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Epidemiological Analysis of a Methicillin-Resistant *Staphylococcus aureus* Outbreak in a Neonatal Intensive Care Unit after Cleaning of Environmental Surfaces in the Unit by Genomic DNA Fingerprinting Using Pulsed-Field Gel Electrophoresis

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Nosocomial infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious problem in neonatal intensive care units (NICUs) (1). Genome typing using pulsed-field gel electrophoresis (PFGE) is a powerful tool for investigating the source, transmission, and spread of MRSA.

An MRSA outbreak occurred in an NICU right after intensive cleaning of the environmental surfaces in the unit and this outbreak continued for 5 months. The hospital had 925 beds and the NICU had 5 beds. MRSA outbreaks had occurred in the NICU twice successively with an interval of 7 months. PFGE analysis revealed that these two outbreaks were due to a single strain (2). After these outbreaks, environmental surfaces in the NICU were intensively cleaned with a hospital-grade disinfectant-detergent (0.2% alkyldiaminoethylglycine hydrochloride).

Thirty-seven isolates from 31 patients affected by the outbreaks were tested for chromosomal DNA typing by using a contour-clamped homogeneous electric field system (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), antibiotic resistance (WalkAway™, Dade Behring, Deerfield, Ill., USA), enterotoxin serotyping (SET-RPLA: Denka Seiken Co., Tokyo), toxic shock syndrome toxin-1 (TSST-1) production (TST-RPLA: Denka Seiken), and coagulase serotyping (Denka Seiken).

Five different PFGE patterns of *Sma*I DNA digests were detected (Fig. 1). A band-based cluster analysis of these patterns (Molecular Analyst™: Bio-Rad) revealed that these five PFGE patterns were more than 95% similarity (Fig. 2). Sensitivity to antibiotics is shown in Table 1; there were four different patterns. All isolates except No. 150 produced enterotoxin type C (Table 2). No. 150 did not produce any type of enterotoxin. All isolates tested produced TSST-1 and type II coagulase (Table 2).

The PFGE pattern, antibiotic pattern, and enterotoxin serotype were identical for 28 isolates (Table 2) and 9 isolates from previous outbreaks in the NICU (1). The dates of isolation of MRSA are illustrated in Fig. 3. Two different strains of MRSA were isolated from the same patients at different periods.

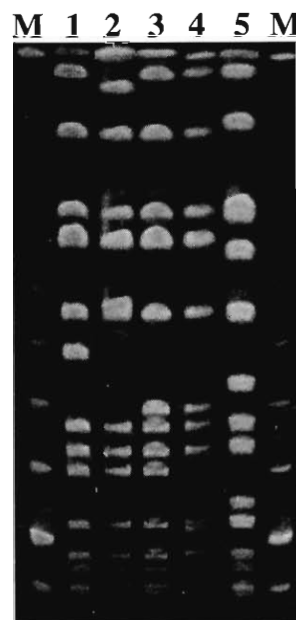


Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. Lane 1: PFGE pattern A, lane 2: H, lane 3: I-1, lane 4: I-2, lane 5: F, M: low range PFG Marker

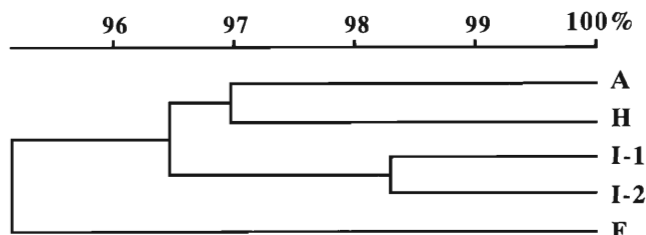


Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns.

These results indicated that this outbreak in the NICU was dominated by a single strain.

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Table 1. Antibiotic pattern classified by MIC pattern of 15 antibiotics against MRSA

Antibiotic pattern	Antibiotics listed in footnote [#]	MIC of:						
		CLDM	FOM	CMZ	GM	MINO	VCM	ST
a-1	R	R	R	R	S	I	S	S
a-2	R	R	R	R	S	S	S	S
a-3	R	R	R	S	S	I	S	S
b	R	S	S	R	R	I	S	S

[#]Listed antibiotics are benzyl-penicillin, oxacillin, ampicillin, piperacillin, cefazolin, cefotiam, erythromycin and imipenem/cilastatin. CLDM: clindamycin, FOM: fosfomycin, CMZ: cefmetazole, GM: gentamicin, MINO: minocycline, VCM: vancomycin, ST: streptomycin, R: resistant, S: susceptible, I: intermediate

Table 2. Phenotypic and genotypic characterization of the *S. aureus* isolates

Isolate No.	PFGE Pattern	Antibiotic pattern	Enterotoxin				TSST-1	Coagulase type
			A	B	C	D		
Dominant strains*	A	a-1	-	-	+	-	+	II
135	H	a-1	-	-	+	-	+	II
136	I-1	a-3	-	-	+	-	+	II
150	F	b	-	-	-	-	+	II
192	A	a-2	-	-	+	-	+	II
214, 293	H	a-3	-	-	+	-	+	II
241	A	a-3	-	-	+	-	+	II
290	I-2	a-3	-	-	+	-	+	II

*Isolate No. of dominant strains: 94, 98, 103, 107, 114, 127, 140, 144, 147, 165, 182, 195, 203, 209, 215, 218, 222, 225, 231, 233, 235, 239, 245, 251, 261, 273, 278, 281 and 292.

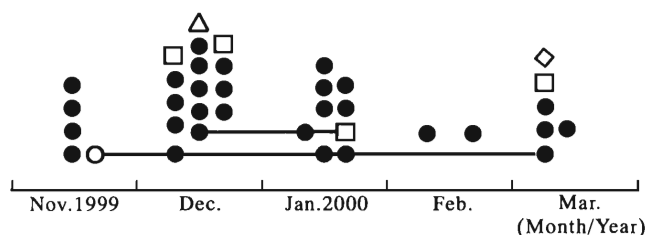


Fig. 3. Dates of isolation of MRSA. Each symbol represents PFGE pattern of each isolate. Closed circle stands for PFGE pattern A, open circle designated pattern F, open square designates pattern H, open triangle refers to pattern I-1, and open diamond refers to pattern I-2. MRSA isolated from the same patient are connected by a line.

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