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Spread of Erythromycin-, Tetracycline-, and Aminoglycoside-Resistant Genes in Methicillin-Resistant Staphylococcus aureus Clinical Isolates in a Kumamoto Hospital

Jun-ichiro Sekiguchi, Tomoko Fujino, Katsutoshi Saruta, Fumio Kawano1, Jun-ichi Takami1, Hisayoshi Miyazaki1, Tadatoshi Kuratsuji, Hiroshi Yoshikura2 and Teruo Kirikae*

International Medical Center of Japan, Tokyo 162-8655,
1Kumamoto National Hospital, Kumamoto 860-0008 and
2National Institute of Infectious Diseases, Tokyo 162-8640

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Various drug-resistance genes with different mechanisms have been identified in methicillin-resistant Staphylococcus aureus (MRSA). Knowing the prevalence of these drug-resistance genes is important for controlling of MRSA spread in hospitals.

In our previous paper (1), 24 MRSA clinical isolates obtained in October 2002 in a hospital with 550 beds in Kumamoto Prefecture were assessed by restriction fragment length polymorphisms (RFLP) of genomic DNA using pulsed-field gel electrophoresis (PFGE), plasmid DNA typing by...
using agarose gel electrophoresis, and antibiotic resistance. The same isolates were analyzed here by PCR and Southern blot to detect drug-resistance genes including gentamicin (GM)-resistant genes aac6'-aph2' and aph(3’)-III; kanamycin (KM)-resistant gene ant(4’)-I(2); erythromycin (EM)-resistant genes ermA, ermB, and ermC(3); and tetracycline (TC)-resistant genes tetK and tetM(4). The PCR results were evaluated based on the expected sizes of PCR products or confirmed by DNA sequencing.

Among the 24 MRSA isolates, 22 isolates, 23 isolates, and 20 isolates were resistant to GM, EM, and TC, respectively (Table 1). The majority of the isolates (18 of 24) were resistant to all of three antibiotics; five of the remaining isolates were resistant to two of the three, i.e., to GM and EM (Nos. 1247, 1254, and 1266), or to EM and TC (Nos. 1261 and 1262); and the last isolate (No. 1268) was resistant to GM. No isolate was sensitive to all three of the antibiotics. Isolate No. 1247 was sensitive to TC but showed an increase in MIC (2 μg/ml) compared with other TC-sensitive isolates (Nos. 1268, 1254, and 1266).

The results of PCR are shown in Table 1. Among the 24 MRSA isolates, 21 were PCR-positive for aac6'-aph2'. 22 were positive for ermA, and 21 were positive for tetM. None of the isolates was positive for aph(3’)-III, ant(4’)-I, ermB, ermC or tetK. The majority of the isolates (19 of 24) were positive for three genes: aac6'-aph2', ermA, and tetM. Two isolates (Nos. 1261 and 1262) were positive for the two genes ermA and tetM. Isolate No. 1254 was positive for the two genes ermA and aac6'-aph2'. Isolate No. 1268 was positive for the gene aac6'-aph2'. Isolate No. 1266 was negative for all genes tested. The existence of aac6'-aph2', ermA, and tetM was consistent with the susceptibility to GM, EM, and TC, respectively, in all MRSA isolates excepting two (Nos. 1247 and 1266). That is, all the isolates resistant to GM, EM, and TC, had aac6'-aph2', ermA, and tetM, respectively. Isolates Nos. 1261 and 1262, resistant to EM and TC but sensitive to GM, had ermA and tetM but not aac6'-aph2'. Isolate No. 1254, resistant to GM and EM but sensitive to TC, had ermA and aac6'-aph2' but not tetM. Isolate No. 1268, resistant to GM but sensitive to EM and TC, had aac6'-aph2' but not ermA or tetM.

There were exceptional isolates in which the existence of the drug-resistance genes was not consistent with the phenotype. Isolate No. 1247, resistant to GM and EM but sensitive to TC (with relatively higher MIC, as above described), had all these three genes. Other TC genes might be affecting the susceptibility to TC, or the detected TC resistance gene was non-functional due to mutation. Isolate No. 1266, resistant GM and EM but sensitive to TC, did not have any of the genes tested, indicating that there are other GM- and EM-resistant genes.

To determine whether the drug-resistant genes aac6'-aph2', ermA, and tetM existed on the plasmid DNA or genomic DNA of these MRSA isolates, Southern blotting was carried out (Fig. 1). The GM-resistance gene aac6'-aph2' was detected on four different-sized plasmids (40 kb, 50 kb, 200 kb, and 280 kb), as well as in a 15 kb Smal-digest chromosome fragment derived from (Fig. 1B and Table 1). The majority of the
isolates had \textit{aac6'-aph2} on the 40 kb and 200 kb plasmids and on the chromosome. Isolates No. 1254 (lane 21) had \textit{aac6'-aph2} on the 50 kb and 280 kb plasmids and also on the chromosome. Isolate No.1267 (lane 19) had \textit{aac6'-aph2} on the 50 kb and 280 kb plasmids. Isolates Nos. 1265 and 1260 (lanes 22 and 23) had \textit{aac6'-aph2} on the 40 kb plasmid. Isolates Nos.1268 and 1266 had neither \textit{ermA} nor \textit{tetM}.

To locate the drug-resistant genes on the chromosome, Southern blotting was done after separation of \textit{Sma}I digests of the genomic DNA by PFGE. The \textit{aac6'-aph2} gene was detected on 110 Kb of the \textit{Sma}I digest in the majority of the isolates with the PFGE pattern A. Notably, \textit{aac6'-aph2} was not detected in isolate No. 1261 (lane 10) even though it was detected in other isolates with the same PFGE pattern. In isolates with other PFGE patterns, \textit{aac6'-aph2} was detected on variously sized \textit{Sma}I digests (100 Kb, 120 Kb, 130 Kb, 180 Kb, 240 Kb, and 280 Kb). The \textit{ermA} was detected on both the 220 Kb and 580 Kb \textit{Sma}I fragments in all isolates with the PFGE pattern A (lanes 1-15), in isolates No. 1249 with the PFGE pattern AH2 (lane 17), and in isolate No. 1262 with PFGE pattern AK (lane 20). In isolates with other PFGE patterns, \textit{ermA} was detected on the 230 Kb and 580 Kb \textit{Sma}I fragments in isolates No. 1253 (lane 18), on the 240 Kb and 530 Kb fragments in No. 1254 (lane 21), and on the 260 Kb and 580 Kb fragments in Nos. 1265 (lane 22) and 1260 (lane 23). The \textit{tetM} was detected on the 290 Kb \textit{Sma}I fragment in all isolates with the PFGE pattern A (lanes 1-15) and in isolates No. 1249 with the PFGE pattern AH2 (lane 17), and in isolate No. 1262 with PFGE pattern AK (lane 20).
Fig. 2. Pulsed-field gel electrophoresis of SmaI-digested genomic DNA from MRSA isolates (A) and Southern blotting hybridized with aac6-aph2’(B), ermA (C), and tetM (D).

M: low range PFG Marker. Lanes 1 to 24: MRSA isolates Nos. were listed in Table 1.
patterns, the tetM was detected on different-sized Smal fragments of 320 Kb, 340 Kb, and 530 Kb.

Based on the PFGE patterns, 14 MRSA isolates among the 24 isolates belonged to one group. Among these isolates, 13 were resistant to GM, EM, and TC. All of them had a multi-drug resistant 40 kb plasmid harboring aac6’-aph2”, ermA, and tetM, and a large plasmid of 200 kb with aac6’-aph2”. They had aac6’-aph2” and tetM each on at least one chromosome site, and ermA on at least two chromosomal sites.

The above molecular analysis of the drug-resistance genes clearly indicates the clonal expansion of MRSA and confirms the data obtained with RFLP, although our previous antibiogram data appears to have given results less convincing than those of RFLP.

REFERENCES