Molecular Typing of Methicillin-Resistant *Staphylococcus aureus* Isolated in a Bahrain Hospital

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Key Words

Methicillin-resistant *Staphylococcus aureus* · Antibiotic resistance · Pulsed-field gel electrophoresis · Staphylococcal cassette chromosome *mec* typing · Fusidic acid resistance

Abstract

Objective: To investigate antibacterial resistance patterns and genetic relatedness of methicillin-resistant *Staphylococcus aureus* (MRSA) obtained at the Salmaniya Medical Complex in Bahrain. **Methods:** A total of 53 consecutive MRSA isolates obtained from 53 patients were studied using antibacterial resistance patterns, coagulase gene polymorphism, staphylococcal cassette chromosome *mec* (SCC*mec*) typing and pulsed-field gel electrophoresis (PFGE). **Results:** There was a high prevalence of resistance to fusidic acid (92.5%), ciprofloxacin (92.5%), erythromycin (90.6%), tetracycline (88.7%), trimethoprim (88.7%), streptomycin (88.7%), kanamycin (83.0%) and gentamicin (73.6%). Coagulase gene typing divided the isolates into five coagulase types comprising coagulase type 36 (86.7%), type 20 (3.8%), type 16 (3.8%), type 38 (1.9%) and type 384 (3.8%). They belonged to SCC*mec* type III (86.7%) and SCC*mec* type IV (13.3%). PFGE identified five pulsotypes (types A–E) with PFGE type A and its subtypes comprising 83% of the isolates. PFGE type A isolates were multiresistant and had the SCC*mec* type III and

coagulase type 36 genotype. The SCC*mec* type IV isolates were nonmultiresistant with different genetic backgrounds. **Conclusions:** The study identified two types of MRSA in the hospital during the study period. One type consisted of a persistent multiresistant PFGE clone with the SCC*mec* type III and coagulase type 36 genotypes. The second type consisted of nonmultiresistant isolates that belonged to different genetic backgrounds and were isolated less frequently.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an established pathogen causing hospital-acquired infections worldwide. *S. aureus* becomes resistant to methicillin by acquiring and integrating into their genome a 21- to 67-kb mobile genetic element designated staphylococcal cassette chromosome *mec* (SCC*mec*) [1]. The SCC*mec* element carries the *mecA* gene that is responsible for the methicillin resistance [1, 2]. The SCC*mec* elements are unique genomic islands which serve as vehicles for gene exchange among staphylococcal species and have been found both in *S. aureus* and in coagulase-negative staphylococci [3]. At least five types of SCC*mec* elements designated types I–V have been reported [3–5].

Since the initial report of methicillin resistance in *S. aureus* from the UK in 1961 [6], MRSA has been reported in many countries with varying degrees of prominence [7–9]. MRSA was initially associated with hospitals and other health care facilities such as nursing homes and long-term care facilities [7, 9]. However, a new type of MRSA, community-acquired or community-associated MRSA (CA-MRSA), has been described in many countries [10–13]. The CA-MRSA is isolated from individuals without risk factors traditionally associated with health-care-acquired MRSA such as recent hospitalization, transfer from another hospital, prior antimicrobial use, invasive procedures, and underlying diseases [7, 13]. The CA-MRSA isolates also differ from health-care-associated MRSA isolates in their resistance patterns and toxin production. Structurally, the CA-MRSA strains harbor the SCCmec types IV or V [1, 4], whereas the hospital-associated MRSA strains carry the SCCmec types I, II, and III [3]. Consequently, SCCmec typing has become an important tool for distinguishing CA-MRSA from hospital-associated MRSA.

Although MRSA strains have been studied extensively in many countries, information on their prevalence, resistance, and clonal distribution is still lacking in some countries. In the Arabian Gulf region, MRSA strains have been reported in Saudi Arabia [14, 15], the United Arab Emirates [16], Kuwait [17–19], and Oman [20]. Although MRSA strains have been isolated from patients in Bahrain hospitals, they have not been studied to define their resistance patterns and clonal types. MRSA constituted 37.5% of all *S. aureus* strains isolated at the Salmaniya Medical Complex (SMC), Manama, Bahrain, in 2005 [unpubl. data of the authors, A.E.J.]. Therefore, there is a need to characterize MRSA isolated from patients admitted to the SMC to establish whether they consisted of single or multiple clones as this would have implications for infection control. This study was conducted on MRSA obtained from patients at the SMC to determine their antibiotic resistance patterns and clonal distribution.

Subjects and Methods

Subjects

In total 53 MRSA isolates were obtained from 53 inpatients consisting of 21 females and 32 males and aged between 8 and 90 years (mean age 54 years). Eight of them were younger than 25 years of age, 12 were aged between 25 and 50 years, 16 between 51 and 70 years and 17 over 70 years. They were nationals of Bahrain (n = 48), India (n = 3), Pakistan (n = 1), and Oman (n = 1). The

majority of MRSA isolates were obtained from wounds (n = 21) and deep tracheal aspirates (n = 19). The other sources were blood (n = 3), nasal swab (n = 3), groin (n = 2) and one sample each from the ear, eye, tissue and peritoneal fluid. Forty-four (83.9%) isolates were from infection and 9 (17%) isolates were from colonized sites (nose, groin and skin).

MRSA Isolates

The 53 consecutive nonduplicate MRSA isolates were obtained from patients at the SMC in Bahrain between January and July 2005. The SMC located in Manama, Bahrain, is a 1,000-bed tertiary care hospital with specialty departments including oncology, surgery, internal medicine, pediatrics, orthopedics, otolaryngology, obstetrics and gynecology and an outpatient clinic. The hospital handles approximately 25,000 admissions annually. The isolates were identified by conventional methods including Gram's stain, tube coagulase, and DNase tests. They were preserved in glycerol 15% (v/v) in brain heart infusion broth (Oxoid, Basingstoke, UK) at –80°C and recovered by subculturing in brain heart infusion broth at 37°C for 24 h followed by two further subcultures on brain heart infusion agar.

Susceptibility Testing

Susceptibility to antimicrobial agents was determined by the disk diffusion method [21] on Mueller-Hinton agar (Oxoid, UK). The following antibiotic disks (Oxoid) were used: methicillin (5 µg), benzylpenicillin (2 U), cefoxitin (30 µg), kanamycin (30 µg), mupirocin (200 µg), gentamicin (10 µg), erythromycin (15 µg), clindamycin (2 µg), chloramphenicol (30 µg), tetracycline (10 µg), trimethoprim (2.5 µg), fusidic acid (10 µg), rifampicin (5 µg), ciprofloxacin (5 µg), teicoplanin (30 µg), vancomycin (30 µg), and linezolid (30 µg). Susceptibility to mupirocin was determined using disks containing 200 and 5 µg of mupirocin. Growth to the edge of the 200-µg mupirocin disk indicated high-level resistance while growth within a 14-mm zone of inhibition with the 5-µg mupirocin disk detected low-level resistance. Disks containing nonantibiotics were prepared in the laboratory with the following concentrations: cadmium acetate (50 µg), propamidine isethionate (100 µg), mercuric chloride (109 µg). The minimum inhibitory concentrations of methicillin, vancomycin, teicoplanin and mupirocin were determined with E-test strips (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. *S. aureus* strain ATCC 25923 was used as the quality control strain for susceptibility testing. Methicillin resistance was confirmed by detecting PBP2a using a rapid latex agglutination kit (Denka-Seiken, Japan) according to the manufacturer's instructions.

Urease Production

Urease production was detected on Christensen's urea agar slope after incubation at 35°C for 48 h.

Pulsed-Field Gel Electrophoresis

Contour-clamped homogenous electric field (CHEF) electrophoresis of *Sma*I-digested chromosomal DNA was performed in agarose (pulsed-field-certified agarose, BioRad, USA) as described previously [22] using a CHEF-DRIII system (BioRad). The pulse times were 5 s initial and 40 s final. *Sma*I-digested chromosomal DNA of *S. aureus* strain NCTC 8325 was used as a molecular size marker. After electrophoresis, the gel was stained with

Table 1. Phenotypic and genotypic characteristics of MRSA isolates

Number of isolates	Resistance patterns	PFGE patterns	SCCmec types	coa-RFLP types
4	Gm, Km, Sm, Em, Tet, Tp, Fa, Cip, Cm, MupL	A (3), Ad (1)	III	36
4	Gm, Km, Sm, Em, Tet, Tp, Fa, Cip, Cm	A (2), Aa (2)	III	36
2	Gm, Km, Sm, Em, Tet, Tp, Fa, Cip, MupL	Aa (1), Ac (1)	III	36
28	Gm, Km, Sm, Em, Tet, Tp, Fa, Cip	Aa (2), A (23), Ab (1), B (1), C (1)	III	36
1	Gm, Km, Tp ¹	C	IV	16
3	Sm, Em, Tet, Tp, Fa, Cip, Cm	Ab	III	36
2	Sm, Em, Tet, Tp, Fa, Cip	Aa	III	36
1	Sm, Em, Tet, Tp, Fa, Cip	Ab	III	36
1	Em, Tet, Tp, Fa, Cip	A	III	36
1	Em, Tet, Tp, Fa, Cip	Ab	III	36
1	Km, Em, Tet, Tp, Cip	C	IV	20
1	Km, Em, Tet, Tp, Cip	D	IV	20
1	Km, Sm, Tet, Fa, Cip	C	IV	38
1	Km, Sm, Fa	D	IV	384
1	Km, Sm	D	IV	384
1	Fa	E	IV	16

Gm = Gentamicin; Km = kanamycin; Sm = streptomycin; Tet = tetracycline; Fa = fusidic acid; Em = erythromycin; Cip = ciprofloxacin; Tp = trimethoprim; Cm = chloramphenicol; MupL = low-level mupirocin. Numbers in parentheses are numbers belonging to the PFGE pattern.

¹ This isolate was urease negative.

ethidium bromide (0.5 µg/ml) and photographed under ultraviolet illumination. The chromosomal patterns were examined visually and assigned PFGE profiles.

Coagulase Gene Typing

The coagulase gene was amplified using published primers [23]. The nucleotide sequences (5'–3') of standard primers purchased were COAG-2: CGA GAC CAA GAT TCA ACA AG, and COAG-3: AAA GAA AAC CAC TCA CAT CA (Gibco BRL, Paisley, UK). The amplified products were digested with 5 U of *AluI* restriction enzyme, incubated at 37°C and separated by agarose gel electrophoresis (2.5% agarose, w/v) in 1 × TBE buffer for 2.5 h at 85 V. The gel was stained with ethidium bromide (0.5 µg/ml) and photographed under ultraviolet illumination. *S. aureus* strain ATCC 25923 and *S. epidermidis* strain ATCC 12228 were used as positive and negative controls, respectively.

SCCmec Typing

SCCmec types were investigated in PCR assays as described previously [18, 24] with strains COL (SCCmec type I), XU642 (EMRSA-16, SCCmec type II), WBG525 (EMRSA-1, SCCmec type III), WBG9465 (EMRSA-15, SCCmec type IV) and WBG8318 (SCCmec type V) as controls provided by Prof. W.B. Grubb of Curtin University of Technology, Perth, Australia.

Detection of Genes for Pantone-Valentine Leukocidin

All isolates were tested for the production of Pantone-Valentine leukocidin (PVL) in PCR assays by detecting the *lukS-lukF* genes using the primers described previously [18].

Results

All 53 isolates were susceptible to vancomycin, teicoplanin, rifampicin, and linezolid. They all yielded positive results for PBP2a and were resistant to the agents summarized in table 1. The majority (n = 46, 86.8%) of them expressed multiresistance to non-β-lactam agents (resistant to more than 4 classes of antibiotics), whereas 7 isolates (13.3%) were nonmultiresistant (resistant to 3 classes of antibiotics or less). The resistance of the isolates to antibacterial agents is presented in table 2. There was a high prevalence of resistance to fusidic acid (92.5%), ciprofloxacin (92.5%), erythromycin (90.6%), tetracycline (88.7%), trimethoprim (88.7%), streptomycin (88.7%), kanamycin (83.0%), and gentamicin (73.6%). Six isolates obtained from deep tracheal aspirates (n = 2), blood (n = 2), groin (n = 1) and peritoneal fluid (n = 1) expressed low-level mupirocin resistance (minimum inhibitory concentration 8–256 mg/l) but none expressed high-level mupirocin resistance (minimum inhibitory concentration ≥ 512 mg/l). Besides resistance to antibiotics, 50 (94.3%) of the isolates were resistant to cadmium acetate and mercuric chloride and 37 (69.8%) isolates were resistant to propamidine isethionate and ethidium bromide. Fifty-two of the 53 isolates produced urease.

Table 2. Antibacterial resistance of MRSA isolates

Antibacterial agents	Number resistant
Gentamicin	39 (73.6)
Kanamycin	44 (83.0)
Streptomycin	47 (88.7)
Erythromycin	48 (90.6)
Clindamycin	46 (86.8)
Chloramphenicol	9 (16.9)
Tetracycline	48 (90.6)
Trimethoprim	47 (88.7)
Fusidic acid	49 (92.4)
Ciprofloxacin	49 (92.4)
Mupirocin (low level)	6 (11.3)
Cadmium acetate	50 (94.3)
Mercuric chloride	50 (94.3)
Propamidine isethionate	37 (69.8)
Ethidium bromide	37 (69.8)

Figures in parentheses indicate percentages.

PFGE Patterns of MRSA Isolates

PFGE analysis of the 53 MRSA isolates yielded 5 different PFGE patterns designated types A–E. However, a majority of the isolates (45 of 53) belonged to one PFGE pattern (types A) or its subtypes (subtypes differed by 1–3 fragments). The remaining 8 isolates consisted of 1 PFGE type B, 3 type C, 3 type D, and 1 type E. Representatives of the PFGE patterns are presented in figure 1.

Coagulase Gene (*coa*-RFLP), *SCCmec* Typing and PVL Detection

The *coa*-RFLP, *AluI* restriction digestion of the amplified coagulase gene products yielded 5 RFLP patterns consisting of 46 (86.7%) coagulase type 36, 2 each of type 16, type 20 and type 384, and 1 of type 38. For the *SCCmec* typing, 46 of the 53 isolates were *SCCmec* type III and 7 were *SCCmec* type IV (table 1). Of the 7 *SCCmec* type IV isolates investigated further for the presence of genes for PVL, 5 contained the PVL genes. These isolates belonged to 4 different genetic backgrounds and were from patients whose ages ranged from 21 to 65 years (table 3).

Discussion

Although the MRSA isolates were obtained from patients with a wide range of age (8–80 years), 15% of them were from patients below 25 years and 62.1% of them were

Fig. 1. PFGE of the dominant MRSA clones. Representatives of PFGE patterns. Lanes a–h: PFGE type A and its subtypes; lane i: PFGE type B; lanes j–l: PFGE type C and subtypes; lane m: PFGE type D; lane n: PFGE type E; lane o: molecular size marker.

from patients over 50 years old, similar to other studies that have shown that old age is a risk factor for MRSA colonization and infection [7]. Most of the MRSA isolates were associated with infected wounds, tracheal aspirates or blood samples and only 17% with colonization emphasizing the importance of MRSA as a nosocomial pathogen.

The high prevalence of multidrug resistance in MRSA observed in Bahrain is comparable to that in Saudi Arabia and Kuwait [14, 18, 25]. A recent study in Saudi Arabia showed that 78.8% of MRSA isolates obtained in 4 hospitals were multiresistant with a high prevalence of resistance to gentamicin, erythromycin, and oxytetracycline [14]. Similarly, multiresistant MRSA constituted 78% of MRSA isolates obtained in Kuwait hospitals with a high prevalence of resistance to gentamicin, kanamycin, erythromycin, tetracycline, ciprofloxacin, and fusidic acid [17, 18, 25]. The high prevalence of fusidic acid resistance in this study was of interest. The proportion of MRSA that expressed fusidic acid resistance in this study (92.5%) was higher than the prevalence reported in European countries [8, 26, 27] and Australia [8]. However, a similarly high prevalence of fusidic acid resistance has been reported among MRSA isolated in Kuwait [17, 19, 28] and Saudi Arabian hospitals [14]. The use of topical fusidic

Table 3. Characteristics of SCCmec IV isolates

Subject	Age	Sex	Specimen	Antibacterial resistance	PFGE pattern	coa-RFLP types	PVL
1	57	female	pus	Gm, Km, Tp, Cd	C	16	tve
2	30	male	pus	Km, Tet, Fa, Cip, Cd	C	38	tve
3	65	female	DTA	Km, Em, Clin, Cip, Cd	C	20	-ve
4	21	male	pus	Km, Em, Clin, Cip, Cd	D	20	tve
5	49	male	wound	Fa	E	16	-ve
6	26	female	pus	Km	C	384	tve
7	64	male	nose	Km, Fa	Ac	384	tve

Gm = Gentamicin; Km = kanamycin; Fa = fusidic acid; Em = erythromycin; Clin = clindamycin; Cip = ciprofloxacin; Tp = trimethoprim; Cd = cadmium acetate; DTA = deep tracheal aspirate; tve = detected; -ve = not detected.

acid to treat impetigo and atopic dermatitis caused by *S. aureus* has been suggested as a cause of fusidic acid resistance in European MRSA isolates [29]. The reason for the high prevalence of fusidic acid resistance in MRSA in Kuwait, Saudi Arabia and Bahrain may also be related to fusidic acid use [29] or the dissemination of a single epidemic MRSA clone as observed in Europe [30, 31]. This line of argument is supported by the demonstration that the fusidic acid-resistant MRSA in Kuwait hospitals belonged to two major clones which had spread to different hospitals [28].

Several molecular methods, including coagulase gene typing (*coa*-RFLP), PFGE, multilocus sequence typing (MLST) and SCCmec typing have been used to type MRSA for epidemiological purposes [23, 32, 33]. PFGE is widely used for bacterial typing because of its stability, high discriminatory power, and reproducibility [23]. PFGE typing differentiated the 53 isolates into two broad groups. One group, consisting of PFGE type A and its subtypes, was the dominant clone being detected in 83% of the isolates. They were also multiresistant (including 38 of the 39 gentamicin-resistant isolates), and had coagulase type 36 and SCCmec type III. The second group consisted of nonmultiresistant isolates with different PFGE and *coa*-RFLP genotypes but all were SCCmec type IV. Six of the SCCmec type IV isolates produced urease. Urease production together with the possession of SCCmec type IV genotype and nonmultiresistance are characteristics of CA-MRSA [12, 13]. They were also resistant to ciprofloxacin as seen in CA-MRSA isolated from Germany [12]. In addition, 5 of the 7 SCCmec type IV isolates contained genes for PVL, which has also been detected in CA-MRSA isolated in some European countries [12]. CA-MRSA isolates have become a major clinical

concern in many countries in recent years [10–13, 18, 33]. Their appearance in this hospital in Bahrain, albeit in small numbers, suggests that they are present in the wider community in the country. CA-MRSA isolates have also been reported in Kuwait [18] and Saudi Arabia [33].

The MRSA clone with the SCCmec type III genotype was obtained throughout the study period from patients of different nationalities suggesting that cross-infection was a factor in the maintenance of this MRSA clone in the hospital. MRSA with the SCCmec type III genotype is common in hospitals in Saudi Arabia, Thailand, and other Asian countries [32, 33]. Although PFGE typing is very useful in determining the relatedness of bacterial isolates locally, it suffers from an inability to compare results from different laboratories. Consequently, the Bahrain MRSA strains could not be compared with those from Saudi Arabia based on their PFGE results. In contrast, a major benefit of MLST is the ability to compare *S. aureus* isolated from different geographical backgrounds [31, 32]. MLST of the SCCmec type III isolates from Saudi Arabia revealed that they belong to sequence type 239 (ST239) [33] similar to the majority of SCCmec type III MRSA isolated in other Asian countries [32]. Although the sequence type of the Bahrain strains has not yet been determined, which would allow comparison to MRSA isolated in Saudi Arabia and other countries, their study with PFGE has yielded important data on the prevailing genotypes of local MRSA clones. The results have added to our limited data on the clonal distribution of MRSA in the Gulf countries. Besides adding to the understanding of the MRSA clones in this hospital, this study has raised the awareness of the introduction of CA-MRSA into the hospital setting from the community.

Analysis of these isolates using MLST will help clarify the relatedness of Bahrain isolates to MRSA in other countries [32].

Conclusion

This study has identified one dominant and several sporadic MRSA clones in a Bahrain hospital. The dominant clone represented the multiresistant health-care-associated MRSA having SCCmec type III with the capac-

ity to persist and spread among patients in hospital. The few sporadic isolates that were nonmultiresistant belonging to different genetic backgrounds were CA-MRSA.

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