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Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Serratia marcescens* in a Long-Term Care Facility for Patients with Severe Motor and Intellectual Disabilities

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Assessing the risk of nosocomial infection is necessary for optimizing the quality of patient care and the practice of infection control in long-term care facilities for patients with severe motor and intellectual disabilities (SMID). We conducted a molecular epidemiological study of pathogens in December 2002 and August 2003 in two wards of such a facility having three wards. Among the 39 inpatients in the wards, 20 had tracheotomy or were cared for with mechanical ventilators. The isolates were tested for chromosomal DNA typing by using a contour-clamped homogeneous electric field system (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA).

In December 2002, 14 of 20 patients carried at least one methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, or *Serratia marcescens* strain (Table 1). MRSA was isolated from 11 specimens from 9 patients, including eight patients' sputa, one patient's abscess, and one patient's eye mucus. Among these, two were obtained on different days from an abscess of patient P5 and two others from different sites of patient P7. *P. aeruginosa* was obtained from nine patients' sputa and *S. marcescens* from five patients' sputa. Three patients, P1, P4, and P6, carried MRSA, *P. aeruginosa*, and *S. marcescens* in the same specimen, and the other three patients, P3, P7, and P5, carried MRSA and *P. aeruginosa*.

The survey was repeated in August 2003. Eighteen patients carried at least one MRSA, *P. aeruginosa*, or *S. marcescens* strain (Table 1). MRSA strains were isolated from six patients, including four patients' sputa and two patients' urine. *P. aeruginosa* was isolated from 13 patients' sputa, and *S. marcescens* from three patients' sputa. No patient simultaneously carried MRSA, *P. aeruginosa*, and *S. marcescens* strains. Only one patient, P15, had both MRSA and *P. aeruginosa*, and two patients, P1 and P11, had *P. aeruginosa* and *S. marcescens*. Nine patients, P1, P2, P3, P4, P7, P8, P11, P13, and P14, carried MRSA, and either *P. aeruginosa* or *S. marcescens* both in December 2002 and in August 2003.

The PFGE patterns of these MRSA isolates are shown in Fig. 1A. From a total of 17 isolates, 12 different PFGE patterns were detected. Band-based cluster analysis of these patterns (Molecular Analyst™: Bio-Rad) revealed a cluster consisting of patterns A1, A3, and A16 (Fig. 1B) (patterns

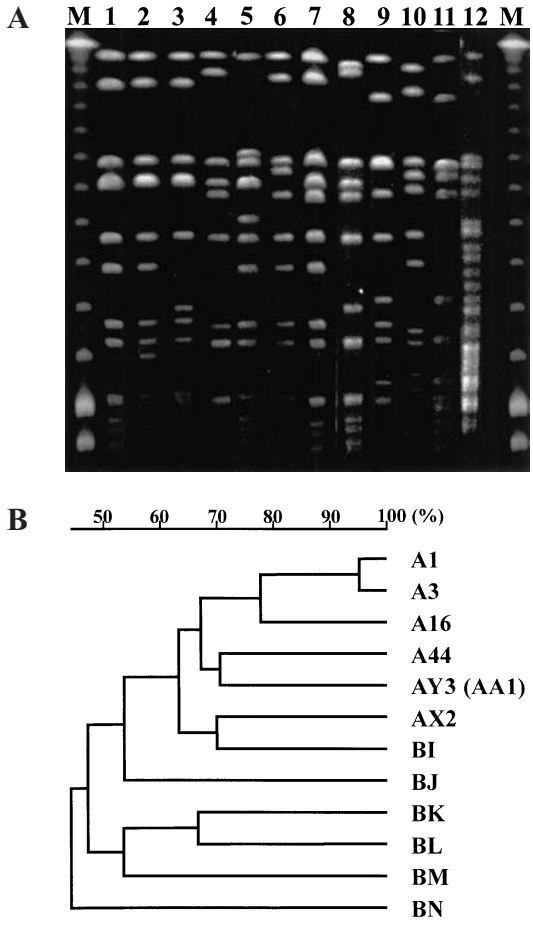

Fig. 1. Molecular analysis of MRSA isolate. A: pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 12 corresponding to the following PFGE pattern; 1: A1, 2: A3, 3: A16, 4: A44, 6: AY3, 7: BJ, 8: BI, 9: BK, 10: BL, 11: BM, 12: BN. B: cluster analysis of MRSA isolates based on PFGE patterns of *Sma* I-digested genomic DNA.

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Table 1. Clinical characteristics of patients with MRSA, *P. aeruginosa* and *S. marcescens*, and PFGE patterns of these isolates

Isolate date	Patient No.	Disease	Tracheotomy	Respirator	Specimen	PFGE pattern		
						MRSA	*P. aeruginosa*	*S. marcescens*
Dec. 2002	P1	Hypoxic encephalopathy	+	+	Sputum	B1	P.D	S.A1
	P2	Mental retardation	+	–	Sputum	AY3(AA1)	–	–
	P3	Hypoxic encephalopathy	+	+	Sputum	A3	P.G1	–
	P4	Cerebral palsy	+	–	Sputum	A3	P.C1	S.A1
	P5	Anoxic encephalopathy	+	+	Abscess	A16/BM	–	–
	P5	Anoxic encephalopathy	+	+	Sputum	–	P.B	–
	P6	Cerebral palsy	+	–	Sputum	A3	P.I1	S.A2
	P7	Cerebral palsy	+	–	Sputum	BM	P.A1	–
	P7	Cerebral palsy	+	–	Eye mucus	BM	–	–
	P8	Developmental disability	+	+	Sputum	A44	–	–
	P9	MELAS[1)]	+	+	Sputum	BK	–	–
	P10	Hypoxic encephalopathy	+	+	Sputum	–	P.E1	–
	P11	Sequelac of encephalitis	+	+	Sputum	–	P.G2	–
	P12	Cerebral palsy	+	–	Sputum	–	P.E2	–
	P13	Hypoxic encephalopathy	+	+	Sputum	–	–	S.B
	P14	Cerebral palsy	+	–	Sputum	–	–	S.A1
Aug. 2003	P1	Hypoxie encephalopathy	+	+	Sputum	–	P.F	S.A1
	P2	Mental retardation	+	–	Sputum	BJ	–	–
	P3	Hypoxic encephalopathy	+	+	Sputum	–	P.H	-
	P4	Cerebral palsy	+	–	Sputum	A3	–	S.A1
	P7	Cerebral palsy	+	–	Sputum	–	P.A1	–
	P8	Cerebral palsy	+	+	Sputum	–	P.K	–
	P11	Sequelae of encephalitis	+	+	Sputum	–	P.G2	S.A1
	P13	Hypoxic encephalopathy	+	+	Sputum	BN	–	–
	P14	Cerebral palsy	+	–	Sputum	–	P.A2	–
	P15	Hypoxic encephalopathy	+	–	Sputum	A1	P.J2	–
	P16	Developmental disability	–	–	Urine	AX2	–	–
	P17	Viral encephalitis[2)]	–	–	Urine	BL	–	–
	P18	Herpatic encephalitis	+	–	Sputum	–	P.G3	–
	P19	Hypoxic encephalopathy	+	+	Sputum	–	P.J1	–
	P20	Cerebral palsy	+	+	Sputum	–	P.I2	–
	P21	Cerebral palsy	+	–	Sputum	–	P.C2	–
	P22	Cerebral palsy	+	–	Sputum	–	P.J1	–
	P23	Herpatic encephalitis	+	+	Sputum	–	P.A3	–

[1)]: MELAS, nutochondrial myopathy and lactic acidosis.
[2)]: caused by measles virus.

sharing a similarity of 70% or higher were grouped into a cluster). No other clustering was observed.

Among 11 MRSA isolates found in December 2002, there were two clusters, one consisting of three isolates of PFGE pattern A3 and the other of three isolates of pattern BM. In contrast, in six isolates found in August 2003, clustering was not detected (Table 1). The PFGE patterns obtained from this study were compared with those identified in previous studies conducted in 2000-2003 in Tokyo (1-4), in 2002-2003 in Kumamoto (5-7), and in 2003 in Sendai (8). Among the patterns detected in the present study, pattern A1 was detected in 2000-2003 both in Tokyo and Kumamoto; pattern A3 in 2000-2003 in Tokyo and in 2003 in Sendai; and pattern A16 in 2001 and 2002 in Tokyo. The other nine patterns we identified were not detected in the previous studies.

The PFGE patterns of *P. aeruginosa* isolates are shown in Fig. 2A. From a total of 22 isolates, 19 different PFGE patterns were detected. Band-based cluster analysis of these patterns revealed six clusters, A, C, E, G, I, and J (Fig. 2B). The isolates from patients P19 and P22 in August 2003 were of the same pattern, P.J1. The isolates in December 2002 and August 2003 from patient P7 were of the same pattern P.A1, and those from patients P11 in the two surveys were also of the same pattern P.G2.

A total eight *S. marcescens* isolates were obtained. These represented three different PFGE patterns (Fig. 3A), two of which were similar to each other (Fig. 3A, 3B). Three of five isolates found in December and all of the three isolates found in August were of pattern S.A1.

Comparison of the August 2003 data with December 2002 data clearly shows reduction of MRSA carriers and disappearance of genetically related MRSA clusters in the second survey. Probably interventions taken after the first survey reduced MRSA transmission among the inpatients. The interventions taken were i) an educational program for the ward staff that dealt with infection control practice, ii) promotion of compliance with hand washing, and iii) replacement of the multi-use catheter with the sterile single-use catheter for suction of respiratory tract secretions. The data also suggested that the above interventions were not as successful for control of *P. aeruginosa* and *S. marcescens* that were present in the environment of the facility.

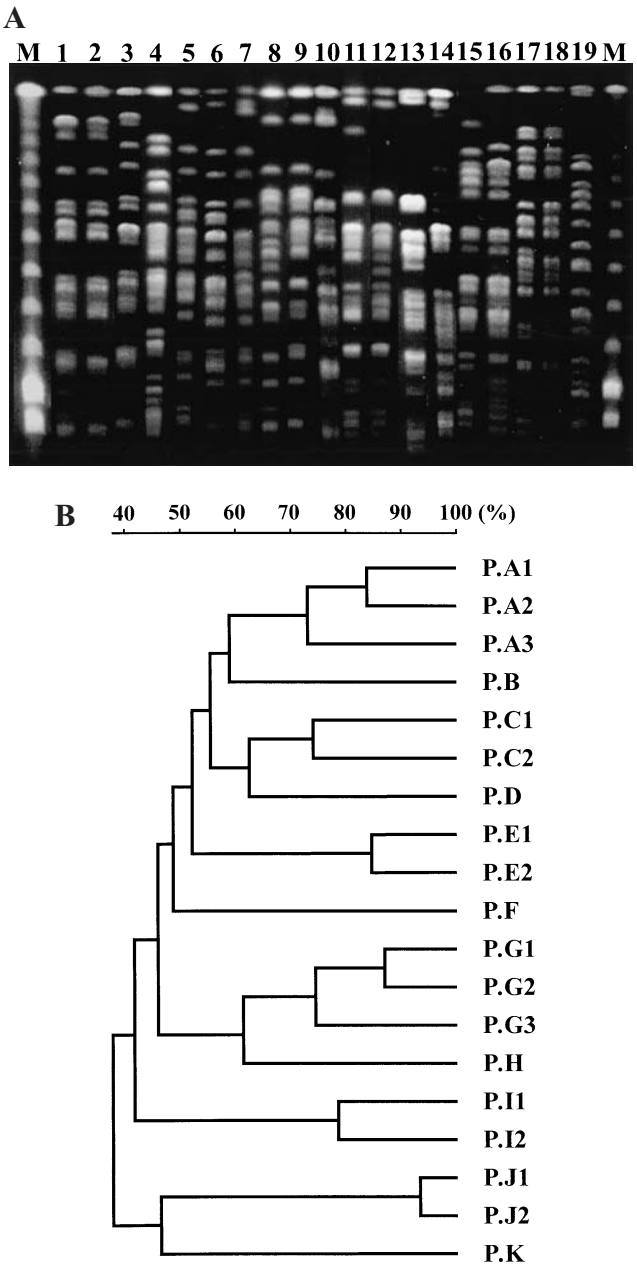

Fig. 2. Molecular analysis of *P. aeruginosa* isolate. A: pulsed-field gel electrophoresis of *Spe*I-digested genomic DNA from *P. aeruginosa* isolates. M: low range PFG Marker. Lanes 1 to 19 corresponding to the following PFGE pattern; 1: P.A1, 2: P.A2, 3: P.A3, 4: P.B, 5: P.C1, 6: P.C2, 7: P.D, 8: P.E1, 9: P.E2, 10: P.F, 11: P.G1, 12: P.G2, 13: P.G3, 14: P.H, 15: P.I1, 16: P.I2, 17: P.J1, 18: P.J2, 19: P.K. B: Cluster analysis of *P. aeruginosa* isolates based on PFGE patterns of *Spe*I-digested genomic DNA.

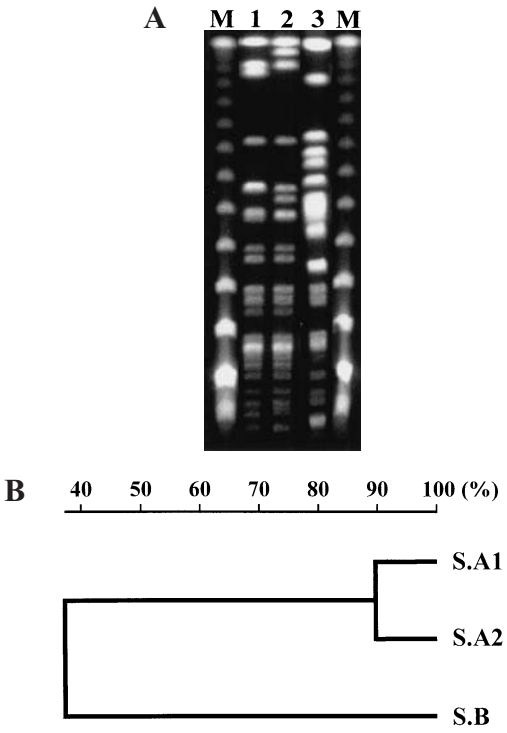

Fig. 3. Molecular analysis of *S. marcescens* isolate. A: pulsed-field gel electrophoresis of *Spe*I-digested genomic DNA from *S. marcescens* isolates. M: low range PFG Marker. Lanes 1 to 3 corresponding to the following PFGE pattern; 1: S.A1, 2: S.A2, 3: S.B. B: cluster analysis of *S. marcescens* isolates based on PFGE patterns of *Spe*I-digested genomic DNA.

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