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Original Article

The *coa*, *mec*, and *spa* Genes Diversity among Methicillin-resistant *Staphylococcus aureus* Strains from Health-care Workers and Patients

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterial pathogen that is frequently isolated in both hospital and community environments. MRSA is considered a major nosocomial pathogen that causes severe morbidity and mortality. **Materials and Methods:** Two hundred and twenty-five nasal swabs were collected (100 from health-care workers and 125 from patients). *S. aureus* was identified by colony morphology in both blood and mannitol salt agars, catalase and coagulase productions, and also by standard biochemical tests. Susceptibility test to several antimicrobial agents was performed by disc diffusion agar according to the Clinical and Laboratory Standards Institute guidelines. The polymerase chain reaction amplification of the *coa*, *mecA*, and *spa* gene was carried out in the clinical isolates showed resistant to oxacillin. **Results:** Among 225 isolates of bacteria, 76 were confirmed to be *S. aureus* by phenotypic characteristics. Thirty isolates were considered MRSA by susceptibility antimicrobial test. Twenty-four were confirmed to be *S. aureus* by the presence of *coa* gene bands. Twenty-one *S. aureus* isolates were confirmed to be MRSA by the presence of *mecA* gene. The *spa* gene in health-care workers was present in 88.88% and for patients was 41.66%. **Conclusions:** This study is suggestive that the PCR for the detection of *coa*, *mecA*, and *spa* gene is a fast, accurate, and valuable diagnostic tool.

Keywords: *Antibiotic susceptibility, coa gene, mecA gene, methicillin-resistant Staphylococcus aureus, spa gene*

Date of Acceptance:
22-Mar-2018

Introduction

Staphylococcus aureus is a major cause of both nosocomial and community-acquired infections. Over the past decades, the incidence of methicillin-resistant *S. aureus* (MRSA) has increased significantly in surgical site infections, bloodstream infections, and pneumonia.[1-3] MRSA strains are considered to be endemic in many hospitals throughout the world and are now responsible for approximately 40%–60% of patients and healthcare-associated infections.[4,5] Accordingly, there is transmission of *S. aureus* from patients to the health-care workers and vice versa easily occurs. Antimicrobial resistance has dramatically increased worldwide due to the widespread use or misuse of antimicrobial agents.[6] Beta-lactam antibiotics are frequently used in the treatment of staphylococcal infections. However, an increasing resistance to beta-lactam antibiotics due to the production of beta-lactamase in *S. aureus* strains has been reported.[7] In the early 1960s, a new type of penicillin antibiotic called methicillin was developed and used to treat infections stemming from beta-lactamase producing strains of *S. aureus*. Today, MRSA strains have become resistant to most common antibiotics. Therefore, treatment of infections in humans caused by MRSA is quite difficult.[6,7]

The methods of antimicrobial susceptibility methods for detection of MRSA were including oxacillin *E*-test and oxacillin and/or cefoxitin screening test using

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Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_301_17

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How to cite this article: Al-Ruwaili MA. The *coa*, *mec*, and *spa* genes diversity among methicillin-resistant *Staphylococcus aureus* Strains from health-care workers and patients. Niger J Clin Pract 2018;21:1065-74.

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disc diffusion method.[8] There were many reports that these conventional antimicrobial tests were associated with false negative and positive results for MRSA identification.[9-11] Therefore, it was necessary to use more exact and specific methods, such as polymerase chain reaction (PCR) that was considered as a DNA-based assay.

Accordingly, molecular identification of MRSA can be performed by PCR amplification of *mecA* gene coding for low-affinity penicillin-binding protein, PBP2,[12] and *coa* gene coding for coagulase protein[3] and also for *spa* gene coding for protein A[13] using specific primers. This technique can offer high efficacy and safety, and it may be considered as a fast and sensitive method, using low amounts of DNA template in a given sample.[8,14] The aim of the present study was to isolate MRSA from nasal swabs obtained from both health-care workers and patients. Then, the identified isolated MRSA strains by antibiotics susceptibility testing were tested genotypically. The *coa*, *mecA*, and *spa* genes from specific strains were amplified using specific primers and identified using PCR.

Materials and Methods

Study design

This is a prospective cohort study. The reason for chosen a prospective cohort study due to its relation to data collection and the events of interest occur after individuals are enrolled (e.g., clinical trials and cohort studies). This prospective collection will enable the use of more solid, consistent criteria, and avoids the potential biases of retrospective recall.

Sample size

The sample size was done by Biostatistics and Modeling Section, Bioinformatics and Research Consulting Services, Al-Jouf University, Saudi Arabia.

Sample size calculation

The sample size was calculated using the prevalence formula(20) in N-Query Advisory Version 4.0 (STATCON Gmbh, Germany).

Estimated sample size

The main outcome of the study is to estimate the prevalence of MRSA among health-care workers/patient individuals in North of Saudi Arabia. As per the literature, the prevalence varies widely between 5% and 95% prevalence, assuming a 95% confidence interval, an alfa of 0.05, a conservative prevalence of 50%, and a precision of 0.05. The estimated number of potentially eligible candidates is between 150 and 250 participants. Population size is 400 samples. The sample size is 197.

Collecting nasal swabs

A dry polyester swab was inserted into the nostril, parallel to the palate, and left in place for a few seconds. It was then slowly withdrawn with a rotating motion. Specimens from both nostrils were obtained with the same swab. The collected nasal swab was transported to the laboratory research as soon as possible if not were kept at 2°C–30°C until transported to the laboratory. If the process of collected nasal swab cannot be performed within 36 h, they were stored in the refrigerator at 2°C–8°C to maximum 5 days.[15]

Two hundred and twenty-five nasal swabs were collected from 100 health-care workers and 125 patients at Prince Mutaib Bin Abdulaziz Hospital, Skaka, Al-Jouf region, Saudi Arabia. The collection of nasal swabs occurred from March 16 to September 9, 2015.

Institutional Review Board approval to perform this study was granted by the Ethical Committee at Al-Jouf University, Skaka, Saudi Arabia.

Isolation of *Staphylococcus aureus*

The nasal swabs were cultured onto blood and mannitol salt agar plates. Only those showing growth of golden-yellow colonies with beta-hemolysis on blood agar and yellow colonies on mannitol salt agar were picked for further testing. *S. aureus* isolates were identified by the Gram-staining and standard biochemical reaction, such as catalase and coagulase tests in the Microbiology Laboratory, College of Applied Medical Sciences, Al-Jouf University, Skaka, Saudi Arabia.

Antimicrobial susceptibility testing

Susceptibility of clinical isolates to seven antibiotics (Mast, Merseyside, UK), including oxacillin (1 μg), Cefoxitin (30 μg), vancomycin (30 μg), clindamycin (2 μg), tetracycline (30 μg), erythromycin (15 μg), and penicillin (10 μg) was evaluated by agar disc diffusion method on Mueller-Hinton agar plates, as recommended by the Clinical and Laboratory Standards Institute.[16] *S. aureus* ATCC 1026 (MRSA strain) was used as control strain for disc susceptibility testing. Only isolates showed resistant to oxacillin were chosen for genotypic tests.

DNA extraction from identified methicillin-resistant *Staphylococcus aureus*

Bacterial genomic DNA was isolated from bacterial suspension cultures using QIAamp DNA Blood Mini Kits (Qiagen, USA) following the protocol in the QIAamp DNA Mini and Blood Mini Handbook (2012).[17]

Genotyping testing

The types and designs of the primers used are shown in Table 1. The concentration and purity of extracted

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DNA were measured using a NanoDrop 8000 spectrophotometer (Thermo Scientific, USA). The DNA concentrations for all *S. aureus* isolates ranged from 7.76–236.7 ng/µl. The DNA purity (260/280) was ≥1.32.

Polymerase chain reaction for *coa* gene

The PCR cycling protocol was applied as following: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 2 min, followed by a final extension at 72°C for 7 min.[18]

Polymerase chain reaction for *mecA* gene

After an initial denaturation step (3 min at 94°C), 30 cycles of amplification were performed: Denaturation at 94°C for one minute, annealing at 56°C for 1 min, and DNA extension at 72°C for 1 min. The reaction was finished with a final extension step at 72°C for 7 min.[14]

Polymerase chain reaction for *spa* gene

The amplification reaction consisted of an initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 3 min, followed by a final extension step at 72°C for 5 min.[19]

Results

Bacterial isolates

The total number of isolated *S. aureus* according to phenotypic characteristics was 76 (33.8%), comprising 35 (46.1%) from health-care workers and 41 (53.9%) from patients. Whereas, 143 (63.6%) and 6 (2.6%) were characterized as coagulase-negative staphylococci and non-*Staphylococcus* [Table 2].

Antibiotic resistance profiles

The antimicrobial susceptibility testing by agar disc diffusion method among *S. aureus* isolates determined that the percentage of resistance to oxacillin, cefoxitin, vancomycin, clindamycin, tetracycline, erythromycin, and penicillin were 100.0%, 60.0%, 33.3%, 46.7%, 53.3%, 56.7%, and 100.0%, respectively [Tables 3 and 4]. The highest rate of resistance among *S. aureus* isolates was related to oxacillin and penicillin with 100% frequency. Thirty out of 76 *S. aureus* isolates showed resistance to oxacillin only but 18 out of 30 showed resistance to both oxacillin and cefoxitin. The latest 30 isolates were considered MRSA and chosen for further genotyping tests.

Coagulase gene typing

The *coa* gene was amplified by PCR for 30 isolated *S. aureus* obtained from health-care workers (n = 11)

Table 1: Types of primers, primer designs, and references

Primer	Primer design	Product size (bp)	Reference
coa gene F	5'-CGA GAC CAA GAT TCA ACA AG-3'	800	Himabindu *et al*., 2009
coa gene R	5'-AAA GAA AAC CAC TCA CAT CA-3'		
mecA gene F	5'-TGG CTA TGT GAC AAT CG-3'	300	Vannuffel *et al*., 1995
mecA gene R	5'-CTG GAA CTT GTT GAG GAG AG-3'		
spa gene F	5'-ATC TGG TGG GGT AAC AACTG-3'		
spa gene R	5'-CGC TGC ACC TAA CGC TAA TG-3'	1100	Wichelhaus *et al*., 2001

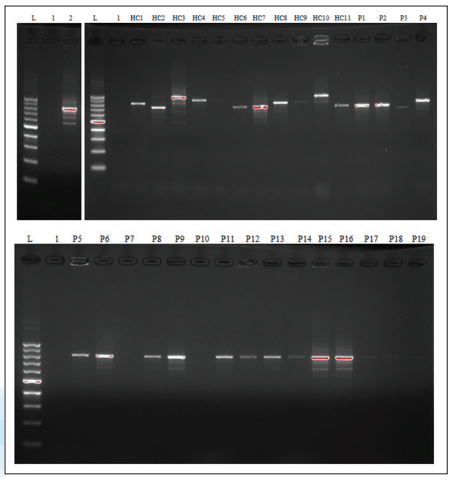

Figure 1: Three percent agarose gel electrophoresis of *coa* gene PCR products where L is DNA molecular marker (100 bp ladder), (a) Lane1: negative control (no DNA template); lane 2: positive control (*coa* positive ATCC1026) showing 4 bands; Lane L: DNA molecular size marker (100 bp ladder), (b) strains for HC1-HC11 showing one band except for HC3, HC7 and HC10 showing four, three and two bands, and (c) strains P1-P19 showing one band except P6, P9, P15 and P16 showing two bands and P7, P10 and P19 showing no bands

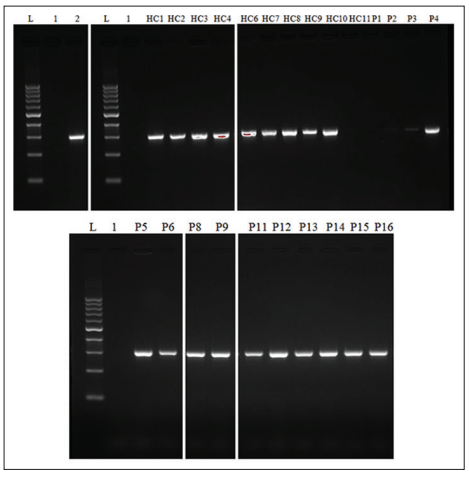

Figure 2: Three percent agarose gel electrophoresis analysis of PCR amplification products of *mecA* gene of 300 bp, extracted from *S. aureus* PCR products for *mecA* gene for positive MRSA samples. Lane1: negative control (no DNA template); lane 2: positive control (*mecA* positive ATCC1026); Lane L: DNA molecular size marker (100 bp ladder)

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Table 2: The prevalence of *Staphylococcus* isolates from the nasal swabs of health-care workers and patients

Participants	Total number of participants, *n* (%)	Coagulase positive[a], *n* (%)	Coagulase negative[a], *n* (%)	Non-*Staphylococcus*, *n* (%)
Health-care workers	100 (44.4)	35 (35.0)	61 (61.0)	4 (4.0)
Patients	125 (55.6)	41 (32.8)	82 (70.4)	2 (1.6)
Total	225 (100.0)	76 (33.8)	143 (63.6)	6 (2.6)

[a]According the results of phenotypic characteristics=Colonial growth in blood agar and mannitol salt agar; type of hemolysis in blood agar; Gram stain; catalase test; and slide and tube coagulase tests

Table 3: Antibiotics susceptibility tests for isolated *Staphylococcus aureus* from both health-care workers and patients

	Susceptibility of antimicrobials						
	OX (1 µg)	FOX (30 µg)	VA (30 µg)	CC (2 µg)	TET (30 µg)	ERY (15 µg)	P (10 µg)
Health-care workers							
HC1	R	R	S	R	R	R	R
HC2	R	R	S	R	R	R	R
HC3	R	R	S	R	R	R	R
HC4	R	R	S	R	S	S	R
HC5	R	R	S	S	S	S	R
HC6	R	R	S	S	S	S	R
HC7	R	R	S	S	R	R	R
HC8	R	R	S	S	S	S	R
HC9	R	S	R	S	R	R	R
HC10	R	S	R	S	S	R	R
HC11	R	S	R	S	S	R	R
Patients							
P1	R	R	R	S	R	R	R
P2	R	R	R	S	R	R	R
P3	R	R	R	S	R	R	R
P4	R	R	R	S	R	R	R
P5	R	R	R	S	S	S	R
P6	R	R	R	S	S	S	R
P7	R	R	R	S	S	S	R
P8	R	R	R	S	S	S	R
P9	R	R	S	S	S	S	R
P10	R	R	S	R	R	R	R
P11	R	S	S	R	R	R	R
P12	R	S	S	R	R	R	R
P13	R	S	S	R	S	S	R
P14	R	S	S	R	S	S	R
P15	R	S	S	R	S	S	R
P16	R	S	S	R	R	R	R
P17	R	S	S	R	R	R	R
P18	R	S	S	R	R	R	R
P19	R	S	S	R	R	R	R
MRSA ATCC 1026	R	R	R	R	R	R	R

Abbreviation for susceptibility: R=Resistance; S=Sensitive. Abbreviation of antimicrobial agents: OX=Oxacillin; FOX=Cefoxitin; VA=Vancomycin; CC=Clindamycin; TET=Tetracycline; ERY=Erythromycin; P=Penicillin. MRSA=Methicillin-resistant *Staphylococcus aureus*

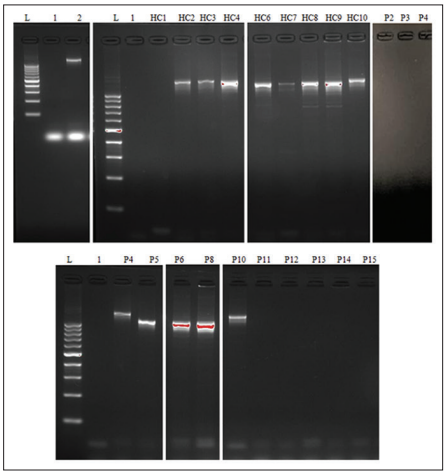

Figure 3: Three percent agarose gel electrophoresis of spa gene PCR products where L is DNA molecular marker (100 bp ladder), (a) Lane1: negative control (no DNA template); lane 2: positive control (*coa* positive ATCC1026) showing 1 band; Lane L: DNA molecular size marker (100 bp ladder), (b) strains HC1-HC11 showing one band except for HC8 and HC9 showing two bands and HC1 showing no bands, and (c) strains P2, P3, P4, P11,P12,P13,P14 and P15 showing no bands but P4, P5, P6, P8 and P10 showing one band

and patient (n = 19). The thirty isolated *S. aureus* were identified according phenotypic characteristics and antimicrobial susceptibility test. The MRSA ATCC 1026 (control) was also amplified for the *coa* gene by PCR. The control showed 4 different bands of different sizes 580, 650, 810, and 900 bp. In health-care workers, HC5 is the only one out of 11 did not show the *coa* gene band. Seven out 10 isolated *S. aureus* showed only one band with different sizes. The HC2, HC6, and HC11 showed one band sized 900 bp. The HC1, HC8, and HC9 showed one band sized 880 bp. The HC2, HC6, and HC11 showed one band sized 810 bp. Whereas 3 out of 10 isolated *S. aureus* showed more than one band. The HC3 showed 4 *coa* gene bands (500, 600, 700, and 810 bp). The HC7 showed 3 bands (500, 600, and 810 bp). The HC10 showed 2 bands (900 and 1000 bp). In patients, 5 out of 19 showed no *coa* gene band. Eleven out of 14 *S. aureus* isolates showed one band with different sizes. The P4 showed one *coa* gene band sized 900 bp. The P5, P11, P13, P14, and P15 showed one band sized 860 bp. The P1, P2, and P3 showed one band sized 810 bp. Two out of 14 showed two bands with different sizes. The P9 and P16 showed two bands

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Table 4: Summary of antibiotics resistance for isolated *Staphylococcus aureus* from both health-care workers and patients

	Number of microbial resistance, *n* (%)						
	OX (1 µg)	FOX (30 µg)	VA (30 µg)	CC (2 µg)	TET (30 µg)	ERY (15 µg)	P (10 µg)
Health-care workers	11 (36.7)	8 (44.4)	3 (30.0)	4 (28.6)	5 (31.3)	7 (41.2)	11 (36.7)
Patients	19 (63.3)	10 (55.6)	7 (70.0)	10 (71.4)	11 (68.57)	10 (58.8)	19 (63.3)
Total	30 (100)	18 (60.0)	10 (33.3)	14 (46.7)	16 (53.3)	17 (56.7)	30 (100)

Abbreviation of antimicrobial agents: OX=Oxacillin; FOX=Cefoxitin; VA=Vancomycin; CC=Clindamycin; TET=Tetracycline; ERY=Erythromycin; P=Penicillin

Table 5: Frequency of coagulase, methicillin, and protein A genotypes in *Staphylococcus aureus* isolates

	Coagulase gene (*coa* gene)		Methicillin gene (*mecA* gene)		Protein A (*spa* gene)	
	Number of bands	PCR products (bps)	Number of bands	PCR products (bps)	Number of bands	PCR products (bps)
Health-care workers						
HC1	1	880	1	300	NB	NB
HC2	1	810	1	300	1	1100
HC3	4	500; 600; 700; 810	1	300	1	1100
HC4	1	900	1	320	1	1100
HC5	NB	NB	ND	ND	ND	ND
HC6	1	810	1	380	1	1100
HC7	3	500; 600; 810	1	380	1	1100
HC8	1	880	1	380	2	800; 1100
HC9	1	880	1	380	2	800; 1100
HC10	2	900; 1000	1	380	1	1200
HC11	1	810	NB	NB	ND	ND
Patients						
P1	1	810	NB	NB	ND	ND
P2	1	810	1	380	NB	NB
P3	1	810	1	380	NB	NB
P4	1	900	1	380	1	1100
P5	1	860	1	300	1	1000
P6	2	700; 860	1	300	1	1000
P7	NB	NB	ND	ND	ND	ND
P8	1	860	1	300	1	1000
P9	2	680; 860	1	300	1	1200
P10	NB	NB	ND	ND	ND	ND
P11	1	860	NB	NB	ND	ND
P12	1	860	1	300	NB	NB
P13	1	860	1	300	NB	NB
P14	1	860	1	300	NB	NB
P15	2	860	1	300	NB	NB
P16	2	680; 860	1	300	NB	NB
P17	NB	NB	ND	ND	ND	ND
P18	NB	NB	ND	ND	ND	ND
P19	NB	NB	ND	ND	ND	ND
MRSA ATCC 1026	4	580; 650; 800; 900	1	300	1	1100

NB=No band; ND=Not determined, PCR=Polymerase chain reaction; MRSA=Methicillin-resistant *Staphylococcus* aureus

sized 680 and 860 bp. The P6 showed also two bands sized 700 and 860 bp. As summary, 6 out of 30 showed no *coa* gene band and 24 out of 30 isolates were confirmed to be *S. aureus* [Table 5 and Figure 1].

The following sized bands presented only in health-care workers *S. aureus* isolates: 500 bp (20%), 600 bp (20%), 880 bp (30%), and 1000 bp (10%), respectively. For those only presented in patients *S. aureus* isolates: 680 bp (15.4%) and 860 bp (69.2%), respectively. There were two sized bands not presented in health-care workers and patients but showed in control were: 580 bp and 650 bp. On the other hand, the following sized bands presented in health-care workers, patients,

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Table 6: The percentage of *coa* gene band presented in health-care workers and patients *Staphylococcus aureus* isolates

	500 bp	580 bp	600 bp	650 bp	680 bp	700 bp	810 bp	860 bp	880 bp	900 bp	1000 bp
Control		Present		Present			Present			Present	
Health-care workers											
HC1									Present		
HC2							Present				
HC3	Present		Present			Present					
HC4										Present	
HC6							Present				
HC7	Present		Present				Present				
HC8									Present		
HC9									Present		
HC10										Present	Present
HC11							Present				
Percentage	20	0.0	20	0.0	0.0	10	40	0.0	30	20	10
Patients											
P1							Present				
P2							Present				
P3							Present				
P4										Present	
P5								Present			
P6						Present		Present			
P9					Present			Present			
P11								Present			
P12								Present			
P13								Present			
P14								Present			
P15								Present			
P16					Present			Present			
Percentage	0.0	0.0	0.0	0.0	15.4	7.7	23.1	69.2	0.0	7.7	0.0

and control: 810 bp (40% and 23.1%) and 900 bp (20% and 7.7%) [Table 6].

Methicillin-resistant typing

The *mecA* gene was amplified by PCR for 24 confirmed isolated *S. aureus* obtained from health-care workers (n = 10) and patients (n = 14). The control showed only one band sized 300 bp. In health-care workers, the HC1 showed no *mecA* gene band. Whereas, 9 isolates showed only one band with different sizes. The HC1, HC2, and HC3 showed one band sized 300 bp which were similar to the control. The HC4 showed one band sized 320 bp but HC6, HC7, HC8, and HC9 showed one band sized 380 bp. In patients, 2 out of 14 showed no *mecA* gene band. The other 12 confirmed isolates showed only one band but with different sizes. The P5, P6, P8, P9, P12, P13, P14, P15, and P16 showed on band sized 300 bp also similar to control. However, P2, P3, and P4 showed one band sized 380 bp. As summary, 3 out of 14 were no *mecA* gene band. Accordingly, 21 *S. aureus* isolates were considered MRSA [Table 5 and Figure 2].

A 320 bp sized *mecA* gene presented only in health-care workers MRSA isolates (12.5%). Similar to control, the 300 bp presented in both health-care workers and patients (37.5% and 75%). The third sized band presented only in health-care workers and patients but not in control: 380 bp (50% and 25%) [Table 7].

Protein A typing

The *spa* gene was amplified by PCR for 21 confirmed MRSA isolates obtained from health-care workers (n = 9) and patients (n = 12). The control showed only one band of *spa* gene of 1100 bp. In health-care workers, HC1 showed no *spa* gene band, whereas 6 out of 8 showed only one band with different sizes. HC2, HC3, HC4, HC6, and HC7 showed one band of 1100 bp similar to control, but HC11 showed one band of 1200 bp. On the other hand, 2 (HC8 and HC9) out of 8 of MRSA showed 2 *spa* gene bands sized 800 and 1100 bp. In patients, 7 out of 12 confirmed MRSA showed no *spa* gene band. The other five MRSA showed one band only with different sizes. The P4 showed one band sized 1100 bp similar to control. The P5, P6, and P8 showed one band sized 1000 bp but P9 showed one

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Table 7: The percentage of *mecA* and *spa* genes band presented in health-care workers and patients methicillin-resistant *Staphylococcus aureus* isolates

	mecA gene			*spa* gene			
	300 bp	320 bp	380 bp	800 bp	1000 bp	1100 bp	1200 bp
Control	Present					Present	
Health-care workers							
HC1	Present					Present	
HC2	Present					Present	
HC3	Present					Present	
HC4		Present				Present	
HC6			Present			Present	
HC7			Present	Present		Present	
HC8			Present	Present		Present	
HC9			Present			Present	
Percentage	37.5	12.5	50	25	0.0	100	0.0
Patients							
P2			Present	NB	NB	NB	NB
P3			Present			Present	
P4			Present	NB	NB	NB	NB
P5	Present				Present		
P6	Present				Present		
P8	Present				Present		
P9	Present						Present
P12	Present			NB	NB	NB	NB
P13	Present			NB	NB	NB	NB
P14	Present			NB	NB	NB	NB
P15	Present			NB	NB	NB	NB
P16	Present			NB	NB	NB	NB
Percentage	75	0.0	25	0.0	42.9	20	20

NB=No band

band sized 1200 bp. As summary, 8 out of 21 MRSA showed no *spa* gene band. The *spa* gene in health-care workers was present in 88.88% and for patients was 41.66% [Table 5 and Figure 3].

The 800 bp-sized *spa* gene band presented only in health-care workers MRSA isolates (25%). Whereas, the two sized bands presented only in patients were 1000 bp (42.9%) and 1200 bp (20%). Similar to control, the 1100 bp presented in both health-care workers and patient MRSA isolates (100% and 20%) [Table 7].

Discussion

Rising colonization rates of MRSA lead to increased infection rates in hospitals. This leads to significant increased morbidity and mortality rates due to invasive MRSA infection.[20] The present study was conducted on health-care workers and patients. Among 225 isolates of bacteria, 76 were confirmed to be *S. aureus* by phenotypic characteristics.

Thirty of *S. aureus* isolates were identified as possible MRSA based on their resistance to oxacillin. They also considered multidrug resistant (MDR), exhibiting resistance to three or more antibiotic classes. In this study, resistance was most frequently observed against oxacillin and penicillin (100%). The cefoxitin showed resistance against 18 (60%). Papadopoulos *et al*.[21] Omar *et al*.,[12] and Baddour *et al*.[22] studies showed different susceptibility patterns. The difference in antibiotic resistance patterns may be due to various factors such as the local environment, selective antibiotic pressure, acquisition and loss of plasmids carrying resistance genes, and various other genetic mechanisms.[23] Other studies documented the association of recovery of MDR-MRSA strains from inpatient clinical samples rather than from outpatients.[24,25]

Genetically, the criterion to identify *S. aureus* is by detecting the *coa* gene by PCR.[26] Tiwari *et al*.[27] study confirmed the fact that this gene is present in all *S. aureus* isolates. The *coa* gene was identified in 21 of MRSA isolates in this study.

In the present study, 11 different band classes of the *coa* gene with band sizes ranging from 500 to 1000 bp were found, generating 2 different types and 7 subtypes of *coa* band patterns. The majority (17/24) of MRSA strains showed single band, 4 (16.7%) showed double

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bands, and the remaining 1 (4.2%) had four and three bands. The 860 bp was the most common band in patients and was found in 9/13 of the isolates (69.2%), whereas the 810 bp was common in health-care workers and was found in 4/10 of the isolates (40%). The presence of more than one band has been explained by the existence of more than one allelic form of coagulase gene, allowing one strain to produce one or more of these variants.[28] This gene polymorphism might be due to deletion or insertion mutations, by which a portion of the 3' end region of the *coa* gene is deleted or several nucleotides are inserted and as a consequence change the *coa* gene size.[29]

The study conducted by Ishino *et al.*[30] showed that the sizes of PCR products obtained after amplification of *S. aureus* from clinical samples ranged from 650 bp to 1000 bp. They categorized 678 *S. aureus* isolates of human specimens into eight classes and the sizes of the PCR products of the *coa* gene ranged from 350 bp to 917 bp. In addition, Ahlam *et al.*[31] described the size of the *coa* gene PCR product of *S. aureus* isolates as 723–913 bp, giving four classes at 723, 812, 648, and 913 bp, of which the 812-bp class was the most common class among the isolates. Afrough *et al.*[13] reported the size of the *coa* gene PCR product of *S. aureus* isolates from patients and carriers in Iran as 650–900 bp, which is similar to the results of the current study. In another study performed by Babu *et al.*[32] in India in 2014.

Omar *et al.*[12] and Himabindu *et al.*,[18] using the same primer, showed that the sizes of *coa* PCR products were classified into three band classes. The majority of isolates belonged to the band class of 812 bp, which was close to our study results, where 23.1% in patients and was 40% in health-care workers isolates belonged to the same band class (810 bp). The difference in coagulase types was found to be subject to geographical variation.[18]

The *mecA* gene is considered to be the gold standard for MRSA diagnosis.[33] This study included only isolates that had *mecA* gene as shown with PCR. Methicillin resistance is mediated by *mecA* gene, this gene is located in *Staphylococcus* Chromosomal Cassette and it is a site-specific transposon-like element that is present only in staphylococcal species.[34] It codes for PBP2 which is present in the cell wall and has low affinity for beta-lactam antibiotics.[35]

According to this study, three different band classes of the *mecA* gene with band sizes ranging from 300 to 380 bp were found, generating two different types and one subtype of *mecA* band patterns. All of MRSA strains showed single band. The 300 bp was the most common band in patients and was found in 9/12 of the isolates (75%), whereas the 380 bp was common in health-care workers and was found in 4/8 of the isolates (50%). Similar to our results, El Shabrawy *et al.*[36] study showed their isolates that were resistant to cefoxitin is a better predictor of methicillin-resistant than oxacillin because it is a stronger inducer of PBP2.

Four different band classes of *spa* gene with band sizes ranging from 800 bp to 1200 bp were found, generating three different types and one subtype of *spa* band patterns. The majority (11/13) showed single band and the remaining 2 (15.4%) showed double bands. Omar *et al.*[12] study showed that majority of patient isolates (63 strains) showed a single band and 7 had two bands with different sizes. Their bands size ranged between 144 and 1392 bp. The present study showed the absence of *spa* gene in patients (58.3%). Similar results were observed by El Shabrawy *et al.*,[36] Shakeri *et al.*,[37] and Adesida *et al.*[38] studies, they showed that *spa* gene was absent in 94.4%, 3.8%, and 5% of their *S. aureus* isolates. The 1100 bp was the most common band in health-care workers MRSA isolates (100%). In the present study, patient isolates showed only one band where the majority were 1000 bp (42.9%). The variations in the size of *spa* gene reflecting the number of 24 bp repeat units contained in the *spa* gene.

Conclusions

Finally, we conclude that MRSA is a serious health problem. Certainly, it is widely spread in our hospital environment. This study is suggestive that the PCR for the detection of *coa*, *mecA*, and *spa* gene is a fast, accurate, and valuable diagnostic tool; it is recommended better prevention and control programs.

Financial support and sponsorship

The project was granted by the Deanship of Scientific Research, Jouf University.

Conflicts of interest

There are no conflicts of interest.

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