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Molecular Analysis of Group A Streptococcal Isolates Associated with Scarlet Fever in Southern Taiwan between 1993 and 2002

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Collected between 1993 and 2002 at a Taiwanese university hospital, 77 group A streptococcus isolates associated with scarlet fever were grouped by *emm* typing and pulsed-field gel electrophoresis. The predominance of an *emm1* clone before 1996 and the presence of genetically diverse *emm1* and *emm4* strains thereafter were found.

The resurgence of invasive infections caused by group A streptococcus (GAS) has been noted worldwide since the mid-1980s (5, 7, 8, 11, 13, 26). Epidemiologic studies of GAS have relied historically on M and T serotyping (10, 16, 18); however, many strains cannot be typed because of the lack of appropriate type-specific antisera, and comprehensive typing serum sets are not available for most of the laboratories in the world. Recently, sequence analysis of the 5′ end of the *emm* gene that encodes the M protein has been widely used to characterize GAS strains (2–4, 9). Since the 5′ *emm* sequences can be efficiently and reliably used to predict M serotypes (2, 4), the typing system has become a useful molecular epidemiologic tool with which to survey and monitor GAS isolate diversity.

Among the GAS M serotypes, M1, M2, M3, M4, M6, M18, and M22 have been associated with scarlet fever epidemics (1, 12, 15, 19, 21–24). Scarlet fever remains a notifiable disease in Taiwan. An outbreak of scarlet fever occurring at a hospital day care center during a 2-month period in 1996 was reported to be caused by one GAS clone (12); however, genetic relationships among GAS strains associated with other scarlet fever epidemics in Taiwan and *emm* types of these strains are still unknown. The purpose of the present study was to investigate the molecular epidemiology of GAS isolates associated with scarlet fever in southern Taiwan. The prevalence and mechanisms of erythromycin resistance in these isolates were also determined.

GAS isolates. Among clinical isolates of GAS randomly collected between 1993 and 1995 and consecutively collected between 1997 and 2002 at the National Cheng Kung University Hospital, Tainan City, Taiwan, a total of 77 isolates were found to be associated with scarlet fever and were investigated in this study. All of the isolates studied were recovered from throat swabs of different patients. Isolates collected between 1993 and 1998 have been tested previously for their resistance phenotypes and genotypes, and among these isolates, erythromycin-resistant isolates have been examined for their pulsed-field gel electrophoresis (PFGE) patterns (30).

***emm* typing.** PCR amplification of the *emm* gene was performed with previously described primers (2). The amplicons were sequenced on an ABI PRISM 310 sequencer analyzer (Applied Biosystems, Foster City, Calif.) with the primer pair for PCR. The *emm* sequences obtained were compared with those deposited in the GenBank database. An isolate was considered to be of a given *emm* type if it had ≥95% identity over the first 160 bases obtained (4, 9).

PFGE. PFGE was carried out with a contour-clamped homogeneous electric field system (CHEF Mapper XA; Bio-Rad Laboratories, Hercules, Calif.) as described previously (6). Genomic DNAs were prepared as described by Piggot et al. (25) and were digested overnight with 10 U of *Sma*I (New England Biolabs, Beverly, Mass.). Restricted DNA samples were electrophoresed through a 1% agarose gel in 0.5× Tris-borate-EDTA buffer at 6 V/cm with switch times ranging from 2.16 to 44.69 s and a lineal switch time ramp for 27 h. Bacteriophage lambda DNA concatemers (Gibco BRL, Gaithersburg, Md.) were used as size standards. The PFGE patterns obtained were interpreted in accordance with the criteria of Tenover et al. (28).

Susceptibility testing and determination of erythromycin resistance phenotypes. MICs of penicillin, erythromycin, and clindamycin (Sigma Chemical Co., St. Louis, Mo.) were determined by the agar dilution method in accordance with the recommendations of the NCCLS (20). Resistance phenotypes of erythromycin-resistant isolates were determined by the double-disk test with erythromycin and clindamycin (Becton Dickinson, Cockeysville, Md.) disks as described previously (27). Blunting of the growth inhibition zone around clindamycin in the area between the two disks indicated an inducible type of macrolide, lincosamide, and streptogramin B (MLS) resistance (27, 29), and resistance to both disks indicated a constitutive type of MLS resistance (17, 27). The M phenotype was characterized by resistance to erythromycin and susceptibility to clindamycin with no blunting of the growth inhibition zone (27).

Detection of erythromycin resistance genes. Detection of erythromycin resistance genes in erythromycin-resistant iso-

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TABLE 1. Susceptibility to erythromycin, resistance genotypes, and strain typing of the 77 GAS isolates associated with scarlet fever

Year (total no. of isolates) and susceptibility to erythromycin (no. of isolates)	*emm* resistance gene/PFGE pattern (no. of isolates)
1993–1995 (20)	
Susceptible (2)	*emm4*/K (2)
Resistant (18)	
ermB (17)	*emm1*/J (17)
mefA (1)	*emm1*/A (1)
1997–1999 (38)	
Susceptible (9)	*emm1*/E (1), *emm1*/G (1), *emm1*/H (3), *emm1*/I (1), *emm4*/C (3)
Resistant (29)	
ermB (7)	*emm1*/A (1), *emm1*/E (1), *emm1*/I (2), *emm1*/J (3)
mefA (22)	*emm1*/A (4), *emm1*/E (1), *emm1*/H (1), *emm1*/J (1), *emm4*/C (2), *emm4*/D (12), *emm25*/B (1)
2000–2002 (19)	
Susceptible (10)	*emm1*/E (2), *emm1*/G (7), *emm1*/L (1)
Resistant (9)	
ermB (4)	*emm1*/J (4)
mefA (5)	*emm1*/F (1), *emm1*/M (3), *emm4*/D (1)

lates by PCR was performed with the oligonucleotide primer pairs specific to *ermB*, *ermTR*, and *mefA* (14, 30). The expected sizes of PCR products were 640 bp for *ermB*, 348 bp for *mefA*, and 530 bp for *ermTR*.

Three *emm* types were obtained for the 77 isolates (Table 1). The most prevalent *emm* sequence type was *emm1* with 56 (72.7%) isolates, followed by *emm4* with 20 (26.0%) isolates. PFGE yielded 13 different patterns among these isolates, and each pattern from a representative isolate is shown in Fig. 1. The most prevalent PFGE patterns represented were pattern J with 25 (32.5%) isolates, pattern D with 13 (16.9%) isolates, and pattern G with 8 (10.4%) isolates. The *emm1* type, prevalent throughout the study period, was predominant among the isolates collected between 1993 and 1995 and after 1998 (Fig. 2A). Only three PFGE patterns were obtained for the isolates collected between 1993 and 1995 (Table 1). All of the *emm1* isolates collected during this period except one showed

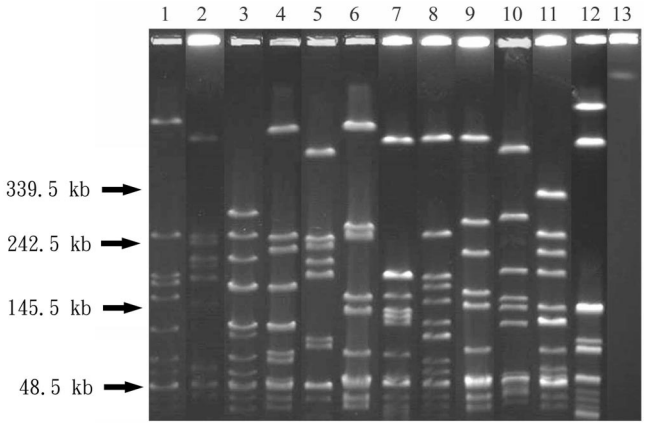

FIG. 1. PFGE profiles of *Sma*I-digested genomic DNAs from 13 representative isolates of GAS. Lanes 1 to 13, PFGE patterns A to M.

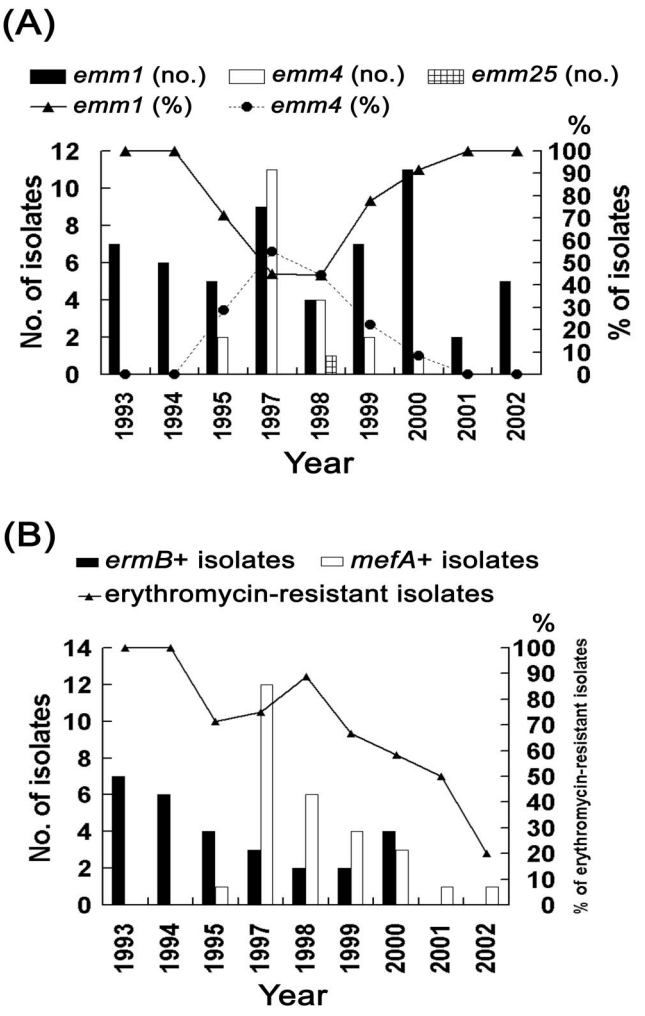

FIG. 2. (A) Changes in the numbers and percentages of GAS isolates of different *emm* types during the period studied. (B) Changes in the percentage of erythromycin-resistant GAS isolates and in the numbers of *ermB*-positive and *mefA*-positive isolates during the study period.

PFGE pattern J, suggesting the occurrences of scarlet fever outbreaks caused by a single *emm1* clone between 1993 and 1995 in southern Taiwan. After 1996, genomic diversity was found among the *emm1* isolates, which displayed nine PFGE patterns (A, E, F, G, H, I, J, L, and M). None of these patterns were found to be prominent. Serotype M1 isolates have caused large outbreaks of scarlet fever in Germany (15). The increase in the numbers of serotype M1 isolates associated with invasive GAS infections has been reported around the world since the mid-1980s (5, 7, 8, 11, 13, 26). The serotype has also been reported to be more prevalent among invasive isolates than among noninvasive isolates in Taiwan (13). Whether the association of *emm1* and scarlet fever outbreaks found in the present study is due to a high prevalence of *emm1* among GAS strains in Taiwan or to specific pathogenic factors is not known and needs further investigation.

The *emm4* type was only found in the isolates collected between 1995 and 2000 and was most prevalent in 1997 and 1998 (Fig. 2A). Only three PFGE patterns were obtained for

the 20 *emm4* isolates, of which 13 (65.0%) exhibited PFGE pattern D (Table 1). These data suggest the occurrence of scarlet fever outbreaks caused by an *emm4* clone between 1997 and 1999 in southern Taiwan. Scarlet fever epidemics associated with M4 strains have also been reported in the United Kingdom and elsewhere (8, 24).

All 77 GAS isolates were susceptible to penicillin (MICs, <0.03 μg/ml), and 56 (72.7%) isolates were resistant to erythromycin (MIC, ≥1 μg/ml). Our previous study has demonstrated a high rate (63.2%) of erythromycin resistance in GAS isolates collected between 1993 and 1998 (30). The percentage of erythromycin resistance during the period was 83.6% for the isolates associated with scarlet fever and 56.8% for the isolates from non-scarlet fever patients (unpublished data). The percentages of erythromycin resistance in GAS associated with scarlet fever were found to decline after 1998 in the present study (Fig. 2B). The increase in the erythromycin-susceptible isolates might be in part due to the occurrence of an outbreak caused by an *emm1* clone with PFGE pattern G (Table 1). Whether the decrease in the number and percentage of erythromycin-resistant isolates represents a trend in erythromycin resistance in GAS associated with scarlet fever or is just a temporal variation in this area deserves further investigation. If such a trend exists, erythromycin may become the drug of choice for the treatment of scarlet fever in penicillin-hypersensitive patients in Taiwan once again.

The erythromycin resistance phenotypes of the 56 erythromycin-resistant isolates were determined by the double-disk test with erythromycin and clindamycin disks. Twenty-eight (50.0%) isolates were of the erythromycin-resistant M phenotype, 28 (50.0%) isolates were of the constitutive MLS resistance phenotype, and none of the isolates exhibited the inducible MLS resistance phenotype. All 28 constitutively MLS-resistant isolates showed high-level resistance to both erythromycin (MICs, ≥128 μg/ml) and clindamycin (MICs, ≥128 μg/ml). All 28 isolates demonstrating the M phenotype of erythromycin resistance showed low-level resistance to erythromycin (MICs, 2 to 32 μg/ml) and were susceptible to clindamycin (MIC, 0.03 to 0.13 μg/ml). Genetic determinants of erythromycin resistance were investigated in all erythromycin-resistant isolates by means of PCR experiments with specific primer sets for *ermB*, *ermTR*, and *mefA*. As predicted, all 28 constitutively MLS-resistant isolates carried the *ermB* gene and all 28 isolates demonstrating the M phenotype of erythromycin resistance carried the *mefA* gene. The resistance genotypes showed a different chronological distribution. The *ermB*-positive isolates were predominant between 1993 and 1995, and the *mefA*-positive isolates were predominant between 1997 and 1999 (Fig. 2B). The numbers of *mefA*-positive isolates appeared to decline year by year after 1997. Only three PFGE patterns were obtained for the 28 *ermB*-positive isolates, and 25 (89.3%) of them showed PFGE pattern J (Table 1). The data indicate that a genetically related *ermB*-positive clone cause outbreaks during 1993 and 1995, and the clone still existed more recently. Genetic diversity was found among the *mefA*-positive isolates, which revealed nine PFGE patterns (A, B, C, D, E, F, H, J, and M). However, 13 (46.4%) of the 28 *mefA*-positive isolates showed pattern D. Thus, the predominance of *mefA* between 1996 and 1999 was in part due to the prevalence of a genetically related clone.

In conclusion, *emm1* was found to be prevalent throughout the past 10 years and the outbreaks of scarlet fever caused by *emm4* isolates were found to occur between 1995 and 2000 in the present study. The outbreaks in the early 1990s were mostly associated with an *ermB*-positive erythromycin-resistant *emm1* clone, and those occurring thereafter were associated with genetically diverse clones. Moreover, erythromycin-resistant isolates seem to be decreasing. This paper is the first report of the genotypes of GAS associated with scarlet fever in Taiwan. Our data provide a basis for future studies on changes in the epidemiology of GAS, outbreak investigation, and development of preventive measures and of recommendations for treatment strategies in Taiwan.

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