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
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 between 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, non-suppurative 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 χ² 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 *emm11* 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

<table>
<thead>
<tr>
<th>Disease category</th>
<th>Isolates, n (%)</th>
<th>Patients infected with the following emm type, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>emm11</td>
</tr>
<tr>
<td>Colonization</td>
<td>6 (1.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Noninvasive diseases</td>
<td>149 (44.6)</td>
<td>20 (13.4)</td>
</tr>
<tr>
<td>Pharyngitis and tonsillitis</td>
<td>41 (12.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Superficial infections</td>
<td>103 (30.8)</td>
<td>20 (19.4)</td>
</tr>
<tr>
<td>URI</td>
<td>3 (0.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>UTI</td>
<td>2 (0.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Invasive diseases</td>
<td>164 (49.1)</td>
<td>16 (9.8)</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>64 (19.2)</td>
<td>12 (18.8)</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>31 (9.3)</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>20 (6.0)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>STSS</td>
<td>2 (0.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>NF</td>
<td>29 (8.7)</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>NF and sepsis</td>
<td>2 (0.6)</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>NF and STSS</td>
<td>2 (0.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Deep tissue infections</td>
<td>14 (4.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Nonsuppurative sequelae</td>
<td>1 (0.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Unknown</td>
<td>14 (4.2)</td>
<td>1 (7.1)</td>
</tr>
</tbody>
</table>

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.
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. 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. Type emm11 has been shown to be associated with the superantigen gene speA2 and the macrolide resistance gene ermB. 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. However, emm11 isolates in Su et al 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 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. In Israel, Wasserzug et al reported erythema 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.

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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References

Streptococcus pyogenes emm type in southern Taiwan


