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Frequent Recovery of a Single Clonal Type of Multidrug-Resistant *Staphylococcus aureus* from Patients in Two Hospitals in Taiwan and China

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One hundred thirty-two methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from patients with *S. aureus* infections between January 1998 and February 1999 in two hospitals, one located in Taipei, Taiwan, and another in Nanjing, People's Republic of China, were examined for antibiotic susceptibility and for clonal type by a combination of three methods: hybridization of *Cla*I restriction digests with *mecA*- and Tn*554*-specific DNA probes and pulsed-field gel electrophoresis of chromosomal *Sma*I digests. Selected isolates representing each clonal type were also analyzed by *spaA* typing, multilocus sequence typing, and a multiplex PCR method capable of identifying the structural type of the staphylococcal cassette chromosome *mec* (SCC*mec*) carried by the bacteria. The overwhelming majority of isolates (126 of 132 or 95%) belonged to minor variants of a single clonal type resembling the Brazilian and Hungarian epidemic MRSA clones, which showed a common *spaA* type and which were either sequence type 239 (ST239) or ST241 (a single-locus variant of ST239) in association with SCC*mec* type III or IIIA.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious problem in both Taiwan and China. The frequency of nosocomial infections caused by MRSA in Taiwan has increased rapidly during the past 10 years (4). In most major hospitals, MRSA accounts for more than 60% of the *S. aureus* isolates (30). According to a study from 1991 to 1996 at the Veterans General Hospital of Taipei, the prevalence of MRSA in this hospital was estimated as 88.2% (28). Furthermore, community-acquired MRSA infections seem to occur frequently in Taiwan among patients (infants and children) with no associated risk factors (31). In China the incidence of MRSA from hospital infection can be over 80% and the incidence of MRSA from community-acquired infections is close to 22% (14).

The Center for Molecular Epidemiology and International Network (CEM/NET) has been created to keep track of the movement of major multidrug-resistant clones of *S. aureus* and other gram-positive pathogens and to identify their reservoirs (6). Under this initiative, clinical isolates of MRSA collected in different countries were analyzed by molecular typing techniques involving *Cla*I-*mecA* polymorphisms, Tn*554* insertion patterns, and pulsed-field gel electrophoresis (PFGE). These techniques have allowed us to identify so far five multiresistant MRSA clones (22). The names assigned to these five pandemic MRSA clones, Iberian (8, 25), Brazilian (27), Hungarian (7, 18), New York/Japan (1, 23), and pediatric (24), reflect the geographic area in which they were first identified or indicate some unique epidemiological property. Enright et al. (10) proposed a different nomenclature for these clones based on their sequence types (ST) (9) and staphylococcal cassette chromosome *mec* (SCC*mec*) types (I through IV) (11, 12). If subtypes IA and IIIA are included (20), the designations of the five pandemic clones are ST247-IA, ST239-IIIA, ST239-III, ST5-II, and ST5-IV, respectively (10, 19).

The aim of this study was to characterize a collection of clinical MRSA strains from Taiwan and China and to evaluate their geographic spread by comparison with information included in the CEM/NET and multilocus sequence typing (MLST) (http://www.mlst.net) databases.

MATERIALS AND METHODS

Bacterial isolates. One hundred thirty-two clinical MRSA isolates were collected from January 1998 to February 1999 from infection sites of individual patients.

Hospitals. Most of the isolates (n = 118) were from the Tri-Service General Hospital/National Defense Medical Center of Taipei, Taiwan, a 1,200-bed medical center. A small sample (14 isolates) was received from the First Teaching Hospital, Nanjing, People's Republic of China, a 950-bed general hospital.

Susceptibility tests. Susceptibility tests were performed by standard disk diffusion method according to National Committee for Clinical Laboratory Standards guidelines (17). Drugs tested included penicillin, oxacillin, trimethoprim-sulfamethoxazole, ciprofloxacin, chloramphenicol, clindamycin, erythromycin, gentamicin, rifampin, tetracycline, vancomycin, and teicoplanin. Spectinomycin and quinupristin-dalfopristin susceptibilities were determined as described previously (1).

Molecular typing. Southern blot hybridization of *Cla*I digests with *mecA* and Tn*554* DNA probes (5), PFGE of *Sma*I digests of chromosomal DNAs (5), *spaA* typing (26), and MLST (9) were performed as previously described. The SCC*mec* types were determined by a multiplex PCR strategy (19) or by PCR amplification

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TABLE 1. Phenotypic and genotypic properties of 132 MRSA isolates from Taipei, Taiwan, and Nanjing, China

*Cla*I-*mecA*::Tn*554* type[a]	PFGE type	No. of isolates	Clonal type[b]	Antimicrobial resistance (of the majority [>50%] of the isolates)[c]	*spaA* type	MLST type	ST	SCC*mec* type
Taiwan isolates								
III′::B	A1	45	III::B::A (n = 97; 82%)	PEN, OXA, CIP, CLI, ERY, TET, GEN, SXT	WGKAOMQ	2-3-1-1-4-4-30	241	III
III′::B	A7	4						
IX::B	A2	36			WGKAOMQ	2-3-1-1-4-4-30	241	IIIA
IX::B	A4	6						
IX::B	A5	1						
IX::B	A9	1						
IX::B	A10	1						
XI::B	A8	3			WGKAOMQ			III
XIV::B	A1	3	XIV::B::A	PEN, OXA, CIP, CLI, ERY, TET, GEN, SXT	WGKAOMQ			III
XIV::B	A3	2						
XIV::B	A6	1						
XIV::B	A9	1						
X′::B	A3	5	X′::B::A	PEN, OXA, CIP, CLI, ERY, TET, GEN	WGKAOMQ			IIIA
III′::AA	A1	1	III′::AA::A	PEN, OXA, CIP, CLI, ERY, TET, GEN, SXT, CHL	WGKAOMQ			III
XI::B	C	2	XI::B::C	PEN, OXA, CIP, CLI, ERY, TET, GEN, SXT, CHL, RIF	WGKAOMQ	2-3-1-1-4-4-3	239	IIIA
II::NH	B1	1	II::NH::B	PEN, OXA, CLI, ERY, TET, GEN, CHL	ZDMDMOB	19-23-15-2-19-20-15	59	IV
II::NH	B2	3						
II::ZZ	D	2	II::ZZ::D	PEN, OXA, CLI, ERY, TET, GEN, CHL, RIF	NT[d]	3-32-1-1-4-4-3	81	II variant
China isolates								
III′::B	A11	7	III::B::A (n = 10; 71%)	PEN, OXA, CIP, CLI, ERY, TET, GEN, CHL	WGKAOMQ	2-3-1-1-4-4-3	239	III
III′::B	A12	1						
III′::B	A16	2						
X′::B	A13	1	X′::B::A	PEN, OXA, CIP, CLI, ERY, TET, GEN, CHL	WGKAOMQ			IIIA
X′::B	A14	2						
X′::B	A15	1						

[a] *Cla*I-*mecA* polymorphs and respective hybridization fragment sizes: III′, 1.9 and 4.5 kb; IX and XI, see reference 8; XIV, 1.9 and 4.3 kb; X′, 1.9 and 6 kb; II, see reference 13. Tn*554* polymorphs: NH, no homology, lack of transposon; B and AA, see reference 13; ZZ, novel pattern (9.2, 7.1, 6.7, 6.1, and 4.1 kb).

[b] *Cla*I-*mecA* polymorphs III′, IX, and XI present minor variations compared to pattern III and were found to be genetically related (21). Therefore strains belonging to these *mecA* polymorphs were grouped under type III for the definition of clonal types.

[c] Abbreviations: PEN, penicillin; OXA, oxacillin; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; TET, tetracycline; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol; RIF, rifampin.

[d] NT, there was no amplification with the set of primers used.

of the *ccr* (cassette chromosome recombinase) gene (12) when ambiguous results were obtained with the previous methodology.

RESULTS AND DISCUSSION

All isolates studied were multiresistant (Table 1). There were, however, differences among strains from the two countries; for instance, resistance or intermediate resistance to chloramphenicol was found in each of the 14 isolates from China but only in about one-half (43%) of the 118 isolates from Taiwan, while resistance to trimethoprim-sulfamethoxazole was found in 89% of the isolates from Taiwan but in only 21% of the isolates from China. None of the strains was resistant to teicoplanin, vancomycin, and quinupristin-dalfopristin. The majority of the isolates were also susceptible to rifampin (94%) and to 500 mg of spectinomycin/liter (94%).

While the 132 MRSA isolates from Taiwan and China could be separated into six *Cla*I-*mecA* polymorphs and three *Cla*I-Tn*554* polymorphs (Table 1), the great majority of isolates were characterized as *Cla*I-*mecA* polymorph III (13) or the genetically related polymorphs IX, XI, and III′ (21) ($n = 110$) and Tn*554* polymorph B (13) ($n = 125$).

The PFGE analysis grouped the 132 MRSA strains into four types (Table 1), of which pattern A was assigned to a surprisingly large proportion of the isolates (Taiwan, $n = 110$, 93%; China, $n = 14$, 100%). This pattern showed 10 different subtypes in Taiwan and 6 in China, but by far the most frequent were A1 in Taiwan ($n = 49$, 42%) and A11 in China ($n = 7$, 50%). There are no common PFGE subtypes shared by the strains from the hospitals in the two countries.

The combination of the three molecular typing methods (*Cla*I-*mecA* and *Cla*I-Tn*554* hybridization and PFGE) distributed the 118 MRSA isolates from Taiwan and the 14 isolates from China into seven and two clonal types, respectively (Table 1). However, the overwhelming majority of the strains from Taipei, Taiwan, and Nanjing, China (82 and 71%, respectively), belonged to the same clone, III::B::A. Interestingly, this

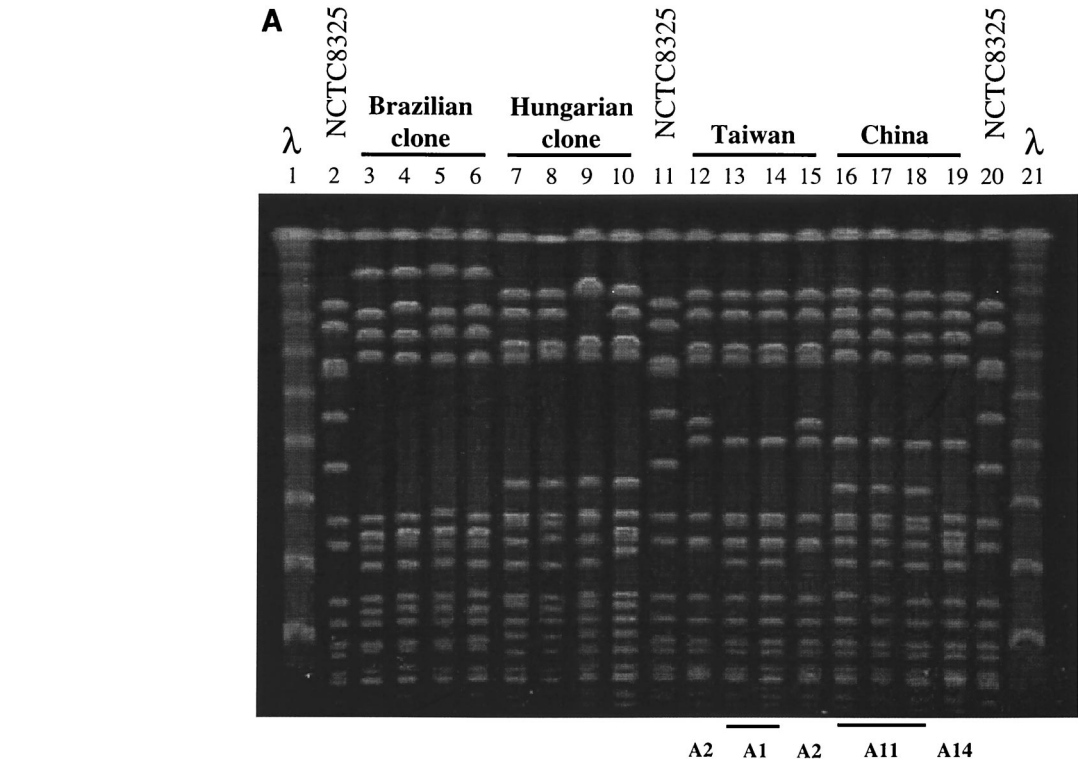

FIG. 1. (A) PFGE of *Sma*I macrorestriction fragments of MRSA clinical isolates belonging to the China/Taiwan, Hungarian (7, 18), and Brazilian clones (2, 3, 27). Lanes 1 and 21, lambda molecular weight marker; lanes 2, 11, and 20, reference strain NCTC 8325; lanes 3 to 6, HU25, HSJ216, BRA5, and BRA101, respectively; lanes 7 to 10, HUSA304, HU101, HUSA88, and HUSA176, respectively; lanes 12 to 15, TAW9 (A2), TAW10 (A1), TAW3 (A1), and TAW5 (A2), respectively; lanes 16 to 19, CHI55 (A11), CHI59 (A11), CHI62 (A11), and CHI61 (A14), respectively. (B) Computer-generated dendrogram based on Jaccard matching of pattern similarity of the PFGE of Fig. 1A. The scale at the bottom of the dendrogram represents similarity.

clone was apparently already present in 1994 in a hospital in another city of Taiwan (15). In addition, the MRSA strain responsible for a hospital-acquired outbreak initiated by a surgeon carrier in the beginning of 1997 in a hospital in northern Taiwan showed the same PFGE pattern, A (29). According to a recent study involving 22 hospitals distributed throughout Taiwan, the high prevalence of MRSA in the country was, at least in past, due to the spreading of a single predominant strain (30) having a PFGE pattern very similar, if not identical, to PFGE pattern A, shown by the majority of MRSA isolates that we studied (Fig. 1). It is probable that clone III::B::A was spread in different cities in Taiwan and had at least a 5-year existence in the country.

Representatives of PFGE pattern A were compared to strains belonging to previously characterized clones (Fig. 1A), namely, the Brazilian and the Hungarian MRSA clones, which share some molecular properties (identical *mecA* and Tn*554* polymorphisms). It was found that PFGE type A showed a high degree of similarity with both clones, in particular with the Hungarian MRSA (Fig. 1A and B).

Several selected isolates from Taiwan and China representing the major clonal lineage (PFGE type A) were also examined by *spaA* typing, MLST, and SCC*mec* typing (Table 1). All shared the same *spaA* repeat motif, WGKAOMQ, which was very similar to the one described for the Hungarian and Brazilian clones (18, 20). MLST analysis showed a single-locus

variant on the basis of a comparison of the ST of isolates from Taiwan (ST241) and China (ST239): ST239 possesses allele 3 at *yqiL*, which is found in several other distantly related lineages, whereas ST241 possesses allele 30. According to information found in the MLST database, allele 30 is only found in ST241 and differs from allele 3 at a single nucleotide site, suggesting that allele 30 arose from allele 3 by a point mutation. Strains from China showed an ST identical to the one described for the Hungarian and Brazilian clones (ST239) (20). All isolates were assigned to SCC*mec* type III or to variant IIIA, two SCC*mec* types that differ mainly by the presence or absence of plasmid pT181 and that are characteristic of the Hungarian and Brazilian clones, respectively (19). ST241 and ST239 have also been detected in Thailand (10), which may indicate a possible spread of the Nanjing/Taipei clone to or from other Asiatic countries.

The application of *spaA* typing, MLST, and SCC*mec* assignment to isolates belonging to the minor clones XIV::B::A, X′::B::A, III′::AA::A, and XI::B::C confirmed that they were related to the major clonal lineage III::B::A. Representatives of two other minor groups of isolates were also characterized: four strains were clonal type II::NH::B, and two strains were clonal type II::ZZ::D. The strain representing clonal type II::NH::B had a rare *spaA* type and belonged to ST59, which was previously detected only in a few MSSA isolates and in a single MRSA isolate from the United States and which is considered one of the most divergent MRSA ST (10). The strain representing clonal type II::ZZ::D was nontypeable by *spaA* and belonged to a new multilocus ST (ST81), which is a double-locus variant of ST239.

Our study documents the dominance of variants of a single multiresistant clone (III::B::A or ST239- and ST241-III and -IIIA) among MRSA isolates (126 of 132 or 95%) from the hospitals in Taipei, Taiwan, and in Nanjing, People's Republic of China. This clone shows high degree of similarity in MLST type, *spaA* type, PFGE pattern, and the structural type of the SCC*mec* element to the Brazilian and Hungarian epidemic MRSA (22). MRSA with an identical genetic background (ST239) has already been recovered in 1982 in Australia and in 1987 in the United States (22), as well as in several countries of Europe, South America, and Asia since the 1990s (2, 10, 16). The results of our study document the dissemination of this highly epidemic MRSA lineage to Taiwan and China.

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