Variation Between Community and Hospital Acquired Methicillin Resistant Coagulase-Positive Staphylococcus Isolates from The Nasal Carriage in The Central Region of Saudi Arabia

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Abstract

Background

The coagulase-positive staphylococcus (CoPS) has become progressively prevailing in both community and hospital. The variation between community and hospital acquired methicillin resistant coagulase positive staphylococcus has been used to monitor decisions about realistic therapy.

Methods

Participants presenting with culture proven coagulase positive staphylococcus from the nose were enclosed during this study. Antibiotic susceptibility test was verified by exploiting the E-test and disk diffusion methods. Eleven toxin genes had been analyzed using a polymerase chain reaction.

Results

The oxacillin-resistant E-test showed extremely vital resistance in hospital-isolates (34.5%) than in community-isolates (19.2%) (p -value = 0.009). The most effective antibiotic against community acquired CoPS is a quinupristin followed by vancomycin and rifampin then clindamycin. Whereas, clindamycin, sulfamethoxazole/trimethoprim, rifampin, and quinupristin seem to be more effective against hospital acquired CoPS. The intermediate effects of both ceftiofur and vancomycin were highly significant in hospital acquired CoPS isolates compared with hospital acquired CoPS isolates (p -value = 0.009 and 0.005). The proven methicillin resistance coagulase positive staphylococcus (MRCoPS) was subjected for detection the following toxin *embp* (480 bp), *aap* (180 bp, 200 bp, 300 bp, 460 bp, and 480 bp), and *aae* (110 bp) genes that were present only in a community acquired MRCoPS. But the following *coa* (400 bp and 800 bp), *spa* (1100 bp and 1120 bp), *aae* (220 bp), and *lcaD* (100 bp) genes were present only in a hospital acquired MRCoPS. The *PVL* gene was absent in both community and hospital acquired MRCoPS isolates.

Conclusion

Hospital acquired coagulase positive staphylococci were highly significant resistant effects against oxacillin and intermediate effects against ceftiofur compared to community acquired coagulase positive staphylococci. All coagulase positive staphylococcus isolates were sensitive to sulfamethoxazole/trimethoprim and quinupristin, but they were resistant to penicillin only. Genotypically, all tested toxin genes showed variation between community and hospital depending on the gene size bands. This study stresses the vital would like for broad screening, precautionary, and educational methods to accomplish the cumulative MRSA prevalence rate.

Background

The first isolation of methicillin-resistant coagulase-positive staphylococcus (MRCoPS) was in 1961. The second wave of epidemic MRCoPS was in 1980. MRCoPS is a major bacterial pathogen that causes a variety of infections in Saudi Arabia and worldwide [1–4]. In 2000, there were increases in the number of reports of community acquired (CA) and hospital acquired (HC) MRCoPS [5–7]. MRCoPS is part of the normal flora of the upper respiratory tract, or especially the nose and nasopharyngeal, thus frequently causing hospital and community-acquired infections. Methicillin resistance in staphylococci is caused by bacterial expression of the *mecA* gene that alters penicillin-binding protein (PBP-2) to PBP-2a resulting in loss of target affinity.

In the Saudi Arabia, only a few studies are performed to date to research the frequency of MRCoPS and documentation of the risk factors related to MRCoPS infection within the healthy and/or hospital patients. In 1991, the first report showed that Saudi and British strains differed in their sensitivity pattern to antibiotics [8]. In 1993, Haddad et al. study documented the first outbreak of MRCoPS in neonatal intensive care unit (ICU) in Riyadh and reported that overcapacity, inadequate space, and inadequate maintenance were implicated as the major risk factors [9].

The first epidemiological report on MRCoPS from the Saudi Arabia was in the year 1994 from the western region (Jeddah) [10]. MRCoPS covered about 7.5% of all coagulases positive staphylococcus isolates studied over three years, with 71% of them obtained from wound sites [10]. Alghaithy et al. [11] have examined the carrier status and antibiotic resistance among hospital and non-hospital personnel in Abha and isolated MRCoPS from 5.1% of the hospital and 18.3% from the non-hospital carriers. Nevertheless, 44% of the hospital carrier isolates revealed multiple resistance as contrasted to 8% of the non-hospital carriers. Moreover, multiple resistance was more frequent among inpatients (8%) as contrasted to outpatients (1%) [11]. Several authors [12, 13, 14, 15] have shown that the prevalence of MRCoPS at a tertiary care hospital is rising in the kingdom, and therefore management measures are required to be developed and efficiently utilized [16, 17].

It is important to distinguish between the isolates from CA-MRCoPS and HA-MRCoPS in the Saudi Arabia. The phenotypical and genotypical of MRCoPS isolates will help researchers to comprehend and supervise the rapidly modifying epidemiology of MRCoPS, and to build a standard from which to assess sequential developments in molecular characteristics of MRCoPS associated with diseases. But there are several obstacles with the use of a method based on the lack of healthcare risk factors in the Saudi Arabia. First, each CA- and HA-MRCoPS strains currently flow within the community. Second, supposed HA-MRCoPS strains area unit bit by bit changing into entrenched as medical building pathogens. The study aims were to indicate and prove associated with variation between nasal discharge isolates from CA- and HA-MRCoPS in the central of the Saudi Arabia characteristically as phenotypically and genotypically.

Method

Sample collection

A total of 200 nasal swabs were collected between April 2017 to December 2018 from two different communities. First hospital samples (n=83) included physicians (n=7), nurses (n=12), radiologists (n=8), laboratory medical specialists (n=8), ECHO (n=1), medical residents (n=1), hospital admins (n=6), clinical nutrition (n=1), internship students (n=35) and medical research assistants (n=4) at King Abdulaziz Medical City (KAMC), Riyadh, Saudi Arabia. Second community samples (n=117), we concentrated on academic faculties (n=10), admins (n=36), and students (n=71) at King Saud Bin Abdulaziz University for Health Sciences (KSAU-HS), Riyadh, Saudi Arabia. The exclusion for hospital were patients, and personnel who worked part-time at KAMC; for the community were students who were in direct contact with patients, and those with incomplete records were excluded from this study.

Specimen collection and transport

A dry polyester swab was inserted into the naris, parallel to the roof of the mouth, and left in situ for some seconds. Specimens from each nostril were obtained with an identical swab. The collected nasal swabs were transported to the lab research as soon as possible. If not were kept at 2-30°C until transported to the lab. If the process of collected nasal swabs could not be performed within 36 hr, they were stored in the refrigerator at 2-8°C for a maximum of five days. The collection of nasal swabs was daily during the working day for up to 3 months.

Isolation and identification of coagulase-positive staphylococcus

Initial screening and identification of CoPS isolates were performed according to the standard laboratory protocols. Briefly, the collected nasal swabs were streaked directly on mannitol salt agar (MSA), and *S. aureus* isolates were tested for methicillin resistance using a standard oxacillin salt agar screening plate procedure and cefoxitin susceptibility as indicated by the Clinical and Laboratory Standards Institute (CLSI) [18]. For confirmation, chromogenic agar was used for rapid MRSA screening since this agar had high sensitivity for MRCoPS (>98%) [19]. Methicillin-resistant and methicillin-sensitive staphylococcus strains were included as controls. Confirmation of methicillin-resistance was done by inoculating a 10 L aliquot of a bacterial suspension with 3×10^6 CFU/mL (McFarland turbidity no. 1) on Mueller-Hinton agar (MHA) supplemented with calcium (50 mg/L), magnesium (25 mg/L), sodium chloride (4%) and oxacillin (4 g/mL). The growth of at least one well-defined colony on these plates incubated at 30°C for 48 hr was suggestive of MRSA. The methicillin-resistance strain was confirmed by microdilution in Muller-Hinton broth supplemented with cations and sodium chloride, using a final inoculation of 7.5×10^4 CFU. MRSA was identified with a minimal inhibitory concentration (MIC) of oxacillin >8 g/mL, and those with a MIC <1g/mL were considered as methicillin-sensitive staphylococcus (MSSA). A MIC of oxacillin between >1g/mL and <8 g/mL was defined as a methicillin-intermediate staphylococcus (MISA) [20].

Antimicrobial susceptibility testing

Antimicrobial susceptibility of isolated coagulase positive staphylococcus towards 7 antimicrobials was determined by the agar dilution method in Mueller-Hinton agar according to the Clinical and Laboratory Standard Institute (CLSI) guidelines [18] and the results were evaluated after incubation at 35°C for 24 hr.

The discs were penicillin (P, 1 µg), cefoxitin (FOX, 30 µg), vancomycin (VA, 30 µg), clindamycin (CC, 2 µg), sulfamethoxazole/trimethoprim (SXT, 25 µg), rifampin (RA, 25 µg), and quinupristin (SYN, 15 µg). A fresh subculture of isolates was prepared on MSA and incubated at 37°C for 18–24 hr. With the help of a wire loop, 4–5 well-isolated colonies of comparable look were picked and transferred into the tube of sterile traditional saline. The clumping of cells was avoided by emulsifying the inoculum inside the tube. The inoculums were adjusted to 0.5 McFarland (McFarland 0.5 equals approximately 10^8 CFU/mL). After adjusting the inoculum within 15 min, a sterile cotton swab was swayback into the inoculum. The swab was patterned over the complete surface of the MHA plate, rotating the plate just about 60° three times to make sure merging growth. Inoculation was completed by running the swab around the surface of the agar. Excess moisture on the agar surface could be absorbed before applying the antimicrobial discs. MSSA ATTC 25923, MSSA ATCC 229213, MRSA ATCC BAA1026, and *Staphylococcus epidermidis* ATCC 1228 were used as control strains.

Genetic characterization of methicillin-resistant coagulase-positive staphylococcus

DNA extraction

Ten microliters of overnight Lysogeny broth culture in a tube were centrifuged at 8000 rpm for 10 min and the supernatant was discarded. The pellet was suspended in phosphate buffer saline using vortex then centrifuged again and the supernatant was discarded. Then the pellet was suspended in 2 mL of TE buffer and mixed by the vortex. A 500 L was transferred to 1.5 ml Eppendorf and 50 L of SDS (10%) and 5 L of proteinase K were added and left in the incubator for 1.5 hr at 37°C. An equal volume of (5 L) phenol, chloroform, isoamyl mixture was added, vortexed for 1 min, centrifuged for 4 min, and the aqueous supernatant containing DNA was transferred to another tube. A fifty-five L of 3 M sodium acetate and double volume of cold alcohol (100%) were added and mixed gently 2-3 times and centrifuged for 10 min. the supernatant was removed and the pellet was washed by mixing gently with 1 mL of 70 % alcohol and centrifuged for 1 min. The supernatant was removed, and the pellet was left to dry then suspended in 10 L of TE buffer and incubated for 30 min at 55°C and stored at -20°C [21].

Detection of the *coa* and *spa* genes

The identified CoPS strains subject to a polymerase chain reaction (PCR) for *coa* gene detection, using the following primers: forward (5'-CGA GAC CAA GAT TCA ACA AG-3') and reverse (5'-AAA GAA AAC CAC TCA CAT CA-3'), which design to amplify the 3' ends hypervariable region containing 81-base pair (bp) tandem repeats of *coa* gene. The amplification reaction consisted of associate degree initial denaturation step at 94°C for 5min, followed by thirty cycles of denaturation at 95°C for 30 sec, tempering at 55°C for 45 sec, extension at 72°C for 2 min, followed by a final extension at 72°C for 7 min [22]. The tested CoPS strains focus on PCR for *spa* gene detection using the following primers: forward (5'-ATC TGG TGG CGT AAC ACC TG-3'), and reverse (5'-CGC TGC ACC TAA CGC TAA TG-3') which scheme to amplify the polymorphic region that contains a variable number of 24 bp tandem repeats of the *spa* gene coding for protein A. Amplification reaction consisted of associate degree initial denaturation step at 94°C for 4 min, followed by thirty-five cycles of denaturation at 94°C for one min, annealing at 56°C for one min, extension at 72°C

for 3 min, followed by a final extension at 72°C for 5 min [23]. The PCR product and restriction digest fragments were detected by electrophoresis in 2% agarose gel. The pictures were pictured and analyzed in Gel Doc (BIORAD).

Detection of the *mecA*, *SCCmecII*, *SCCmecIVa*, and *SCCmecIVb* genes

All MRCoP isolates were tested for confirmation of the presence of the 310 bp PCR product of the *mecA* gene, using the following primers: forward (5'-TGG CTA TCG TGT CAC AATCG-3') and reverse (5'-CTG GAA CTT GTT GAG CAG AG-3'). The amplification reaction was carried out in 25 L volumes, under the following conditions: initial denaturation at 92°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 30 seconds, and extension at 72°C for 30 sec, followed by a final extension at 72°C for 3 min [24]. Identification of the *SCCmec* complex was performed by a PCR screen for *SCCmecII* (287 bp) and *SCCmecIVa* (776 bp) and *SCCmecIVb* (1000 bp) using the DNA extract prepared above as a template. DNA samples were tested for the presence of the *SCCmec* complex, using the following primers: for *SCCmecII* gene, forward 5'-CAA AAG GAC TGG ACT GGA GTC CAA A-3' and reverse 5'-CAA GTG AAT TGA AAC CGC CT-3'; for *SCCmecIVa* gene, forward 5'-TTT GAA TGC CCT CCA TGA ATA AAA T-3' and reverse 5'-AGA AAA GAT AGA AGT TCG AAA GA-3'; for *SCCmecIVb* gene, forward 5'-AGT ACA TTT TAT CTT TGC GTA-3' and reverse 5'-AGT CAT CTT CAA TAT CGA GAA AGT A-3'. Samples were heated for 1 min at 95°C, 30 cycles of [30 sec at 94°C, 60 sec at 50°C and 120 sec at 72°C], and 2 min at 72°C [25]. The PCR product and restriction digest fragments were detected by electrophoresis in 2% agarose gel. The pictures were pictured and analyzed in Gel Doc (BIORAD).

Detection of the *aae*, *aap*, *emb*, *lcaD*, and *PVL* genes

DNA samples were tested for the presence of the 110 to 220 bp PCR product of *aae* gene, using the following primers: forward 5'-AAC AAA TTG ATA AAG CAA CG-3' and reverse 5'-GTT GTC TTT CCT TTA GTG TC-3'. Samples were heated at 95°C for 10 min before cycling for 45 cycles of 95°C for 10 sec, 5°C for 20 sec, and 72°C for 25 sec [26]. The *aap* gene was detected using PCR with a specific primer: forward 5'-TCA CTAAAC AAC CTG TTG ACG AA-3' and reverse 5'-AAT TGA TTT TTA TTA TCT GTT GAA TGC-3'. Samples were heated at 94°C for 4 min, followed by 25 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min, and 72°C for 5 min [27]. All isolated MRSA were tested for the presence of the 455 bp PCR product of the *embp* gene, using the following primers: forward 5'-AGC GGT ACA AAT GTC AAT ATC-3' and reverse 5'-AGA AGT GCT CTA GCA TCA TCC-3'. Amplification reaction consisted of an initial denaturation step (96°C for 2 min), 40 amplification cycles of 94°C for 1 min, 55°C for 30 sec and 72°C for 1 min, with a final 10-min extension step at 72°C [28]. DNA samples were tested for the presence of the 188 bp PCR product of *lcaD* gene, using the following primers: forward 5'-ATG GTC AAG CCC AGA CAG AG-3' and reverse 5'-CGT GTT TTC AAC ATT TAA TGC AA-3'. Samples were heated at 94°C for 5 min, followed by 50 cycles at 94°C for 30 sec, 55.5°C for 30 sec, 72°C for 30 sec, and 72°C for 1 min after the conclusion of the 50 cycles [29]. The primer sequence for the *PVL* gene was described previously by McClure et al. [30]. The primer was used for the co-amplification of *PVL* genes: forward 5'-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3' and reverse 5'-GCA TCA ACT GTA TTG GAT AGC AAA AGC-3' procedure as indicated by the

CLSI [18, 30]. Amplification reaction consisted of an initial denaturation step at 94°C for 3 min followed by 30 cycles of the following steps: denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min, and a final extension at 72°C for 5 min [30]. The PCR products and restriction digest fragments were identified by electrophoresis in 2% agarose gel. The images were visualized and analyzed in Gel Doc (BIORAD).

Statistical analysis

Statistical analysis was performed with SPSS (v.22.0) statistics software. The variation between Healthy and healthcare communities by several variables. Observed differences were assessed for statistical significance by chi-square. A significant difference was statistically accounted for at a p-value of <0.05.

Ethical considerations

The Institutional Review Board (IRB) at King Abdullah International Medical Research Centre (KAIMRC) granted permission to conduct the project after reviewing the ethical aspects of the proposal (File#3190/2015).

Result

In total 200 nasal swabs were collected for this study. Where 58.5% of collected nasal swabs were from CA infection and 41.7% of collected nasal swabs were from HA infection.

Isolation of Staphylococcus species using mannitol salt agar (Table 1)

In total 200 nasal swabs, 24 (12%) of them showed growth of CoPS only. Where 11.1% of isolated CoPS were from CA infection and 13.3% from HA infection. Sixty (30%) coagulase-negative staphylococci (CoNS) were isolated out of the total number of collected nasal swabs. The only factor significantly among CA infection was the nasal swabs growth of both CoPS and CoNS (77.8%) (p -value = 0.007) compared to HA infection 21.7%. Whereas the only factor significantly among HA was the nasal swabs growth of only CoNS (57.8%) (p -value = 0.006) compared to CA (10.3%). Only 1.2% of HA nasal swabs showed bacterial growth other than staphylococcus. Whereas 3% of nasal swabs showed no bacterial growth: 0.9% from CA and 6% from HA, respectively. In summary, a total of 133 CoPS were isolated from mannitol salt agar, 104 CoPS from CA, and 29 from HA.

Table 1
Isolation of microorganisms by using mannitol salt agar.

	Number (n)	Percentage (%)	Total (CA + HA) [n (%)]	Proportion (n/Number of participants)	<i>p</i> - Value
Number of participants	117	58.5	200 (100)	-	0.010
CA	83	41.7		-	
HA					
CoPS	13	11.1	24 (12)	0.111	0.737
CA	11	13.3		0.133	
HA					
CoNS	12	10.3	60 (30)	0.103	0.006
CA	48	57.8		0.578	
HA					
CoPS and CoNS	91	77.8	109 (54.5)	0.778	0.007
CA	18	21.7		0.217	
HA					
Other bacteria	0	0.0	1 (0.5)	0	0.344
CA	1	1.2		0.012	
HA					
No bacterial growth	1	0.9	6 (3)	0.009	0.180
CA	5	6.0		0.060	
HA					

Antimicrobial susceptibility test and Oxacillin E-test of isolated coagulase-positive staphylococcus (Table 2)

The oxacillin E-test was used against the 133 CoPS isolates. Thirty (22.6%) out of 133 CoPS isolates were resistant to oxacillin. The resistance to oxacillin was highly significant in HA-CoPS isolates (34.5%) than in CA-CoPS isolates (19.2%) (p -value = 0.009). Whereas 10.5% showed intermediate effects to oxacillin, but it was not statistically significant (p -value = 0.464). Eighty-nine out of 133 showed sensitivity results to oxacillin, but it was not statistically significant (p -value = 0.182). All coagulase-positive staphylococcus isolates in our study were susceptible to sulfamethoxazole/trimethoprim and quinupristin but resistant to

penicillin. In CA-CoPS, the antimicrobial susceptibility test showed resistance against cefoxitin (28.9%), clindamycin (9.5%), and rifampin (4.8%). The test also showed intermediate results against cefoxitin (14.4%), vancomycin (4.8%), and clindamycin (9.5%). In addition, statistical studies showed that CA-CoPS isolates were highly significant susceptible to cefoxitin (p -value = 0.003) and vancomycin (p -value = 0.008). For HA-CoPS isolates, the antimicrobial susceptibility test showed resistance against only cefoxitin (38.5%). The test also showed intermediate results against cefoxitin (30.8%) and vancomycin (46.2%). The three factors that statistically significant for HA-CoPS isolates were susceptibility to clindamycin (p -value = 0.011), and intermediate effects against both cefoxitin (p -value = 0.009) and against vancomycin (p -value = 0.005).

Table 2

Antimicrobial susceptibility test and Oxacillin E-test of isolated coagulase-positive staphylococcus.

Variables	Number [n (%)]			Proportion (number/Total Number)			p-Value		
CA-SA (n = 104)									
HA-SA (n = 29)	R	I	S	R	I	S	R	I	S
E-Test Method (MIC)									
Oxacillin	20 (19.2)	12 (11.5)	72 (69.2)	0.192	0.115	0.692	0.009	0.464	0.182
CA-CoPS				0.345	0.069	0.586			
HA-CoPS	10 (34.5)	2 (6.9)	17 (58.6)						
Disc Diffusion Method									
1) β -Lactam Antibiotics									
FOX30	30 (28.9)	15 (14.4)	59 (56.7)	0.288	0.144	0.567	0.165	0.009	0.003
CA-CoPS				0.379	0.818	0.310			
HA-CoPS	11 (38.5)	9 (30.8)	9 (31)						
P10	104 (100)	0 (0)	0 (0)	1	0	0	ns	ns	ns
CA-CoPS		0 (0)	0 (0)	1	0	0			
HA-CoPS	29 (100)								
2) Non- β -Lactam Antibiotics									
VA30	0 (0)	5 (4.8)	99 (95.2)	0	0.048	0.952	ns	0.005	0.008
CA-CoPS	0 (0)	13 (46.2)	16 (55.2)	0	0.462	0.552			
HA-CoPS									
CC2	10 (9.5)	10 (9.5)	84 (81)	0.096	0.096	0.808	0.163	0.163	0.011
CA-CoPS			29 (100)	0	0	1			
HA-CoPS	0 (0)	0 (0)							
SXT25	0 (0)	0 (0)	104 (100)	0	0	1	ns	ns	ns
CA-CoPS	0 (0)	0 (0)		0	0	1			
HA-CoPS			29 (100)						

Penicillin 10 μ g (P10); Cefoxitin 30 μ g (FOX30); Vancomycin 30 μ g (VA30); Clindamycin 2 μ g (CC2); Sulfmeth/Trimeth 25 μ g (SXT25); Rifampin 25 μ g (RA25); Quinupristin 15 μ g (SYN15).

Variables	Number [n (%)]			Proportion (number/Total Number)			<i>p</i> -Value		
CA-SA (n = 104)									
HA-SA (n = 29)	R	I	S	R	I	S	R	I	S
RA25	5 (4.8)	0 (0)	99 (95.2)	0.048	0	0.952	0.485	ns	0.485
CA-CoPS	0 (0)	0 (0)		0	0	1			
HA-CoPS			29 (100)						
SYN15	0 (0)	0 (0)	104 (100)	0	0	1	ns	ns	ns
CA-CoPS	0 (0)	0 (0)		0	0	1			
HA-CoPS			29 (100)						
Penicillin 10 µg (P10); Cefoxitin 30 µg (FOX30); Vancomycin 30 µg (VA30); Clindamycin 2 µg (CC2); Sulfmeth/Trimeth 25 µg (SXT25); Rifampin 25 µg (RA25); Quinupristin 15 µg (SYN15).									

Detect *mec* genes for isolated methicillin-resistant coagulase-positive staphylococcus using PCR (Table 3)

Genotypically, all MRCoPS isolates showed no bands for *SCCmecII*, *SCCmecVIa*, and *SCCmecVIb* genes. In a total of 30 CA-MRCoPS, 25 (83.3%) showed *mecA* gene band (300 bp). But 9 (81.8%) *mecA* gene bands (300 bp) were observed in a total of 11 HA-MRCoPS. In total 34 (82.9%) out of phenotypically confirmed 43 MRCoPS isolates showed *mecA* gene bands. They were considered and confirmed as MRCoPS and used for further genotypic testing.

Table 3
Detect *mec* genes for suspected isolated methicillin resistant coagulase-positive staphylococcus using PCR.

	CA-MRSA (n = 30)	HA-MRSA (n = 11)	Total (n = 41)
<i>mecA</i> gene (300 bp)	25 (83.3)	9 (81.8)	34 (82.9)
<i>SCCmecII</i> gene	0 (0)	0 (0)	0 (0)
<i>SCCmecVIa</i> gene	0 (0)	0 (0)	0 (0)
<i>SCCmecVIb</i> gene	25 (83.3)	9 (81.8)	34 (82.9)

Typing *coa* and *spa* genes for isolated methicillin-resistant coagulase-positive staphylococcus using PCR (Table 4)

Both CA- and HA-MRCoPS showed different size bands for the *coa* and *spa* genes. For *coa* gene, CA-MRCoPS showed three different *coa* gene bands: 81 bp (100%), 120 bp (4%) and 720 bp (8%). Whereas HA-MRCoPS showed presence five different *coa* gene bands: 81 bp (100%), 120 bp (44.4%), 400 bp (22.2%), 720 bp (22.2%) and 800 bp (33.3%). For *spa* gene, CA-MRCoPS showed only one band size of 1100 bp (64%). Whereas HA-MRSA showed three different bands size: 1020 bp (11.1%), 1100 bp (33.3%), and 1120 bp (11.1%).

Table 4
Detecting patterns of *coa* and *spa* genes from isolated methicillin resistant coagulase-positive staphylococcus using PCR.

	<i>coa</i> gene (n (%))				<i>spa</i> gene (n (%))			
	81 bp	120 bp	400 bp	720 bp	800 bp	1020 bp	1100 bp	1120 bp
CA-MRSA (n = 25)	25 (100)	1 (4)	0 (0)	2 (8)	0 (0)	0 (0)	16 (64)	0 (0)
HA-MRSA (n = 9)	9 (100)	4 (44.4)	2 (22.2)	2 (22.2)	3 (33.3)	1 (11.1)	3 (33.3)	1 (11.1)

Detecting other genes for isolated methicillin-resistant coagulase-positive staphylococcus using PCR (Table 5)

All MRCoPS isolates showed no bands for the *PVL* gene. Whereas, for *aae* gene, a different band size observed in CA-MRCoPS compared to HA-MRCoPS: 220 bp (24%), and 110 bp (22.2%), respectively. While five bands of *aap* gene showed only in CA-MRCoPS of different sizes (180 bp, 200bp, 460 bp, and 480 bp). For the *emb* gene, CA-MRCoPS showed two different bands size: 50 bp (100%) and 480 bp (24%); whereas HA-MRCoPS showed only one band size: 50 bp (100%). For the *lcaD* gene, CA-MRCoPS showed only one band size: 200 bp (44.8%); whereas HA-MRCoPS showed two different bands size: 100 bp (11.1%) and 200 bp (33.3%), respectively.

Table 5

Detecting patterns of other genes from isolated methicillin resistant coagulase-positive staphylococcus using PCR.

	<i>aae</i> gene		<i>aap</i> gene			<i>emb</i> gene			<i>lcaD</i> gene		<i>Luk-PV</i> gene	
	110 bp	220 bp	180 bp	200 bp	300 bp	460 bp	480 bp	50 bp	480 bp	100 bp	200 bp	433 bp
CA-MRSA (n = 25)	0 (0)	6 (24)	6 (24)	6 (24)	6 (24)	6 (24)	6 (24)	25 (100)	6 (24)	0 (0)	13 (44.8)	0 (0)
HA-MRSA (n = 9)	2 (22)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	9 (100)	0 (0)	1 (11.1)	3 (33.3)	0 (0)

Discussion

MRCoPS is still an important health problem in all counties because it is transmissible and difficult to eliminate and treat. The variation between HA- and CA-MRCoPS has been used to monitor decisions about realistic therapy.

Prevalence of CA-MRCoPS and HA-MRCoPS

Al-Hamad et al. [31] study showed that CA-MRCoPS isolation increased steadily from 23% in 2006 to 60% in 2015, exceeding the rate of isolation of HA-MRCoPS in the Kingdom of Saudi Arabia. Other studies related to the prevalence of MRCoPS in the central region of the Kingdom of Saudi Arabia from 2017 to 2019 were selected according to the selection criteria in comparison with our study, two reported data from the community settings and five from hospital settings. In this study a total of 133 CoPS were isolated from nasal swabs of participants: 104 were from CA and 29 from HA infections. In HA infections, the prevalence of methicillin-resistance among CoPS was 21.4% nearly similar reports in the central regions of Saudi Arabia (2013 to 2020), such as 28% by Sirkhazi et al. [32], 24% by Naeem et al. [33] and 22% by Shady et al. [34] and Alrabiah et al. [12]; but it was higher than Albarrag et al [35] study (9%) and lower than Abujhesha et al. [36] study (43.4%). Whereas in CA infections, the prevalence of MRCoPS was 10.8% higher than similar studies, such as Albusayes et al. [37] study was 3.3% and Alaklobi et al. [13] study was 4.6% prevalence.

Antibiotic susceptibility test

The oxacillin E-test showed highly significant resistance in HA-CoPS isolates (34.5%) than in CA-CoPS isolates (19.2%) (p -value= 0.009). Whereas 10.5% showed intermediate effects to oxacillin, but it was not statistically significant (p -value= 0.464). Eighty-nine out of 133 showed sensitivity results to oxacillin, but it was not statistically significant (p -value= 0.182). In our study, the overall susceptibility of CA-CoPS

isolates to the various antibiotics tested was: cefoxitin (56.7%), vancomycin (95.2%), clindamycin (81%), rifampin (95.2%), and quinupristin (100%), respectively. Accordingly, the most effective antibiotic against CA-CoPS is quinupristin followed by vancomycin and rifampin then clindamycin. Our results for CA-CoPS were consistent with the previous studies [38,39]. HA-CoPS isolates, the overall susceptibility to the various antibiotics tested was: cefoxitin (31%), vancomycin (55.2%), clindamycin (100%), sulfamethoxazole/trimethoprim (100%), rifampin (100%), and quinupristin (100%), respectively. Bai et al. [40] and Shariati et al. [41] studies showed similar results. Accordingly, clindamycin, sulfamethoxazole/trimethoprim, rifampin, and quinupristin seem to be more effective against HA-CoPS. Like other studies, our findings also suggested that quinupristin is the most effective antibiotic against CA-CoPS and HA-CoPS isolates [41, 42]. On the other hand, the intermediate effects of both cefoxitin and vancomycin were highly significant in HA-CoPS isolates compared with CA-CoPS isolates (p -value = 0.009 and 0.005). The overall intermediate effects of CA-CoPS isolate to the various antibiotics tested was: cefoxitin (30.8%), and vancomycin (46.2%), respectively. In HA-CoPS isolates, the overall intermediate effects to the various antibiotics tested were: cefoxitin (14.4%), vancomycin (4.8%), and clindamycin (9.5%), respectively. No intermediate effects showed against clindamycin in CA-CoPS isolates. Whereas the following antibiotics showed no intermediate effects against both CA-CoPS and HA-CoPS isolates were penicillin, sulfamethoxazole/trimethoprim, rifampin, and quinupristin. In several places like the United State, Africa, Middle East, and southeast Asia have successively discovered multiple intermediate CoPS against several tested antibiotics such as vancomycin [43, 44, 45].

Genotypic characterization of CA-MRCoPS and HA-MRCoPS

Most of our tested genes showed the presence of more than one band. Several studies confirmed similar results such as Omar et al. [46], Goh et al. [47], Saei et al. [48], and Frenay et al. [49]. The explanation for the existence of more than one band was due to the presence of more than one allele's form. This polymorphic gene might be due to removal or supplement transformations by which a slice of the 3' end area of the gene is erased or several nucleotides are implanted and therefore causes a modification in the gene [46, 47, 48]. Another explanation for the gene polymorphism is due to containing several base pair repeats, whose number and sequence differ among strains as seen with the X-region for the *spa* gene [46, 49]. Regarding the *SCCmec* types, it was spotted that the type IVb was abundant in CA- and HA-MRCoPS isolates (83.3%, 81.8%), while the type II and VIa were not spotted. Previous studies showed that HA-MRCoPS strains carry *SCCmec* types I and II, whereas CA-MRCoPS strains carry *SCCmec* IV and V [50, 51, 52]. An extensive variety of surface proteins with adhesive properties developments the ability of MRCoPS to stick to different surfaces [53]. The autolysin/adhesin *aae* and *aap* intervene the early adhesion through hydrophobic connections [54]. Almost at the same time, extracellular matrix-binding protein (*embp*) can precisely impasse to collagen, fibrinogen, and fibronectin respectively [55, 56, 57]. Additionally, *aap* and *aae* proteins bind non-specifically to fibrinogen, and fibronectin [58, 59]. Many MRCoPS isolates transport the *lcaD* operon that converts proteins elaborated in the creation of the polysaccharide intercellular adhesin (PIA), which links the bacteria cells in the biofilm [60, 61]. PIA together with *embp*, *aae*, and *aap* is accountable for the intercellular adhesion and accretion, permitting biofilm development [62, 63, 64]. Our study showed variation in the presence of the above toxin gene proteins in CA-MRCoPS

and HA-MRCoPS. The following *coa* (81 bp) and *embp* (50 bp) genes were present in similar prevalence among CA-MRCoPS and HA-MRCoPS (100%). The following *embp* (480 bp), *aap* (180 bp, 200 bp, 300 bp, 460 bp, and 480 bp), and *aae* (110 bp) genes were present only in CA-MRCoPS, 24%, 24%, and 22.2%, respectively. The following *coa* (400 bp and 800 bp), *spa* (1100 bp and 1120 bp), *aae* (220 bp) and *lcaD* (100 bp) genes were present only in HA-MRCoPS, 22.2% and 33.3%, 11.1% and 11.1%, 24%, and 11.1%, respectively. The following *coa* genes (120 bp and 720 bp) were present in higher prevalence among HA-MRCoPS than in CA-MRCoPS, 44% vs 4% and 22.2% vs 8%, respectively. An additional unique genetic feature of CA-MRCoPS is that a high percentage of strains convey genes for *PVL*, which is mostly inattentive from HA-MRCoPS strains [65, 66, 67]. Therefore, *PVL* is expected to be answerable for the boosted pathogenic CA-MRCoPS strains. Our study showed that the *PVL* gene was negative not just in HA-MRCoPS but also in CA-MRCoPS isolates. The absence of *PVL* gene results in HA-MRCoPS were consistently recorded in Iraq [68], Poland [69], and Portugal [70].

Conclusion

Our study is the first to show variation in phenotypic and genotypic between CA- and HA-MRCoPS in the central region of the Kingdom of Saudi Arabia. The MRCoPS occurrence rate in the Kingdom of Saudi Arabia is extensively high, with a substantial amount of the cases noticed in the central region of the country, specified the multi-national environment of the population in that area and the circumstance that this region signifies a very demanding marketable for people from all over the world. The conclusions of this study stress the vital would like for broad screening, precautionary, and educational methods to accomplish the cumulative MRSA prevalence rate. Meanwhile, this present study comprised studies that concealed the central region of the country, forthcoming studies that cover the whole country are suggested to spectacle the complete picture of MRCoPS prevalence among CA- and HA-MRCoPS in the Kingdom of Saudi Arabia.

Abbreviations

CA

Community acquired; HA:Hospital acquired; CoPS:Coagulase positive staphylococcus; CoNS:Coagulase negative staphylococcus; MRCOPS:methicillin resistant coagulase positive staphylococcus; SDS:Sodium dodecyl sulfate; MHA:Muller Hinton agar; CFU:Colony forming unit; PVL:Panton-Valentine leukocidin; spa:Staphylococcus protein A.

Declarations

Ethics approval and consent to participate The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of King Abdullah International Medical Research Center (Ref. No. IRBC/1010/18). Approved informed consent was used and obtained from all participants involved in this study.

Consent publication- Individual data such as images and videos did not accompany this particular manuscript and hence consent for publication is not applicable.

Availability of Data and materials- The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests- The authors declare that they have no competing interests for this work.

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