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Molecular differentiation of coagulase-positive staphylococcal isolates carrying *mec*A- and PVL-encoding genes among healthy males

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ABSTRACT

Aims: This study was aimed to monitor the asymptomatic carriage of coagulase-positive staphylococcal bacteria among university male students and detect the prevalence of virulence marker genes that encode methicillin resistance (*mec*A) and Panton-Valentine leukocidin (PVL) toxin among the isolates.
Methodology and results: Single nasal swaps were collected from 144 participating students who resided at four different locations within Al-Madinah city. A total of 112 Gram-positive staphylococcal isolates were recovered from the 144 participants (carriage rate of 77.8%). Coagulase-positive staphylococci were differentiated using duplex PCR amplification of the 16S rRNA and *nuc* genes and accounted for 30 isolates (carriage rate of 20.8%). These isolates were most prevalent in the northern and southern parts of Al-Madinah city, while the lowest numbers of isolates were detected in students of the eastern part. Coagulase-positive isolates were further phenotypically characterized for methicillin resistance by the disc diffusion method. Uniplex PCR assays were conducted to screen for *mec*A- and PVL toxin-encoding genes. The *mec*A gene was amplified from all 15 (50%) methicillin-resistant coagulase-positive isolates, while the PVL toxin-encoding gene was detected in 19 isolates (63.3%), 10 (33.3%) of which contained the *mec*A gene. Lastly, PCR amplification of the *NRPS* gene from coagulase-positive isolates revealed the absence of *Staphylococcus argenteus,* the recently discovered genetically divergent lineage of *Staphylococcus aureus*.
Conclusion, significance and impact of study: An elevated prevalence of coagulase-positive isolates harboring *mec*A and PVL virulence genes was observed compared with previous investigations. This poses a potential threat if they spread among the population, resulting in outbreaks of community-acquired infections.

Keywords: Coagulase-positive staphylococci, methicillin resistance, PCR, university male students, virulence marker genes

INTRODUCTION

Staphylococcus spp. are categorized based on their coagulase enzyme activity into coagulase-positive staphylococci, comprising *S. aureus* and its recently defined genetically divergent lineage *S. argenteus*, and coagulase-negative staphylococci, including all remaining species of the genus *Staphylococcus* (Tong *et al*., 2015; Argemi *et al*., 2019).

Many species of the genus *Staphylococcus* are typically found among the microflora of the skin and upper respiratory tract (URT). Nevertheless, they are also known to be opportunistic colonizers and may turn into serious pathogenic microorganisms due to several factors, such as perturbation (e.g., disease, wound, medication), evolution of virulence factors, immunodeficiency, and aging. Opportunistic staphylococci have been linked to a wide list of human infections, including upper and lower respiratory tract infections, wound infections, nosocomial infections, food poisoning and community-acquired illnesses. This increase in staphylococci-related infections is primarily due to the rise in the number of multidrug-resistant strains in addition to the growing incidence of immunocompromised patients (Chen *et al*., 2018; Lourtet-Hascoët *et al*., 2018; Parlet *et al*., 2019).

Traditional microbiological analysis, which involves a wide variety of selective media and biochemical tests followed by antibiotic sensitivity assays, is still considered the standard protocol for confirming the identity of *Staphylococcus* spp. However, they tend to be laborious and time-consuming (Frickmann *et al*., 2017; Tunsjø *et*

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al., 2017). Molecular techniques based mainly on PCR assays were developed as an alternative to increase sensitivity and reduce detection time. The conserved region within the bacterial 16S rRNA genome was described and later used in developing PCR primers for fast detection of *Staphylococcus* species 16S rDNA in specimens (McClure *et al*., 2006). Furthermore, the thermonuclease (*nuc*) gene was reported as a species-specific target for the detection of *S*. *aureus* (Zhang *et al*., 2004). Since then, several reports have successfully applied duplex PCR to direct *Staphylococcus* genus-specific 16S rRNA and *nuc* gene amplification in the differentiation of coagulase-negative staphylococci from coagulase-positive staphylococci (McClure *et al*., 2017; McClure *et al*., 2020; Teixeira *et al*., 2021).

The universally reported *S. argenteus* is phenotypically very similar to *S. aureus* to the extent that traditional biochemical methods used in diagnosis and the 16S rRNA gene sequence fail to distinguish them separately (Dupieux *et al*., 2015). Although all reported *S*. *argenteus* isolates grew white colonies due to the lack of staphyloxanthin (carotenoid pigment) biosynthesis genes that are located in the *crtOPQMN* operon (Tong *et al*., 2013), inhibition of *crtOPQMN* operon expression could sometimes occur in response to intracellular factors, indole derivatives, or interactions with other species, causing the appearance of a nonpigmented phenotype for some *S. aureus* isolates (Antonic *et al*., 2013; Lee *et al*., 2013; Ding *et al.*, 2014). This adds more difficulties in distinguishing *S. argenteus* from *S. aureus*. Recently, a simple PCR assay based on the length of a nonribosomal peptide synthetase (*NRPS*) gene sequence was developed and found to be responsible for their speciation (Zhang *et al*., 2016; Aung *et al*., 2019).

Pathogenic *Staphylococcus* spp. produces many essential virulence factors, including methicillin resistance and Panton-Valentine leukocidin (PVL) pore-forming toxin (Jenul and Horswill, 2018). Resistance to methicillin is correlated with *mecA*. This gene is found on the mobile genetic staphylococcal cassette chromosome element, usually acquired through horizontal transfer. Methicillin resistance occurs by producing a protein that modifies the antibiotic binding sites on the bacterial cell wall, which decreases its affinity toward β-lactams (Hiramatsu *et al*., 2013). On the other hand, the PVL-associated genes are two cotranscribed genes (*lukS* and *F-PV*) responsible for producing a toxin involved in deep skin, soft tissue, and necrotizing pneumonia infections (Lina *et al*., 1999; Okolie *et al*., 2013). These toxin-producing genes were found to be easily transmitted between staphylococcal strains by bacteriophages (Oliveira *et al*., 2019). Methicillin resistance and PVL have been strongly associated with community-acquired staphylococcal isolates (Shore *et al*., 2014; Karmakar *et al*., 2018). Therefore, the detection and identification of *mecA* and PVL gene distribution among *Staphylococcus* spp. samples is of important concern.

The nasopharynx of most humans is colonized with staphylococci without developing clinical illness. However, some of these staphylococci species have been noted as important opportunistic pathogens and their colonization is considered a risk factor for infection. Hence, the present study aimed to reveal the distribution of coagulase-positive *Staphylococcus* spp. among Taibah University male students using a PCR-based assay and link their prevalence to the geographical location of the students within Al-Madinah districts. Explore the presence and association of PVL and *mecA* virulence-encoding genes within the collected isolates.

MATERIALS AND METHODS

Study population

Sample collection took place during the autumn of 2019 over one month (from the beginning until the end of October). The study included a set of 144 undergraduate male university students (age group 19-21 years). This total number of undergraduate students was selected from the four departments (Biology, Chemistry, Physics and Mathematics) of the College of Science at Taibah University (the largest university in Al-Madinah city). All 144 students under study were Saudi nationals who resided at different geographic places within the city of Al-Madinah (Table 1 and Figure 1). Based on a preformed questionnaire used to collect the health-related information of the participants selected for this study, students showing respiratory infection symptoms or recovering from one and those receiving antibiotic treatment were excluded from the sampling.

Sample collection and isolation

A single nasal specimen was collected from each participant using a sterile swab. Specimens were labeled with the relevant information regarding each participant, including resident location. Specimens were immediately streaked after collection on nutrient and mannitol salt agar growth medium and then incubated at 37 °C overnight. Following incubation, traditional microbiological identification was conducted by catalase and coagulase tests, cell morphology using Gram staining and culture plate examination for colony characteristics on mannitol salt agar (Wertheim *et al*., 2001). The identified bacterial

Table 1: Geographical distribution of the selected study population within Al-Madinah city.

Number of students	Geographic position	Percentage (%)
31	North of Al-Madinah	21.5
39	South of Al-Madinah	27
43	East of Al-Madinah	30
31	West of Al-Madinah	21.5

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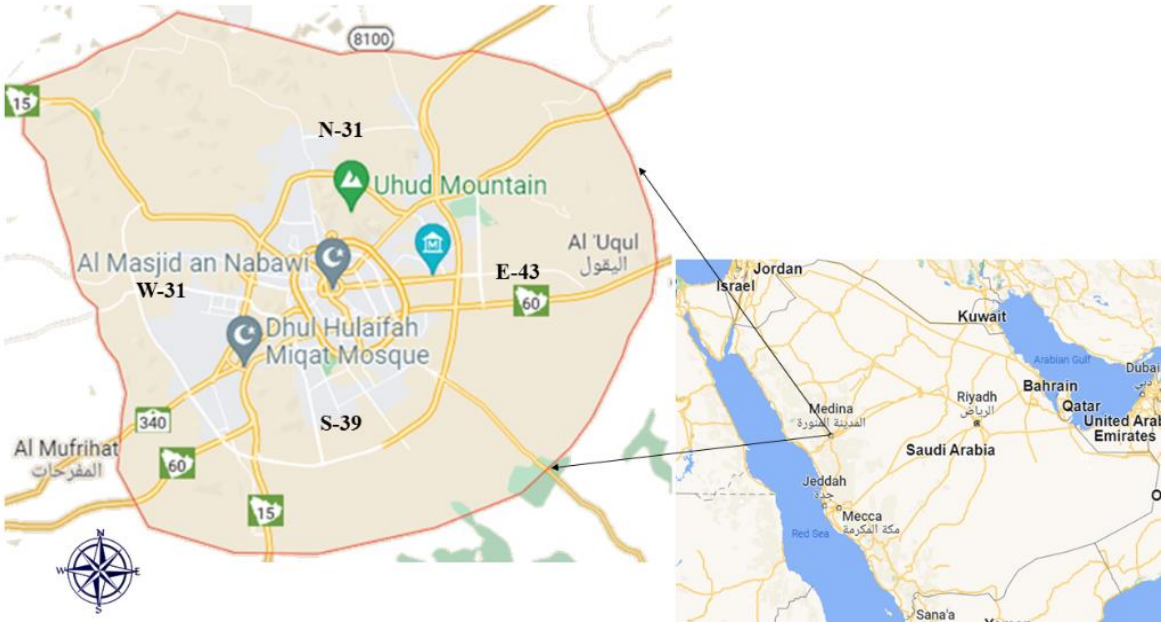

Figure 1: Google map view of Al-Madinah city, KSA, marked with the geographic locations and the number of participating students from each location (Google Earth, image Landsat/Copernicus, Data SIO, NOAA, U.S. Navy, NGA, GEBCO, Map Data © 2022 AND).

Table 2: PCR primers used in the duplex and conventional PCRs for the detection of 16S rRNA, *nuc*, *mec*A, *PVL* and *NRPS* genes.

Target gene	Primer	Oligonucleotide sequences (5'-3')	Amplicon size (bp)	Reference
16S rRNA	*Staph*-756 F	5'-AAC TCT GTT ATT AGG GAA GAA CA-3'	756	McClure *et al*. (2006)
	Staph-750 R	5'-CCA CCT TCC TCC GGT TTG TCA CC-3'		
nuclease	*nuc*-F	5'-GCG ATT GAT GGT GAT ACG GTT-3'	279	Zhang *et al*. (2004)
	nuc-R	5'-AGC CAA GCC TTG ACG AAC TAA AGC-3'		
*mec*A	*mec*A-F	5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3'	533	McClure *et al*. (2006)
	*mec*A-R	5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3'		
PVL	*Luk-PV*-1	5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3'	433	McClure *et al*. (2006)
	Luk-PV-2	5'-GCATCAAGTGTATTGGATAGCAAAAGC-3'		
NRPS	*NRPS*-F	5'-TTG ARW CGA CAT TAC CAG T-3'	340*	Zhang *et al*. (2016)
	NRPS-R	5'-ATW RCR TAC ATY TCR TTA TC-3'	160**	

*Where the template was from *S. argenteus*; **where the template was from *S. aureus*.

colonies were further subcultured on Luria-Bertani (LB) agar for further analysis using molecular approaches.

Molecular differentiation of staphylococci using duplex PCR

Genomic DNA was extracted by the boiling method (Rollo *et al*., 1990) as follows: two bacterial colonies from an overnight culture were picked up and suspended in 200 µL of sterile distilled water by vortexing and then boiled for 10 min. After boiling, the mixture was centrifuged for 3 min at 1000 rpm and the supernatant was collected for use in the subsequent PCRs. A duplex PCR assay to separate coagulase-positive staphylococci from coagulase-negative staphylococci was performed using two pairs of PCR primers (Table 2) targeting the 16S rRNA (*Staphylococcus* genus-specific conserved sequence) and the thermonuclease *nuc* (*S. aureus* species complex-specific gene). The duplex PCR was performed with an automated DNA thermal cycler using a 2 µL aliquot of extracted DNA supernatant in a volume of 25 µL along with Go*Taq* DNA Polymerase PCR master mix (Promega, USA) containing 50 mM KCl, 20 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1.25 units of the Go*Taq* polymerase 10 pmol of the 16S rRNA primers and 5 pmol for *nuc* primers. The amplification cycles were as follows: a primary denaturation cycle at 94 °C for 5 min; 15 cycles at 94 °C for 45 sec (denature); 55 °C for 45 sec

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(annealing); and 72 °C for 1 min (extension); then 20 cycles at 94 °C for 1 min; 50 °C for 1 min; and 72 °C for 2 min before a final extension step at 72 °C for 10 min.

Antibiotic sensitivity testing

Methicillin resistance was determined *in vitro* for coagulase-positive isolates using the Kirby-Bauer assay with 1 µg oxacillin discs (Oxoid LTD, UK). The results were inferred to correspond to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017).

Molecular screening for virulence-encoding genes

Amplification of both *mec*A- and PVL-encoding genes was performed with *mec*A- and *lukS*/*F-PV*-specific primers (Table 2) using the previously mentioned PCR master mix. Amplification of *mec*A was conducted for 3 min at 95 °C (initial denaturation), followed by 30 amplification cycles of 94 °C for 1 min, 55 °C for 45 sec and 72 °C for 1 min, with a final extension cycle at 72 °C for 7 min. Amplification of PVL was performed under the same initial denaturation cycle, followed by 35 amplification cycles of 94 °C for 1 min, 50 °C for 45 sec, and 72 °C for 75 sec, with a final extension at 72 °C for 10 min.

Nonribosomal peptide synthetase amplification

To validate or reject the presence of the newly recovered divergent lineage *S*. *argenteus* among the supposed *S*. *aureus* isolates, PCR amplification of the nonribosomal peptide synthetase (*NRPS*) biosynthetic gene cluster using the *NRPS* primer pair (Table 2) was performed. The PCR amplification of the *NRPS* gene fragment was executed as follows: an initial cycle of 94 °C for 4 min, followed by 35 cycles of 94 °C for 45 sec, 53 °C for 45 sec and 72 °C for 1 min, followed by 72 °C for 10 min (final extension cycle). The amplified PCR products were separated on a 1.5% (w/v) agarose gel, stained with ethidium bromide (0.5 µg/mL) and then visualized and photographed in a gel documentation system. The sizes of the resulting PCR fragments were determined using a 100-bp DNA ladder (Invitrogen, USA).

RESULTS

Characterization of the isolates

A total of 112 bacterial isolates that showed catalase-positive activity and appeared as Gram-positive cocci under a microscope were collected from all 144 participants. The proportion of these Gram-positive *Staphylococcus* bacterial isolates in relation to the total number of swabs gave an overall carriage rate of 77.8%.

All 112 isolates were categorized according to their culture characteristics on mannitol salt agar and the duplex PCR method into two distinct groups (coagulase-positive and coagulase-negative staphylococci). A total of 30 coagulase-positive bacterial isolates showing yellow- and/or white-colored colonies encircled by yellow zones were detected on mannitol salt agar plates, while the remaining 82 were defined as mannitol non fermenter coagulase-negative staphylococci isolates producing pink-red colonies enclosed by red to purple zones.

When the 112 staphylococcal isolates were analyzed using duplex PCR, the results revealed that amplification of both 16S rRNA and the *nuc* genes was obtained from the 30 presumed coagulase-positive isolates only. The remaining 82 coagulase-negative staphylococci showed PCR amplification of the 16S rRNA gene only (Figure 2). Neither the staphylococci-specific 16S rRNA gene nor the

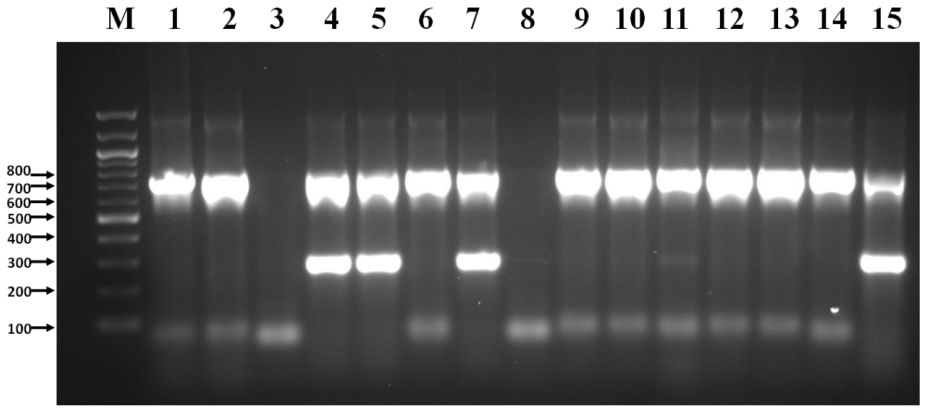

Figure 2: Duplex PCR assay to differentiate between coagulase-positive and coagulase-negative staphylococci isolates. Lanes 1, 2, 6 and 9-13 show the duplex PCR profile of representative coagulase-negative staphylococci isolates containing staphylococci-specific 16S rRNA genes without the *S. aureus*-specific *nuc* gene. Lanes 4, 5 and 7 are representative coagulase-positive staphylococci isolates containing both staphylococci-specific 16S rRNA genes and *S. aureus*-specific *nuc* genes. Lanes 3 and 8 negative control reference strain *Pseudomonas aeruginosa* ATCC 9027 and *Bacillus cereus* ATCC 10876. Lanes 14 and 15 are positive control reference strains *Staphylococcus epidermidis* ATCC 12228 and *Staphylococcus aureus* ATCC 6538. Lane M, DNA marker (100 bp ladder).

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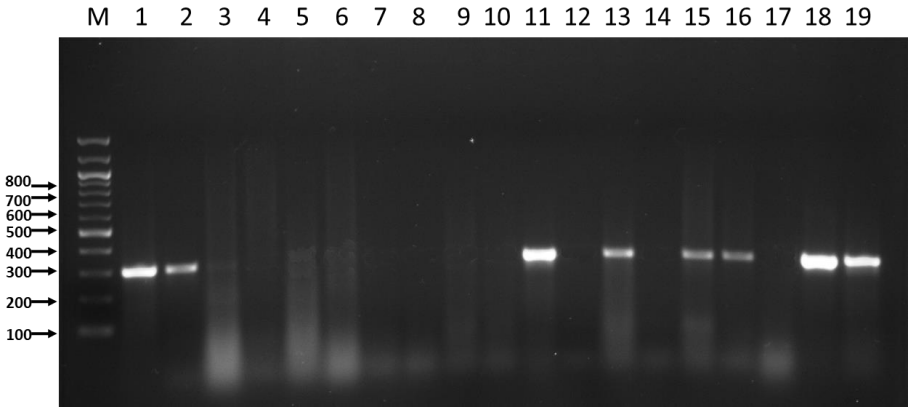

Figure 3: Specific PCR assay to reveal methicillin resistance among coagulase-positive bacterial isolates. Lanes 1, 2, 11, 13, 15, 16, 18 and 19 are the isolates containing the *mec*A methicillin resistance gene. Lane M, DNA marker (100 bp ladder).

nuc gene were amplified from nonstaphylococci bacterial isolates that were used as a negative control in the reaction (Figure 2). The results of the duplex PCR matched precisely with those from the phenotypic characterization, confirming its reliability.

Prevalence of staphylococci groups among the students

Of the 112 isolates, coagulase-positive staphylococci accounted for 30 (a carriage rate of 20.8%), while the remaining coagulase-negative *Staphylococcus* spp. accounted for 82, with a carriage rate of 56.9% (Table 3). Coagulase-positive and -negative *Staphylococcus* spp. were geographically distributed among university students to varying degrees. Coagulase-positive isolates were most prevalent among students in the northern and southern parts of Al-Madinah city. On the other hand, coagulase-negative coagulases were almost equally distributed within the four residential areas of the city. The prevalence investigations also showed that the lowest numbers of coagulase-positive and coagulase-negative staphylococci isolates were found in students residing in the eastern part of Al-Madinah compared to the remaining areas (Table 3).

Detection of methicillin resistance among coagulase-positive isolates

The presence of methicillin resistance among all 30 coagulase-positive bacterial isolates was investigated using a specific PCR primer pair to amplify the methicillin resistance marker (*mec*A gene). The primer pair used in the reaction amplified single-targeted PCR amplicons that matched the expected size of the methicillin resistance *mec*A gene (Figure 3).

The Kirby Bauer disc diffusion assay detected Methicillin resistance in 15 (50%) of the 30 coagulase-positive isolates corresponding to their phenotypic classification as MRSA strains. The southern area

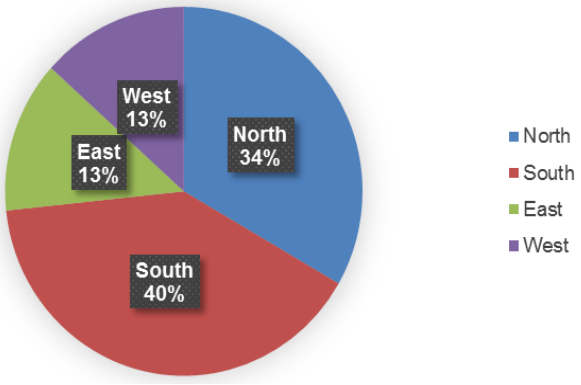

Figure 4: Frequency and geographic distribution of coagulase-positive bacterial isolates harboring the *mec*A gene among the study population residential locations.

contained the highest number of methicillin-resistant isolates (six isolates), followed by the northern part, which came in second with five isolates. Finally, the western and eastern areas accounted for two isolates each (Figure 4).

Considering the number of coagulase-positive bacteria isolated from each area, it is worth mentioning that the prevalence of methicillin resistance in the *mec*A gene in this study was higher in students living in the eastern part (two out of the three isolates) than in the remaining three areas.

Detection of PVL-encoding genes among coagulase-positive isolates

Virulence genes encoding PVL tissue necrotizing toxin (*lukS*/*F-PV*) were abundantly detected in 19 (63.3%) out of the 30 coagulase-positive staphylococci isolates by conventional PCR amplification (Figure 5).

Both the southern and northern areas contained the highest number of isolates harboring PVL-encoding genes, with seven isolates in each, followed by the

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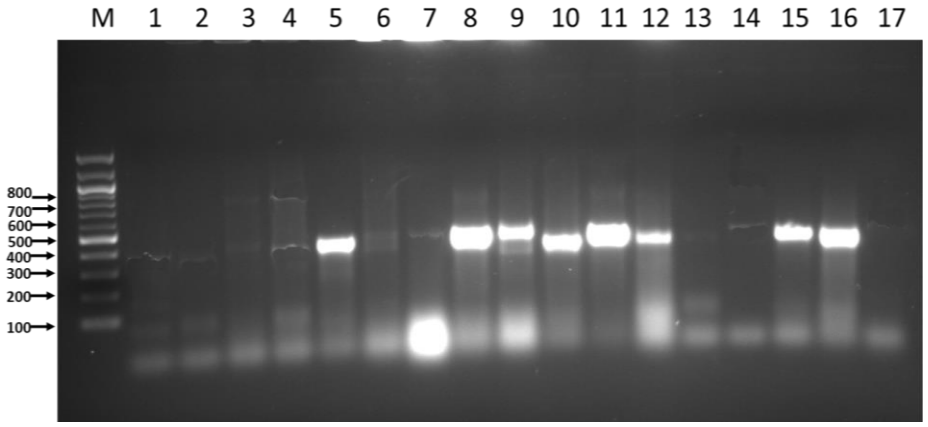

Figure 5: PCR amplification of the PVL toxin (*lukS/F-PV*)-encoding genes among coagulase-positive bacterial isolates. Lanes 5, 8-12, 15 and 16 represent the isolates containing the *PVL*-encoding gene. Lane M, DNA marker (100 bp ladder).

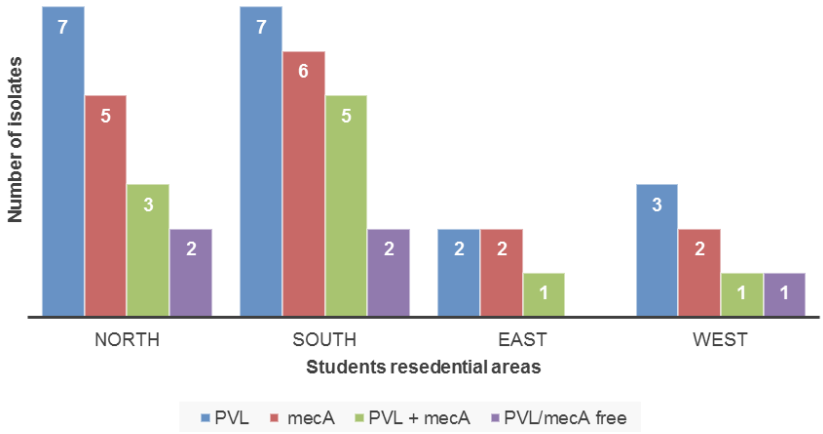

Figure 6: Frequency of bacterial isolates harboring *mec*A- and PVL-encoding genes among the study population and their geographical distribution within the residential locations.

Table 3: Prevalence and geographic distribution of bacterial isolates among the study population residential locations.

Student resident location in Al-Madinah city	No. of participants	No. and (%) of coagulase-positive isolates	No. and (%) of coagulase-negative isolates
North part	31	11 (7.6%)	19 (13.2)
South part	39	10 (6.9%)	21 (14.6)
East part	43	3 (2.1%)	20 (13.8)
West part	31	6 (4.2%)	22 (15.3)
Total	144	30 (20.8%)	82 (56.9)

western part, accounting for three isolates and finally, two isolates in the eastern area (Figure 6). Ten isolates (33.3%) were found to contain PVL and methicillin-resistant *mec*A-encoding genes. Nine of the coagulase-positive bacterial isolates (30%) were found to be positive for PVL-encoding genes only and six (20%) isolates contained neither methicillin resistance *mec*A- nor PVL-encoding genes (Figure 6).

PCR amplification of the *NRPS* gene

The presence of *S. argenteus* among coagulase-positive isolates with colonies that remained white after 48 h of growth was investigated. This was carried out through PCR amplification of the nonribosomal peptide synthetase (*NRPS*) gene cluster. The results showed that the DNA of all tested isolates amplified PCR products of ~160 base

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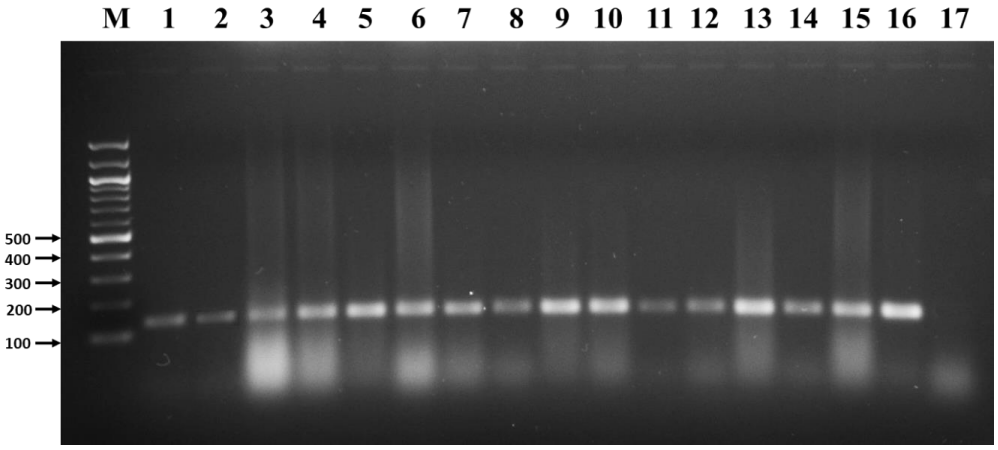

Figure 7: PCR amplicons of the nonribosomal peptide synthetase gene from non-pigmented white colony coagulase-positive isolates. Lanes 1-15, the *S. aureus* isolates with white colony morphology showing PCR products of ~160 base pairs. Lane 16-17, positive control reference strains *Staphylococcus aureus* ATCC 6538 and negative control reference strains *Staphylococcus epidermidis* ATCC 12228, respectively. Lane M, DNA marker (100 bp ladder).

pairs (Figure 7). This established that all the isolates showing white colony morphology yielded a short PCR fragment (~160) from the *NRPS* gene and, hence, are most likely to be *S. aureus* and not *S. argenteus,* which usually yields a PCR fragment of ~340 base pairs. The remaining isolates that produced yellow colonies were excluded from this investigation based on colony morphology.

DISCUSSION

Many bacteria inhabit the human nasal cavity, some deemed normal flora, while others are believed to be possible pathogens (Hoang *et al*., 2019). The genus *Staphylococcus* includes many distinctive coagulase-positive and coagulase-negative species implicated in causing community-acquired and nosocomial infections (Weterings *et al*., 2019; Becker *et al*., 2020).

Most of the studies conducted in the Kingdom of Saudi Arabia (KSA) underline the pathogenic role of *Staphylococcus* spp. among the population by either focusing on methicillin-resistant *S. aureus* infections in children (Alaklobi *et al*., 2015; Alrabiah *et al*., 2016; Alzomor *et al*., 2017) or targeting mature working adults over thirty years of age, who were suggested to have the highest prevalence of *Staphylococcus* species carriage (Abie *et al*., 2020; Akinduti *et al*., 2021). On the other hand, only a few studies took place to highlight the distribution of staphylococci nasal carriage among teenage and young adult (from 19 to 21 years of age) university students in the KSA (Zakai, 2015), which should have been the center of previous research since they are the most likely group to be heavily colonized by being subjected to the congested urban life setting.

In view of this study's results, nasal colonization by asymptomatic coagulase-positive and coagulase-negative staphylococci among university male students was found to be high. The prevalence of coagulase-negative *Staphylococcus* species was higher (73.2%) than coagulase-positive ones (26.8%) among the 112 collected isolates from the participating male students. These percentages of the two staphylococcal groups are consistent with most of the previous studies that showed a lower carriage rate of coagulase-positive staphylococci in the nasal cavities of teenagers compared to elders, who are at higher risk of exposure, probably because of their weakened immune system (Decousser *et al*., 2015; El Aila *et al*., 2017; Abimana *et al*., 2019). This study also showed that the colonization rate of both staphylococci groups varied according to the residential location of the participating students. It was noticed that participants who resided in the eastern part of Al-Madinah city had the lowest number of isolates (16%) from the two staphylococcal groups. Elevated numbers were noticed in the remaining geographical areas (19-22%). It is quite known from diverse studies that carriage rates of respiratory staphylococci vary considerably in relation to numerous factors, such as age, competency of the immune system, geographical site, sampling techniques, climate, and socioeconomic conditions (Alharbi *et al*., 2020; Akinduti *et al*., 2021). In this study, however, since most of the previous factors were standardized, one explanation behind such differences could be linked to the overcrowding of specific geographical sites over others. This will consequently result in a higher transmission rate of respiratory staphylococci among university students residing at such locations during most of their activities. Another explanation may be related to socioeconomic conditions, meaning that students residing in the eastern part of Al-Madinah could benefit from the higher living standards that reflect their proper hygiene practices.

The detection of methicillin resistance among coagulase-positive isolates, as described in our study along with several other studies, is crucial, specifically when it involves individuals often targeted by these bacteria, such as university students (Demirel *et al*., 2014;

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Abroo *et al*., 2017; Alzoubi *et al*., 2020). In this ongoing survey, it was observed that 50% (15/30) of the phenotypically identified methicillin-resistant coagulase-positive isolates also showed PCR amplification of the *mec*A gene. Similar results in which most of the examined methicillin-resistant isolates during other studies also had the *mec*A gene indicated a strong correlation between methicillin resistance and the presence of this gene (Santosaningsih *et al*., 2014; Wang *et al*., 2017; Takadama *et al*., 2018; Albarrag *et al*., 2020). Furthermore, asymptomatic nasal cavity colonization with potential methicillin-resistant coagulase-positive pathogens is widespread in healthy individuals (El Aila *et al*., 2017; Abimana *et al*., 2019). Nineteen of the 30 coagulase-positive isolates tested positive for the PVL (*lukS/F-PV*)-encoding gene, giving a prevalence rate of 63.3%. This is the highest PVL prevalence rate compared to those observed in other Asian country studies: Iran prevalence of 20% (Ranjkesh *et al*., 2020) and 24.1% in China (Wu *et al*., 2019), as well as the ones reported in some African countries: Egypt prevalence of 16.1% (Thabit *et al*., 2017), Nigeria prevalence of 27% (Orji *et al*., 2016) and up to 61% in Gambia (Darboe *et al.*, 2019).

In this study, when methicillin-resistant isolates were associated with PVL-positive isolates, more than half (10 out of 19/52.6%) of the PVL-positive isolates were also resistant to methicillin. However, the prevalence of *mec*A-positive isolates that harbor the PVL-encoding gene in this study was found to be 33.3%, which is much higher than PVL prevalence detected in other countries, such as Malaysia (Suhaili *et al*., 2018) with a 5.3% prevalence, 24% in Central Europe (Rebić *et al*., 2019) and in Africa ranging between 9 and 29% (Abdulgader *et al*., 2015). Nevertheless, it was lower than those recorded in other Asian countries (~56.8%) as well as African countries (57-73%) (Dalman *et al*., 2019; Darboe *et al*., 2019). Similar to a study carried out by Amin *et al*. (2020) in Northern Cyprus, there was no association observed between PVL and *mec*A prevalence and the students' residential locations since *mec*A and/or PVL gene-positive isolates were uniformly distributed across all the study areas.

Surprisingly, the swap cultures from 32 of the participating students in this study showed growth of only nonstaphylococci bacterial isolates. Such an absence of coagulase-positive and coagulase-negative staphylococcal isolates from the nasal swaps of these students may comprise several factors, such as the participants' immune response in addition to direct competitive relations between staphylococcal isolates and other bacterial species found within the same habitat, all of which play an essential role in influencing their isolation (McMurray *et al*., 2016; Iversen *et al*., 2020).

Furthermore, a PCR assay using the hypothetical *NRPS* gene primers was applied to distinguish between *S. aureus* and its interspecies divergent lineage, *S. argenteus*, among coagulase-positive isolates with white colony morphology. The results of the PCR amplification revealed that the presence of *S. argenteus* within the ecological niche of *S. aureus* in this part of the KSA appears to be absent or very rare. Such data support several previous studies that stated that, until recently, there was only a limited number of *S. argenteus* isolates recognized among a much larger population of *S. aureus* in several cases (Thaipadungpanit *et al*., 2015; Argudín *et al*., 2016; Chantratita *et al*., 2016; Moradigaravand *et al*., 2017).

Finally, it is quite known that the results from surveillance studies related to the colonization rates of potential respiratory pathogens among selected populations vary significantly from one study to another, making them sometimes difficult to compare. Such variation between different studies is related to the influence of numerous factors apart from age, such as geographical distribution, climate changes, level of immunity, sampling technique and socioeconomic standards (Chen *et al.*, 2017).

The only limitation of this study was the small number of participants (144) from the entire College of Science in Al-Madinah city. This happened because some of the students who took part in the study hesitated after signing the consent forms and had to be removed from the study. Another limitation was that the study only included male students. Due to the lack of trained female researchers among the authors, it was challenging to include female students in the study because the College of Science in Al-Madinah City has a single-gender education policy that has female students attending separate classes on separate campuses that can only be accessed by females. Nevertheless, the findings of this study have given the authors confidence that an accurate investigation was carried out.

CONCLUSION

The overall results obtained in this study suggest that the prevalence of coagulase-positive isolates harboring *mec*A and PVL was elevated compared to the levels observed in earlier studies and neighboring countries. Moreover, asymptomatic carriers of coagulase-positive isolates harboring methicillin resistance and PVL toxins pose a potential threat to the community and may result in outbreaks of community-acquired infections. Hence, such highly observed prevalence raises a superior concern, requiring further investigation in a broader study by analyzing a larger sample size and incorporating participants with different health conditions. This will allow for additional exploration of the potential association between these two traits and what is required for their management and control going forward.

ETHICAL APPROVAL

The research plan was approved by Taibah University, College of Dentistry, Research Ethics Committee (approval number TUCDREC/20181017). Informed approval was obtained from the participating students before sample collection. A consent form was presented to each participating student, clarifying all the specifics concerning the study. Additionally, the authors insisted on

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informing the participants that they could withdraw at any time and that their information would remain confidential.

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CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest to disclose.

AUTHORS CONTRIBUTIONS

All authors have met the qualifying criteria for authorship by substantially contributing to the work done in this manuscript through conceptualizing and implementing the methodology, analyzing and interpreting the data, and drafting, reviewing and editing the manuscript prior to journal submission. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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