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Brief Original Article

Phenotypic and molecular characterization of HA-MRSA in Taif hospitals, Saudi Arabia

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Abstract

Introduction: Methicillin-resistant *S. aureus* (MRSA) is one of the most important organisms causing hospital-acquired infections worldwide. Molecular analysis of MRSA strains from Taif, Saudi Arabia, had not been previously done. Phenotypic and molecular characteristics of MRSA isolated from Taif hospitals were investigated.

Methodology: This study involved *S. aureus* strains isolated from different clinical samples from Taif hospitals. MRSA strains were identified and antimicrobial susceptibility profiles were determined. Multiplex polymerase chain reaction (PCR) was used to identify *S. aureus*-specific sequence, *mecA* genes, and type of staphylococcal cassette chromosome *mec* (SCC*mec*). MRSA strains were typed using coagulase gene polymorphism.

Results: In total, 390 strains of *S. aureus* were isolated, and 58 MRSA strains – 40 hospital-acquired MRSA (HA-MRSA) and 18 community-acquired MRSA (CA-MRSA) – were detected. HA-MRSA strains included three SCC*mec* types, while CA-MRSA strains included two SCC*mec* types. PCR amplification and restriction of the coagulase gene of the 58 MRSA isolates showed nine different patterns, while three strains were non-typable. HA-MRSA strains showed seven distinct restriction fragment length polymorphism (RFLP) patterns; the most frequent was pattern 2 (15 isolates), followed by patterns 1 and 4 (5 isolates each). CA-MRSA showed five RFLP patterns; the most frequent was pattern 3 (7 isolates) followed by pattern 8 (6 isolates).

Conclusions: HA-MRSA strains were more common than CA-MRSA strains. SCC*mec* typing and coagulase gene polymorphism analysis may be useful methods for studying clonal relatedness of isolates and for discriminating between HA-MRSA and CA-MRSA.

Key words: HA-MRSA; CA-MRSA; SCC*mec*; *mec*A gene.

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Introduction

Healthcare-associated infections (HAIs) are considered among the most prevalent complications of hospital care [1]. *Staphylococcus aureus* (*S. aureus*) is responsible for a wide spectrum of infections ranging from superficial skin infections to fatal invasive infectious diseases including pneumonia, septicemia, and endocarditis [2]. Methicillin-resistant *S. aureus* (MRSA) was first reported in the early 1960s and has become a major nosocomial pathogen worldwide [3]. In the Gulf region, MRSA has been considered to be one of the most prevalent pathogens by Gulf Corporation Council (GCC) countries in the last two decades [4].

Methicillin resistance results from introduction of staphylococcal cassette chromosome *mec* (SCC*mec*) into methicillin-susceptible *S. aureus* strains. SCC*mec* is a genomic island that contains the antibiotic resistance gene *mecA* [5]. SCC*mec* also contains *ccrA* and *ccrB*; both genes encode recombinases that mediate the site-specific integration and excision of the SCC*mec* element from the *S. aureus* chromosome [6,7]. The *mec*A gene codes for a variant type penicillin-binding protein (PBP2a), which has a lower affinity for the β-lactam antibiotics because its active site does not bind methicillin or other β-lactam antibiotics. Therefore, the transpeptidation reaction required for peptidoglycan cross-linking will continue, enabling cell wall synthesis [8]. Seven main types of SCC*mec* (type I to VII) are recognized. Types IV, V, VI, and VII code for β-lactam antibiotic resistance only, while SCC*mec* I, II, and III cause resistance to

multiple classes of antibiotics, due to additional integrated drug resistance genes [9].

There are two major strains of MRSA: hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA). They have been proven to be genetically distinct with respect to the SCC*mec* type; most HA-MRSA strains carry one of three types of SCC*mec* (types I, II, or III). On the other hand, CA-MRSA strains carry mainly SCC*mec* type IV and occasionally type V [10].

In Saudi Arabia, few studies have been conducted to investigate the epidemiology of MRSA in the community or in hospitals. In 1991, a study showed that Saudi and British strains differed in their sensitivity patterns to antibiotics [11]. In Riyadh, it was found that overcrowding of patients and inadequate infection control measures were the major risk factors for MRSA acquisition [12]. In Abha (south region of Saudi Arabia), MRSA accounted for 5.1% of hospital and 18.3% of non-hospital isolates [13]. To our knowledge, molecular analysis of MRSA strains in Taif area of Saudi Arabia has not been previously done. This study was conducted to investigate the phenotypic and molecular characteristics of MRSA isolates in Taif region, Saudi Arabia, and to compare CA-MRSA and HA-MRSA strains.

Methodology

Specimen collection and phenotypic testing

This study was conducted on MRSA strains collected form Taif hospitals between January 2013 and February 2014. Demographic, clinical, and laboratory data were collected after written consent was obtained. The clinical specimens of MRSA isolates included sputum, pus, pharyngeal swabs, urine, blood, and peritoneal fluid. The study proposal was approved by the Faculty of Applied Medical Sciences Ethics Committee of Taif University.

Identification and antibiogram of S. aureus

Initial screening and identification of *S. aureus* were performed according to the standard laboratory protocols. Isolates were further confirmed by detection of staphylococcal-specific genes by polymerase chain reaction (PCR). Antibiotic susceptibility profiles were determined and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Methicillin resistance was tested by standard oxacillin salt agar screening plate procedure and cefoxitin susceptibility as indicated by the CLSI. Methicillin-resistant and methicillin-sensitive strains were included as controls [14]. Methicillin resistance was further confirmed by the detection of the *mecA* gene by the PCR method.

DNA extraction

Bacterial DNA was extracted by PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, Foster city, USA) according to the manufacturer's instructions. Briefly, 1 mL of overnight lysogeny LB broth culture was centrifuged for 2 minutes. The pellet was re-suspended in phosphate buffer saline, then centrifuged again and the supernatant was discarded. PrepMan Ultra Sample Preparation Reagent (100 µL) was added. The tubes were placed in a heat block at 100°C for 10 minutes. The tubes were allowed to cool to room temperature and centrifuged at the highest speed for 2 minutes. Then, 50 µL of the supernatant was stored at -20°C and 5 µL was used for each PCR assay.

Detection of S. aureus-specific sequence gene and mecA gene

Multiplex PCR was used to amplify both the *S. aureus*-specific (107 bp) and *mecA* (532 bp) genes. The *mecA* gene was amplified using primers 5'- AAA ATC GAT GGT AAA GGT TGGC - 3' and 5'- AGT TCT GCA GTA CCG GAT TTG C-3'. The *S. aureus*-specific gene was amplified using primers 5'- AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG -3' and 5'- CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA -3'.The PCR reaction mixture and cycling conditions were done using the method of Martineau *et al*. [15].

Staphylococcal cassette chromosome mec typing

SCC*mec* typing was done by the multiplex PCR method [16]. The multiplex PCR included eight loci (A through H) selected on the basis of the previously described *mec* element sequences. The multiplex PCR was performed using the GeneAmp PCR kit (Applied Biosystems).

PCR-restriction fragment length polymorphism (RFLP) typing of MRSA

MRSA strains were typed using coagulase gene polymorphism. The 3' end region of the coagulase gene was amplified by PCR as previously described by Lawrence *et al*.[17]. PCR amplification of the variable region of the coagulase gene was performed using primers COAG2-5'CGAGACCAAGA TTCAACAAG3' and COAG3-5'AAAGAAAACCACTCACATCA3'.

Table 1. Clinical sources of the isolated MRSA andMSSA strains

Type of infection	MSSA (n = 332)		MRSA (n = 58)		Total
	No	%	No	%	
Bloodstream infection	121	36.4	22	37.9	**143**
Wound infection	130	39.1	19	32.8	**149**
UTI	22	6.6	4	6.9	**26**
LRT infection	31	9.3	11	19.0	**42**
URT infection	18	5.4	0	0	**18**
Other	10	3.0	2	3.4	**12**
Total	**332**	**85.1%**	**58**	**14.9%**	**390**

Table 2. Characterization of HA-MRSA strains, SCC*mec* type, PCR-RFLP of *coa* and *mecA* genes

Strain no.	Source	*SCCmec* type	*mecA*	*coa*-RFLP profile (in bp)	Antibiotic resistance pattern
MRSA1	Wound swab	I	+ /+	(2) 81, 567	(2) PEN, OX, CEF, GN, TET, TS, RF
MRSA2	Wound swab	I	+ /+	(2) 81, 567	(2) PEN, OX, CEF, GN, TET, TS, RF
MRSA3	Blood	III	+ /+	(4) 234, 486	(1) PEN, OX, CEF, MU, ERY, CHL, TET, TS, CIP
MRSA4	Urine	I	+ /+	(1) 324, 405	(2) PEN, OX, CEF, GN, TET, TS, RF
MRSA5	Wound swab	III	+ /+	(9) 81, 230, 480	(1) PEN, OX, CEF, MU, ERY, CHL, TET, TS, CIP
MRSA6	Wound swab	I	+ /+	(2) 81, 567	(4) PEN, OX, CEF, GN, TS, RF
MRSA7	Blood	I	+ /+	(2) 81, 567	(6) PEN, OX, CEF, GN, ERY, TET, TS, CIP
MRSA8	Wound swab	I	+ /+	(2) 81, 567	(10) PEN, OX, CEF, ERY, CHL, TET, TS, CIP
MRSA9	Eye swab	I	+ /+	(2) 81, 567	(2) PEN, OX, CEF, GN, TET, TS, RF, TZP
MRSA10	Blood	III	+ /+	(9) 81, 230, 480	(6) PEN, OX, CEF, GN, ERY, TET, TS, CIP
MRSA11	Urine	III	+ /+	(4) 234, 486	(10) PEN, OX, CEF, ERY, CHL, TET, TS, CIP
MRSA12	Blood	III	+ /+	(4) 234, 486	(10) PEN, OX, CEF, ERY, CHL, TET, TS, CIP
MRSA13	Wound swab	III	+ /+	(4) 234, 486	(11) PEN, OX, CEF, GN, ERY, CHL, TET
MRSA14	Urine	I	+ /+	(1) 324, 405	(2) PEN, OX, CEF, GN, TET, TS, RF TZP
MRSA15	Wound swab	III	+ /+	(9) 81, 230, 480	(9) PEN, OX, CEF, GN, ERY, TET, TS
MRSA16	Blood	III	+ /+	(9) 81, 230, 480	(12) PEN, OX, CEF, GN, ERY, TET, TS
MRSA17	Wound swab	I	+ /+	(2) 81, 567	(4) PEN, OX, CEF, GN, TS, RF
MRSA18	Blood	II	+ /+	(6) 81, 146, 178, 340	(12) PEN, OX, CEF, GN, ERY, TET, TS
MRSA19	Blood	II	+ /+	(7) 81, 234, 405	(1) PEN, OX, CEF, MU, ERY, CHL, TET, TS, CIP
MRSA20	Peritoneal fluid	II	+ /+	(7) 81, 234, 405	(3) PEN, OX, CEF, GN, MU,ERY, TET, TS, CIP
MRSA21	Blood	III	+ /+	None	(9) PEN, OX, CEF, GN, ERY, TET, TS
MRSA22	Sputum	I	+ /+	(2) 81, 567	(3) PEN, OX, CEF, GN, MU, ERY, TET, TS, CIP
MRSA23	Wound swab	III	+ /+	(9) 81, 230, 480	(9) PEN, OX, CEF, GN, ERY, TET, TS
MRSA24	Blood	III	+ /+	(9) 81, 230, 480	(12) PEN, OX, CEF, GN, ERY, TET, TS
MRSA25	Wound swab	I	+ /+	(2) 81, 567	(2) PEN, OX, CEF, GN, TET, TS, RF
MRSA26	Wound swab	I	+ /+	(2) 81, 567	(2) PEN, OX, CEF, GN, TET, TS, RF
MRSA27	Blood	II	+ /+	(7) 81, 234, 405	(5) PEN, OX, CEF, GN, ERY, CHL, TET, TS, CIP
MRSA28	Blood	II	+ /+	(1) 324, 405	(12) PEN, OX, CEF, GN, ERY, TET, TS
MRSA29	Blood	III	+ /+	(4) 234, 486	(5) PEN, OX, CEF, GN, ERY, CHL, TET, TS, CIP
MRSA30	Blood	III	+ /+	None	(2) PEN, OX, CEF, GN, TET, TS, RF
MRSA31	Sputum	II	+ /+	(7) 81, 234, 405	(8) PEN, OX, CEF, GN, SM, CHL, TET, CIP
MRSA32	Wound swab	I	+ /+	(2) 81, 567	(2) PEN, OX, CEF, GN, TET, TS, RF, TZP
MRSA33	Sputum	I	+ /+	(2) 81, 567	(4) PEN, OX, CEF, GN, TS, RF
MRSA34	Urine	III	+ /+	(9) 81, 230, 480	(7) PEN, OX, CEF, GN, MU, ERY, TET, TS, RF
MRSA35	Sputum	I	+ /+	(2) 81, 567	(9) PEN, OX, CEF, GN, ERY, TET, TS
MRSA36	Wound swab	I	+ /+	(2) 81, 567	(3) PEN, OX, CEF, GN, MU, ERY, TET, TS, CIP
MRSA37	Blood	III	+ /+	(8) 162, 230, 324	(5) PEN, OX, CEF, GN, ERY, CHL, TET, TS, CIP
MRSA38	Wound swab	II	+ /+	(1) 324, 405	(5) PEN, OX, CEF, GN, ERY, CHL, TET, TS, CIP
MRSA39	Sputum	I	+ /+	(2) 81, 567	(2) PEN, OX, CEF, GN, TET, TS, RF
MRSA40	Sputum	I	+ /+	(1) 324, 405	(4) PEN, OX, CEF, GN, TS, RF

PEN: penicillin, OX: oxacillin, CEF: cefoxitin, GN: gentamicin, SM: streptomycin, ERY: erythromycin, CHL: chloramphenicol, TET: tetracycline, TS: trimethoprim, RF: rifamipicin, CIP: ciprofloxacin, TZP: piperacillin-tazobactam and MU: mupirocin.

After amplification, 15 μL of the PCR products was digested for 15 minutes with 6 IU of restriction endonuclease *Alul* (Fermentas, Sunderland UK) according to the manufacturer's protocol. Restriction digest fragments were separated by 2% agarose electrophoresis [18].

Results

The studied strains included 390 *S. aureus* that were isolated from male (n = 249) and female (n = 141) Saudi (n = 334) and non-Saudi (n = 56) patients (Table 1). Of them, 58 MRSA strains (40 HA-MRSA and 18 CA-MRSA) were detected and characterized. All the isolated *S. aureus* strains were susceptible to vancomycin and linezolid. All the MRSA isolates were resistant to penicillin, oxacillin, and cefoxitin. Various resistance patterns were demonstrated for the other antibiotics (Table 2). HA-MRSA strains were more resistant than CA-MRSA to the most tested non–β-lactam antibiotics, including ciprofloxacin, gentamicin, tetracycline, chloramphenicol, and tetracycline.

HA-MRSA strains (n = 40) were grouped into three SCC*mec* types: type I (n = 18), type II (n = 7), and type III (n = 15). CA-MRSA strains were grouped into two SCC*mec* types: type IV (n = 11) and type V (n = 6). However, one isolate was non-typeable using the multiplex SCC*mec* (Tables 2 and 3).PCR products of coagulase gene amplification ranged from 648 to 810 bp. The restriction DNA digests were estimated by comparison with a 100-bp DNA marker. PCR amplification and restriction of the coagulase gene of the 58 MRSA isolates showed nine different patterns (Tables 2 and 3), while three strains were non-typable. HA-MRSA strains showed seven distinct RFLP patterns; the most frequent was pattern 2 (15 isolates, 37.5%), followed by patterns 1 and 4 (5 isolates each, 12.5%). CA-MRSA showed five RFLP patterns; the most frequent was pattern 3 (7 isolates, 38.9%) followed by pattern 8 (6 isolates, 33.3%).

Discussion

MRSA is an increasing problem and its burden continues to rise in healthcare facilities [19]. Rising colonization rates lead to increased infection rates in hospitals. This leads to significant increased morbidity and mortality rates due to invasive MRSA infection [20,21]. In our study, MRSA accounted for 14.8% (n = 58) of 390 *S. aureus* strains. This rate is similar to a previous report in New South Wales, Australia [21]. However, a more recent report showed a much lower rate [22]. In Saudi Arabia, an earlier study demonstrated that MRSA accounted for 7.5% of all *S. aureus* isolates in a three-year epidemiological study in Jeddah [23].

Antibiogram allows quick and early recognition of a previously defined epidemic strain in a particular hospital setting. In our study, the antibiotic resistance patterns were similar to those previously reported in Korea by Goh *et al*. [24]. Multiplex PCR SCC*mec* typing of 40 MRSA isolates obtained from clinical cultures showed that 18 isolates were SCC*mec* type I, 15 isolates were type III, and 7 isolates were type II. Among the CA-MRSA strains, the most common

Table 3. Characterization CA-MRSA strains, SCC*mec* type, PCR-RFLP of *coa* and *mecA* genes

Strain no.	Source	*SCCmec* type	*mec*A	*coa*-RFLP profile	Antibiotic resistance pattern
MRSA41	Wound swab	IV	+ /+	(8) 162, 230, 324	(13) PEN, OX, CEF, ERY, TS
MRSA42	Blood	IV	+ /+	(3) 81, 324, 405	(13) PEN, OX, CEF, ERY, TS
MRSA43	Wound swab	V	+ /+	(1) 324, 405	(14) PEN, OX, CEF, TET, TS, RF
MRSA44	Wound swab	V	+ /+	(8) 162, 230, 324	(17) PEN, OX, CEF, ERY, TET, TS
MRSA45	Sputum	V	+ /+	(8) 162, 230, 324	(13) PEN, OX, CEF, ERY, TS
MRSA46	Blood	V	+ /+	(1) 324, 405	(14) PEN, OX, CEF, TET, TS, RF
MRSA47	Sputum	IV	+ /+	(3) 81, 324, 405	(13) PEN, OX, CEF, ERY, TS
MRSA48	Blood	V	+ /+	(8) 162, 230, 324	(17) PEN, OX, CEF, ERY, TET, TS
MRSA49	Blood	non	+ /+	(8) 162, 230, 324	(13) PEN, OX, CEF, ERY, TS
MRSA50	Blood	IV	+ /+	None	(13) PEN, OX, CEF, ERY, TS
MRSA51	Blood	IV	+ /+	(3) 81, 324, 405	(17) PEN, OX, CEF, ERY, TET, TS
MRSA52	Sputum	IV	+ /+	(3) 81, 324, 405	(17) PEN, OX, CEF, ERY, TET, TS
MRSA53	Wound swab	V	+ /+	(8) 162, 230, 324	(17) PEN, OX, CEF, ERY, TET, TS
MRSA54	Blood	V	+ /+	(6) 81, 146, 178, 340	(13) PEN, OX, CEF, ERY, TS
MRSA55	Wound swab	IV	+ /+	(5) 81, 162, 567	(16) PEN, OX, CEF, TET, TS, RF
MRSA56	Sputum	IV	+ /+	(3) 81, 324, 405	(16) PEN, OX, CEF, TET, TS, RF
MRSA57	Blood	IV	+ /+	(3) 81, 324, 405	(15) PEN, OX, CEF, ERY, TS, CIP
MRSA58	Sputum	IV	+ /+	(3) 81, 324, 405	(15) PEN, OX, CEF, ERY, TS, CIP

PEN: penicillin, OX: oxacillin, CEF: cefoxitin, ERY: erythromycin, TET: tetracycline, TS: trimethoprim, RF: rifamipicin, CIP: ciprofloxacin, TZP: piperacillin-tazobactam and MU: mupirocin

SCC*mec* type was type IV (10 isolates) followed by type V (7 isolates), a finding similar to that of Dejing *et al*. [25]. These results suggest that CA- and HA-MRSA are likely to coexist in our hospitals, as previously reported in the United States [26].

In this study, the SCC*mec* types correlated well with the major antibiotic susceptibility patterns. Although previous studies have shown that SCC*mec* types IV and V, which are CA-MRSA strains, were generally susceptible to non β-lactam antibiotics and more susceptible to antibiotics than were HA-MRSA strains [3,20]. Moreover, our results demonstrated that SCC*mec* type IV (2 isolates) and SCC*mec* type V (3 isolates) were resistant to many non-β-lactam antibiotics. This finding was noted in previous studies, suggesting that SCC*mec* types IV and V strains can acquire resistance to non-β-lactam antibiotics in order to tolerate hospital environments and excessive antibiotic exposure [27].

Pulsed-field gel electrophoresis (PFGE) is considered the most discriminatory and reliable method of typing. However, it is more complex and time consuming. Furthermore, it was not available in our laboratory. PCR-RFLP typing of the coagulase gene (*coa*) can be used for *S. aureus* typing on the basis of DNA variation within the 3' end coding region of the gene [28]. Since the isolation of the staphylococcal *coa* gene in 1987, *AluI* and, to a lesser extent, *Hue*,III-RFLP digestion of the *coa* gene has been extensively used for differentiation of MRSA strains with excellent correlation between RFLP patterns and multi-locus enzyme electrophoresis [2]. PCR amplification and restriction of the *coa* gene of the studied 58 MRSA isolates generated nine different patterns. Four RFLP patterns (2,4,7,9) were detected only in HA-MRSA, and two RFLP patterns (3,5) were detected only in CA-MRSA, while other RFLP patterns (1,6,8) were shared by both types of MRSA. Our results are in accordance with those of Kobayashi *et al*. [29], who found that MRSA and MSSA were classified into 6 and 12 RFLP patterns, respectively, with 5 patterns detected frequently in both MRSA and MSSA. Walker *et al*. [18] showed that *AluI* digestion of the *coa* gene PCR products of 356 MRSA strains yielded 13 different RFLP patterns. In contrast to our results, Lawrence *et al*. [17] isolated MRSA strains from various hospitals and showed that the strains were closely related and that 91.8% of the isolated strains had a unique RFLP pattern when analyzed by coagulase gene typing.

Conclusions

In conclusion, HA-MRSA strains were more common than CA-MRSA strains in Taif. Coagulase gene polymorphism requires only small quantities of DNA, and isolates can be compared by both the number of PCR products and the size of their *AluI* restriction fragments**.** MRSA RFLP analysis yielded nine distinct types, and HA-MRSA and CA-MRSA were discriminated to a great extent. Coagulase gene polymorphism is an easy and accurate method for studying clonal relatedness of isolates.

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