Methicillin-resistant *Staphylococcus aureus* carriage, infection and transmission in dialysis patients, healthcare workers and their family members

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Abstract

**Background.** Carriage and subsequent infection with methicillin resistant *S. aureus* (MRSA) and its transmission between hospital and community settings have not been studied in dialysis patients and their contacts.

**Methods.** Surveillance for nasal MRSA carriage and infection among dialysis patients, healthcare workers (HCWs) and their family members in a dialysis centre was prospectively undertaken during three time periods within 1 year. Molecular typing was used to determine epidemiological relationship.

**Results.** Among 1687 samples collected, MRSA colonization rates were 2.41% (2/83) for peritoneal dialysis patients and 2.36% (12/509) for haemodialysis patients. Five (5/14) subjects subsequently had MRSA infection. The clinical MRSA isolates had the same molecular type as the colonized strains of the same person, indicating MRSA colonization preceded clinical infection. Significantly higher MRSA nasal carriage rates were observed among family members of HCWs than family members of dialysis patients (*P* = 0.0024). Only three major clones were observed. Pulmonary diseases (OR: 4.873, 95% CI: 1.668–14.235), recent admission to a hospital (OR: 2.797, 95% CI: 1.291–6.059) and recent antibiotics usage (OR: 2.319, 95% CI: 1.053–5.104) were also significantly associated with MRSA carriage.

**Conclusion.** Transmission of MRSA among dialysis patients, HCWs and their family members in a dialysis unit could be inferred. Monitoring and eradication of MRSA from patients, HCWs and their family members should be considered to prevent continuous spread between healthcare facilities and the community.

Keywords: dialysis; methicillin resistance; *Staphylococcus aureus*

Introduction

Infection is a major cause of morbidity and mortality among patients undergoing haemodialysis and continuous ambulatory peritoneal dialysis (CAPD) for chronic renal failure [1–4]. *Staphylococcus aureus* is a major pathogen in this patient group [5–8], and colonization of *S. aureus* is associated with a four-fold higher risk of blood stream infection [9,10]. For hospitalized patients in one study, a proportion of patients (1.095%) with nasal carriage of *S. aureus* subsequently developed bacteremia during a 14-month follow-up period, and most clinical isolates (85.7%) were identical to the nasal colonization isolates as demonstrated using molecular typing methods [11]. The *S. aureus* strain causing infection among patients has been shown to be the same as the colonization strain among 80 to 90% of patients in other studies [10,12].

Dialysis therapy, including haemodialysis and peritoneal dialysis, results in frequent contact with the hospital environment, despite the patients being ambulatory in the community setting. Few studies have addressed the issue that the colonization strains may spread from patients or healthcare personnel of a dialysis centre to the community [13]. In Taiwan, there is a high prevalence rate (84%) of methicillin-resistant *S. aureus* (MRSA) among hospital-acquired infections with *S. aureus* [14]. In the community setting, the MRSA burden is also considerable. A proportion of 3.4% of community dwellers with MRSA nasal carriage have no predisposing risk factors [15]. The emergence of community-acquired (CA-) MRSA infection has been reported in various studies [15,16]. The reportedly higher MRSA colonization rate in dialysis patients...
were designed according to Ito previously described [19].

complex (site specific recombinase) of SCC

For according to the methods of Oliveira and de Lencastre

S. aureus

Study duration and population

Surveillance of nasal S. aureus carriage together with the administration of a questionnaire were undertaken prospectively at a dialysis unit in a regional hospital in Kaohsiung City, Taiwan during three study periods within 1 year (September 2002, January 2003 and May 2003). All dialysis cases and HCWs in the dialysis centre and their family members living in the same household were included. Written consent was obtained from all subjects before the study.

Microbiological study

All study participants underwent swabbing of the anterior 1.5 cm of the nasal vestibule of both nares with a sterile swab (CultureSwab Transport System, Difco, Detroit, USA). Each swab specimen was streaked onto two mannitol salt agar plates (Difco, Sparks, MD, USA), one of which was supplemented with oxacillin (4 µg/mL). These inoculated plates were incubated at 35°C for 48 h, after which morphological and Gram-stain examination were conducted. Colonies were inoculated onto sheep blood agar plates (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) and cultured at 37°C overnight. The coagulase test (Coagulase Plasma System; Difco) was employed to identify S. aureus strains. Methicillin-susceptible S. aureus (MSSA) strains were preliminarily detected by characteristic growth on mannitol salt agar and absence of growth in the presence of oxacillin. Growth on both agar plates was presumed to indicate the presence of MRSA. All isolates were inoculated onto Mueller–Hinton agar (Becton Dickinson Microbiology Systems) containing 6 µg/mL oxacillin and 4% NaCl to confirm methicillin resistance.

SCCmec, agr and ccr typing by multiplex PCR

Multiplex PCR for SCCmec typing was performed according to the methods of Oliveira and de Lencastre [17]. For ccr gene typing, two pairs of PCR primers for the detection of ccrA3 and ccrB3 belonging to the Φecr complex (site specific recombinase) of SCCmec type III (AB037671) were designed according to Ito et al. [18] The primers for detection of ccrC of SCCmec type V were as previously described [19].

The detection of the Panton–Valentine leukocidin (PVL) gene was performed according to a published protocol [20]. The agr types were determined by the PCR method as reported by Shopsin et al. [21]

Genomic fingerprinting by pulsed-field gel electrophoresis (PFGE)

The total DNA was prepared and PFGE was performed as described previously using the restriction enzyme SmaI [22]. The band patterns were visually compared and classified as indistinguishable (no differences), closely related (clonal variants, one to three band differences), possibly related (four to six band differences) and unrelated (more than six band differences) according to previously described criteria [23]. isolates with banding patterns that differed from the main pattern by up to three bands were considered to represent subtypes within the main type.

To identify PFGE polymorphism, each sample was analysed using Molecular Analyst Fingerprinting, Fingerprinting Plus and Fingerprinting DST Software (Bio-Rad Laboratories, Richmond, CA, USA). A dendrogram was deduced from the matrix using the Unweighted Pair Group Method with the arithmetic average clustering technique, after the calculation of similarities using the Pearson correlation coefficient between pairs of organisms. PFGE patterns were distinguished at an 80% similarity level.

Questionnaire and statistical analysis

Each adult participant completed a standardized questionnaire form. Questionnaires for children were completed by their parents. The participant’s age, gender and medical history over the preceding year, including previous hospitalization, medication history prior to receiving the screening test and any underlying diseases, were correlated with the S. aureus colonization status. Chart review was conducted for nursing home, haemodialysis and hospitalized subjects.

Since some subjects might have participated in more than one of the three study periods, the data might lack independence. To overcome this difficulty, generalized estimating equations (GEE), which are similar to general regression models but account for dependence among observations [24], were used to determine the various study parameters.

We used the SAS PROC GENMOD procedure (Version 8.2, SAS Institute Inc., Cary, NC, USA) to fit GEE models. Odds ratio for each risk factor and 95% confidence intervals were also calculated. Statistical significance was defined as \( P < 0.05 \).

Results

Isolation of S. aureus in recruited subjects

We studied 629, 525 and 533 subjects (total 1687) during the three surveillance periods within the duration of 1 year, with 1687 samples collected from 748 individuals. A total of 379 (20.7%) individuals participated in all three study periods; 181 individuals participated in two and 188 in one. Among the 379 individuals taking part during all three
Community MRSA carriage in dialysis

Table 1. Dialysis patients, healthcare workers and their family members enrolled in three surveillance periods

<table>
<thead>
<tr>
<th>Study parameter</th>
<th>I (n = 629)</th>
<th>II (n = 525)</th>
<th>III (n = 533)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case groupa</td>
<td>P</td>
<td>P-FM</td>
<td>HCW</td>
</tr>
<tr>
<td>Case no.</td>
<td>196</td>
<td>336</td>
<td>23</td>
</tr>
<tr>
<td>Age range (mean, standard deviation)</td>
<td>16–84 (54.5, 14.4)</td>
<td>1–86 (36.8, 19.8)</td>
<td>29–48 (34.4, 19.8)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>54.8</td>
<td>54.8</td>
<td>95.7</td>
</tr>
<tr>
<td>S. aureus colonization Cases (%)</td>
<td>55 (28.1)</td>
<td>89 (0.6)</td>
<td>6 (43.1)</td>
</tr>
<tr>
<td>MRSA colonization Cases (%)</td>
<td>5 (2.6)</td>
<td>2 (0.6)</td>
<td>6 (43.1)</td>
</tr>
</tbody>
</table>

aP: dialysis patients, P-FM: patients’ family, HCW: healthcare worker, HCW-FM: healthcare workers’ family. HD, haemodialysis patients; PD, peritoneal dialysis patients.

Table 2. MRSA carriage among dialysis patients, healthcare workers and their family members

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Groupsa</th>
<th>Case no. in each surveillance period (proportion carrying MRSA)</th>
<th>OR (95% CI)</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>P versus P-FM</td>
<td>P</td>
<td>I (n = 629)</td>
<td>II (n = 525)</td>
<td>III (n = 533)</td>
</tr>
<tr>
<td>P-FM versus HCW-FM</td>
<td>P</td>
<td>196 (2.6%)</td>
<td>201 (4.0%)</td>
<td>213 (2.4%)</td>
</tr>
<tr>
<td></td>
<td>P-FM</td>
<td>336 (0.6%)</td>
<td>242 (1.2%)</td>
<td>232 (2.2%)</td>
</tr>
<tr>
<td></td>
<td>HCW-FM</td>
<td>74 (6.8%)</td>
<td>63 (3.2%)</td>
<td>65 (7.9%)</td>
</tr>
</tbody>
</table>

aP: dialysis patients, P-FM: patients’ family, HCW: healthcare worker, HCW-FM: healthcare workers’ family. HD, haemodialysis patients; PD, peritoneal dialysis patients.
bComparisons among P versus HCW, HCW versus HCW-FM and HD versus PD showed no significant difference with OR (95% CI) of 0.887 (0.112–7.026), 0.672 (0.079–5.681), and 1.052 (0.225–4.915) respectively among three groups.

study periods, 38 (10.0%) were positive for S. aureus nasal carriage (including 35 MSSA and 3 MRSA) at all periods of surveillance while 222 (58.6%) were never colonized by S. aureus. The demographics and carriage rates of S. aureus and MRSA of the four groups, including dialysis patients (P), patients’ family members (P-FM), HCWs, and HCWs’ family members (HCW-FM) are presented in Table 1.

The S. aureus carriage rate was significantly higher among peritoneal dialysis patients (43.4%, 36/83) than haemodialysis patients (21.8%, 111/509) (P < 0.0001, OR: 2.746, 95% CI: 1.695–4.449). However, their MRSA carriage rates were similar: 2.41% (2/83) for peritoneal dialysis patients and 2.36% (12/509) for haemodialysis patients.

There was no significant difference in the percentages of nasal S. aureus and MRSA carriage between patients and HCWs in each surveillance period. A significantly higher MRSA nasal carriage rate was observed among HCW-FMs than family members of dialysis patients (P = 0.0024). The nasal carriage rate of the dialysis patients was higher than that of the P-FM (P = 0.0171) (Table 2).

Risk factors for S. aureus and MRSA colonization

The association of underlying diseases and receiving medical treatment within 1 year as risk factors for MRSA carriage is shown in Table 3. Pulmonary diseases (OR: 4.873, 95% CI: 1.668–14.235), recent admission to a hospital (OR: 2.797, 95% CI: 1.291–6.059) and recent antibiotics usage (OR: 2.319, 95% CI: 1.053–5.104) were significantly associated with MRSA carriage (Table 3). No significant association with MRSA carriage (P > 0.05) was observed for sex distribution, diabetes mellitus, hypertension, renal diseases, gastrointestinal diseases, nasal diseases and recent outpatient visit. For S. aureus carriage, only recent antibiotic usage was significantly associated during the third surveillance period (P = 0.017, OR: 2.07, 95% CI: 1.13–3.8).

Microbiology

Forty-three MRSA colonization isolates were recovered from 30 subjects including 12 haemodialysis patients, 5 family members of haemodialysis patients, 2 peritoneal dialysis patients, 2 family members of peritoneal dialysis patients, 1 HCW and 8 HCW-FMs (Figure 1). During the 1-year study period, four haemodialysis patients (H1, H4, H5 and H6) and one peritoneal dialysis patient (P1) had infections due to MRSA and 10 clinical isolates were collected from them. Regarding the infection foci, two had catheter related infection; one had cellulitis; one had psoas abscess and one had infection related to percutaneous biliary drainage. Except for the cellulitis case with nasal colonization detected after infection, the remaining dialysis patients had prior nasal MRSA colonization, constituting...
Table 3. Risk factors for MRSA colonization during each surveillance period

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Surveillance period (no. of events)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (MRSA 13)</td>
<td>Non-MRSA 616</td>
<td>II (MRSA 14)</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Recent admission</td>
<td>2</td>
<td>67</td>
<td>7</td>
</tr>
<tr>
<td>Recent antibioticsa</td>
<td>7</td>
<td>71</td>
<td>8</td>
</tr>
<tr>
<td>Diabetic mellitus</td>
<td>2</td>
<td>67</td>
<td>3</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>5/8</td>
<td>260/356</td>
<td>5/9</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2</td>
<td>81</td>
<td>3</td>
</tr>
<tr>
<td>Renal disease</td>
<td>5</td>
<td>192</td>
<td>8</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>0</td>
<td>58</td>
<td>4</td>
</tr>
<tr>
<td>Nasal diseases</td>
<td>2</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Recent out-patient visit</td>
<td>10</td>
<td>392</td>
<td>9</td>
</tr>
<tr>
<td>Recent medication</td>
<td>10</td>
<td>363</td>
<td>10</td>
</tr>
</tbody>
</table>

*a85, 20 and 2 cases respectively could not indicate whether they received antimicrobial agents in the three surveillance periods.

Non-MRSA cases are those study participants with negative culture for MRSA, including those with MSSA and negative culture for *S. aureus*.

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Fig. 1. For each isolate, the capital letters denote the following: H: haemodialysis case, P: peritoneal dialysis case, N: healthcare worker, HF: haemodialysis case’s family member, PF: peritoneal dialysis case’s family member and NF: healthcare worker’s family member. Each number between letters indicates one person. The small case letter denotes the following: a: first, b: second and c: third surveillance period. XI, XII and XIII indicate the first, second and third clinical isolate respectively recovered from episodes of infection, for example, H1a, H1XI and H1XII were recovered from the same patient. Similarity of >80% was defined to represent clonal spreading of strains. For the SCCmec types, six non-typable strains were named N1 to N5 and N3-1.
an infection rate of 28.6% (4/14) among dialysis patients. The durations from colonization to infection were 27, 28, 83 and 99 days for the four cases. Two MRSA colonization isolates could not be revived after storage; hence, a total of 51 MRSA isolates were further examined.

Analysis of the PFGE typing results of the colonization and infection strains revealed three major clusters circulating in the dialysis centre (Figure 1). These three major PFGE types comprised 88.2% (45/51) of all isolates.

All the MRSA isolates of PFGE type III were of SCCmec type IV but were non-typable with ccr gene typing. Isolates of PFGE types I and II respectively were of ccr types A1B2 and A1B2C but were non-typable by SCCmec typing (Figure 1). Considering all typing results, the existence of three clones with specific SCCmec and ccr types corresponding to each PFGE type indicated the occurrence of MRSA transmission among individuals associated with the dialysis centre. Five family members (HF1, HF2, NF5, NF6 and PF1) carrying MRSA strains of PFGE type III denied receiving medical attention in the past year. Their MRSA isolates had the same PFGE, ccr and SCCmec types as those from the respective dialysis patients and HCWs, suggesting cross-transmission. It is noteworthy that one HCW (N1) and her two family members (NF3 and NF4) had isolates of the same PFGE, SCCmec, ccr and agr types, indicating the spread of MRSA among HCWs in dialysis units and their family members.

All the isolates from clinical MRSA infections belonged to PFGE types I (H1) and II (P1, H4, H5 and H6) and these clinical isolates were all non-typable for SCCmec. None of these MRSA isolates possessed the PVL virulence gene.

Discussion

Colonization and infection by *S. aureus* are known to be significantly associated with infection among hospitalized patients [10,11]. In the era of high prevalence of methicillin resistance among *S. aureus*, a relatively high percentage (11–19%) of MRSA nasal carriage among hospital patients on admission will increase the likelihood of MRSA infection during the same episode of hospitalization [25,26]. Studies on MRSA in intensive care units have also demonstrated that MRSA colonization predisposed to MRSA infection during the same hospitalization [27]. Upon discharge back to the community, a high percentage (29%) of previously infected patients were documented to develop infection after 18-month follow-up [28]. This greatly raises concerns about community infections caused by healthcare-associated strains of MRSA. In the present study, we found a high proportion (28.6%) of dialysis patients with MRSA carriage who developed MRSA infection subsequently. This rate may actually be an underestimation due to the relatively short study period of 1 year. Such a high rate indicates that dialysis patients who carry MRSA nasally are highly susceptible to MRSA infection. These findings also suggest that colonization was due to virulent *S. aureus* strains, accounting for the high rate of infection among dialysis patients, and supporting measures to eradicate MRSA carriage in this group of patients [11].

Occult transmission of MRSA in the dialysis unit between HCWs and patients was strongly suggested by PFGE, SCCmec and ccr genotyping, demonstrating the existence of endemic strains among the patients, HCWs and their family members. These results clearly showed transmission of MRSA from the dialysis centre, a hospital-related environment, through family members of patients and HCWs and into the community. This was further supported by the fact that there were family members who were colonized by the endemic MRSA strains but had no other recent medical contact.

Infections in haemodialysis and peritoneal dialysis patients are generally not community acquired and thus are classified as healthcare-associated infections according to Friedman’s classification [29]. Our results showed that MRSA isolates of the dialysis group had several characteristics of healthcare-associated strains, while the traits of CA-MRSA, such as harbouring the PVL gene [16] and being of SCCmec type IV [30], were not present unlike the USA300 clone in USA [26]. Epidemiological analysis also showed that hospital admission and receiving antimicrobial therapy within 1 year were two important risk factors for MRSA colonization in the dialysis unit cohort, although recall bias accounted for a proportion of individuals who could not ascertain whether they have received prior antimicrobials (Table 3). Whether the particular PFGE type III, which is SCCmec type IV positive but PVL negative, represents a new endemic CA-MRSA clone that is different from previously reported strains in northern Taiwan [16] requires further investigation. The situation of CA-MRSA clones being transmitted to healthcare facilities, thus leading to healthcare-associated infections in USA [31–33], Australia [33] and UK [34], warrants vigilance in our setting.

The estimated long half-life of MRSA colonization of 40 months [35], the possibility for horizontal genetic exchange among nasal colonization strains [36] and the propensity for subsequent infection after MRSA colonization as seen in this study underline the significance of MRSA colonization as an infection control problem. Although the follow-up rate was high in the current study, the possibility of missed cases still exists. The sampling interval (every 4 months) was determined according to the feasibility of sampling and workload. This might have underestimated the proportion of individuals having *S. aureus* or MRSA colonization since more frequent sampling might have identified a substantially greater number of individuals with transient nasal colonization. The role of transient flora on hands in causing transmission of infection [37,38] was unable to be defined in this study. This may be one of the causes of occult spread of endemic strains in the dialysis unit.

In the present study, the *S. aureus* colonization rate (28.1%) in haemodialysis patients was relatively lower than that in a previous study (56.7%) in Turkey [39] but was comparable to the study (27.3%) for the American Indian population [40]. In one study from Saudi Arabia, an overall nasal carriage of 38.0% was observed, and the carriage rate varied in different age groups [41]. Similarly our MRSA carriage rate in peritoneal dialysis patients was also relatively lower than that in a previous study [42]. Since nasal carriage of MRSA was associated with prior MRSA
exposure [42]. Taken together, these studies indicate that MRSA carriage rates may vary among different geographic regions and may not depend solely on the dialysis state. In an earlier study in Taiwan [15], HCWs had higher MRSA carriage rates when compared with the community setting (3.5%). Our study also showed a higher MRSA carriage rate, ranging from 4.0 to 5.3%. The rates of MRSA colonization among HCW-FMs (3.2–6.8%) were significantly higher than those among P-FMs. This indicates the necessity for further emphasis on hand hygiene for HCWs especially before going home, similar to recommendations currently given to patients and their families, to avoid transmission of MRSA to family members. These findings also point to the importance of eradicating MRSA colonization in HCWs and their family members, in addition to applying this strategy to dialysis patients and their family contacts. Since MRSA infection is a potentially severe event, early diagnosis is essential. However, especially among dialysis patients, catheter-related infections may arise from silent endoluminal contamination or low-grade infection, which are difficult to identify and may result in significant complications. Prevention is thus important and relies on hygienic measures and strict protocols based on aseptic manipulation by HCWs [43]. In nasal carriers of MRSA, eradication of bacteria by a topical antimicrobial ointment has been associated with a significant reduction of the incidence of bacteremia [43].

In conclusion, the nasal carriage of MRSA among dialysis patients is significant not only in terms of predisposing to subsequent infections, but also plays an important role in transmission among dialysis unit staff and their family members. Monitoring and eradication of MRSA from patients, HCWs and their family members. Monitoring and eradication of MRSA colonization in HCWs and their family members, in addition to applying this strategy to dialysis patients and their family contacts. Since MRSA infection is a potentially severe event, early diagnosis is essential. However, especially among dialysis patients, catheter-related infections may arise from silent endoluminal contamination or low-grade infection, which are difficult to identify and may result in significant complications. Prevention is thus important and relies on hygienic measures and strict protocols based on aseptic manipulation by HCWs [43]. In nasal carriers of MRSA, eradication of bacteria by a topical antimicrobial ointment has been associated with a significant reduction of the incidence of bacteremia [43].

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Conflict of interest statement. None declared.

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