

Does Nasal Cocolonization by Methicillin-Resistant Coagulase-Negative Staphylococci and Methicillin-Susceptible *Staphylococcus aureus* Strains Occur Frequently Enough To Represent a Risk of False-Positive Methicillin-Resistant *S. aureus* Determinations by Molecular Methods?

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Received 12 October 2005/Accepted 17 October 2005

By analyzing the colonization of the anterior nares in cardiothoracic surgery patients on admission, nasal cocolonization by methicillin-susceptible *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci was detected in 8/235 (3.4%) specimens. Consequently, in a low-methicillin-resistant *S. aureus* (MRSA) setting, a molecular MRSA screening test targeting the *mecA* gene and an *S. aureus*-specific gene in parallel and applied directly to clinical specimens would be associated with an unacceptable positive predictive value of about 40%.

Infections caused by methicillin-resistant (MR) *Staphylococcus aureus* (MRSA) strains have become one of the most commonly acquired types of nosocomial infections, resulting in increased morbidity, mortality, length of hospital stay, and health care costs (6, 12, 18). Recently, MRSA has also become an established cause of community-acquired infections. Consequently, there is a need for rapid, reliable, and cost-effective methods for the detection of MRSA. Methicillin resistance in staphylococci is encoded by the *mecA* gene, which is part of the staphylococcal cassette chromosome *mec* (SCC*mec*) family. Because many of the MRSA clones exhibit a heteroresistance phenotype, with only a few staphylococcal cells of the population expressing methicillin resistance (4), detection of the *mecA* gene by molecular methods has become the reference method for confirmation of MRSA strains. However, SCC*mec* elements including *mecA* are also found in MR, coagulase-negative staphylococci (CoNS). Therefore, molecular assays for the detection of *mecA* alone are not sufficient for definite identification of MRSA unless an *S. aureus*-specific gene marker is included. This is fulfilled, e.g., by testing for the *nuc* gene (5) encoding the staphylococcal thermonuclease found in all isolates of *S. aureus* and different from sequences of thermonuclease genes of other staphylococcal species (3).

Unfortunately, *S. aureus* (including MRSA strains) and CoNS, such as *S. epidermidis* and *S. haemolyticus*, share the same habitats and permanently or transiently colonize the anterior nares and further regions of skin and mucous membranes which may act as sources of subsequent bacteremia and other infections (7, 22). Thus, both *S. aureus* and CoNS may often be recovered in parallel in the same clinical specimen. Whereas this situation is of minor diagnostic relevance if pure

bacterial cultures are tested (following cultivation and isolation of bacteria), results based on molecular methods for detection of MRSA directly from clinical specimens may be influenced by the coexistence of methicillin-susceptible (MS) *S. aureus* (MSSA) and MR-CoNS. Such a combination may result in false-positive diagnostic findings with the assumption of pseudo-MRSA and the consequence of infection control measures that usually have medical, as well as psychological, consequences for the patient and that often have dramatic organizational and financial impact on the health care unit. Although limitations of direct diagnostic MRSA tests have already been mentioned (20, 21), the design of previous studies did not address this problem adequately. Consequently, studies systematically investigating the frequency of MSSA and MR-CoNS cocolonization of the skin and mucous membranes with the potential risk of false-positive results in molecular MRSA detection tests are needed. In addition, published data on nasal colonization by CoNS were mostly collected in the pre-molecular era, not reflecting their present prevalence, recent taxonomic emendations, and the descriptions of novel (sub)species.

In order to determine nasal colonization by staphylococci as the epidemiological basis for the introduction of novel molecular screening assays to detect MRSA directly in clinical specimens, this study aimed to analyze the frequency of cocolonization of MSSA and MR-CoNS. In addition to *S. aureus*, the prevalence of further staphylococcal species involved in nasal colonization and their resistance to methicillin were studied.

A total of 235 nasal swabs were collected from patients on admission to the cardiothoracic surgery department at the University Hospital of Münster, where the prevalence of MRSA is known to be low (22). Only one swab per patient, with sample collection from both nares, was included. The swabs were streaked onto Columbia sheep blood agar and enriched in dextrose broth.

Gallery API ID 32 Staph (bioMérieux, Marcy l'Etoile,

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TABLE 1. Analysis of nasal colonization of 235 patients by staphylococci

Patients colonized	Only MS isolates	No. (%) of specimens bearing:			Total no. (%)
		MR isolates			
		Only MR isolates	MR and MS isolates	Total	
Only by <i>S. aureus</i>	16 (6.8)	2 (0.9)	1 (0.4)	3 (1.3)	19 (8.1)
Only by CoNS ^a	78 (33.2)	47 (20.0)	42 (17.9)	89 (37.9)	167 (71.1)
By <i>S. aureus</i> and CoNS	22 (9.4)	1 (0.4)	9 (3.8) ^b	10 (4.2)	32 (13.6)
Total	116 (49.4)	50 (21.3)	52 (22.1)	102 (43.4)	218 (92.8)^c

^a Including one coagulase-positive, methicillin-negative *S. intermedius* isolate.

^b Comprising eight (3.4%) patients cocolonized by MSSA and MR-CoNS and one patient cocolonized by MRSA and MS-CoNS.

^c Seventeen (7.2%) patients were observed without staphylococcal colonization of the anterior nares.

France) and the ID-GBP card of the VITEK 2 system (bioMérieux) were used for biochemical identification as recommended by the manufacturer. *S. aureus* isolates were confirmed by testing the thermonuclease (*nuc*) genes (3, 5). If the identification of CoNS isolates by the use of biochemical procedures was ambiguous or categorized as unacceptable, 16S rRNA gene sequencing was performed as previously described (2). Sequences were queried in the RIDOM and GenBank databases (2). For all *S. aureus* and CoNS isolates, methicillin resistance was determined by *mecA* PCR (17). For amplification procedures, staphylococcal DNA was isolated as previously described (1). All *S. aureus* isolates were *spa* typed as described elsewhere (13).

Nasal swabs were found to be colonized with at least one staphylococcal species in 92.8% of the cases ($n = 218$). Overall, 52 *S. aureus* isolates encompassing 47 MSSA and 5 MRSA isolates (exhibiting different *spa* types) and 311 isolates of CoNS (*S. epidermidis*, $n = 219$; *S. haemolyticus*, $n = 32$; *S. warneri*, $n = 15$; *S. hominis* subsp. *hominis* and *S. lugdunensis*, each $n = 10$; *S. capitis* and *S. chromogenes*, each $n = 6$; *S. lentus*, $n = 5$; *S. kloosii*, $n = 4$; *S. intermedius*, *S. schleiferi* subsp. *schleiferi*, *S. sciuri* subsp. *sciuri*, and *S. simulans*, each $n = 1$) were recovered. Of the CoNS isolates, 130 (41.8%) were shown to be MR (MR *S. epidermidis*, $n = 98$; MR *S. haemolyticus*, $n = 20$; MR *S. hominis*, $n = 5$; MR *S. warneri*, $n = 3$; MR *S. lentus*, $n = 2$; MR *S. lugdunensis* and MR *S. kloosii*, each $n = 1$).

The anterior nares were found to be colonized only with CoNS in 71.1% of the cases ($n = 167$), including 89/167 (53.3%) cases of colonization with at least one MR strain (only MR-CoNS, $n = 47$; MR-CoNS plus MS-CoNS, $n = 42$). Only *S. aureus* but not any cocolonizing CoNS was found in 19 (8.1%) of the nasal swabs, comprising 16 patients with MSSA, 2 patients with MRSA, and 1 patient cocolonized by MRSA and MSSA.

Cocolonization of the anterior nares by *S. aureus* and CoNS was observed in 32 cases (Table 1). Of these, 2 patients' nares were colonized by MRSA and those of 30 (93.7%) were colonized by MSSA. Nasal swabs of eight patients were characterized by a combination of cocolonizing MSSA and MR-CoNS (MR *S. epidermidis*, $n = 5$ [1 of these in combination with an additional MS isolate]; MR *S. haemolyticus*, $n = 1$; MR *S. sciuri*, $n = 1$; MR *S. epidermidis* plus MR *S. haemolyticus*, $n = 1$). Consequently, in 3.4% of the cases an incorrect conclusion of MRSA detection would have occurred.

Assuming a given molecular detection assay targeting genes

encoding methicillin resistance and an *S. aureus*-specific marker in parallel, statistical analysis (chi square test) based on the results of our study (false-positive MRSA testing, $n = 8$; true-positive MRSA testing, $n = 5$; false-negative MRSA testing, $n = 0$; true-negative MRSA testing, $n = 222$) reveals a positive predictive value of 39.3% (negative predictive value, 100%; sensitivity, 99%; specificity, 96%; $P = <0.00001$).

To shorten the time for detection of MRSA without cutting back on sensitivity and specificity is a key challenge for the improvement of MRSA diagnostics as a basic requirement for any effective MRSA prevention strategy. Compared to molecular methods, classical cultivation and identification methods, followed by phenotypic determination of antimicrobial resistance, are often hampered by lower specificity and sensitivity and they are usually more time-consuming. In addition, traditional approaches are based on the use of pure cultures. In contrast, amplification-based methods introduced to overcome the disadvantages of classical methods may be applicable for the specific detection of nucleic acid target structures independently of a previous isolation of an assumed pathogen. Thus, they also may be used directly for specimens contaminated with the resident or transient flora of the skin or mucous membranes. However, such an approach may fail in specificity if the target structure is part of the genomes of various species, as exemplified by SCC*mec*, which also occurs in other staphylococcal species (24). Since both *S. aureus* and CoNS are known to colonize the same habitats, a mixed population of MSSA (providing the *S. aureus*-specific target) and MR-CoNS (providing the target of methicillin resistance) might be diagnostically misleading when respective molecular methods are applied directly to clinical specimens (pseudo-MRSA). Even in the case of specimens recovered from primarily sterile body fluids or tissues, contamination with MR-CoNS (or at least with staphylococcal DNA) during the transection of the skin or of mucous membranes may occur.

In a low MRSA rate setting, a molecular test applied directly to clinical specimens which targets the species-unspecific *mecA* gene (or other parts of SCC*mec*) and an *S. aureus*-specific marker gene in parallel would be characterized by an unacceptable positive predictive value despite a high sensitivity (99%), as shown in our study. Consequently, such molecular screening assays might not be appropriate for detection of MRSA carriers in this setting. Moreover, an underlying 100% sensitivity and specificity, as used here for calculation of the predictive values for a given assay, is unlikely to be achieved in practice due to PCR inhibitors and other factors influencing

the scenario posed. Recently, the implication of initially unrecognized MRSA in a low-prevalence area was reported. In The Netherlands, the annual number of new MRSA isolates tripled in 2002 compared with previous years due to an MRSA clone with low-level resistance to oxacillin which was able to spread unnoticed and became endemic (23).

In our study, a relatively low rate of MR-CoNS (41.8%) was observed, compared to much higher rates reported for Central Europe or other regions, ranging from about 70 to 80% (10, 16). Whereas in past studies mostly nosocomial isolates were tested, our isolates were collected on admission, thus representing CoNS predominantly acquired in the community. Consequently, a further increase in cocolonization with MR-CoNS may be assumed with an increasing duration of the patients' hospital stay. Thus, MRSA detection strategies combining detection of the *mecA* gene in parallel with *S. aureus*-specific targets might be useful primarily for screening to determine patients without MRSA colonization and for preisolated cultures, respectively.

Recently, more sophisticated molecular methods such as selective immunomagnetic enrichment of *S. aureus* prior to PCR amplification (11) or PCR strategies providing a link between *SCCmec* and the *S. aureus* chromosome by targeting the *orfX* region (8, 14) were introduced. However, instead of the *mecA* gene itself, other regions near the integration site of *SCCmec* were targeted that are known to be more heterogeneous than assumed so far (14, 19). Furthermore, the occurrence of the *orfX* region among other members of the staphylococcal genus is still unstudied. Also, it was found that *SCCmec* may be unstable and strains that lost this region but further bear the flanking sequences of the cassette were described (9). In addition, *SCC* is not strictly associated with the *mec* genes and other genes (e.g., *cap1*) were found to replace them (15). Indeed, *SCCcap1* was shown to cause false-positive MRSA results (8).

In summary, simultaneous nasal cocolonization with MSSA and MR-CoNS was found in a low percentage of the patients tested. However, in particular in a low-MRSA setting, false-positive MRSA detection (pseudo-MRSA) may lead to unneeded efforts to eliminate patients' putative MRSA colonization, as well as to additional infection control measures, and consequently to a substantial increase in costs and personnel workload. Besides the obvious need for timely detection of MRSA, the accuracy of respective screening tests must not be disregarded. Finally, the sensitivity and specificity of MRSA screening approaches should be assessed in the light of the concrete local or regional MRSA prevalence.

We are grateful to B. Schuhen and M. Schulte for excellent technical assistance.

This study was conducted under the auspices of the Paul Ehrlich Society (Staphylococcus Working Group).

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