JOURNAL OF CLINICAL MICROBIOLOGY, July 2005, p. 3491–3493 Vol. 43, No. 7
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.7.3491–3493.2005

Molecular Evidence that Nasal Carriage of *Staphylococcus aureus* Plays a Role in Respiratory Tract Infections of Critically Ill Patients

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Received 19 October 2004/Returned for modification 17 November 2004/Accepted 21 March 2005

The relationship between nasal *Staphylococcus aureus* carriage and lower respiratory tract infections was studied in 16 critically ill patients. *S. aureus* strains from nasal and bronchial samples were characterized by pulsed-field gel electrophoresis. In all but one case, nasal and bronchial strains were genetically identical in the same patients.

Staphylococcus aureus is a major cause of severe community-acquired and nosocomial pneumonia in intensive care units (ICU) (13). The anterior nares are the main reservoir of *S. aureus*, and nasal *S. aureus* carriage appears to play a key role in the pathogenesis of *S. aureus* infection (5, 7, 11, 19). The aim of this study was to explore if nasal *S. aureus* carriage is the source of lower respiratory tract infections in critically ill patients.

During the study period (4 November 2000 to 4 January 2002), 402 patients were hospitalized in the medical ICU and 49 had *S. aureus* lower respiratory tract infections, 28 with community-acquired infections and 21 with nosocomial infections defined by the onset of the first clinical manifestations 72 h after hospital admission. For each patient, *S. aureus* nasal carriage was investigated by nasal swab on admission and weekly. Pneumonia was diagnosed on clinical, biological, and radiologic criteria and the presence of a colony count of $\geq 10^4$ CFU/ml from culture of bronchoalveolar fluid (BAL) or $\geq 10^7$ CFU/ml from culture of a tracheobronchial aspirate (TBA) sample. Under these thresholds, a diagnosis of distal bronchitis was considered.

Of the 27 patients with both *S. aureus* nasal carriage and pulmonary infection, 16 patients (11 men, 5 women; mean age, 51 ± 17 years; simplified acute physiology score II, 51 ± 13 [9]; 15 under mechanical ventilation by the oropharyngeal route) were studied and 32 isolates were taken, 16 from the anterior nares and 16 from bronchial samples. Standard microbiological methods were used for identification and antimicrobial susceptibility testing according to the national recommendations (15). Methicillin resistance was confirmed by the presence of the *mecA* gene, determined by PCR (12). Molecular analysis using pulsed-field gel electrophoresis (PFGE) was performed on the 32 isolates. DNAs were digested with SmaI (New England Biolabs, Hertfordshire, United Kingdom), and PFGE was performed with a CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules, CA).

Methicillin-susceptible *S. aureus* (MSSA) strains were isolated in both nares and bronchial samples of eight patients and methicillin-resistant *S. aureus* (MRSA) in the other eight patients. No patient had simultaneous MSSA and MRSA. MSSA nasal carriage was detected on admission in seven patients. MRSA nasal carriage was acquired in the ICU in six and imported in two patients (Table 1). Pneumonia and distal bronchitis were community acquired in seven patients and nosocomial in nine. In 10 cases, *S. aureus* was isolated in nasal and bronchial samples simultaneously. In five patients, *S. aureus* was isolated first in nasal samples and secondarily in bronchial samples. In patient 10, *S. aureus* was recovered in nasal samples 18 days after pneumonia (Table 1).

Antibiotic susceptibility profiles of isolates recovered from both nares and the respiratory tract of the same patient were identical for the 17 antibiotics used in 12 patients and differed by no more than two agents for the other 4 patients (Table 1).

PFGE profiles are shown in Fig. 1. In 15 cases, *S. aureus* strains obtained from nasal and bronchial samples of the same patients were indistinguishable (Table 1). In the remaining patient (patient 1), the MSSA strains isolated from the nares and bronchial sample differed from each other by 17 DNA fragments in PFGE and displayed different antibiotypes.

Several studies focused on the link between *S. aureus* nasal carriage, oropharyngeal and tracheal colonization, and pneumonia, but the majority used phenotypic markers such as serotyping or antibiotic susceptibility testing (1, 3, 4, 8, 18). As shown in this study, antibiotyping is often inadequate for differentiating strains. Antibiotic susceptibility patterns were different between strains isolated from nares and strains isolated from the respiratory tract in three patients while these strains were genetically identical using molecular tools. These differences could be explained by acquisition of plasmids or mobile genetic elements carrying antibiotic resistance genes or differences in gene expression between identical genotypes (10).

To our knowledge, only four studies using molecular markers focused on relatedness between *S. aureus* strains isolated in nasal or oropharyngeal or gastric and bronchial samples in noncritically or critically ill patients (2, 6, 16, 20). All four of

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TABLE 1. Characteristics of *S. aureus* strains isolated in both nares and bronchial samples

Patient no.	Nasal carriage	Type of bronchial sample, bacterial count, type of *S. aureus*	Type of infection[b]	Time from colonization to infection	Antibiotype[c]	Pulsotype
1	MSSA	TBA, 10^9, MSSA	P	Simultaneous	Different	Unrelated
2	MRSA	BAL, 10^6, MRSA[a]	P	Simultaneous	Identical	Indistinguishable
3	MRSA	TBA, 10^7, MRSA[a]	P	Simultaneous	Identical	Indistinguishable
4	MRSA	BAL, 10^4, MRSA	P	Simultaneous	Identical	Indistinguishable
5	MSSA	TBA, 10^6, MSSA	DB	Simultaneous	Different	Indistinguishable
6	MRSA	BAL, 10^5, MRSA[a]	P	7 days before	Different	Indistinguishable
7	MSSA	TBA, 10^8, MSSA	P	7 days before	Identical	Indistinguishable
8	MRSA	TBA, 10^6, MRSA[a]	DB	Simultaneous	Identical	Indistinguishable
9	MRSA	BAL, 10^4, MRSA	P	Simultaneous	Identical	Indistinguishable
10	MSSA	TBA, 10^7, MRSA	P	18 days after	Identical	Indistinguishable
11	MSSA	TBA, 10^8, MSSA	P	22 days before	Identical	Indistinguishable
12	MSSA	BAL, 10^3, MSSA	DB	11 days before	Identical	Indistinguishable
13	MRSA	TBA, 10^8, MRSA[a]	P	19 days before	Different	Indistinguishable
14	MRSA	TBA, 10^6, MRSA[a]	DB	Simultaneous	Identical	Indistinguishable
15	MSSA	BAL, 10^2, MSSA	DB	Simultaneous	Identical	Indistinguishable
16	MSSA	TBA, 10^7, MSSA	P	Simultaneous	Identical	Indistinguishable

[a] MRSA acquired in the ICU.
[b] P, pneumonia; DB, distal bronchitis.
[c] Antibiotype differences: penicillin and erythromycin resistance in patient 1, penicillin resistance in patient 5, rifampicin resistance in patient 6, and pristinamycin resistance in patient 13.

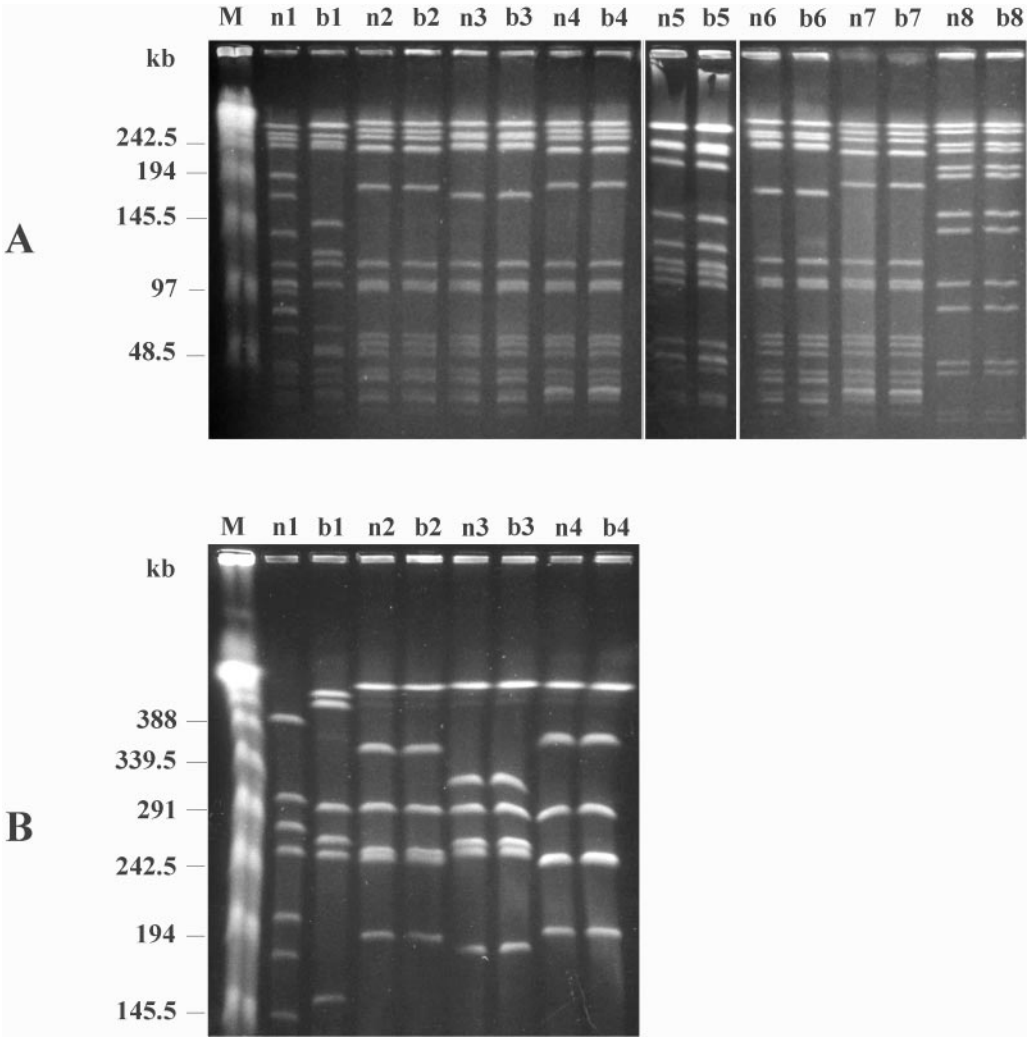

FIG. 1. PFGE of *S. aureus* strains isolated in nasal (n) and bronchial (b) samples in patients 1 to 8. Lane M, phage lambda DNA ladder. Panels: A, whole PFGE patterns; B, details of upper bands.

them used the PFGE method, but the designs of these studies were different. In our study, we assessed a larger sample of critically ill patients who had nasal carriage and community-acquired or nosocomial respiratory tract infections with MRSA or MSSA. Our PFGE results showed that the *S. aureus* strain isolated from nares was genetically identical to that isolated from the bronchial sample of the same patient in 15 out of 16 cases. This genetic identity demonstrates a link between *S. aureus* nasal carriage and *S. aureus* pneumonia or bronchitis in the majority of critically ill patients. In only one patient were MSSA strains isolated simultaneously in bronchial and nasal swab samples genetically different according to the criteria of Tenover et al. (more than seven different DNA fragments) (17). In studies by Garrouste-Orgeas et al. and Watanabe et al., MRSA strains isolated from oropharyngeal or nasal and bronchial samples were unrelated in one and three cases, respectively (6, 20). Thus, in some cases, nasal carriage does not imply a pulmonary infection by the same strain and nasal and bronchial strains can present different phenotypic characteristics, especially antibiotic susceptibility patterns. Several hypotheses could explain the differences between nasal and bronchial samples: (i) a minority of patients can harbor several different strains in different parts of the body (14); (ii) some strains might have been underdetected because only one colony was selected for PFGE analysis; or (iii) one different strain might have been inoculated by health care workers during tracheal aspiration.

These results are important with regard to understanding the route of *S. aureus* pneumonia in critically ill patients. Most of the *S. aureus* strains from pneumonia and bronchitis are derived from the nasal cavity. Oropharyngeal secretions are probably contaminated by *S. aureus* strains from the nasal cavity, and patients aspirate oropharyngeal secretions when consciousness is altered or during intubation and mechanical ventilation. The severe consequences of *S. aureus* pneumonia heighten the importance of prevention. Thus, strategies that can eliminate *S. aureus* nasal carriage may prevent staphylococcal pneumonia.

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