Carriage rates of methicillin-resistant *Staphylococcus aureus* (MRSA) depend on anatomic location, the number of sites cultured, culture methods, and the distribution of clonotypes

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**Abstract** The present study was carried out to determine how active surveillance for methicillin-resistant *Staphylococcus aureus* (MRSA) could be improved by the use of enrichment broth and the inclusion of extra-anatomic sites with nasal cultures. Molecular typing was also performed to identify colonization by single or multiple strains. Surveillance cultures for MRSA were obtained from 650 patients on admission to a medical and surgical intensive care unit (ICU) in Taiwan. MRSA was detected on directly plated vs.

broth-enrichment cultures in any site at 10.0% vs. 24.2%, nares 8.2% vs. 17.5%, throat 4.8% vs. 13.4%, axilla 1.2% vs. 9.1%, and perineum 1.8% vs. 9.5%, respectively. Nares cultures alone detected only 81.5% and 72.5% of all colonized patients by direct and broth-enriched cultures, respectively. The molecular typing of 68 isolates from 17 patients revealed that multiply infected isolates were largely indistinguishable within each patient, but four patients had multiple subtypes and another three patients had different clonotypes. The detection of MRSA carriers was considerably enhanced by broth-enrichment cultures at multiple anatomic sites and simultaneous colonization by multiple strains at different sites can occur. Epidemiological studies are needed to determine the likelihood of subsequent nosocomial infection among colonized patients detected via direct nasal versus broth-enriched cultures from multiple sites.

**Introduction**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of severe, invasive nosocomial infections. The worldwide prevalence of MRSA has increased in association with the widespread occurrence of community-acquired MRSA. Patients colonized with MRSA are not only at higher risk of developing subsequent infection [1, 2], but they may also serve as a reservoir for transmitting MRSA to other hospitalized patients and healthcare workers. Recommended control measures include hand hygiene,
colonized patients may serve as a potential source for transmitting the organism to others. Therefore, active surveillance of high-risk patients to identify asymptomatic carriers and placing them under contact precautions has been standard practice and is mandated in some countries [3, 4]. Although there have been studies comparing the recovery of MRSA from different anatomical sites [5–8], most surveillance testing has focused only upon surveying for nasal carriage. In addition, although broth enrichment had been suggested as a means to improve the recovery of S. aureus [9–12], the use of enrichment broth in surveillance testing for MRSA has not been universally embraced [6, 7, 13, 14].

MRSA accounts for 70 to 75% of S. aureus infections in intensive care units (ICUs) and more than 50% of nosocomial S. aureus infections in Taiwan. To better understand the carriage of MRSA in high-risk patients, we conducted an active surveillance project in two hospitals in Taiwan. Surveillance cultures of the nares, throat or sputum, axilla, and perineum were performed on ICU patients at admission. This report compares the recovery of MRSA from the four body sites and determined the effect of enrichment broth on the culture results. Molecular characterization was also performed on isolates from patients who were colonized on the four sites to determine if different MRSA strains colonize a patient at different body sites simultaneously.

Materials and methods

Patients

The study population consisted of patients admitted to the ICUs at two large (more than 700 beds) hospitals in Taipei, Taiwan. The medical ICU (3 beds) of hospital A and the surgical ICU (12 beds) of hospital B were included in the study. The survey was conducted over a period of 6 months between August 2005 and February 2006. It was approved by the Institutional Review Board (IRB) committees of both hospitals.

Specimens

Surveillance cultures were performed within 24 h of admission to the ICUs. A sample set of nose, throat or sputum, axilla, and perineum cultures were obtained using separate EZ Cultettes (BBL, Becton Dickinson, Sparks, MD). The swabs were plated onto a sheep blood agar (SBA) and a CHROMagar MRSA (direct culture). The swabs were then inserted into a tube of enrichment broth containing 5 ml TrypsEase soy broth with 7.5% NaCl (broth-enrichment cultures). The plates and broth cultures were transported to the National Health Research Institutes (NHRI) for subsequent workup. After overnight incubation, the enrichment broth was subcultured on CHROMagar Staph aureus plates. The plates were incubated at 35°C in ambient air. The media and identification reagents were purchased from BD Diagnostics.

Isolation, identification, and enumeration

The plates were inspected at 24 and 48 h. S. aureus was confirmed by coagulase latex agglutination. S. aureus isolates from SBA and CHROMagar Staph aureus plates were checked for methicillin resistance using the cefoxitin disk following the Clinical and Laboratory Standards Institute (CLSI) guidelines [15]. S. aureus isolates that grew on CHROMagar MRSA plates were assumed to be MRSA. All culture results were also enumerated. If MRSA colonies were observed on direct culture plates, we recorded the growth as few when colonies were seen in the first quadrant only. If colonies were seen in up to the second, third, and fourth quadrants, we recorded the growth as occasional, moderate, and numerous, respectively. If MRSA was found in subculture from enrichment broth only, we recorded the growth as rare. All isolates were subcultured on SBA and stored at −80°C for further evaluation.

Molecular studies

Molecular typing of the genomic DNA was performed by pulsed-field gel electrophoresis (PFGE) according to published protocols [16]. PFGE patterns were analyzed using BioNumerics software (Applied Maths NV, Sint-Martens-Latem, Belgium). The pulotype were assigned to clusters of isolates with ≤ 8 bands differences from each other [17]. Isolates of the same pulotype shared >80% or higher similarity from the dendrogram, except for pulotype A isolates, which shared >75% similarity with each other. Polymerase chain reaction (PCR) was performed to determine the staphylococcal cassette chromosome mec (SCCmec) types I to V and the presence of pvl (Panton-Valentine leukocidin) toxin genes according to published protocol [18, 19]. Multilocus sequence typing (MLST) was performed on selected isolates from each pulotype and the sequence type (ST) was assigned using the MLST database (http://www.mlst.net) [20].

Statistical analysis

Statistical analysis was performed using Epi Info 6.04 (CDC, Atlanta, GA). The Fisher exact two-tailed χ² test was used to determine significant differences in frequencies and proportions. A p-value<0.05 was considered to be significant.
Results

Study population

The initial population consisted of 679 patients. These included 235 from hospital A and 444 from hospital B. Twenty-nine patients were excluded because they lacked cultures from one or more sites. The remaining 650 patients were included in the analysis. The rates of MRSA colonization at the various anatomical sites at both hospitals were found to be similar (data not shown). Therefore, the data from the two hospitals were combined in the final analysis.

Enumeration of MRSA from different body sites

Among the four anatomical sites, more MRSA isolates were present in nares, while the lowest number of colonies was present in axilla. Slightly over half (51.3%) of the positive nare cultures and 60.7% of the positive throat/spit cultures had rare MRSA as defined by growth from enrichment broth subculture only, whereas this was the case for 72.3% of positive perineum and 82.2% of positive axilla cultures. In positive cultures from primary plates, more organisms were also found in nares and throat/spit (Fig. 1).

Detection of MRSA by direct and broth-enrichment cultures

MRSA carriage in the nares, throat, axilla, and perineum was 8.2, 4.8, 1.2, and 1.8% by direct culture, and 17.5, 13.4, 9.1, and 9.5% by broth enrichment, respectively (Table 1). The percentage of MRSA carriers overall (MRSA detected in any site) was 10.0% (65 patients) by direct culture and 24.2% (157 patients) by broth enrichment (p<0.001). Broth enrichment detected all of the MRSA detected by direct culture. Although the detection sensitivity was highest in the nares at 81.5% (53/65) by direct culture and 72.6% (114/157) by broth enrichment, the direct culture of nasal specimens missed MRSA colonization in over half (53.5%, 61/114) of patients detected by broth enrichment. The broth enrichment method was particularly effective in detecting MRSA in the axilla (7.4-fold, 59 vs. 8 patients) and perineum (5.2-fold, 62 vs. 12 patients) (broth vs. direct culture), a reflection on the low numbers of organism load in these two sites (Fig. 1).

Table 1 Recovery of methicillin-resistant Staphylococcus aureus (MRSA) according to anatomical site and culture methods for 650 intensive care unit (ICU) patients

<table>
<thead>
<tr>
<th>Site cultured</th>
<th>MRSA isolates by direct culture</th>
<th>MRSA isolates by broth enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% positive</td>
</tr>
<tr>
<td>Nasal</td>
<td>53</td>
<td>8.2</td>
</tr>
<tr>
<td>Throat/spit</td>
<td>31</td>
<td>4.8</td>
</tr>
<tr>
<td>Axilla</td>
<td>8</td>
<td>1.2</td>
</tr>
<tr>
<td>Perineum</td>
<td>12</td>
<td>1.8</td>
</tr>
<tr>
<td>Nasal+throat/spit</td>
<td>60</td>
<td>9.2</td>
</tr>
<tr>
<td>Nasal+axilla</td>
<td>55</td>
<td>8.5</td>
</tr>
<tr>
<td>Nasal+perineum</td>
<td>57</td>
<td>8.8</td>
</tr>
<tr>
<td>One or more sites</td>
<td>65</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*Statistical comparison of each site colonized by direct culture enrichment found all to be p<0.001

**=Number of patients detected by each site combined
The highest detection sensitivity was obtained when nare was combined with throat cultures (92.3% by direct culture and 85.4% by broth enrichment), followed by combining with perineum (87.7% by direct culture and 84.1% by broth enrichment).

The distribution of MRSA according to various combinations of colonized sites is shown in Table 2. Broth enrichment, but not direct cultures, detected 17 patients who were colonized at all four sites. Only four patients with a negative direct nasal culture had a positive direct perineal culture. In contrast, 18 patients with a negative broth-enrichment nasal culture had a positive broth-culture perineal culture. Similar disparities between nasal and distal-site cultures were observed with throat and axillary cultures.

Molecular characterization of MRSA strains colonizing all sites

MRSA strains obtained from 17 patients found to be colonized at all four sites were further characterized by molecular methods. A dendrogram of the PFGE patterns, plus the sequence type (ST), pvl, and SCCmec, of the 68 isolates is shown in Fig. 2. The isolates were grouped into four main clusters (pulotypes). Isolates of the same pulotype also shared the same genetic background (ST) and carried the same SCCmec type. Pulotype A was ST239:SCCmec III, pulotype B was ST5:SCCmec II, pulotype C was ST59:SCCmec V and pvl-positive, and pulotype D was ST59:SCCmec IV. The majority of isolates (46/68) belonged to pulotype A. However, four patients were found to carry multiple subtypes of MRSA simultaneously, including three (K0134, K0154, and W0723) carrying more than one subtype of pulotype A and one (W0768) carrying two subtypes of pulotype D. In addition, three patients carried distinct pulotypes of MRSA on different anatomical sites, including one (W0207) carrying pulotypes A and B, one (K0109) carrying pulotypes A and D, and another (W0662) simultaneously carried three distinct pulotypes (A, D, and non-A-D).

Discussion

The value of active surveillance cultures (ASCs) to detect MRSA carriers combined with measures to block transmission continues to generate considerable controversy.Proponents point to the remarkable success in countries in Northern Europe and Western Australia in controlling infections caused by MRSA [21]. Others are less enthusiastic [22]. McGinigle et al. recently reviewed the literature on ASCs [23]. They state that, “Existing evidence may favor the use of ASCs, but the evidence is of poor quality, and definitive recommenda-
tions cannot be made.” The review generated vigorous responses from the proponents of ASCs [24, 25].

The current study may help clarify some of the technical issues inherent in studies of ASCs for MRSA. Most epidemiological studies rely on direct nasal cultures to generate prevalence rates. It is clear from the current observations that direct nasal culture is not a particularly sensitive marker for colonization by MRSA. We found that direct nasal culture detected only 33 MRSA carriers among 650 ICU patients tested (carriage rate 8.2%). Broth-enrichment cultures increased the nasal carriage rate to 17.5% (114 patients). The carriage rate rose to 24.2% (157 patients) by including the broth-enrichment cultures of the throat, axilla, and perineum. Therefore, 66.2% (104/157) of MRSA-colonized patients would have gone undetected if only direct cultures of nasal swabs were used.

Prior investigators have also shown that the broth enrichment increases the ability to detect colonization by MRSA. Gnek-Korsak et al. found that the inclusion of broth enrichment improved MRSA detection by 25.8% compared to direct plating [5]. van Ogtrop found that broth enrichment reduced the false-negative Staphylococcus culture rate by 44.6% and van Loo et al. reported that inclusion of the broth enrichment identified 12% more MRSA isolates in a pilot study and 30% more in a subsequent study [26, 27]. A recent study by Noshiro et al. found that broth enrichment nearly doubled the detection sensitivity of three MRSA chromogenic media (from around 45% to 85%) [28], while Van Heistraen et al. also found a significant increase in the number of MRSA-positive samples after overnight pre-enrichment incubation [29]. It remains to be seen whether rapid detection methods such as the multiplex PCR are equally or more sensitive than broth-enrichment cultures [30]. The nare has been long considered to be the major ecological niche for Staphylococcus. Nasal cultures are easy to obtain and are considered to be the specimen of choice for surveillance cultures [1, 31]. An early study suggested that the axilla and perineum had limited value to detect MRSA carriers [30]. This notion was not supported in a study of nursing home residents in which 35% of MRSA carriers were colonized only at extra-nasal sites [5]. A recent review by Acton et al. also pointed out that axillary (including perineum screen) Staphylococcus and MRSA carriage may be underestimated, since multiple studies have found various rates (5–77%) of perineal colonization [32]. The variation in the detection rates may be due to differences in the organism load and methods used. The MRSA organism load was lower in the axilla and perineum in the present study. In the study by Van Heistraen et al. the authors also found the average MRSA colony count in the groin swabs to be nearly one log lower than that of nasal swabs [29]. Although the nare was the major site of colonization by MRSA in the current study, broth-enrichment cultures of extra-nasal sites detected an additional 43 patients who would otherwise have gone undetected by direct nasal culture only.

Broth-enrichment cultures allowed us to isolate MRSA at all four sites (nose, throat, axilla, and perineum) in 17 patients. Molecular studies confirmed that the same strain of MRSA was isolated from all sites in most patients, but some patients carried more than one strain at multiple sites. Pulotype A (ST239 SCCmec III) isolates predominated in the ICUs of the two hospitals in this study. This is consistent with our previous reports, in which we found that ST239 SCCmec III pulotype A isolates were the main clonal type (as indicated by pulotype, MLST, and SCCmec type) of MRSA in hospitalized patients in Taiwan [19, 33]. Pulotype C (ST59: SCCmec V, pul-positive) isolates were considered to be community strains in Taiwan [19, 33]. The presence of these community MRSA strains in ICU patients indicates that they have established in our hospital environment. Some patients were found to simultaneously carry multiple subtypes of MRSA. This phenomenon has also been described in half of the ICU patients in Australia [14]. Simultaneous carriage of distinct MRSA clonotypes is important from the epidemiological perspective and may be useful in outbreak investigations. Pooling specimens could reduce laboratory costs and workload in MRSA carriage surveillance, but may miss patients carrying multiple MRSA strains.

In conclusion, the current study provides substantial evidence that the optimal detection of MRSA-colonized patients requires the use of broth-enrichment cultures and inclusion of the nose and extra-nasal sites. To establish that broth-enrichment cultures are clinically meaningful and cost-effective, it will be necessary to demonstrate that low-level MRSA colonization, detected by broth enrichment, increases the risk of autogenous infections and the transmission of MRSA to other patients and healthcare workers.

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References


