Trafficking of Methicillin-Resistant Staphylococci and Co-Colonization with Vancomycin-Resistant Enterococci

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Key Words

Methicillin-resistant *Staphylococcus aureus* •
Methicillin-resistant coagulase-negative staphylococci •
Vancomycin-resistant enterococci

Abstract

Objectives: To determine the trafficking of methicillin-resistant staphylococci between the hospital and community as well as the occurrence of co-colonization with vancomycin-resistant enterococci (VRE). **Subjects and Methods:** From November 2005 to April 2006, methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative Staphylococcus (MRCoNS)-positive patients at the Salmaniya Medical Complex, Bahrain were assessed for VRE co-colonization. Characterization of vancomycin resistance genotype by PCR was carried out. Close family contacts were screened for MRSA and pulsed-field gel electrophoresis (PFGE) analysis of MRSA isolates from patient-family member pairs was conducted. **Results:** One hundred and eighty-two patients (93 MRSA; 89 MRCoNS) and 356 family members were enrolled. Seven MRSA and 41 MRCoNS strains were isolated from the family members. PFGE analysis re-

vealed the presence of variants of a single MRSA clone among patients and their relatives. A total of 112 patients (62 MRSA; 50 MRCoNS) provided stool for VRE screening. Of these 13 stool specimens (11.6%) were VRE-positive. All the VRE isolates were from MRSA-positive patients, thus positivity rate among MRSA patients was 20.9% (n/N = 13/62). These were predominantly *Enterococcus gallinarum* with vanC1 genotype and one strain was *Enterococcus faecium* (vanB genotype). Two *E. gallinarum* isolates harbored an additional *vanB* gene. The majority of VRE isolates were from patients in medical and surgical units (n/N = 10/13; 77%). Male gender, prolonged hospitalization and presence of co-morbidities were significantly associated with MRSA/VRE co-colonization ($p < 0.05$). **Conclusion:** MRSA/VRE co-colonization with MRSA trafficking between the hospital and community environment is a public health concern occurring in our setting.

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Introduction

Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase-negative staphylococci (MRCoNS) and vancomycin-resistant enterococci have emerged as important etiological agents in serious infections. Both MRSA and vancomycin-resistant enterococci (VRE) are now endemic in many health care institutions with an increased risk of patients being co-colonized by these two microorganisms. In vitro studies have shown that genes can be transferred from enterococci to *S. aureus*, *Staphylococcus epidermidis*, and other Gram-positive organisms via plasmid-mediated conjugation [1, 2]. Thus, cross transfer of resistance genes from VRE to MRSA strains in such co-colonized patients represents a significant risk for the emergence of vancomycin-resistant *S. aureus* (VRSA). In fact vancomycin-resistant *S. aureus* reported so far arose due to the transfer of the vancomycin resistance determinant *vanA* from enterococcus to *S. aureus* [3]. These would potentially pose greater challenges in terms of treatment options and elimination strategies.

This study was carried out to determine the occurrence of VRE co-colonization among MRSA/MRCoNS-positive patients as well as determine the molecular fingerprinting of the MRSA isolates circulating in the hospital and the community.

Materials and Methods

Setting and Patients

The study was carried out at the Salmaniya Medical Complex (SMC), Kingdom of Bahrain over a 6-month period from November 2005 to April 2006. SMC serves as the main national secondary and tertiary referral center for specialist care, laboratory diagnosis and admissions. Ethical clearance was obtained from the Hospital Research and Ethics Committee and informed consent was given by all participants. Consecutive patients with culture-proven MRSA or MRCoNS colonization/infection during the study period were enrolled and stool specimens for VRE screening obtained. Specimens from two body sites (anterior nares and axilla) were obtained from close family contacts of these patients for MRSA/MRCoNS screening. Close family contacts were defined as those living in the same house or having prolonged contact with the patient. A total of 356 family members representing 113 families were enrolled in the study giving an average of 3 family members per patient. For 69 patients, we were unable to screen family members, because of refusal to participate or in some cases the family members were not domiciled in the country. Relevant demographic data, medical and surgical history for the preceding 6 months including previous hospitalization, antibiotic usage and presence of co-morbidities was obtained from patients and family members.

Detection of MRSA/MRCoNS Isolates

This was carried out using Diagnostic Susceptibility Test media (HiMedia Labs, India) supplemented with 4% (wt/vol) NaCl and containing 6 µg/ml oxacillin. Colonies showing characteristic staphylococcal morphology, microscopic appearance and positive catalase test were inoculated on Mueller-Hinton agar (Oxoid, Basingstoke, UK) supplemented with 5% sheep blood and incubated at 37°C for 24 h. Colonies were confirmed to be *S. aureus* by positive tube coagulase test and DNase activity. Isolates negative for these tests were classified as MRCoNS. *S. aureus* strain ATCC33591 (oxacillin-resistant) was used as control. All MRSA isolates were confirmed by BD PHOENIX™ automated microbiology system (Becton Dickinson, USA). MRCoNS isolates were identified to species level using the API STAPH identification system (bioMérieux, Marcy l'Etoile, France) and BD PHOENIX™.

Pulsed-Field Gel Electrophoresis

To compare MRSA isolates obtained from the patients and their corresponding MRSA-positive family members, typing of isolates using pulsed-field gel electrophoresis (PFGE) was carried out at the Department of Microbiology, Kuwait University, Kuwait. Cells were treated as reported previously and digested with *Sma*I (Gibco BRL, Gaithersburg, Md., USA) according to the manufacturer's instructions [4]. Electrophoresis of *Sma*I-digested chromosomal DNA was performed using a CHEF-DRIII system (BioRad, Hercules, USA). A *Sma*I digest of *S. aureus* strain NCTC 8325 was used to normalize the gel as size standard [5]. Interpretation of gels was performed by visual inspection using the criteria of Tenover et al. [6]. The band patterns were compared and classified as indistinguishable (no band differences), closely related or clonal variants (one to three band differences) and subtypes of each other, possibly related (four to six band differences) and unrelated (more than six band differences) [6].

Detection of VRE Isolates

Stool specimens were inoculated directly on bile-esculin agar plates containing 0.15 g/l sodium azide and incubated at 37°C for 48 h in ambient air. Enterococcus isolates were identified using standard microbiological methods and up to 10 colonies per plate were screened. Susceptibility to vancomycin was assessed by subculture of isolates on Mueller-Hinton agar containing 6 µg/ml vancomycin (Gulf Pharmaceutical Industries, United Arab Emirates), incubated at 37°C for 48 h. *Enterococcus faecalis* ATCC29212 (vancomycin-sensitive) and *E. faecalis* ATCC51299 (vancomycin-resistant) were used as controls. VRE isolates were identified to species level using API 20 STREP (bioMérieux) and BD PHOENIX™. Vancomycin minimum inhibitory concentration as determined by BD PHOENIX™ was recorded. Isolates were preserved at -80°C in stock culture trypticase medium (Becton Dickinson, USA) containing 20% glycerol until further analysis.

Detection of Vancomycin Resistance Genotype by PCR

Preserved isolates were thawed and inoculated on Mueller-Hinton agar. DNA for PCR was obtained from overnight cultures as follows: a colony was picked from the plate using a tooth pick and suspended in 400 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and the suspension was heated for 15 min at 100°C, then centrifuged at 14,000 g at room temperature for 10 min. The supernatant containing DNA was stored at -20°C until further use. The genes for *vanA*, *vanB*, *vanC1*, *vanC2/C3*,

Fig. 1. Study flow chart.

ddl (*Enterococcus faecium*), and *ddl* (*E. faecalis*) were amplified using previously described primers [7]. PCR was carried out with initial denaturation at 94°C for 5 min, followed by 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min), with a final extension at 72°C for 10 min in a GeneAmp® PCR system 9700 (Perkin-Elmer 9700, Applied BioSystem, Singapore). Gel electrophoresis was carried out using 15 µl PCR products mixed with 1 µl of the loading dye and run on a 1.2% agarose gel (Sigma Chemical Co. type 1-A, USA). A 100-bp DNA ladder (Bio-Rad EZ Load™, USA) was used as the molecular size marker.

Statistical Analysis

Data were analyzed with Statistical Package for Social Science (SPSS), version 13. The χ^2 -test and Fisher's exact two-tailed test were used for statistical analysis.

Results

MRSA/MRCoNS

Of the 182 consecutive patients positive for MRSA/MRCoNS infection or colonization enrolled in the study, male patients were 113 (62%) and female 69 (38%). The difference was statistically significant ($p < 0.01$). Mean duration of hospitalization was 100 days and co-morbidity with chronic illnesses was present in 67 (37%) patients. Of the 182 patients, 93 (51%) had MRSA and 89 (49%) had MRCoNS (fig. 1). The age range for MRSA patients was 4 weeks to 97 years (mean $\pm$ SD: 55 $\pm$ 21.4 years) and for MRCoNS patients 10 days to 98 years (mean $\pm$ SD: 59 $\pm$ 19 years).

Fig. 2. PFGE of *Sma*I-digested total DNA from 12 MRSA strains. M = *S. aureus* strain NCTC 8325 standard; lanes 1–5 = MRSA isolates from patients; lanes 6–12 = MRSA isolates from family members. Comparison of isolates from patient-family pairs: lane 1 vs. lane 6, closely related or clonal variants; lane 2 vs. lane 7, indistinguishable; lane 3 vs. lane 8, closely related or clonal variants; lane 4 vs. lanes 9 and 10, indistinguishable; lane 5 vs. lanes 11 and 12, unrelated.

The highest percentage of both MRSA and MRCoNS isolates were from the medical wards 35 (37.6%) and 27 (30.3%), respectively. In the pediatric ward and intensive care unit (ICU), the proportion of MRCoNS isolates (pediatrics 16, 18.0%; ICU 18, 20%) was significantly higher compared to MRSA (pediatrics 1, 1.1%; ICU 8, 8.6%). In contrast in the burns unit, the MRSA isolates (n/N 14/93, 15.1%) were significantly higher compared to MRCoNS (n/N 3/89, 3.4%; $p < 0.05$). For all other hospital wards, there was no significant difference in MRSA and MRCoNS isolates. Of 89 MRCoNS isolates 41 (46.1%) were from blood which was significantly higher compared to 6.4% (n/N = 6/93) for MRSA ($p < 0.05$). In contrast for wound swabs the isolation of MRSA (n/N = 39/93; 42%) was significantly higher compared to MRCoNS (n/N = 18/89; 20.2%; $p < 0.05$). There was no significant difference in the detection of MRSA and MRCoNS in other specimen types. However, all catheter tips yielded only MRCoNS.

From the 356 family members screened, 7 MRSA- and 41 MRCoNS-positive individuals were identified. The MRSA-positive family members were related to MRSA patients (fig. 1).

PFGE Patterns

The comparison of the banding patterns in patients with that of their corresponding family members is shown in figure 2 (lanes 1–5: MRSA isolates from patients, while lanes 6–12 MRSA isolates from family member). The patient-family pairs compared are as follows: lane 1 vs. lane 6; lane 2 vs. lane 7; lane 3 vs. lane 8; lane 4 vs. lanes 9 and 10; lane 5 vs. lanes 11 and 12. This comparison of patient-family member pairs showed that isolates from 2 patients and their corresponding family members were indistinguishable (lane 2 vs. lane 7 and lane 4 vs. lanes 9 and 10, fig. 2). Isolates from 2 patients and their corresponding family members were related (lane 1 vs. lane 6 and lane 3 vs. lane 8). The MRSA isolate from the patient in lane 5 was not related to MRSA from the family members represented in lanes 11 and 12.

MRSA/VRE Co-Colonization

Of the 112 patients (62 MRSA; 50 MRCoNS) who provided stool specimens for VRE screening, 13 (11.6%) stool specimens were positive for VRE by PCR and all were obtained from MRSA-positive patients (fig. 1). The VRE positivity rate among the MRSA-positive cohort was 20.9% (n/N = 13/62). A total of 14 isolates were characterized as 1 patient had two VRE isolates with different genotypes (*Enterococcus gallinarum* vanC and *E. faecium* vanB). The VRE isolates identified were *E. faecium* with vanB genotype (n = 1); *E. gallinarum* with vanC1 genotype (n = 11); *E. gallinarum* with vanC1 and vanB genotype (n = 2). The minimum inhibitory concentration for all isolates by the BD Phoenix system was 16 µg/ml (intermediate resistant). The majority of VRE isolates were from patients in medical and surgical units (n/N = 10/13; 77%). Male gender, prolonged hospitalization and presence of co-morbidities were significantly associated with MRSA/VRE co-colonization ($p < 0.05$).

Discussion

We have investigated the risk factors for MRSA/MRCoNS infection, trafficking of MRSA between the hospital and the community as well as the occurrence of VRE co-colonization among patients at SMC Bahrain. The identified risk factors include gender, older age, hospital unit, duration of hospitalization and co-morbidity with chronic illnesses, similar to previous reports [8]. In many tertiary hospitals, the medical ward tends to be populated with older patients with chronic illnesses and prior prolonged stay at healthcare-related facilities. A

similar scenario exists at SMC, which explains the high rates of isolation of MRSA and MRCoNS from the medical ward. Coagulase-negative staphylococci have been described as accounting for a large number of nosocomial bacteremias, especially those related to intravascular catheters as the bacteria are often carried from the site of insertion into the bloodstream [9]. The high rates of MRCoNS isolation in blood and catheter tips suggest that intravenous catheters are important sources of bacteremia in our setting.

To explore possible trafficking of MRSA between the community and the hospital environments the molecular fingerprinting of MRSA isolates from patients and their corresponding family member was carried out using PFGE. The findings indicate the presence of the same MRSA clone circulating in the hospital and the community. It also demonstrated the transmission of the same MRSA clones between patients and family members. Current evidence indicates that some MRSA clones were capable of persisting for long periods in hospitals and spreading among patients [10]. Additionally, familial transmissions of an MRSA clone have been described [11, 12]. A recent study from Bahrain has shown that the majority of MRSA isolates identified at SMC were persistent multiresistant PFGE clones with the SCCmec type III [13]. As none of the family members had a history of previous stay in a healthcare facility or any other risk factor for MRSA colonization, we speculate that patients could be the possible source of the MRSA trafficking into the community. This dissemination of MRSA from the hospital into the community poses a significant threat to public health as the family members serve as reservoirs of infection, thus undermining efforts at containing the spread of MRSA. However, as it could still be argued that MRSA trafficking occurs from the community to the hospital, we recommend further work on the molecular characterization of the MRSA isolates circulating in our setting.

Within this context of trafficking of MRSA between the hospital and the community, the greater problem is that of co-colonization with VRE in MRSA patients. It has been suggested that hospitalized patients with VRE colonization are at increased risk for MRSA colonization or infection and the clinical significance of this is highlighted by the emergence of VRSA [14, 15]. Our findings indicate a high prevalence of co-colonization with VRE among MRSA-positive patients particularly in the medical and surgical ward. It is of concern that 11.6% of all patients screened (20.9% of the MRSA-positive cohort) were co-colonized with these pathogens, which is higher

compared to the 2.7–9.5% in other studies [16–18]. Although no case of VRSA has yet been identified in our setting, the combination of similar risk factors for MRSA/VRE colonization, high occurrence of co-colonization and the trafficking between the hospital and the community sets the stage for the possible emergence of VRSA within the hospital with the potential for its transfer into the community. It is interesting that no VRE co-colonization was observed in MRCoNS patients, the reason for this is unclear and we suggest further studies to clarify this finding. As the occurrence of VRE co-colonization among family members with MRSA colonization was not investigated, research work to assess co-colonization in the community and to determine factors that influence MRSA and VRE transmission in our setting is recommended.

In this study, the majority of VRE isolates (93%) were *E. gallinarum* with vanC genotype and no vanA VRE was detected. This is significant as transfer of resistance determinants from chromosomally encoded vanC is extremely unlikely as opposed to plasmid-mediated transfer of vanA. The reason for the predominance of *E. gallinarum* with absence of *E. faecalis* in our population remains unclear. However, antibiotic therapy, particularly use of cephalosporins and vancomycin, has been suggested as being important in the upward trend of colonization with *E. gallinarum* [19]. In addition, a significant increase in the emergence of *E. gallinarum* in healthy subjects who were administered oral vancomycin has been reported [20]. Although *E. gallinarum* with vanC genotype had hitherto not been considered to be of public health significance because of their intrinsic resistance to glycopeptides and low pathogenicity, new evidence indicates that these organisms can cause serious invasive infections, and should be regarded as nosocomial pathogens [21, 22]. Two of the *E. gallinarum* isolates identified in this study harbored the vanB gene as an additional resistance gene. A similar report of *E. gallinarum* isolates with the vanB gene as additional glycopeptide resistance gene has previously appeared in the literature and transfer of the vancomycin resistance genes between *E. faecalis*, *E. gallinarum* and *Enterococcus casseliflavus* has also been documented [22–25].

Conclusion

These data demonstrate high levels of co-carriage of MRSA and VRE among patients in a tertiary care facility with evidence suggestive of trafficking of MRSA between

the hospital and community environment. This sets the stage for possible emergence of VRSA that could be disseminated into the community. Healthcare personnel should be cognizant of this possibility and appropriate methods for the early detection of co-colonized patients

should be identified. In addition, measures to limit patient-to-patient transmission and prevent endemic colonization in the healthcare institutions and community should be implemented.

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