Molecular Characterization of the Transmission between the Colonization of Methicillin-resistant Staphylococcus aureus to Human and Environmental Contamination in Geriatric Long-term Care Wards

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Abstract

Objective **Transmission between human and environmental contamination from colonized methicillin-resistant *Staphylococcus aureus* (MRSA) remains a controversial issue. We, therefore, investigated the differences between MRSA types which colonize in humans and in the environment.**

Methods **A 4-week prospective culture survey for MRSA was performed for 12 patients as well as for the environment of the room of MRSA carriers in quarantine in the geriatric long-term care ward of a 270-bed hospital.**

Results **A total of 97 *S. aureus* strains (80 MRSA and 17 methicillin-sensitive *Staphylococcus aureus* [MSSA]) was isolated during the periods of September 8 to 10, 23 to 25 and October 5 to 7, 1998; 25 strains were from the respiratory tract, 4 strains from feces and 11 strains from decubitus ulcers. Fifty-seven strains were from the patients' environment. Molecular typing by pulsed-field gel electrophoresis (PFGE) with the Sma I restriction enzyme demonstrated that the predominant type of MRSA isolated from the environment changed by the minute. The patterns of 42 MRSA strains isolated from the environment were identical in 26 (61.9%), closely related in 15 (35.7%) and possibly related in 1 (2.4%) of the cases of those isolated from patients simultaneously. There was no correlation between patients and the environment with the 17 MSSA isolates.**

Conclusion **Our results demonstrated that MRSA from patients can contaminate the environment, whereas MRSA from the environment might be potentially transmitted to patients via health care workers under unsatisfactory infection control.**
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Key words: Methicillin-resistant *Staphylococcus aureus*, MRSA, pulsed-field gel electrophoresis, PFGE, environment, geriatric long-term care ward

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen and a major cause of nosocomial infections (1). MRSA strains colonize easily in the host particularly in immunodeficient patients which can cause a variety of serious difficult-to-control infections including septicemia, pneumonia, endocarditis, meningitis and postoperative intra-abdominal infection (2–5). The transmission of MRSA occurs through direct person-to-person contact, usually via the hands of health care workers. However, the prevalence of the infection caused by MRSA remains relatively high despite the intensification of preventive measures (6–8). Of the many possible factors, the most likely factor is poor compliance with recommended infection control policies by health care workers (9). On the other hand, some authors have suggested that contaminated environmental surfaces may serve as a reservoir for MRSA in hospitals (10–18). However, this issue remains a controversial one.

To assess the transmission of MRSA between patients and environmental contamination in a hospital setting, we compared MRSA isolated from patients with that from the envi-

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ronmental contamination using a molecular technique by pulsed-field gel electrophoresis (PFGE) at an intervals of 2 weeks.

Materials and Methods

Setting

Aino Memorial Hospital (AMH), which is affiliated with Nagasaki University, is a 270-bed long-term care facility consisting of 180 beds in internal medicine wards including geriatric medicine and 90 orthopedics beds. The average length of hospital stay in the geriatric wards is approximately 110 days. In this study, an 8-bed cohorting room for seriously affected patients in a geriatric long-term care ward was used for the investigation of possible relationships, including a longitudinal study, between the type of *S. aureus* isolated from patients and that isolated from their hospital room.

Patients

This study was performed with a total of 12 seriously affected patients (5 males and 7 females, mean age, 77.4 years) who had been admitted to AMH on September 8 to 10, 23 to 25 and October 5 to 7, 1998. Decubitus ulcers were observed in 4 cases (33.3%). In this study interval, no patients became infected with *S. aureus* when infection control measures were used, which included hand washing, disinfection of the upper respiratory tract and decubitus ulcers by treatment with povidone iodine, and cleaning the floor at daily intervals.

Sampling and culture of S. aureus

Cultures were obtained simultaneously from the nares, pharynx, and rectum with a sterile swab. At the same time, sputum samples were collected using sterile suction tubes. Opened rabbit blood agar plates were placed on the foor of the room of the MRSA carriers in quarantine for 4 or 12 hours in 6 locations throughout the room (4 in each corner, one in the center and one in the entrance). Surveillance was performed three times at 2-week intervals at a specific time. Cultures were performed using TSA II medium (Becton Dickinson, San Jose, CA) supplemented with 5% rabbit blood agar and *S. aureus*-selective medium (mannitol salt agar, Eiken Chemical Co., Tokyo, Japan) for 18 hrs at 35°C. The identification of *S. aureus* was based on the morphology of the colonies and the use of specific kits (Staphylo-LA by slide latex agglutination, Denka Seiken Co., Tokyo, Japan). MRSA were identified by the oxacillin disk diffusion method (Kirby-Bauer) according to the guidelines of the National Committee for Clinical Laboratory Standards (19). Ninety-seven strains of *S. aureus* , 40 from patients (7 from nares, 9 from pharynx, 9 from sputum, 4 from feces and 11 from decbitus ulcer) and 57 from hospital environment, were identified.

Pulsed-field gel electrophoresis

MRSA isolates were grown overnight in Brain Heart Infusion (BHI) broth at 35°C, and PFGE by Sma I chromosomal digestion was performed as described previously (20). The DNA was digested with 10U of *Sma*I (Takara Shuzo Co., Shiga, Japan) at 30°C overnight. A CHEF Mapper pulsed-field electrophoresis system (Bio-Rad Life Science) was used for electrophoresis, with the potential set at 6 V/cm, switch times set at 0.47 and 63 seconds, and the run time set at 20 hours 18 minutes. After staining with ethidium bromide, the band patterns were compared according to the criteria for bacterial strain typing, as described by Tenover et al (21).

Results

Cultures of patients

Each of the 24 cultures in the nares, pharynx, sputum and faeces and 14 cultures in the decubitus ulcer revealed that 7 (29.2%) in the nares, 9 (37.5%) in the pharynx, 9 (37.5%) in the sputum, 4 (16.7%) in the faeces and 11 (78.6%) in the decubitus ulcer were contaminated with *S. aureus*. Thirty-eight (95%) of these 40 strains were determined to be MRSA.

Cultures of environment surface

Forty (44.4%) of 90 environment surfaces were contaminated with *S. aureus*. Eight cultures yielded fewer than ten colonies, 27 cultures yielded from ten to ninty-nine colonies and the remaining 5 cultures yielded more than one hundred colonies of *S. aureus*. In addition, one strain of *S. aureus* in 26 plates, two strains of those in 11 plates and three strains of those in 3 plates were examined for difference in colonial characteristics. Therefore, a total of 57 environment surface cultures yielded *S. aureus*. Forty-two (73.7%) of these 57 strains were determined to be MRSA.

Interpretation of PFGE

The total of 97 strains of *S. aureus* (80 MRSA) were analyzed by PFGE (Fig. 1). Molecular typing demonstrated that the patterns of the 42 MRSA isolates from the environment were identical in 26 (61.9%), closely related in 15 (35.7%) and possibly related in 1 (2.4%) with 38 MRSA isolated from the patients simultaneously, despite the fact that no correlation between human and environment in 17 methicillin-sensitive *S. aureus* (MSSA) was found (Table 1). The genotypes of *S. aureus* isolated from the environment changed by the minute even if they were in same area on September 8 to 10, 23 to 25 and October 5 to 7, 1998, respectively (Table 2).

Discussion

Some previous reports have suggested that environmental reservoirs might be a potential route of transmission for epidemic strains (22–24). However, others have concluded that the environment may not be important because of differences in the phenotypes in *S. aureus* isolated from the hospital

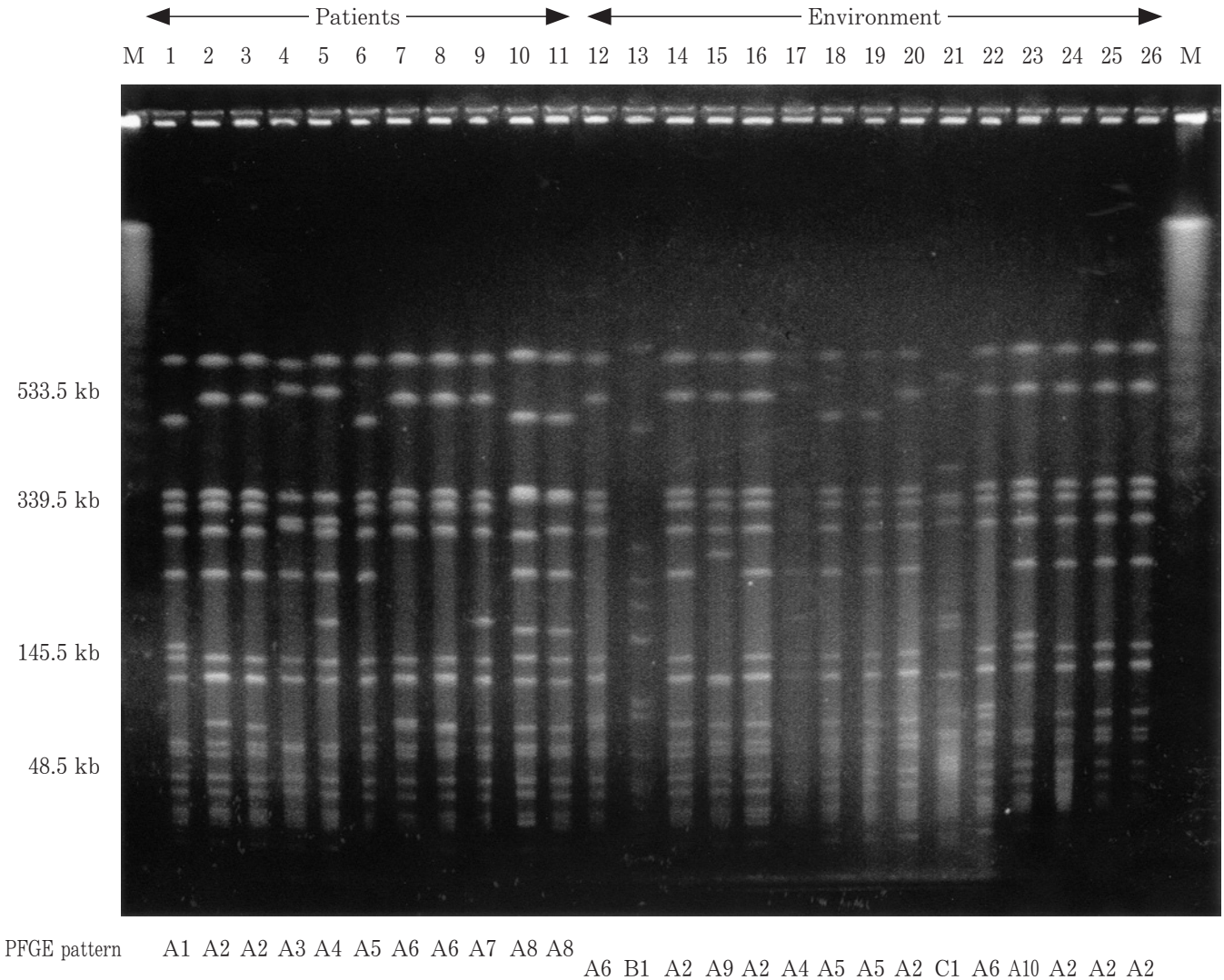

Figure 1. Pulsed-field gel electrophoresis (PFGE) patterns of Sma I digested DNA of *S. aureus* isolated from patients and the environment. Lanes 1–11, isolated from patients; lanes 12–26, isolateed from the environment on September 8 to 10, 1998. M indicates molecular weight markers (lambda ladder DNA size standards; Bio-Rad Laboratories). Of these 26 lanes, lanes 13 and 21 were methicillin-sensitive *S. aureus* (MSSA). The other lanes were Methicillin-resistant *Staphylococcus aureus* (MRSA). The genotype of the MRSA between patients and environment is identical (lanes 2, 3, 14, 16, 20, 24–26; 5, 17; 6, 18, 19; 7, 8, 12, 22) or closely related.

environment and patients (25, 26). Recently, the association between the dispersion of MRSA strains into the environment by colonized patients and MRSA epidemics has been increasingly reported via the use of molecular typing methods (10–18). However, the transmission between colonies of MRSA to human and environmental contamination continues to be a controversial issue and remains uncertain. In the present study, we examined the relationship between the environment and seriously affected elderly patients who had some risk factors for MRSA infection (27), such as bedridden status, and the presence of decubitus ulcers. In particular, we repeatedly examined environmental cultures, whereas previous studies have investigated these only on a temporary basis. Our molecular analysis demonstrates that the DNA patterns of MRSA from the environment were identical or closely related to those from patients in a geriatric long-term ward, and the predominant type of MRSA isolated from the environment changed by the minute under conditions of intensified preventive measures including cleaning the floors. In our hospital, we have achieved a significant reduction of MRSA bacteremia in geriatric wards after the introduction of infection control measures against nosocomial infections (28), and these infection control measures have continued to the present time. During this study period, MRSA infections, including bacteremia, did not occur despite the isolation of MRSA strains from the environment.

Table 1. Genetic Relationships of MRSA Strains Isolated from Patients and the Hospital Environment

PFGE interpretation \ Date	No. of environment			Total (%)
	September 8 to 10. 1998	September 23 to 25. 1998	October 5 to 7. 1998	
Identical	15	10	1	26 (61.9)
Closely related	2	12	1	15 (35.7)
Possibly related	0	1	0	1 (2.4)
Different	0	0	0	0 (0)
Total	17	23	2	42

Table 2. Distribution of PFGE Patterns of S. aureus Isolated from Each Area on September 23 to 25

	Area. 1	Area. 2	Area. 3	Area. 4	Area. 5	Area. 6
Period 1			MRSA (A12) MRSA (A13)	MRSA (A12) MRSA (A13)		MRSA (A8)
Period 2	MRSA (A12)	MRSA (A8) MRSA (A8)	MRSA (A11)	MSSA (D) MRSA (A2)	MRSA (A8) MRSA (A8) MRSA (A8)	MRSA (A8) MSSA (K)
Period 3	MSSA (J)					
Period 4				MRSA (A8)	MRSA (A2)	
Period 5			MSSA (E) MRSA (A12)	MRSA (A8) MRSA (A14) MRSA (A16)	MRSA (A15) MRSA (A15)	MRSA (A8)

Period 1: 8 PM on September 23–8 AM on September 24, Period 2: 8 AM on September 24–12 AM on September 24, Period 3: 12 AM on September 24–4 PM on September 24, Period 4: 4 PM on September 24–8 PM on September 24, Period 5: 8 PM on September 24–8 AM on September 25.

In conclusion, our results demonstrated that MRSA from patients can contaminate the environment, whereas MRSA from the environment might be potentially transmitted to patients via health care workers under unsatisfactory infection control.

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