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An Electronic System Combining MIC2000™ and Antibiogram Cluster Analysis for Surveillance of Methicillin-Resistant *Staphylococcus aureus* in Hospitals

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Pulsed-field gel electrophoresis (PFGE) is the method of choice for typing *Staphylococcus aureus* (1). However it requires a processing time of more than several days, trained specialists, special equipment, and reagents such as restriction enzymes (2). We previously reported that cluster analysis based on antibiotic susceptibilities gave results that agreed with PFGE in 79% of all cases (3). To expedite data processing of methicillin-resistant *Staphylococcus aureus* (MRSA), we here introduce an electronic system that combines MIC2000™ (Nagase Enterprise, Tokyo) and cluster analysis. MIC2000™ provides electronic data on minimum inhibitory concentration (MIC) in a tabulated form, such as in the file type of a comma separated value (.csv), which are used directly for computerized analyses.

MRSA isolates, obtained from October to December 1999, were used for the analysis. The MIC values (μg/ml) for 17 drugs (see Table for the drugs used) were converted into $\log_2$ scale; if MIC exceeded 2^n but the inhibitory effect at a higher concentration was not determined, the converted value was provisionally set to $n+1$. The transforming program can be downloaded from the web site (http://www.yoshidaj.com). The transformed data were directly processed by the statistical software SPSS™ Version 10.0 (SPSS Inc., Chicago, Ill., USA) for hierarchical cluster analysis on the operating system Windows™ (Microsoft Corp., Redmond, Wash., USA). The variables were $\log_2$ transformed data of MIC for 17 drugs and the labels were code numbers corresponding to the patients, wards, and specimens. For computation, the default settings of SPSS™ was used, except for the output, as a dendrogram. Any isolates for which the "Rescaled Distance Cluster Combine" (see the scale in Figure) was 2 or less were considered to form a cluster group. The computing time to obtain dendrograms was 2-3 min.

We obtained 76 isolates from 48 patients. As shown in Figure, excepting six, all of the isolates were grouped into the five groups A-E: A consisted of 27 isolates, B of 10 isolates, C of 18 isolates, D of 10 isolates, and E of 5 isolates. The excepted six isolates, 5, 74, 11, 9, 42 and 71, respectively derived from patients 03, 46, 08, 07, 26 and 43, did not belong to any of the above groups. Some patients harbored MRSAs from different groups; for example, patient 12 had group A (isolates 17 and 18) and group C (isolate 16) in different sites, and patient 41 had group A (isolates 68, 66, and 69) and group B (isolate 65) in the same infection site.

Isolates from the same ward demonstrated a tendency to belong to the same group; for example, isolates from Ward 4E were from groups A and C, isolates from Ward 3E were from groups A and B, isolates from Ward PED were from group E, etc. Isolates obtained on the day of admission from four pediatric (Ward PED) patients 19, 23, 25, and 44, who were referred from three different practitioners, were all in group E. This finding may suggest the extramural spread of one MRSA strain.

Table. MIC (μg/ml) and susceptibility of representative strains from MIC2000™

G	Num	MPIPC	ABPC	CEZ	CTM	CMZ	FMOX	IPM	CFDN	S/A	GM	ABK	CAM	CLDM	MINO	VCM	FOM	LVFX
A	68	>4 R	>4 R	>32 R	>32 R	32 R	32 R	8 R	>4 R	12 R	>16 R	1 S	>8 R	>4 R	16 R	<=1 S	>32 R	4 I
B	53	>4 R	>4 R	>32 R	>32 R	32 R	32 R	8 R	>4 R	24 R	>16 R	1 S	>8 R	>4 R	16 R	<=1 S	>32 R	4 I
C	16	>4 R	>4 R	>32 R	>32 R	32 R	32 R	16 R	>4 R	24 R	<=.5 S	<=.5 S	>8 R	>4 R	<=.5 S	<=1 S	<=8 S	>8 R
D	15	>4 R	>4 R	>32 R	>32 R	32 R	16 R	8 R	>4 R	12 R	<=.5 S	<=.5 S	>8 R	>4 R	4 S	<=1 S	>32 R	>8 R
E	33	>4 R	>4 R	2 R	2 R	8 R	8 R	<=.5 R	4 R	12 R	<=.5 S	<=.5 S	>8 R	>4 R	<=.5 S	<=1 S	<=8 S	<=.25 S

G, cluster grouping (see Figure); Num, number of strains (see Figure); MPIPC, oxacillin; ABPC, ampicillin; CEZ, cefazolin; CTM, cefotiam; CMZ, cefmetazole; FMOX, flumoxef; IPM, imipenam; CFDN, cefdinir; S/A, sulbactam and ampicillin; GM, gentamycin; ABM, arbekacin; CAM, clarithromycin; CLDM, clindamycin; MINO, minocycline; VCM, vancomycin; FOM, fosfomycin; LVFX, levofloxacin; R, resistant; I, intermediate; S, sensitive.

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Hierarchical Cluster Analysis
Dendrogram using Average Linkage (Between Groups)
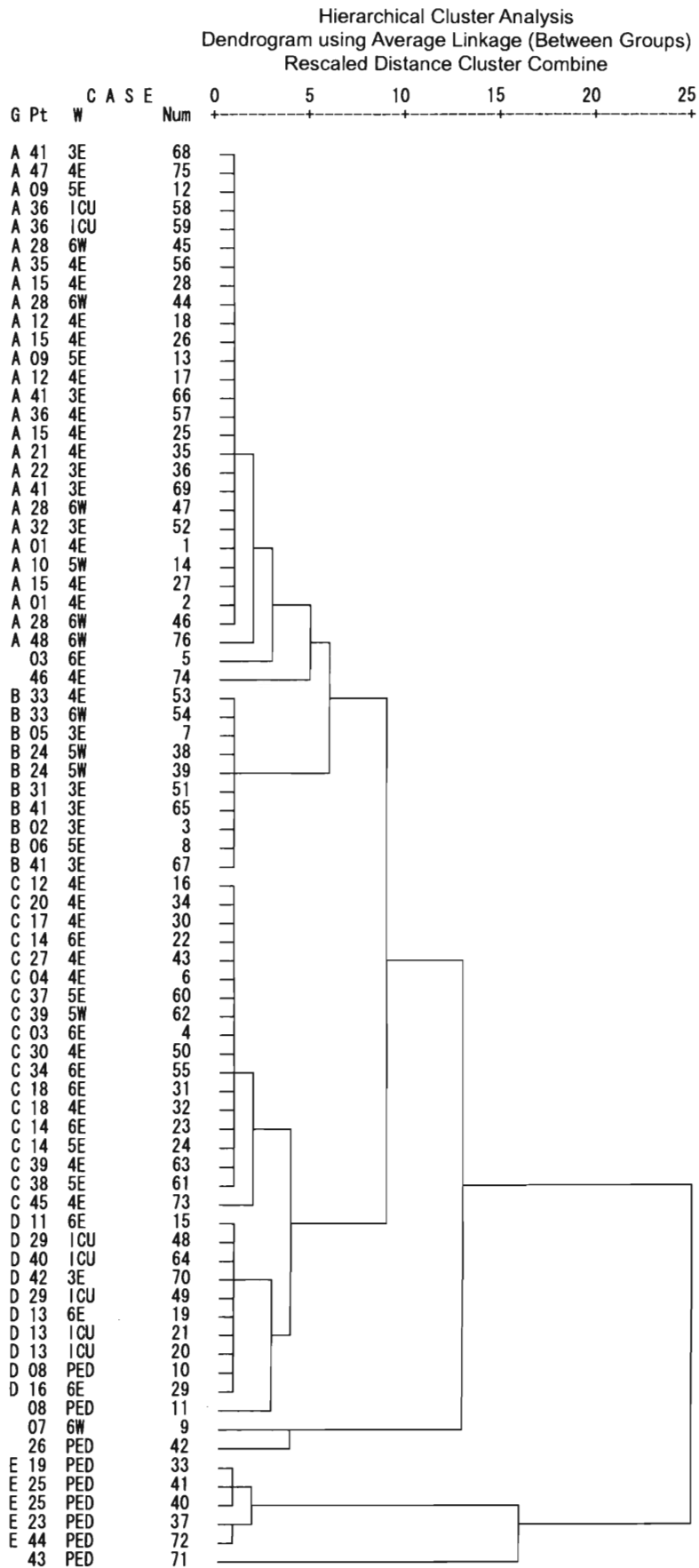Rescaled Distance Cluster Combine

Figure. Computer output of the cluster analysis superimposed with cluster grouping (G) and patient codes (Pt) in the left columns. W, ward from which MRSA strains were isolated; ICU, intensive care unit; PED, pediatric ward; Num, number of isolates.

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