Molecular and Clinical Characteristics of Invasive Group A Streptococcal Infection in Sweden

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Background. The incidence and severity of invasive group A streptococcal infection demonstrate great variability over time, which at least, in part, seems to be related to group A streptococcal type distribution among the human population.

Methods. An enhanced surveillance study of invasive group A streptococcal infection (746 isolates) was performed in Sweden from April 2002 through December 2004. Noninvasive isolates from either the throat or skin (773 isolates) were collected in parallel for comparison. Clinical and epidemiological data were obtained from 88% of patients with invasive disease and were related to isolate characteristics, including T type, emm sequence type, and the presence of 9 superantigen genes, as well as pulsed-field gel electrophoresis pattern comparisons of selected isolates.

Results. The annual incidence was 3.0 cases per 100,000 population. Among the patients with invasive disease, 11% developed streptococcal toxic shock syndrome, and 9.5% developed necrotizing fasciitis. The overall case-fatality rate was 14.5%, and 39% of the patients with streptococcal toxic shock syndrome died (P < .001). The T3/P13/B3264 cluster accounted for 33% of invasive and 25% of noninvasive isolates. Among this most prevalent type cluster, emm types 89 and 81 dominated. Combined results from pulsed-field gel electrophoresis, emm typing, and superantigen gene profiling identified subgroups within specific emm types that are significantly more prone to cause invasive disease than were other isolates of the same type.

Conclusions. This study revealed a changing epidemiology of invasive group A streptococcal infection in Sweden, with emergence of new emm types that were previously not described. The results also suggest that some clones may be particularly prone to cause invasive disease.

Since the mid 1980s, group A streptococci (GAS) have been identified as a reemerging cause of severe invasive infections, such as septic shock, puerperal sepsis, soft-tissue infections (including necrotizing fasciitis [NF]), and streptococcal toxic shock syndrome (STSS) [1–3]. Despite increased awareness and improved treatment of the most severe disease manifestations (i.e., NF and STSS), the mortality rate remains high, often exceeding 50% [3, 4].

The M protein is a major GAS virulence factor, evoking a type-specific host immune response. To date, >150 different M protein sequence types (emm types) have been described [5]. Both emm and T typing are important tools in epidemiological studies. T and M/emm types correlate to each other [6], and certain type combinations have been associated with certain clinical manifestations and disease severity [7–11]. However, the prevalence of specific GAS types in a community shows high variability depending on geographic area and over time [12–15]. The GAS superantigens (SAgs) are also important for virulence, participating in the induction of the systemic toxicity associated with severe infections [16, 17]. Currently, there are at least 12 known streptococcal SAgs [4, 9, 18].

In the present national surveillance study, performed for 2 years and 8 months, essentially all invasive GAS isolates in Sweden were collected from the microbio-
logical laboratories and characterized using molecular techniques. The results were correlated to clinical parameters. Also, a representative noninvasive material was collected. The study revealed a significant change in GAS epidemiology in the Swedish population, with a predominance of emm types not previously shown to cause invasive disease to any large extent. Furthermore, certain clonal subgroups of isolates with higher invasiveness than others were identified.

**MATERIALS AND METHODS**

**Study Materials**

**Invasive isolates.** A national active surveillance of invasive GAS infections was conducted in Sweden from 23 April 2002 through 31 December 2004. Invasive disease was defined by isolation of GAS from blood samples or from samples obtained from other normally sterile sites. All 29 Swedish clinical microbiological laboratories, with a national catchments area of ∼9 million inhabitants, participated. The attending physicians completed a questionnaire regarding clinical information about the infection, such as probable port of entry, symptoms, focus, treatment, and outcome, as well as about recent use of antibiotics and underlying conditions. STSS cases were classified according to the consensus definition proposed by The Working Group on Severe Streptococcal Infections [19]. The study was approved by the ethics committee at Karolinska Institutet (Stockholm, Sweden).

**Noninvasive isolates.** From February 2003 through June 2004, 6 of the microbiological laboratories, which were geographically spread in Sweden, submitted 10 consecutive noninvasive isolates each month, of which 5 were isolated from skin samples and 5 were isolated from throat samples. Two laboratories were located in Stockholm (Karolinska University Hospital Solna and Karolinska University Hospital Huddinge), and the others were located at the University hospitals in Lund, Göteborg, Uppsala, and Umeå.

**Characterization of Isolates**

**T typing.** All invasive and noninvasive isolates were T typed using conventional poly- and monospecific T antisera (Sevapharma).

**emm Typing.** Genomic DNA from all isolates was prepared, as described elsewhere [20]. The emm types were determined by direct sequencing of the N-terminal hypervariable portion of the emm gene, as described elsewhere [21]. The type was assigned using the emm type database [22].

**SAg genotyping.** All isolates were genotyped for their SAg profile using multiplex PCR with 8 specific primer pairs for the streptococcal SAg gene and the pyrogenic exotoxin genes speA, speB, speC, speF, speG, speH, and speJ, as described in detail elsewhere [14]. We used a single PCR for the streptococcal mitogenic exotoxin (sme) Z gene, with primer pairs covering the gene’s allelic variations (5′-CAGATAGTAATTGATT-TTA and 3′-AGCTAGAACCAGAAGATAT).

**PFGE.** Invasive and noninvasive isolates of emm types 89, 81, 77, 28, 12, and 1 (992 isolates in total) were subjected to PFGE analysis, using the restriction enzyme Smal (New England Biolabs), as described elsewhere [23]. BioNumerics, version 3.5 (Applied Maths), was used for analysis; isolates with 85% similarity (≤3 bands difference) were considered to belong to the same clone.

**Statistical Analysis**

Data were analyzed using Stata Statistical Software, release 8.0 (Stata), and GraphPad Prism, version 4 (GraphPad Software). For nominal data, χ² test or Fisher’s exact test were used when appropriate. For continuous data, either Mann-Whitney U test or Kruskal-Wallis test were used. Multinomial logistic regression analysis [24] with dichotomous and continuous independent variables was fitted using SAS, version 9.1.3 (SAS Institute), proc logistic. The independent variables were removed from the model in a backwards procedure using the likelihood ratio test as exclusion criteria.

**RESULTS**

**Characteristics of invasive GAS infections.** Seven hundred forty-six invasive cases were identified, corresponding to a mean annual incidence of 3.0 cases per 100,000 inhabitants during the study period. The incidence increased with age (figure 1), and seasonal fluctuations were observed, with an increased number of cases occurring during the winter months. Questionnaires with clinical information were obtained for 88% of the patients. Among the patients with invasive cases, 11% developed STSS, and 9.5% developed NF. A majority of the patients (69%) reported underlying chronic disease (of which diabetes and cardiovascular disease were most common), predisposing factors, or drug abuse. The overall case-fatality rate was 14.5%, compared with 39% among STSS cases (P < .001). Univariate analysis revealed that fatal outcome was significantly associated with the presence of underlying conditions, multiorgan failure, and STSS (P < .001) (table 1).

The majority (94%) of invasive isolates were obtained from blood samples, and the remaining isolates sampled were obtained from other normally sterile sites. The T3/13/B3264 type cluster was the most common T type, accounting for one-third of the isolates. The most abundant emm types were, in descending order, emm types 89, 81, 28, 1, 12, 77, and 4, accounting for 74% of the invasive infections (figure 2 and table 2). Skin was the most common site of entry (63% of patients), followed by the respiratory tract (13%), urogenital tract (6%), and other/unknown sites (19%). Patients with skin and/or tissue involvement were older (P < .001) and, hence, had more underlying diseases and/or conditions reported (P < .001) (table...
1). The most frequent emm type in this group was emm type 81, which was not the case for patients without the manifestation ($P < .05$) (table 2). In contrast, emm type 1 was the most prevalent type among both patients with STSS and patients with NF ($P < .001$ and $P < .05$, respectively, compared with patients outside these groups). STSS was significantly more prevalent among NF cases than among cases with other skin and/or tissue involvement (51% vs. 7.4%; $P < .001$). A significantly higher fatality rate was noted among patients with NF who had STSS than among patients with NF who did not have STSS (28% vs. 7.7%; $P = .044$) (table 1). However, no type was significantly associated with fatal outcome (table 2).

Women with puerperal sepsis accounted for 3% of all patients with invasive cases, none of whom had fatal outcome. The most common emm types were emm types 28 and 89, with emm type 28 accounting for 35% of these infections, compared with 14% of other cases ($P = .018$) (table 2). Also, the urogenital tract as site of entry of the infection was more common among women than among men and was significantly associated with emm type 28 ($P = .037$).

**Noninvasive GAS disease.** In total, 773 isolates were collected from skin or throat specimens and were considered to represent the distribution of noninvasive isolates in the country, because the 6 laboratories involved were geographically spread. Patients with noninvasive disease were younger than those with invasive disease, and there was also a significant age difference between patients with skin infection and patients with throat infection (table 1). The same 7 emm types that were found to be most prevalent among patients with invasive disease were also the most prevalent among patients with noninvasive disease, accounting for 72% of the isolates. However, the emm type distribution differed significantly between invasive and noninvasive isolates ($P < .001$) (figure 2B and table 2). The emm type distribution also differed between throat and skin isolates ($P < .001$) (table 2).

**emm Type distribution differed with sex, geographic area, and time.** Among all invasive and noninvasive cases, 60% of emm type 81 isolates were from male patients ($P = .001$), whereas 59% of emm type 28 isolates were from female patients ($P = .026$). The difference for emm type 28 was even stronger among invasive cases alone ($P = .008$). Furthermore, the T and emm type distribution of invasive isolates varied over time (figure 2A and data not shown). In contrast, no distinct fluctuations over time were found among noninvasive isolates, among which emm type 28 isolates dominated during the whole study period. Geographical differences in type distribution were also found and recognized between larger urban areas.

**Invasive disease clinical characteristics as predictors of emm type.** To correlate clinical information to specific emm types, a multinomial regression analysis was performed. Only the 7 most prevalent emm types were considered in the analysis, and all others were grouped into a single category (“others”), which served as a reference in the model. The following characteristics were included as explanatory variables in the model: (1) skin and/or tissue infection, (2) puerperal sepsis, (3) NF without STSS, (4) STSS, (5) adult respiratory distress syndrome, (6) cancer and/or immunosuppressive treatment, (7) age, and (8) sex. The statistically significant variables that we found were skin and/or tissue infection, STSS, and age (log likelihood, 2531.9; $P < .05$). These variables could, to a certain degree, be used as emm type predictors. Among patients with skin and/or tissue infection, the predicted probability of an emm type 4 infection depended on age; the highest probability of having emm type 4 was among younger patients, and the lowest probability was among elderly patients. In contrast, the probability of an emm type 89 infection was high among elderly patients. Irrespective of age, the predicted probability of an emm type 81 infection was highest among patients with skin and/or tissue infections. Also, the predicted probability was highest for emm type 1 among patients with STSS but decreased somewhat with age.

**Clonal analysis correlated with emm types.** PFGE analysis
Table 1. Demographic characteristics in relation to group A streptococcal disease manifestation.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Invasive disease</th>
<th>Noninvasive disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Skin and/or tissue infectionsb</td>
</tr>
<tr>
<td>No. (%) of patients</td>
<td>654 (100)</td>
<td>461 (70.4)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>63.6</td>
<td>66.8</td>
</tr>
<tr>
<td>Median (range)</td>
<td>68 (0–99)</td>
<td>71 (0–99)</td>
</tr>
<tr>
<td>Male sex</td>
<td>48.7</td>
<td>51.6</td>
</tr>
<tr>
<td>Overall CFR</td>
<td>14.5</td>
<td>15.7</td>
</tr>
<tr>
<td>Underlying condition</td>
<td>68.6</td>
<td>73.8</td>
</tr>
<tr>
<td>Mean no. of organ failuresf</td>
<td>0.91</td>
<td>1.01</td>
</tr>
<tr>
<td>NF (CFR)</td>
<td>9.5 (18.9)</td>
<td>13.4 (19.0)</td>
</tr>
<tr>
<td>STSS (CFR)</td>
<td>11.3 (38.9)</td>
<td>13.2 (37.3)</td>
</tr>
</tbody>
</table>

NOTE. Data are percentage of patients, unless otherwise indicated. CFR, case-fatality rate; NA, not applicable; NF, necrotizing fasciitis; STSS, streptococcal toxic shock syndrome.

a Based on information from returned questionnaires for 654 of 746 patients with invasive disease. The patient groups for invasive disease are overlapping, which has been taken into consideration during statistical analysis results not shown in the table.

b Patients with symptoms from skin and/or soft-tissue infection or skin stated as the probable entry of infection.

c Information available for 753 patients.

d Information available for 374 patients.

e Information available for 379 patients.

f Per case presenting with a specific disease manifestation.
Figure 2. Major emm and T types for invasive and noninvasive isolates. A, The emm type distribution for invasive isolates in 4-month intervals. B, The proportions and combinations of emm and T types for the 746 invasive isolates and 773 noninvasive isolates collected during the study period. Types including <5% of either invasive or noninvasive isolates are not specified in detail but are grouped in the “other” category. T types are shown as the percentage of combined invasive and noninvasive isolates.

of all invasive and noninvasive isolates of emm types 89, 81, 77, 28, 12, and 1 (992 isolates total) revealed that most of the isolates tested (97%) grouped in concordance with their emm type, and no discrimination between PFGE patterns of invasive and noninvasive isolates could be noted. Strikingly, emm types 1 and 81 isolates were highly homogenous, clustering into 1 PFGE group each. In contrast, emm types 89 and 28 formed 4 groups each, and 2 groups were noted for both emm type 12 and 77. No specific disease manifestation or outcome correlated to any specific PFGE group.

SAg gene profiles as markers for invasiveness. The mean number of SAg genes present in invasive and noninvasive isolates ranged from 4.0 to 6.6 (table 3), and differences in SAg gene profiles were noted between emm types and their PFGE determined subgroups (table 4). The speA gene was commonly detected among both invasive and noninvasive emm type 1 isolates (83% and 79%, respectively) but was rarely found among other types (P < .001). speH was particularly linked to emm type 12, speH was linked to emm types 1 and 28, and the gene for the streptococcal SAg was most common among emm type 4 isolates. The speC gene was less common among emm types 89, 81, and 1 than among other types (43% total positive isolates among emm types 89, 81, and 1 vs. 73% among the other types; P < .001). The speB, speE, speG, and smeZ genes were found in the majority of isolates. Thus, some invasive emm type 81 isolates lacked the same smeZ gene or had an allelic variant not detected by our primers. These isolates showed similar PFGE banding patterns as isolates with the gene present (table 4). Similar results were found for speG among emm type 177 and emm type 4 isolates (table 3); however, the emm type 77 negative isolates did not belong to the same PFGE subgroup.

The emm type subgroups, as identified by PFGE, could be divided by SAg genotyping. As shown in table 4, 2 specific SAg profiles within groups 89A and 81A were significantly more prevalent among invasive than noninvasive isolates. No specific SAg profile within any of the 14 PFGE subgroups, was statistically more prevalent among noninvasive isolates.

DISCUSSION

The present nationwide active surveillance study of invasive GAS infections in Sweden includes >1500 isolates from patients with invasive and noninvasive GAS disease. Clinical information was related to molecular characteristics of all isolates, aiming at identifying GAS virulence markers and other attributes
Table 2. *emm* type distribution in relation to invasive and noninvasive disease, invasive disease manifestation, and origin of noninvasive infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Invasive disease</th>
<th>Noninvasive disease</th>
<th>Total(^b)</th>
<th>Skin and/or tissue infection</th>
<th>Puerperal sepsis</th>
<th>NF without STSS</th>
<th>STSS</th>
<th>Fatal outcome</th>
<th>Skin</th>
<th>Throat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of isolates</td>
<td>746</td>
<td>773</td>
<td>654</td>
<td>461</td>
<td>17</td>
<td>30</td>
<td>62</td>
<td>74</td>
<td>95</td>
<td>384</td>
</tr>
<tr>
<td><em>emm</em> Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>15.7</td>
<td>8.1</td>
<td>16.4</td>
<td>15.2</td>
<td>23.5</td>
<td>6.7</td>
<td>8.1</td>
<td>13.5</td>
<td>19.0</td>
<td>8.6</td>
</tr>
<tr>
<td>81</td>
<td>14.5</td>
<td>10.6</td>
<td>15.3</td>
<td>17.1</td>
<td>0</td>
<td>23.3</td>
<td>14.5</td>
<td>12.2</td>
<td>11.6</td>
<td>18.5</td>
</tr>
<tr>
<td>28</td>
<td>13.9</td>
<td>16.2</td>
<td>13.5</td>
<td>11.3</td>
<td>35.3</td>
<td>20.0</td>
<td>12.9</td>
<td>10.8</td>
<td>10.5</td>
<td>15.6</td>
</tr>
<tr>
<td>1</td>
<td>11.9</td>
<td>7.4</td>
<td>12.1</td>
<td>12.8</td>
<td>11.8</td>
<td>10.0</td>
<td>22.6</td>
<td>28.4</td>
<td>14.7</td>
<td>5.5</td>
</tr>
<tr>
<td>12</td>
<td>6.3</td>
<td>14.2</td>
<td>6.4</td>
<td>6.3</td>
<td>11.8</td>
<td>10.0</td>
<td>6.5</td>
<td>6.8</td>
<td>8.4</td>
<td>8.3</td>
</tr>
<tr>
<td>77</td>
<td>5.9</td>
<td>6.1</td>
<td>5.8</td>
<td>7.0</td>
<td>0</td>
<td>6.7</td>
<td>4.8</td>
<td>5.4</td>
<td>8.4</td>
<td>7.0</td>
</tr>
<tr>
<td>4</td>
<td>5.9</td>
<td>9.1</td>
<td>6.0</td>
<td>6.5</td>
<td>0</td>
<td>13.3</td>
<td>9.7</td>
<td>4.1</td>
<td>3.2</td>
<td>8.1</td>
</tr>
<tr>
<td>Other</td>
<td>25.9</td>
<td>28.3</td>
<td>24.6</td>
<td>23.9</td>
<td>17.6</td>
<td>10.0</td>
<td>21.0</td>
<td>18.9</td>
<td>24.2</td>
<td>28.4</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.001(^c,d)</td>
<td>...</td>
<td>&lt;.029(^e)</td>
<td>&lt;.084(^a)</td>
<td>&lt;.090(^c)</td>
<td>.128(^c)</td>
<td>.003(^c)</td>
<td>.537(^c)</td>
<td>&lt;.001(^c,f)</td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** Data are percentage of isolates, unless otherwise indicated. NF, necrotizing fasciitis; STSS, streptococcal toxic shock syndrome.

\(^a\) Based on information from returned questionnaires for 654 of 746 patients with invasive disease.

\(^b\) The type distribution within each patient group is compared with the distribution among all patients who did not present with the specific disease manifestation.

\(^c\) Determined using \(x^2\) test.

\(^d\) Comparison of invasive and noninvasive isolates.

\(^e\) Determined using Fisher’s exact test.

\(^f\) Comparison of skin and throat isolates.

that are important for disease severity and outcome. Although several clinical studies involving invasive GAS infections have been conducted around the world, few reports have included noninvasive isolates (e.g., from tonsillitis, impetigo, or asymptomatic carriage) [25–28]. The present study compares isolates from invasive and noninvasive GAS disease, collected during the same time period and from the same geographic area, to correlate molecular characteristics to disease severity and to estimate the invasiveness of different GAS isolates and/or clones.

Previous national epidemiological studies of invasive GAS infections in Sweden revealed an increasing incidence, from 1.8 cases per 100,000 population in 1987 [29] to ≥2.9 cases per 100,000 population during 1994–1995 and 1996–1997 [27, 30]. This is in accordance with the increase noted in several European countries during the 1990s [31]. In our study, the mean annual incidence was 3.0 cases per 100,000 population.

Skin was found to be the major port of entry of infection, and skin infection was the major clinical feature among patients with invasive disease. The majority (69%) of all patients had underlying medical conditions, except for those with puerperal sepsis. The overall fatality rate was 14.5%, but the rate was higher (39%) among the 11% of patients who developed STSS. These findings are consistent with previous findings from the period 1996–1997 [27]. However, patients with STSS did not differ with regard to age or prevalence of underlying disease, compared with all patients with invasive disease. This suggests that host susceptibility plays an important role in determining severity of disease. Kotb et al. [32] reported an association between outcome of invasive GAS infection and HLA class II haplotype, which was linked to the influence of HLA class II and the magnitude of SAg-mediated inflammatory responses.

In contrast to the Swedish surveillance studies performed during 1994–1995 and 1996–1997, in which T types 1 and 28, respectively, dominated the T type cluster T3/13/B3264 dominated among both invasive and noninvasive cases in our study. This cluster was mainly associated with the recently described *emm* types 89 and 81, which is a new finding, because *emm* type 89 has previously been most commonly associated with T11 [6]. Thus, *emm* types 89 and 81 accounted for 30% of invasive isolates, and to our knowledge, it is the first time in western countries that 2 *emm* types of high numbers (>80) have dominated a national surveillance study. Furthermore, the type distribution also fluctuated over time and with geographic area.

In agreement with previous reports, associations between *emm* types and particular disease manifestations were observed [7–11]. *emm* type 28 was associated with puerperal sepsis and, also, was more prevalent among female patients. As expected, *emm* type 1 was strongly associated with STSS. Furthermore, *emm* type 81 was more prevalent among male patients and was significantly associated with skin or tissue involvement and/or
Table 3. Superantigen (SAg) gene distribution within major emm types of invasive and noninvasive isolates.

<table>
<thead>
<tr>
<th>emm Type</th>
<th>No. (%) of isolates</th>
<th>Mean no. of SAg</th>
<th>speA</th>
<th>speC</th>
<th>speG</th>
<th>speH</th>
<th>speJ</th>
<th>ssa</th>
<th>smeZ</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>NI</td>
<td>I</td>
<td>NI</td>
<td>NI</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>89</td>
<td>117 (16)</td>
<td>62 (8)</td>
<td>4.5</td>
<td>4.8</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>81</td>
<td>108 (14)</td>
<td>63 (11)</td>
<td>4.0</td>
<td>4.6</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>104 (14)</td>
<td>125 (16)</td>
<td>5.9</td>
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<td>0</td>
<td>5</td>
<td>97</td>
<td>92</td>
<td>100</td>
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<td>1</td>
<td>89 (12)</td>
<td>57 (7)</td>
<td>5.9</td>
<td>6.1</td>
<td>83</td>
<td>79</td>
<td>36</td>
<td>54</td>
<td>100</td>
</tr>
<tr>
<td>12</td>
<td>47 (6)</td>
<td>110 (14)</td>
<td>5.8</td>
<td>5.6</td>
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<td>7</td>
<td>75</td>
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<tr>
<td>77</td>
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<td>47 (7)</td>
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<td>77</td>
<td>30</td>
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<tr>
<td>4</td>
<td>44 (6)</td>
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<td>43</td>
</tr>
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<td>Other</td>
<td>297 (26)</td>
<td>219 (28)</td>
<td>5.1</td>
<td>5.2</td>
<td>1</td>
<td>1</td>
<td>52</td>
<td>68</td>
<td>98</td>
</tr>
</tbody>
</table>

NOTE. The vast majority of the isolates harboured the genes for speB (>95%) and speF (>98%). I, invasive isolates; NI, noninvasive isolates; smeZ, streptococcal mitogenic exotoxin Z; ssa, streptococcal SAg.

a Mean number of SAg genes for all invasive isolates and noninvasive isolates was 5.1 and 5.4, respectively.

Table 4. Superantigen (SAg) gene profiles among emm81 and 89 PFGE groups.

<table>
<thead>
<tr>
<th>PFGE group, profile</th>
<th>Spe</th>
<th>Alla</th>
<th>Invasiveb</th>
<th>Noninvasivec</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
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NOTE. smeZ, streptococcal mitogenic exotoxin Z; spe, streptococcal pyogenic exotoxin; ssa, streptococcal SAg.

a All isolates within the PFGE group with identical SAg profile.
b Invasive isolates within the PFGE group with identical SAg profile.
c Noninvasive isolates within the PFGE group with identical SAg profile.
d Invasiveness of a PFGE group (i.e., difference in prevalence of invasive and noninvasive isolates with identical SAg profiles and belonging to the same PFGE group) were assessed by Fisher’s exact test, 2-sided P<.001.
to prophages, phage-like elements, and other exogenous sequences [37, 38]. Several of these prophages encode extracellular virulence factors, such as SAGs (SpeA, SpeC, SpeH, SpeL, SpeK, SpeL, SpeM, and streptococcal SAG), whereas other SAGs (SpeB, SpeF, SpeG, and SmeZ) are chromosomally encoded. We characterized the clinical isolates with respect to 9 SAG genes and found associations between emm/ PFGE subgroups and SAG profiles. Importantly, some of these profiles, in combination with the PFGE subgroups, differed depending on invasiveness, which argues that the mechanisms for differences in invasive disease potential among different GAS isolates may, at least in part, be attributed to horizontally acquired prophages and other mobile DNA elements. However, the mechanisms for the increased invasiveness of a particular PFGE subgroup with a certain SAG profile may not be because of SAG expression per se, because SAG profiles that were overrepresented among invasive isolates of PFGE groups 89A and 81A harbored fewer SAG genes than did other profiles within the same groups (table 4).

Overall, noninvasive isolates harbored slightly more SAG genes than did invasive isolates. The gene profiles were, to a large extent, also linked to emm types of the isolates (table 3). Importantly, the use of these combined typing methods allowed identification of particular clones with higher invasive disease potential. It will be interesting to make a comparative genomic analysis between the clones identified, to possibly identify genes of direct relevance for invasive disease.

In conclusion, the present surveillance study reveals a shift in the epidemiology of invasive GAS infection in Sweden, with a dominance of emm types that, previously, were not observed to have a high incidence. No correlation was found between invasiveness and the presence of single SAG genes. However, certain SAG profiles within clonal clusters, as determined by PFGE and emm type, were found to be more prominent among invasive isolates, suggesting an increased invasive disease potential. If we can identify clones that are particularly invasive and, in the future, also understand which properties affect virulence, we will enable better and more focused strategies for treatment and intervention, as well as for prevention (by creating a basis for vaccine development). Both the M protein and the recently described pilus-like structure [39], which has been associated with T types, constitute potential vaccine candidates, emphasizing the need for knowledge of emm and T type distributions among GAS isolates. In addition, studies of GAS isolate SAG profiles may represent a useful strategy to identify subclones that are particularly prone to cause invasive disease, to the benefit of surveillance and prevention of these important infections.

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