Changing Epidemiology of *Streptococcus pyogenes* *emm* Types and Associated Invasive and Noninvasive Infections in Southern Taiwan[▽]

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A total of 242 isolates were recovered from 76 patients with invasive diseases, 89 with scarlet fever, and 77 with pharyngitis. The most frequent *emm* types were types 12 (43.4%), 4 (18.2%), and 1 (16.9%). *emm*12 reemerged in 2005 and peaked in 2007. *emm*11 was recovered only from patients with invasive disease.

Streptococcus pyogenes, a group A *Streptococcus* (GAS) species, produces many diseases, ranging from impetigo, pharyngitis, and scarlet fever to life-threatening sepsis, necrotizing fasciitis, and streptococcal toxic shock syndrome (STSS) (8). M protein that is encoded by the *emm* gene is a major virulence factor of GAS. Different M protein types are epidemiologically related to particular clinical syndromes; e.g., *emm*28 was isolated more frequently than other types among women with puerperal sepsis (6, 10, 14). M1, M2, M3, M4, M6, M18, and M22 strains are associated with outbreaks of scarlet fever (1, 11, 15, 18, 20, 22–24), while M1 and M3 are highly associated with severe invasive disease (19, 25, 27) and M18 is associated with acute rheumatic fever (7).

The distribution of *emm* types in cases of invasive diseases tends to vary over time and within different geographic regions. In the United States, the most common *emm* types were 1, 28, 12, 3, and 11 during the period from 1995 to 1999 (19). *emm* types 1, 3, and 12 were predominant from 2000 to 2004 (21). In Europe, the distribution of the *emm* types differs among countries (16). For example, in Denmark, *emm*1 and *emm*28 were the most prevalent types from 2001 to 2004, whereas in Greece, *emm*1 and *emm*12 were predominant from 2003 to 2005. In Sweden, four *emm* types, 89, 81, 28, and 1, accounted for 56% of 746 patients with invasive GAS diseases during the period from 2002 to 2004. In Taiwan, *emm*1 associated with invasive GAS disease, while *emm*12 was more often associated with noninvasive GAS disease (13). The purpose of the current study was to investigate the changing epidemiology, genetic diversity, and epidemic virulence of GAS infections over a 10-year period in southern Taiwan, utilizing M genotyping and pulsed-field gel electrophoresis (PFGE) analysis.

Clinical isolates of *S. pyogenes* was obtained from patients

seen at the National Cheng Kung University Hospital, Tainan City, Taiwan, from 1998 to 2007. They were divided into strains isolated from patients with invasive diseases or those from patients with noninvasive diseases. Invasive isolates were defined as those obtained from sterile body sites (blood, cerebrospinal, joint, pleural, peritoneal, or pericardial fluids) or from nonsterile sites (wounds associated with STSS or necrotizing fasciitis). Noninvasive strains were defined as GAS isolates obtained from patients with asymptomatic nasopharyngeal colonization, pharyngitis, scarlet fever, erysipelas, and impetigo.

A total of 242 nonduplicate GAS isolates recovered from normally sterile sites (165 from throat swabs, 30 from wounds, 25 from pus, 12 from blood, 10 from other materials) were collected. The mean age of patients with invasive diseases was significantly greater than that of those with noninvasive diseases (28.5 ± 27 years versus 8.7 ± 7.9 years [$P < 0.0001$]). Among the 76 patients with invasive diseases, 58 (76.3%) patients had skin and soft-tissue infections. Three had necrotizing fasciitis, eight had STSS, four had severe sepsis, and three had complicated infections including pneumonia or mastoiditis. Among the 166 patients with noninvasive diseases, 77 (46%) had pharyngitis (including one with lymphadenitis), and 89 (54%) had scarlet fever (including 59 patients with pharyngitis).

The distribution of *emm* types among patients with invasive and noninvasive diseases (subdivided into those with scarlet fever and those with pharyngitis) is shown in Table 1. GAS *emm* sequence typing was based on the 5' end of the *emm* gene within the *emm* chromosomal region (2, 3). A unique *emm* type was defined as having $\geq 95\%$ sequence identity to any other known *emm* type over 160 bp near the 5' end of the gene, using a BLAST search (<http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm>). Twenty *emm* sequence types were identified among the 242 GAS isolates, including types 1, 4, 6, 11, 12, 13, 22, 33, 49, 57, 77, 81, 82, 85, 87, 92, 94, 101, 102, and 123. The leading *emm* types were 12 (43.4%), 4 (18.2%), 1 (16.9%), 11 (4.5%), 6 (3.7%), and 22 (2.5%) (Table 1). All of the *emm* types were associated with both invasive and noninvasive dis-

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TABLE 1. Distribution of *Streptococcus pyogenes* *emm* types isolated from 1998 to 2007 according to clinical characteristics

<i>emm</i> type	No. (%) of indicated isolates				χ^2 ^c	<i>P</i> value ^b
	Invasive	Noninvasive		Total		
		Scarlet fever	Pharyngitis			
1	14 (34.1)	14 (34.1)	13 (31.7)	41 (16.9)	0.06	0.8
4	11 (25)	21 (47.7)	12 (27.3)	44 (18.2)	0.03	0.86
6	1 (11.1)	3 (33.3)	5 (55.6)	9 (3.7)	2.87	0.09
11	11 (100.0)	0 (0.0)	0 (0.0)	11 (4.5)	18.38	<0.0001
12	15 (14.3)	47 (44.8)	43 (41.0)	105 (43.4)	20.22	<0.0001
22	4 (66.7)	2 (33.3)	0 (0.0)	6 (2.5)	4.38	0.04
Other ^a	20 (76.9)	2 (7.7)	4 (15.4)	26 (10.7)		
Total	76 (31.4)	89 (36.8)	77 (31.8)	242 (100.0)		

^a The "Other" group includes ≤ 5 isolates of *emm*13, *emm*33, *emm*49, *emm*57, *emm*77, *emm*81, *emm*82, *emm*85, *emm*87, *emm*92, *emm*94, *emm*101, *emm*102, and *emm*123.

^b Variation in the proportion of each *emm* type, which is compared with the remaining *emm* types combined; determined by χ^2 test. Two-sided *P* values of <0.05 are statistically significant.

^c The χ^2 for trend statistic was used to assess differences in proportions and to test for departures from linear trends associated with clinical manifestations of GAS acquisition.

eases except for *emm*11, which was recovered only from patients with invasive disease. *emm*22 and other less frequent types were more likely to be associated with invasive diseases ($P < 0.0001$). The *emm* types 1, 4, 6, and 12 accounted for 82.2% of all patients with GAS infections, 95.2% of the patients with noninvasive diseases, and 53.9% of the patients with invasive disease. The most common type, *emm*12, was also significantly associated with noninvasive disease (85.7%) ($P < 0.0001$).

The most prevalent *emm*12 type was analyzed by PFGE after digestion of total DNA with *Sma*I or *Sgr*AI for determining the clonal relatedness. The PFGE protocol for GAS was developed on the basis of Gautom's *Escherichia coli* rapid PFGE protocol, with minor modifications (9). Six PFGE patterns were observed among the isolates during the 10-year period from 1998 to 2007 (Fig. 1). Pattern A was shown as the most common pattern during the entire study period (Fig. 2). This clonal pattern has also been identified in northern and central Taiwan (4, 5). The reemergence of *emm*12 strains throughout Taiwan may explain the reported increase in cases of scarlet fever noted since 2005.

There is considerable controversy concerning the virulence of the various GAS *emm* types. There appear to be major differences in the frequency of purportedly disease-specific *emm* types in Taiwan (4, 5, 28). These apparent differences can be explained, in part, by the changes in the relative abundance of different *emm* types over time, herd immunity following epidemic peaks, epidemic virulence, age, sex, and location in the community. In the current study, *emm* type 12 accounted for 43.4% of all strains. Because of its relatively great abundance, it was also the most common invasive strain, accounting for 15 of 76 (19.7%) of all invasive strains. But only 14.3% of the *emm* type 12 strains were associated with invasive infections. Most were associated with noninvasive infections. *emm* types that were significantly associated with invasive infections

were types 11 and 22 and several less-common types. These findings are in concert with a report of an outbreak of invasive infections in a nursing home in which *emm*11 was one of two distinct *emm* types identified in the outbreak (26). An *emm*11 strain expressing the *ermA* gene was identified in Spain (17). Nevertheless, 10 *emm* types (types 1, 6, 11, 12, 13, 22, 33, 77, 92, and 101) were covered by the 26-valent GAS vaccine (12). Therefore, this vaccine may provide antibodies to protect vulnerable groups from GAS infection.

In conclusion, *emm*12 was found to be prevalent throughout the past 10 years. All of the *emm* types were associated with both invasive and noninvasive diseases, except for type 11, which was associated only with invasive disease. The predominant *emm* type during this period was type 12, clonal pattern A. It was the major *emm* type associated with scarlet fever and pharyngitis. Types 12, 22, and a few less-common *emm* types appeared to have special epidemic virulence for invasive diseases. Long-term surveillance studies combined with *emm* sequence typing and clonal analysis are needed to

FIG. 1. Illustration of the various PFGE patterns of *Sma*I (A to E)- or *Sgr*AI (F)-digested chromosomal DNA of *emm*12 GAS isolates. Six major PFGE patterns are shown.

FIG. 2. PFGE patterns of *emm12* strains isolated from patients with GAS invasive diseases, scarlet fever, and pharyngitis. Chromosome DNA was digested with *Sma*I (lanes 1 to 5, 7 to 10, and 12) or *Sgr*AI (lanes 6 and 11). The study periods were divided into the following: period I, 1998 to 1999; period II, 2000 to 2002; period III, 2003 to 2005; and period IV, 2006 to 2007. Lane 1, 5, and 9 represent period I. Lanes 2, 6, and 10 represent period II. Lanes 3, 7, and 11 represent period III. Lanes 4, 8, and 12 represent period IV. Lanes 1 to 4 are the patterns of *emm12* strains isolated from patients with invasive diseases; lanes 5 to 8 are those from patients with scarlet fever; lanes 9 to 12 are those from patients with pharyngitis; and lane M shows the lambda DNA reference size markers (size range, 50 to 1,000 kb).

understand the natural history and epidemic virulence of GAS infections.

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