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Genetic Lineages of Methicillin-Resistant *Staphylococcus aureus* Acquired during Admission to an Intensive Care Unit of a General Hospital

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Keywords
Methicillin-resistant *Staphylococcus aureus* · Intensive care unit · Colonization · Infection

Abstract

Objectives: The objectives of this study were to determine the frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization or infection while on admission to the intensive care unit (ICU), and examine the genetic backgrounds of the MRSA isolates to establish transmission among the patients. ***Subjects and Methods:*** This study involved screening 2,429 patients admitted to the ICU of Farwania Hospital from January 2005 to October 2007 for MRSA colonization or infection. The MRSA isolates acquired after admission were investigated using a combination of molecular typing techniques to determine their genetic backgrounds. ***Results:*** Of 2,429 patients screened, 25 (1.0%) acquired MRSA after admission to the ICU. Of the 25 MRSA, 19 (76%) isolates belonged to health care-associated (HA-MRSA) clones: ST239-III ($n = 17$, 68%) and ST22-IV ($n = 2$, 8%). The remaining 6 MRSA isolates belonged to community-associated clones: ST80-IV ($n = 3$, 12%), ST97-IV ($n = 2$, 8%), and ST5-IV ($n = 1$, 4%). The ST239-III-MRSA clone was associated with infection as well as colonization, and was isolated from patients from 2005 to 2007. ***Conclusions:*** The HA-MRSA clone ST239-III persistently colonized patients admitted to the ICU, indicating the possibility of its transmission among the patients over time.

Introduction

Since its first description reported from the UK in 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major cause of infections in both hospitals and the community [1–5], although the prevalence seems to vary according to the geographic location, type of health care facility, and the specific population being studied [5]. The prevalence of MRSA in a hospital setting, such as a tertiary care facility or intensive care unit (ICU), had been reported to be approximately 60–70% of all *S. aureus* isolates [5, 6]. The ICU is among the most affected areas in a hospital where patients are at a higher risk of acquiring MRSA [2, 7]. In contrast to the prevalence of MRSA colonization or infection in the ICU, which has been reported to be 4–8% [2, 8], the prevalence in general in-patient setting varies from 0.18 to 7.2% [5–9].

Surveillance studies have shown that old age, male gender, and previous hospital admission are risk factors for acquisition of MRSA in patients undergoing surgical

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treatment [10]. Some of the important risk factors for acquisition and colonization of MRSA are use of extended-spectrum antimicrobial agents [4] and prolonged duration of antimicrobial therapy [3], whereas inappropriate antimicrobial therapy, comorbid conditions, and advanced patient age cause increased mortality associated with systemic MRSA infections [5]. Risk factors associated with MRSA acquisition in the ICU setting include prolonged stay, use of intravascular devices, and the intensity of exposure to colonized or infected patients [5].

Molecular epidemiology of MRSA has enhanced the understanding of the dynamics of acquisition and spread of community-acquired (CA-MRSA) and health care-acquired MRSA (HA-MRSA) in health care facilities. The CA-MRSA strains are distinguished by their carriage of types IV or V staphylococcal cassette chromosome *mec* (SCC*mec*) elements [11, 12], whereas HA-MRSA strains carry the SCC*mec* types I, II, and III [13].

Although MRSA are regularly isolated from patients at Farwania Hospital, there are no data on MRSA colonization of patients admitted to the ICU of this hospital. This study was conducted to determine the prevalence of MRSA colonization at admission or during ICU stay, and its impact on subsequent MRSA infection. The study also investigated the genetic backgrounds of the MRSA isolates to understand whether they were being transmitted among patients admitted to the ICU.

Subjects and Methods

Active Surveillance, Decolonization, and Infection Control Measures

Farwania Hospital serves a population of approximately 700,000 in Kuwait. The ICU has 17 beds, including 2 side rooms, and admits adult in-patients from all specialties within the hospital, emergency room, and those transferred from other hospitals. The medical records of 2,429 patients admitted to the ICU from January 1, 2005 to October 31, 2007 were reviewed for colonization and/or infection with MRSA. Three sites, anterior nares, axillae, and groin, were screened at the time of admission to the ICU and weekly thereafter. If MRSA was isolated from any of the 3 screening specimens at the time of admission, the patient was identified as having been initially colonized. If the admission cultures were negative but subsequent cultures from any site during the course of ICU stay grew MRSA, the patient was identified as having acquired MRSA after admission. MRSA-positive patients were isolated in one of the side rooms or cohorted with other positive patients. Decolonization was attempted for MRSA-positive patients who were treated for 5 days with 2% mupirocin ointment for anterior nares, whereas repeated bathing with chlorhexidine was used when the other 2 body sites tested positive for MRSA. Patients were then retested for 3 consecutive days to ensure clearance. Patients with positive MRSA from blood cultures or those who were diagnosed with ventilator-associated pneumonia due to MRSA were treated with intravenous vancomycin or teicoplanin. Nurses who attended MRSA-positive patients did not care for MRSA-negative patients, and the staff attending positive patients followed strict contact precautions according to hospital infection control policy. At the time of the patient's discharge from the ICU, terminal cleaning was undertaken using 1% hypochlorite solution for bed rails, floor, walls, and curtains.

Culture and Identification of MRSA Strains

Surveillance specimens from anterior nares, axillae, and groin as well as clinical samples from other diagnostically relevant sites were cultured on 5% sheep blood agar and mannitol salt agar plates for the isolation of *S. aureus*. Both media were incubated for 24 h at 37°C. Conventional methods including Gram's stain, tube coagulase, and DNase tests and the automated identification system VITEK 2 (bioMerieux, Marcy l'Etoile, France) or Phoenix (Becton Dickinson, USA) were used to identify suspected *S. aureus* colonies. Isolates were preserved in glycerol 15% (v/v) in brain-heart infusion broth (Oxoid, Basingstoke, UK) at –80°C for further analysis.

Antibiotic Susceptibility Testing

The disk diffusion method was used to perform antimicrobial susceptibility and interpreted according to the Clinical and Laboratory Standard Institutes (CLSI) guidelines [14] with the following antimicrobial disks (Oxoid): benzyl penicillin (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), and linezolid (30 μg). The minimum inhibitory concentration for cefoxitin, vancomycin, and teicoplanin was determined with Etest strips (bioMerieux) according to the manufacturer's instructions. *S. aureus* strain ATCC25923 was used as a quality control strain for susceptibility testing. Methicillin resistance was confirmed by detecting PBP 2a using a rapid latex agglutination kit (Denka-Seiken, Japan) according to the manufacturer's instruction.

Molecular Typing of MRSA Isolates

Pulsed-field gel electrophoresis, coagulase gene typing, SCC*mec* typing, Spa typing, and multilocus sequence typing were used to perform genotypic characterization of the MRSA isolates. Pulsed-field gel electrophoresis was performed as described previously [15]. Coagulase gene typing was performed using published primers and protocols as described by Goh et al. [16]. SCC*mec* typing was performed by PCR assays as described previously [17, 18]. Spa typing was performed as described by Harmsen et al. [19] for all MRSA isolates. Multilocus sequence typing was performed on all isolates as described by Enright et al. [20].

Results

Of the 2,429 patients admitted to the ICU for MRSA carriage, 18 (0.74%) were colonized and 2 (0.08%) were infected (one was diagnosed with pneumonia and the other with gluteal abscess) on admission. Of the 18 colo-

Table 1. Demographic characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA)-positive patients

Characteristic	Frequency, *n* (%)
Gender	
Male	37 (82.2)
Female	8 (17.7)
Clinical condition	
Medical	31 (68.9)
Surgical	14 (31.1)
Pattern of MRSA affection	
Colonization only	35 (77.8)
Infection and colonization	7 (15.6)
Infection only	3 (6.7)
Total number of colonization sites per patient	
1 site	37 (82.2)
2 sites	3 (6.7)
3 sites	1 (2.2)
4 sites	1 (2.2)
Type of MRSA colonization	
Community acquired	17 (55.6)
Nosocomial	25 (37.8)
Type of MRSA infection	
Community acquired (pneumonia 1, gluteal abscess 1)	2 (4.4)
Nosocomial (BSI 6, pneumonia 2)	8 (17.8)

BSI, blood stream infection.

nized patients, 1 (5%) developed blood stream infection on day 21 of the ICU stay. Of 2,409 patients not colonized or infected with MRSA at admission, 25 (1.0%) acquired MRSA in the ICU after stays of 7–54 days. Of these 25 patients, 18 (72%) were identified with colonization only, whereas 7 (28%) were colonized and or infected. The demographic characteristics of the MRSA-positive patients are shown in Table 1. The 25 MRSA isolates acquired after admission were investigated further to determine their genetic relatedness.

Molecular Typing of MRSA Acquired in the ICU

The genetic backgrounds and antibiotic resistance patterns of those isolates are presented in Table 2. Seventeen (68%) of the 25 isolates belonged to the ST239-III-MRSA clone (a health care-associated MRSA clone), while the remaining 8 (32%) isolates belonged to 4 different community-associated MRSA clones consisting of three ST80-IV-MRSA, two ST-22-IV-MRSA, two ST97-IV-MRSA, and one ST5-IV-MRSA. Spa typing revealed that the seventeen ST239-III-MRSA isolates belonged to 2 dominant subtypes consisting of eight t421 and six t945 and two minor subtypes consisting of two t388 and one t4410. The dominant ST239-III-MRSA strains were isolated from both colonized and infected patients. The ST239-MRSA isolates were resistant to multiple antibiotics including tetracycline, aminoglycosides, erythromycin, clindamycin, and fusidic acid. One isolate obtained in 2006 expressed high-level mupirocin resistance. The other MRSA clones, ST80-IV, ST22-IV, and ST97-IV, were non-multidrug-resistant. All isolates were susceptible to vancomycin, teicoplanin, linezolid, and rifampicin.

Discussion

The results of this study revealed that 1.0% of patients admitted to the ICU became colonized or infected with MRSA 7–54 days after admission, which confirmed the findings of previous studies [21, 22] that admission to the ICU is a risk factor for MRSA colonization and infection. The study also revealed that more patients (1%) acquired MRSA after admission to the ICU than those colonized prior to admission (0.74%), further strengthening the notion that admission to the ICU is a risk factor for MRSA acquisition [21, 22]. The 0.74% prevalence of MRSA among patients at the time of admission to the ICU in this study was comparable to an earlier study from our geographical region (Saudi Arabia) that reported prevalence of MRSA colonization at the time of admission to be 1.1% [23]. However, other studies have shown different prevalence rates of MRSA colonizing patients at the time of admission to ICU, which ranged from 2.5 to 46% [10, 24–26], probably reflecting a higher rate of MRSA colonization in the community prior to hospital admission. However, a study from Brazil which reported a higher frequency (46%) of colonization with MRSA at the time of admission with 52% of the patients acquiring it in the ICU, did not find any association with identifiable risk factors although the unusually higher rates of MRSA acquisition among their patient population in the ICU was attributed to improper hand-washing, environmental surface cleaning, and barrier protection from infected patients, unlike infection control procedures adopted in our ICU [26].

MRSA acquisition often results in increased length of hospital stay as seen in the present study. The patients in this study acquired MRSA after 7 days of ICU admission which resulted in 30.5 days average length of hospital stay. This was much longer than the 7.2 days stay in the ICU reported by Marshall et al. [27] with 12.8% of their patients acquiring MRSA (colonization/infection) after 5 days of admission to the ICU.

Table 2. Antimicrobial resistance pattern and molecular typing results of 25 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) acquired in the ICU

Strain	Source	Antibiotic resistance	Coagulase	PFGE	SCC*mec*	Spa type	ST
1	NS	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip	36	1	III	t421	239
2	NS	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip	36	1	III	t421	239
3	Nasal	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip	36	1	III	t421	239
4	Nasal	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip, Cm	36	1	III	t421	239
5	Groin	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip	36	1	III	t421	239
6	Sputum	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip	36	1	III	t421	239
7	Nasal	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip	36	1	III	t421	239
8	Sputum	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip	36	1	III	t421	239
9	Nasal		16	2	IV	t6665	97
10	Axilla	Tet	264	2a	IV	t1234	97
11	Blood	Em, Clin, Tet, Cm	16	3	IV	t688	5
12	Pus	Km, Fd	128	4	IV	t044	80
13	Nasal	Km, Sm	128	4	IV	t044	80
14	Nasal	Tp	16	5	IV	t223	22
15	Nasal	Gm, Km, Sm, Em, Clin, Tet, Cip	36	6	III	t4410	239
16	Nasal	Gm, Km, Em, Clin, Tet, Fd, Cip	36	6a	III	t945	239
17	Blood	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip	36	6a	III	t945	239
18	Axilla	Gm, Km, Sm, Em, Clin, Tet, Cip	36	6a	III	t945	239
19	Axilla	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip, MupH	36	6a	III	t945	239
20	Axilla	Gm, Km, Tp, Fd, Cip	36	6a	III	t945	239
21	NS	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip, Tp	36	6a	III	t945	239
22	NS	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip, Tp	36	6b	III	t388	239
23	Axila	Gm, Km, Sm, Em, Clin, Tet, Fd, Cip, Tp	36	6b	III	t388	239
24	Sputum	Km, Sm, Fd	384	7	IV	t5393	80
25	Nasal	Tp	36	8	IV	t3010	22

Cip, ciprofloxacin; Cm, chloramphenicol; Clin, clindamycin; Em, erythromycin; Fd, fusidic acid; Gm, gentamicin; Km, kanamycin; MupH, high-level mupirocin; PFGE, pulsed-field gel electrophoresis; SCC*mec*, staphylococcal cassette chromosome *mec*; Sm, streptomycin; ST, MLST sequence type; Tet, tetracycline; Tp, trimethoprim

In this study, the dominant MRSA clone acquired in the ICU belonged to a well-known health care-associated clone, ST239-III MRSA. In contrast, Kwon et al. [6], who investigated the relationship between MRSA strains isolated from ICU patients with bacteremia and nasal colonization, observed that a clone belonging to the pulsed-field gel electrophoresis type B (SCC*mec* type II/ST5) genotype, which represented another hospital-acquired genotype, was the dominant clone acquired in the ICU in Korea.

This study also revealed that the same MRSA clone was involved in colonization as well as in infection, and was isolated from patients admitted to the ICU from 2005 to 2007, indicating a persistence of the ST239-III clone in the ICU. Persistence of the MRSA clone in the ICU could be due to environmental contamination or carriage of the MRSA clone by health care workers in the ICU or both. Unfortunately, neither the environment nor health care workers were screened as part of this study.

Conclusions

In this study, 1.0% of patients admitted to the ICU of a Farwania General Hospital in Kuwait acquired MRSA while on admission. Most of the patients were colonized or infected by a health care-associated ST239-III-MRSA clone which persisted in the facility over time. Hence, screening patients for MRSA is strongly advocated to detect carriers and enforce decontamination procedures, which can reduce infections and prevent transmission to others. Further research is needed to identify effective methods for sustained eradication of MRSA carriage to reduce the probability of subsequent infection in the high-risk population.

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