such as black-light indicators and proper disinfectant application, effectively reduce MRSA colonization and transmission risk from previously occupied rooms [55].

A total of three primary clusters of hospital-acquired strains have been identified. The first cluster predominantly comprises MRSA isolated from soft tissue and skin infections. The second cluster is primarily associated with respiratory infections, while the third cluster is mainly linked to UTIs and other minor infections. This distribution is likely attributable to the adaptation of each bacterial cluster to its respective environment: the first cluster is adapted to the skin, the second to the respiratory system, and the third to the urinary tract. In outpatient samples, two predominant clusters of strains were identified. The first cluster comprises the majority of strains associated with soft tissue infections, while the second cluster is characterized by MRSA strains obtained from UTIs.

This study evaluated the prevalence of MRSA in clinical samples obtained from hospitals in Jeddah, Saudi Arabia as an indicator for assessing the effectiveness of infection prevention and control measures. A low prevalence of MRSA, with susceptibility to commonly used antimicrobials, was detected, indicating well-implemented infection control measures at Jeddah hospitals. However, the presence of some MRSA strains with varying degrees of reduced susceptibility to certain antibiotics should alert hospital administrators to strengthen the appropriate use of antibiotics in accordance with local hospital policy to minimize selective drug pressure on *S. aureus* strains and reduce exposure to risk factors by continuing to implement proper infection control policies. Control of the highly adaptable MRSA organism requires continuous teamwork among all healthcare workers. Further studies on MRSA prevalence in various regions of Saudi Arabia are necessary, along with comparing results to assess the burden of MRSA disease among the Saudi population and improve overall infection control measures to limit the spread of such deadly organisms.

To enhance our understanding of the dynamics of MRSA transmission in outpatient settings, additional research is warranted. By analyzing electronic healthcare records that encompass multiple routinely collected isolates per patient, phenotypic antibiograms, and data on hospitalization and antibiotic consumption, we can examine the diversity of *S. aureus* antimicrobial resistance across the major hospitals in Jeddah over the past decade.

The highest prevalence of isolated MRSA strains occurred in skin and soft tissue infections, followed by respiratory tract infections, UTIs, bloodstream infections, ocular swabs, optic infections, and other tissue infections. Antimicrobial susceptibility testing showed significant resistance to ceftriaxone, ampicillin, and amoxicillin-clavulanic acid, while vancomycin and gentamicin had the highest susceptibility rates. Multiplex PCR detected all positive MRSA strains within hours, achieving 100% sensitivity and specificity. Most MRSA isolates were SCCmec type III (HA-MRSA), followed by type IV (CA-MRSA). The PVL gene was found in all *S. aureus* isolates, especially in SCCmec type IV and methicillin-sensitive strains, but absent in SCCmec type III isolates. RAPD-PCR analysis revealed distinct profiles among strains from outpatient and inpatient clinics, indicating high similarity.

Limitations of the study

This study recognizes PFGE as a valuable method for typing and subtyping MRSA, however some limitations are found. Sampling biases may restrict the generalizability of the data due to unclear hospital caseloads, so the findings should not be extrapolated nationally. Additionally, the differentiation between HA-MRSA and CA-MRSA is unclear, which complicates identifying infection sites. Key information, such as hospital stay duration, residence location, antibiotic history, and livestock exposure, was not collected, and there was no molecular characterization of antimicrobial resistance.

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Conflicts of interest

The authors declare no conflicts of interest.

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