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# Epidemiological Analysis of Methicillin-Resistant *Staphylococcus aureus* Outbreaks in a Neonatal Intensive Care 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 critical problem in neonatal intensive care units (NICUs) (1). Genome typing using pulsed-field gel electrophoresis (PFGE) is useful for investigating the source, transmission, and spread of MRSA infection (2).

An MRSA outbreak, though with no clinical manifestations, occurred in NICU twice successively with an interval of 7 months. The hospital had total of about 900 beds and the NICU had five beds. Isolates from individual patients affected by the two outbreaks and those from other wards during the

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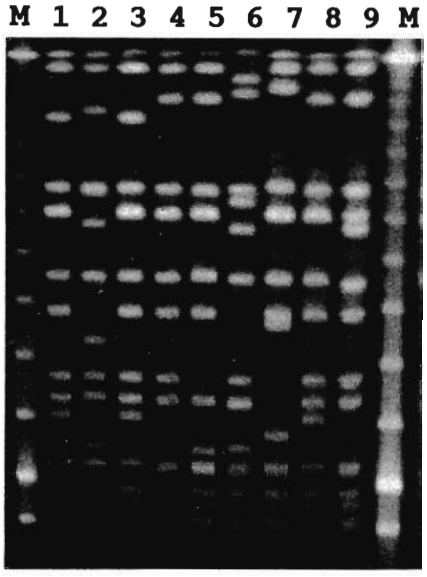


Fig. 1. Pulsed-field electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates.
Lane 1: isolate No.9 (pattern A), lane 2: No. 27 (F), lane 3: No. 28 (A), lane 4: No. 50 (B1), lane 5: No. 43 (D), lane 6: No. 44 (G), lane 7: No. 45 (E), lane 8: No. 49 (B2), lane 9: No. 52 (C), M: low range PFG Marker.

second outbreak were tested for chromosomal DNA type (contour-clamped homogeneous electric field system, CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), antibiotic resistance (WalkAway™, Dade Behring, Deerfield, Ill., USA), enterotoxin serotype (SET-RPLA: Denka Seiken Co., Tokyo), toxic shock syndrome toxin-1 (TSST-1) production (TST-RPLA: Denka Seiken), and coagulase serotype (Denka Seiken).

Eight different PFGE patterns of *Sma*I DNA digests were detected (Fig. 1). Band-based cluster analysis (Molecular Analyst™: Bio-Rad) revealed that these eight PFGE patterns shared more than 93 % similarity (Fig. 2). Sensitivity to antibiotics is shown in Table 1; there were five different patterns. All isolates except No. 27 produced enterotoxin type C (Table

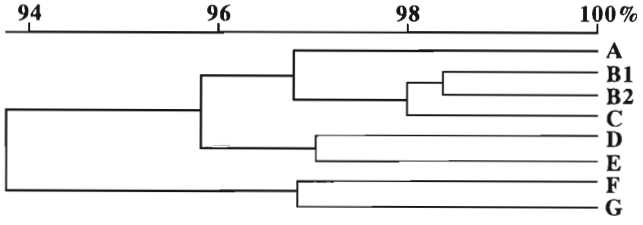


Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns.

Table 1. Antibiotic pattern classified by MIC pattern of 16 antibiotics against MRSA

| Antibiotic pattern | MIC ($\mu$g/ml) of: | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PCG | MPIPC | ABPC | PIPC | CEZ | CMZ | CTM | IPM | EM | GM | OFLX | MINO | VCM | CLDM | ST | FOM |
| a | R | R | R | R | R | R | R | R | R | S | R | I | S | R | S | R |
| b | R | R | R | R | R | R | R | R | R | S | R | S | S | R | S | R |
| c | R | R | R | R | R | R | R | R | R | R | R | I | S | R | S | R |
| d | R | R | R | R | R | R | R | R | R | R | R | I | S | R | S | I |
| e | R | R | R | R | R | R | R | R | R | R | R | S | S | S | S | S |

PCG: benzyl-penicillin, MPIPC: oxacillin, ABPC: ampicillin, PIPC: piperacillin, CEZ: cefazolin, CMZ: cefmetazole, CTM: cefotiam, IPM: imipenem/cilastatin, EM: erythromycin, GM: gentamicin, OFLX: ofloxacin, MINO: minocycline, VCM: vancomysin, CLDM: clindamycin, ST: streptomycin, FOM: fosfomycin, R: resistant, S: susceptible, I: intermediate

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

| Ward | Isolation month (1999) | Isolate No. | PFGE pattern | Antibiotic pattern | Enterotoxin | | | | TSST-1 | Coagulase type |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | A | B | C | D | | |
| NICU | Feb. | 1 | A | a | – | – | + | – | + | II |
| | | 2 | A | a | – | – | + | – | + | II |
| | | 5 | A | c | – | – | + | – | + | II |
| | | 9 | A | a | – | – | + | – | + | II |
| | | 19 | A | a | – | – | + | – | + | II |
| NICU | Oct. | 27 | F | e | – | – | – | – | + | II |
| | | 28 | A | a | – | – | + | – | + | II |
| | | 29 | A | a | – | – | + | – | + | II |
| | | 31 | A | a | – | – | + | – | + | II |
| | | 40 | A | a | – | – | + | – | + | II |
| 8N | Oct. | 43 | D | d | – | – | + | – | + | II |
| 10N | | 44 | G | a | – | + | + | – | + | II |
| 8N | | 45 | E | c | – | – | + | – | + | II |
| 10S | | 49 | B2 | a | – | – | + | – | + | II |
| 6S | | 50 | B1 | a | – | – | + | – | + | II |
| 5S | | 52 | C | b | – | – | + | – | + | II |

2). In addition, No. 44 produced type B enterotoxin. No. 27 did not produce any type of enterotoxin. All the isolates produced TSST-1 and coagulase type II (Table 2).

PFGE pattern, antibiotic pattern and enteterotoxin serotype were identical for all the 5 isolates from the first outbreak in the NICU and for 4 in 5 isolates from the second outbreak (Table 2), while isolates from other wards showed larger diversity. These results indicated that, though there was an interval of 7 months, the two MRSA outbreaks in the NICU was due to a single strain.

## REFERENCES

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