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ORIGINAL ARTICLE

Emergence of uncommon *emm* types of *Streptococcus pyogenes* among adult patients in southern Taiwan

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Background: *Streptococcus pyogenes* isolated from adult patients during a 12-year period in southern Taiwan were analyzed to estimate the distribution of *emm* types and their correlation with disease manifestations and patient age.
Methods: Three hundred thirty-four invasive and noninvasive isolates collected from patients older than 20 years between 1997 and 2008 at National Cheng Kung University Hospital were included for *emm* typing. A correlation between *emm* type, disease manifestations, and patient ages was analyzed.
Results: The nine most prevalent types were *emm11*, *emm12*, *emm4*, *emm1*, *Sp9458/VT8*, *emm81*, *emm106*, *emm13*, and *emm75*. Formerly rare *emm* types, including *emm11*, *emm81*, and *emm102*, emerged dramatically after 2004 in southern Taiwan. Type *emm11* was significantly associated with both superficial infections and cellulitis. In addition, types *emm13*, *emm81*, and *emm106* were more prevalent in patients older than 50 years and significantly associated with specific invasive disease manifestation.
Conclusion: These results suggest new *emm* types (*emm11*, *emm81*, and *emm102*) of *S pyogenes* were introduced into the adult population in southern Taiwan after 2004. The

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rarely reported *emm* types, including *emm13*, *emm81*, and *emm106*, caused invasive diseases more often in adult patients.

Introduction

Streptococcus pyogenes (group A streptococcus, GAS) causes a broad spectrum of diseases, including pharyngitis, tonsillitis, impetigo, cellulitis, necrotizing fasciitis, streptococcal toxic shock syndrome, rheumatic fever, and heart disease.[1] Severe GAS infections reemerged in the mid-1980s.[2] However, the factors underlying the resurgence of GAS severe infections are still unclear.

M protein is a GAS surface fibril protein and is an important virulence factor of GAS.[3] The *N*-terminal sequence heterogeneity of the *emm* gene (coding for M protein) is used to characterize GAS isolates in epidemiological studies.[4–6] More than 200 *emm* types and 750 *emm* subtypes have been identified to date.[7] Luca-Harari et al[8] showed that the prevalent *emm* types in 11 countries across Europe (during 2003–2004) were *emm1*, *emm28*, *emm3*, *emm89*, and *emm87*. In the United States, the predominant types were *emm1*, *emm3*, *emm28*, *emm12*, and *emm89* during 2000–2004.[9] Types *emm1*, *emm12*, *emm8*, *emm18*, and *emm80* were predominant in China from 2000 to 2004.[10] Although the distribution of *emm* types in different geographic regions is diverse, *emm1* and *emm3* (in Western countries) are among the most common and important types associated with invasive diseases.

GAS epidemiological surveillance in Taiwan is mainly focused on isolates of patients with scarlet fever.[11–14] Until now, only two reports provided GAS epidemic information for invasive and noninvasive diseases.[15,16] *emm12*, *emm4*, and *emm1* were the most prevalent types in central (1993–2003) and southern Taiwan (1998–2007).[15,16] However, Su et al[16] showed that 82% of isolates were collected from patients younger than 20 years (personal communications). In the present study, we provide epidemiological information on 334 GAS isolates collected from patients older than 20 years from 1997 to 2008, showing that uncommon *emm* types are emerging among the adult population in southern Taiwan.

Methods

Bacterial isolates and disease categories classifications

Three hundred thirty-four GAS isolates were consecutively collected from patients older than 20 years between 1997 and 2008 at National Cheng Kung University Hospital, Tainan, Taiwan. Twenty-two isolates used in a previous surveillance were included in this study.[16] The ranges of patients' ages were 20–29, 11.4%; 30–39, 12%; 40–49, 12.9%; 50–59, 10.5%; 60–69, 12.9%; 70–79, 11.4%; 80–89, 7.8%; 90–99, 0.3%; and unknown, 21% (59% of isolates were collected in 1997). The disease categories were defined as colonization, noninvasive diseases, invasive diseases, nonsuppurative sequelae, and unknown (Table 1).

emm typing

PCR amplification and sequencing were performed according to the protocol described by Beall et al.[6] The *emm* sequences (at least the first 220 base pair of sequence) were compared with those deposited in the NCBI Genbank database. The isolate was given a specific *emm* type if it had a 180 base pair exact match with the majority query result (http://www.cdc.gov/ncidod/biotech/strep/assigning.htm).

Pulsed-field gel electrophoresis analysis

Pulsed-field gel electrophoresis (PFGE) typing was performed as previously described.[14] The PFGE fragment patterns were analyzed by using visual comparison, and all different PFGE fragment patterns were compared with the use of GelCompar II software (Unimed Healthcare Inc., Houston, TX, USA). By using the criteria of Tenover et al[17] and unweighted pair group method with arithmetic mean based on the Dice coefficient with a position tolerance of 1.5%, PFGE-based clusters were defined as isolates with a genetic relatedness of more than 80% on a dendrogram.

Statistical analysis

Statistical analysis was performed by Prism, version 4 (GraphPad software, San Diego, CA, USA) and SPSS software, version 10.0 (SPSS Inc., Chicago, IL, USA). The χ^2 test or Fisher's exact test was used to assess differences in proportions when appropriate. Stepwise unconditional logistic regression was performed to examine the independence of explanatory variables in the development of the outcome of interest. A p value less than 0.05 was taken as significant.

Results

Trends in the yearly fluctuation of type prevalence

From 1997 to 2008, a total of 42 *emm* types were identified among 334 isolates. The nine most prevalent types were *emm11* (11.1%), *emm12* (9.9%), *emm4* (8.4%), *emm1* (7.2%), *Sp9458/VT8* (6.6%), *emm81* and *emm106* (6.3%), and *emm13* and *emm75* (4.5%), accounting for 64.8% of total isolates (Table 1). Infections by type *emm12* peaked in 2001, causing 50% of all invasive infections; the same was true for type *emm81* in 2007 (Fig. 1A and C). Invasive diseases caused by *emm*11 isolates peaked in 2005 and 2008 (Fig. 1C). In addition, type *emm102*, which was not

Table 1 Distribution of 334 GAS strains isolated from 1997 to 2008 characterized by *emm* type according to disease category

Disease category	Isolates, *n* (%)	Patients infected with the following *emm* type, *n* (%)									
		emm11 (*n* = 37)	*emm12* (*n* = 33)	*emm4* (*n* = 28)	*emm1* (*n* = 24)	*Sp9458/VT8* (*n* = 22)	*emm81* (*n* = 21)	*emm106* (*n* = 21)	*emm13* (*n* = 15)	*emm75* (*n* = 15)	Other (*n* = 118)
Colonization	6 (1.8)	0 (0)	2 (33.3)	2 (33.3)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)
Noninvasive diseases	149 (44.6)	20 (13.4)	17 (11.4)	14 (9.4)	8 (5.4)	8 (5.4)	7 (4.7)	7 (4.7)	3 (2.0)	7 (4.7)	58 (38.9)
Pharyngitis and tonsillitis	41 (12.3)	0 (0)	10 (24.4)	7 (17.1)	6 (14.6)	1 (2.4)	1 (2.4)	1 (2.4)	0 (0)	3 (7.3)	12 (29.3)
Superficial infections	103 (30.8)	20 (19.4)	6 (5.8)	5 (4.9)	2 (1.9)	6 (5.8)	6 (5.8)	6 (5.8)	3 (2.9)	4 (3.9)	45 (43.6)
URI	3 (0.9)	0 (0)	0 (0)	2 (66.7)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
UTI	2 (0.6)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)
Invasive diseases	164 (49.1)	16 (9.8)	13 (7.9)	11 (6.7)	11 (6.7)	13 (7.9)	14 (8.5)	12 (7.3)	12 (7.3)	6 (3.7)	56 (34.1)
Cellulitis	64 (19.2)	12 (18.8)	3 (4.7)	2 (3.1)	1 (1.6)	7 (10.9)	7 (10.9)	2 (3.1)	4 (6.3)	4 (6.3)	22 (34.3)
Bacteremia	31 (9.3)	1 (3.2)	4 (12.9)	2 (6.5)	3 (9.7)	3 (9.7)	1 (3.2)	2 (6.5)	4 (12.9)	0 (0)	11 (35.5)
Sepsis	20 (6.0)	1 (5)	1 (5)	1 (5)	4 (20)	1 (5)	4 (20)	2 (10)	0 (0)	0 (0)	6 (30)
STSS	2 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)
NF	29 (8.7)	2 (6.9)	3 (10.3)	1 (3.4)	1 (3.4)	2 (6.9)	2 (6.9)	5 (17.2)	4 (13.8)	0 (0)	9 (31)
NF and sepsis	2 (0.6)	2 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)
NF and STSS	2 (0.6)	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Deep tissue infections	14 (4.2)	0 (0)	1 (7.1)	5 (35.7)	1 (7.1)	0 (0)	0 (0)	1 (7.1)	0 (0)	2 (14.3)	4 (28.6)
Nonsuppurative sequelae	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown	14 (4.2)	1 (7.1)	1 (7.1)	1 (7.1)	3 (21.4)	1 (7.1)	0 (0)	2 (14.3)	0 (0)	1 (7.1)	4 (28.6)

Superficial infections include genital tract infection, wound infection, erysipelas, ecthyma, impetigo, paronychia, dermatitis, folliculitis, and carbuncle.
NF = necrotizing fasciitis; STSS = streptococcal toxin shock syndrome; URI = upper respiratory tract infection; UTI = urinary tract infection.

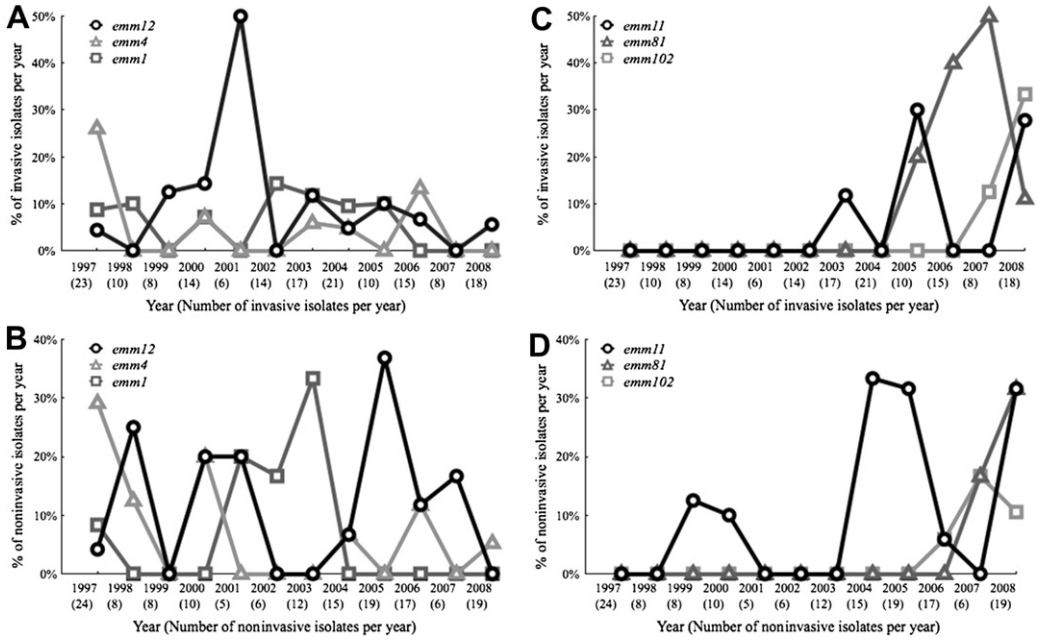

Figure 1. Distribution of the prevalence (*emm1*, *emm4*, and *emm12*) and emergence (*emm11*, *emm81*, and *emm102*) types in invasive (A and C) and noninvasive diseases (B and D) from 1997 to 2008. The percentage of invasive or noninvasive isolates per year was calculated from the number of each *emm* type isolate divided by the total number of invasive or noninvasive isolates in each year.

detected before 2006, increased steadily from 2007 to 2008 (Fig. 1C).

emm12, *emm4*, and *emm1* were the predominant types isolated in noninvasive diseases in 2005, 1997, and 2003, respectively (Fig. 1B). In 2004 and 2005, 33.3% and 31.6% of noninvasive diseases were caused by *emm11* isolates, respectively (Fig. 1D). The *emm81* and *emm102* isolates collected from noninvasive diseases increased after 2007 and 2006, respectively (Fig. 1D).

PFGE type of *emm11*, *emm81*, and *emm102* isolates

Types *emm11*, *emm81*, and *emm102* emerged after 2004. PFGE was applied to analyze the clonal distribution of these isolates collected after 2004. Two clusters were found among *emm11* isolates, with 83.8% (31/37) of strains belonging to the major cluster. Both *emm81* and *emm102* isolates had a single cluster each (data not shown).

Correlation between disease severity and *emm* types

Types *emm11*, *emm12*, and *emm4* were more prevalent in noninvasive diseases, accounting for 13.4%, 11.4%, and 9.4% of noninvasive isolates, respectively (Table 1). Type *emm12* was significantly correlated with pharyngitis and tonsillitis ($p = 0.004$). No other *emm* types were significantly correlated with specific noninvasive disease manifestation.

The prevalent *emm* types in invasive diseases included *emm11*, *emm81*, *emm12*, *Sp9458/VT8*, *emm13*, and *emm106* (Table 1). Type *emm11* and *emm81* was significantly associated with cellulitis ($p = 0.025$) and sepsis ($p = 0.025$), respectively. Type *emm13* and *emm106* were significantly associated with necrotizing fasciitis ($p = 0.028$ and 0.027, respectively). Furthermore, type *emm13* was also significantly associated with bacteremia ($p = 0.035$).

Correlation between age and *emm* types

Among 334 isolates, 264 isolates (79%) had patient age information available. Types *emm11* and *Sp9458/VT8* were significantly more prevalent among patients older than 40 and 30 years, respectively ($p = 0.01$ and 0.036, respectively). Types *emm13*, *emm81*, and *emm106* were more prevalent among patients older than 50 years ($p = 0.002$, 0.005, and 0.012, respectively). Furthermore, type *emm106* was found to be prevalent among patients older than 60 years ($p = 0.007$).

Discussion

In this study, we showed the 12-year surveillance results for our adult population characterized by *emm* type. Changing type prevalence during the years of surveillance was found; and *emm11*, *emm12*, *emm4*, and *emm1* were the most prevalent types among adult patients in southern Taiwan. *emm11*, *emm81*, and *emm102* emerged after 2004 and were the dominant types in 2008. In addition, *emm13*, *emm81*, and *emm106* were significantly associated with specific invasive disease manifestations and more prevalent in patients older than 50 years.

Su et al[16] analyzed 242 isolates, mostly collected from patients younger than 20 years, representing the young adult and child population in southern Taiwan. Twenty different *emm* types were found in their surveillance, whereas 42 different *emm* types were found among adult

patients in our study. The uncommon *emm11* was found to be a prevalent type among both young and adult populations.[16] Type *emm11* has been shown to be associated with the superantigen gene *speA2* and the macrolide resistance gene *ermB*.[18–20] Although two outbreaks caused by *emm11* isolates have been reported in France and the United States, it is associated with invasive infections in less than 5% of cases.[9,21–25] However, *emm11* isolates in Su et al[16] and our study are not only responsible for superficial infections (noninvasive diseases, $p = 0.001$) but are also significantly associated with invasive disease. Although the representativeness of the study population in Su et al[16] and our study are different, the trend of the significant emergence of type *emm11* is found in southern Taiwan.

Surveillance reports from Poland, Romania, and Sweden showed *emm81* is one of the predominant types in invasive GAS diseases.[26–28] In Israel, Wasserzug et al[29] reported ecthyma outbreaks caused by an *emm81* clone in different infantry units. In the present study, we found the invasive diseases caused by *emm81* isolates emerged after 2005. PFGE analysis showed clones isolated from Israel and our collections have similar band patterns (data not shown), suggesting this particular clone has spread to different geographic regions around the world.[29]

The rarely reported *emm* types, including *emm13*, *emm81*, and *emm106*, were not only significantly associated with invasive diseases but also more prevalent among patients older than 50 years. The lack of the immune functional analysis for adult patients make it difficult to connect the correlation between rarely encountered *emm* types and invasive diseases directly. However, our results suggest that these rarely encountered types caused invasive diseases more often in adult patients in southern Taiwan.

In conclusion, this study shows significantly changing *emm* type prevalence during 12-year surveillance in southern Taiwan. In addition, the rarely reported *emm* types in Taiwan, including *emm11*, *emm13*, *emm81*, and *emm106*, were significantly associated with specific invasive disease manifestation in adult population. Long-term epidemiological surveillance of GAS infections will help us to understand the epidemic trend for the adult population.

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