Clinical and Microbiological Characteristics of Severe Streptococcus pyogenes Disease in Europe

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Received 11 November 2008/Returned for modification 17 December 2008/Accepted 9 January 2009

In an attempt to compare the epidemiology of severe Streptococcus pyogenes infection within Europe, prospective data were collected through the Strep-EURO program. Surveillance for severe cases of S. pyogenes infection diagnosed during 2003 and 2004 was undertaken in 11 countries across Europe by using a standardized case definition and questionnaire. Patient data as well as bacterial isolates were collected and characterized by T and M/emm typing, and selected strains were analyzed for the presence of superantigen genes. Data were analyzed to compare the clinical and microbiological patterns of the infections across the participating countries. A total of 4,353 isolates were collected from 5,521 cases with severe S. pyogenes infections who were identified. A wide diversity of M/emm types (n = 104) was found among the S. pyogenes clinical isolates, but the M/emm type distribution varied broadly between participating countries. The 10 most predominant M/emm types were M/emm type 1 (M/emm1), M/emm28, M/emm3, M/emm89, M/emm87, M/emm12, M/emm4, M/emm83, M/emm81, and M/emm5, in descending order. A correlation was found between some specific disease manifestations, the age of the patients, and the emm types. Although streptococcal toxic shock syndrome and necrotizing fasciitis were caused by a large number of types, they were particularly associated with M/emm1 and M/emm3. The emm types included in the 26-valent vaccine under development were generally well represented in the present material; 16 of the vaccine types accounted for 69% of isolates. The Strep-EURO collaborative program has contributed to enhancement of the knowledge of the spread of invasive disease caused by S. pyogenes within Europe and encourages future surveillance by the notification of cases and the characterization of strains, which are important for vaccination strategies and other health care issues.

Streptococcus pyogenes (group A streptococcus [GAS]), a major human pathogen (9), has been studied for decades and may give rise to common throat and skin infections as well as to invasive diseases, such as arthritis, septicemia, cellulitis, puerperal fever, necrotizing fasciitis (NF), and streptococcal toxic shock syndrome (STSS) (14). Since the mid-1980s there have been increasing numbers of reports describing severe manifestations of GAS infections; however, the factors underlying the worldwide resurgence of this pathogen remain unknown (20).

The M protein, which is encoded by the emm gene, is an important virulence factor and is also an epidemiological marker that is used throughout the world to characterize GAS isolates (5, 21–23). The type specificity of the M protein, of which more than 100 different types are known, is largely determined by the epitope located in 40 to 50 amino acid residues at the amino terminus (4, 16, 27). These regions of M proteins have been shown to evoke antibodies that have strong bactericidal activity and that are not likely cross-reactive with other proteins have been shown to evoke antibodies that have strong bactericidal activity and that are not likely cross-reactive with...
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<th>No. of isolates</th>
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human tissues (3, 16). Hence, an approach to the development of a GAS vaccine has been to combine small amino-terminal M-protein peptides to make multivalent vaccines that would elicit opsonic antibodies against epidemiologically important GAS serotypes (15). Other surface proteins, like the serum opacity factor and the T protein, are also used to characterize different GAS types. In addition to the known linkage between the T serotype, serum opacity factor production, and theemm type (25, 26), several studies also indicated correlations betweenemm types, disease manifestations, and other virulence factors, especially the superantigens (SAgs) (7, 10, 40, 42).

Epidemiological studies, which provide the distributions of the types of streptococci prevalent in communities, are of basic importance for the identification and control of streptococcal infections. Furthermore, by tracing selected virulence features of the isolates causing disease, understanding of the pathogenic mechanisms of the various disease manifestations would be enhanced. In order to improve knowledge about severe GAS infections, the Strep-EURO program was implemented in 2003 and 2004. The overall epidemiological findings of the program were reported recently (29). The present paper describes the type characteristics and the SAg repertoires of the streptococcal isolates and also their possible association with clinical findings.

**MATERIALS AND METHODS**

**Clinical data and isolates.** Through a collaboration among 11 European countries (Cyprus, the Czech Republic, Denmark, Finland, France, Germany,
Greece, Italy, Romania, Sweden, and the United Kingdom), enhanced surveillance for the epidemiology of invasive GAS disease was undertaken between 1 January 2003 and 31 December 2004. The methods employed to identify cases varied by country but mostly involved the invited submission of isolates from local microbiology laboratories to the national streptococcal reference center. Demographic and clinical data, as well as risk factor information, were collected through a standardized questionnaire in all countries except Denmark and Sweden, where surveillance with questionnaires designed earlier was already operational.

**Case definition and isolate identification.** For invasive GAS disease and STSS, the consensus definition proposed by the Working Group on Severe Streptococcal Infections in 1993 was used (48). The identities of the GAS isolates were confirmed by using morphological and growth characteristics, bacitracin susceptibility, or pyrolidonyl-arylamidase testing and latex agglutination with commercially available group A antiserum.

**Typing of isolates.** The isolates were T typed by using commercial poly- and monospecific T antiserum, according to the manufacturer’s recommendation (Sevapharma, Prague, Czech Republic). M/emm typing was performed by some-
what different methods in each country, and thus, the typing results were evaluated and are further described in an external quality assurance study (33). Although both serological and/or genotypic methods were used to determine the M/\(emm\) types, the results are hereafter referred to as \(emm\) types. The \(emm\) sequences obtained by sequencing-based methods were identified by comparison to the sequences available in the CDC database (ftp://ftp.cdc.gov/pub/infectious\_diseases/biotech/tsemm/). Unusual combinations of \(T\) and \(emm\) types (rare or previously not reported) were verified blindly by another participating reference center.

**SAg gene detection.** A total of 1,127 isolates from five countries (Czech Republic, Denmark, Finland, France, and Romania) were tested for the presence of SAg genes in Lund, Sweden, including \(speA\), \(speB\), \(speC\), \(speF\), \(speG\), \(speH\), \(speI\), \(ssa\), and \(smeZ\) (30). Isolates from the remaining countries were tested at the respective national centers: Swedish isolates were tested for all the SAg genes mentioned above except \(speA\) (18); Greek and Italian isolates were tested for the \(speF\), \(speB\), and \(speC\) genes (13); and German isolates were tested for the \(speC\), \(ssa\), and \(smeZ\) genes (47). A fraction consisting of 256 United Kingdom isolates (18% of all strains whose \(emm\) types were determined) were tested locally for the presence of \(speA\), \(speB\), and \(speC\) genes. In addition, 193 isolates (covering 38 of the 74 different \(emm\) types identified in the United Kingdom) were tested in Lund, Sweden, as described above.

**Statistical analysis.** Data were analyzed by using Prism (version 4) software (GraphPad Software) and SAS (version 9.1.3) proc logistic software (SAS Institute). For nominal data, the \(x^2\) test or Fisher’s exact test were used when appropriate. Logistic regression was performed by using the \(emm\) type as the outcome and clinical conditions or risk factors as predictors. The analyses were performed separately for each of the 10 most prevalent \(emm\) types, and the results were compared to those for the group consisting of cases caused by all other types (i.e., except the 10 most prevalent ones). Each model was reduced by backward elimination, and the significance level was set at 5%. In the logistic regression analyses, only cases with age, gender, and clinical or risk factor information available were included.

**RESULTS**

From a total of 5,521 patients with invasive streptococcal disease, 4,353 (79%) bacterial isolates were submitted to the reference centers in the participating countries. Clinical information was available for 3,404 isolates (62% of all cases).

**T types.** In total, 4,171 isolates were subjected to \(T\) typing, and 408 (10%) of these were nontypeable. Fifty different \(T\) types or type profiles were recognized; the most prevalent of these were \(T\) type 1 (T1; 19%); T28 (18%); T3/13/B3264 (23%); T12 (8%); T4 (5%); T5 (3%); and T6, T11, and T8/25/Imp19 (2% each) (Table 1).

**\(emm\) types.** One hundred four different types were identified among 4,353 isolates whose \(emm\) types were determined. The most prevalent (\(\geq 2\%) were \(emm\) type 1 (\(emm1\); 19%); \(emm28\) (12%); \(emm3\) (10%); \(emm89\) (8%); \(emm87\) (6%); \(emm12\) (5%); \(emm4\) (5%); \(emm83\) (3%); \(emm81\) (3%); and \(emm5\), \(emm77\), \(emm6\), \(emm22\), and \(emm18\) (2% each) (Table 1). The type distribution varied significantly among the 11 countries, but the overall prevalence was strongly influenced by the large proportion of isolates originating from the United Kingdom (Fig. 1) and also from Sweden. Although \(emm87\) and \(emm83\) were the fifth and eighth overall most common types, respectively, the majority of these isolates were from the United Kingdom (93% and 90% of isolates, respectively). In total, 34 different \(emm\) types encompassed the 10 most prevalent types in the 11 countries. Importantly, \(emm1\) was the most abundant type in the majority of countries, with the proportion ranging from 15% to 33% of isolates. In contrast, in Denmark, Finland, and Sweden, \(emm28\) was the most prevalent type, ranging in prevalence from 16% to 45% of isolates. As shown in Fig. 1B, certain types among the overall 10 most prevalent \(emm\) types were absent in some of the countries; e.g., in Romania, only 3 of the overall 10 most prevalent types were found. Type \(emm3\) was infrequent in the Czech Republic, Finland, Greece, and Sweden, with the prevalence ranging from 1% to 5%; and it was absent in Romania. Type \(emm43\) was found exclusively in the United Kingdom. Other types almost confined to the United Kingdom were \(emm82\) (93% from the United Kingdom), \(emm5\) (91%), \(emm83\) (90%), and \(emm68\) (81%). Type \(emm53\) was found only in the Czech Republic, Greece, and the United Kingdom. All the \(emm118\) isolates (\(n = 34\)) originated from either Denmark or Sweden.

**\(T-\emm\) type combinations.** As shown in Table 1, the number of \(T-\emm\) type combinations was high (\(n = 314\); some of these
were infrequent and others were not previously reported. The most prevalent T type was 3/13/B3264 (or combinations thereof, e.g., 3/13, 13/B3264, and 3/B3264), and it was associated with no less than 40 different emm types. In general, emm1 was limited to T1 (98%); but a small number of these isolates expressed T type 3, 3/13/B3264, or 4.

**Correlation between age, gender, and emm types.** Among 600 isolates collected from children (ages 0 to 17 years), the most frequent emm types were, in descending order, emm1 (26%), emm12 (11%), emm4 and emm3 (10% each), and emm28 (7%). Among patients aged 18 years and older, the most prevalent type was also emm1 (19%); this was followed by emm28 (13%), emm3 (10%), and emm9 (9%).

A significant predominance of emm87 and emm28 among females (58%; P < 0.001 for both) was found. Type emm28 was also more prevalent in the age groups 30 to 39 years (17%) and 70 to 79 years (19%); in the younger age group, type emm28 was strongly associated with females (80%; P < 0.001). Types emm81 and emm83 were significantly overrepresented among males (62% [P < 0.05] and 68% [P < 0.001], respectively).

**Seasonal fluctuations.** During the study period, several emm types presented a steady seasonal prevalence, whereas other showed fluctuations (Fig. 2). Overall, 59% of the cases were reported in the 6 winter months (January to April, November, and December) in both years. In contrast, a tendency toward a higher frequency of emm12 was noted during the warmer months (May to August; P < 0.05).

**Disease manifestations, risk factors, and emm types.** The most severe manifestations, STSS and NF, were caused by 45 different types, of which emm1 was the most prevalent, accounting for 37% and 31% of cases, respectively (Table 2); in addition, considerable proportions of cases of STSS and NF were caused by emm3 isolates (17% and 14%, respectively). In the statistical regression model, when each of the 10 most prevalent types was compared with the other types combined, STSS was found to be caused statistically more often by emm1 or emm3 (P < 0.001 for each type).

Patients without focal symptoms were less often infected by emm1 (17%; P < 0.05), in contrast to emm81 (45%) and emm77 (47%) (P < 0.001 for each type) and in contrast to emm83 (34%) and emm87 (26%) (P < 0.05 for each type), which were more common among patients without focal symptoms. Furthermore, patients with arthritis were less prone to be infected by emm28 isolates (5%; P < 0.05), and cellulitis was more often caused by either emm87 (32%; P < 0.0001) or emm83 (30%; P < 0.05) than by types other than the 10 most prevalent types. Although puerperal sepsis was caused by 16 different types and only 8% of patients with puerperal sepsis were infected with isolates of emm28, a clear correlation between puerperal sepsis and emm28 was noted (31% of cases; P < 0.001). Other emm types significantly involved as a cause of puerperal sepsis were emm89 and emm87 (4% each; P < 0.001 for emm89 and P < 0.05 for emm87).

Data regarding risk factors as well as the infecting emm types were available for 2,796 patients (Table 3). Patients with diabetes were statistically more prone to infections caused by either emm81 (P < 0.001) or emm12 (P < 0.05) than to infections caused by the other types in the logistic regression analysis.

**Information on the emm type distribution among patients who were injection drug users (IDU) was available for 359 of 471 (76%) cases; a majority of these (93%) were identified in the United Kingdom.** The 10 most prevalent types among these patients were, in descending order, emm83, emm87, emm82, emm89, emm81, emm43, emm33, emm101, emm1, and emm53; and these emm types accounting for 70% of infections among IDUs. Conversely, as many as 70% of the emm33, emm82, and emm83 infections and 54% of the emm43 infections were related to injection drug use.

Among 242 health care institution-associated infections, the same types as the overall 10 most prevalent ones caused the majority of infections (71%). However, emm1 and emm3 infections were less commonly related to surgery before disease onset, as determined by the regression model (P > 0.05 for each type).

Among patients with chicken pox, the probability that isolates of emm1 and emm12 were the cause was high (P < 0.001 for each type), which is in concordance with the high frequency of both types among children.

**CFRs and emm types.** The overall case fatality rate (CFR) over 7 days among cases whose isolates were typed was 19%; and the CFR was the highest among those with infections caused by emm3 (36%), followed by emm5, emm1, emm43, and emm77 (Table 2). Furthermore, the highest CFRs were noted, as expected, among cases with STSS (44%) and NF (31%) and, as already mentioned, were correlated with emm1 and emm3 infections. For patients with cellulitis, the overall CFR was
18%, but it was considerably higher for infectious caused by emm77 and emm3 (33%; P < 0.001 for each type) or emm1 (25%; P < 0.05) isolates. Among infections without a focus, the overall CFR was 15%; and the deaths were predominantly caused by emm3 (32%), emm83 (19%), emm87 (17%), emm1 (16%), and emm28 (15%) isolates (Table 2).

**SAg gene patterns and emm types.** As expected, speB, speF, and speG were detected in the vast majority of strains, although speG was lacking from the emm4 and emm77 isolates from several countries.

Data regarding speA and speC were available for 2,321 isolates. Overall, 30% and 54% of the isolates were positive for speA and speC, respectively. As shown in Table 4, speA was primarily associated with emm1 and emm3 (P < 0.001 for both types), whereas speC was common in several other types, such as emm4, emm5, emm6, emm28, and emm77 (P < 0.001 for each type) and emm18 (P < 0.01). Both emm1 and emm3 isolates harbored speC to a lesser extent (P < 0.001 for both types), and the same was true for emm81 and emm12 isolates (P < 0.05 for both). The speA gene was less prevalent among Finnish and Swedish strains (10% and 13%, respectively), which is ascribable to the emm type distribution in those countries, where both emm1 and emm3 isolates were less common than in the other countries (Fig. 1). However, among the emm1 and emm3 isolates from the Czech Republic, Denmark, and Finland, the frequencies of speA were lower (about 70% and 50% for each type, respectively), whereas the frequencies of speA were more than 90% among isolates of these types from the remaining countries (data not shown). Conversely, the high proportion of emm28 in Finland was reflected in an overall higher prevalence of speC-positive isolates (80%).

More than 800 isolates from five countries were investigated for the presence of speF, and only 1% of these isolates harbored the gene. The speH gene was detected in 10% of 1,667 isolates tested, most notably, in isolates of emm12 (65%; P < 0.001) and emm81 (19%; P < 0.01) (Table 4). The highest prevalence of speH among emm12 isolates was noted for Swedish (97%) and United Kingdom (91%) isolates, but surprisingly, speH was not detected among emm12 isolates from either Denmark or Finland. The ssa gene was detected in 31% of the isolates tested, primarily among isolates of emm3 and emm4 (P < 0.001 for both) but also among isolates of emm87 (P < 0.05). However, ssa was less frequently found among emm1, emm81, and emm89 isolates (P < 0.001 for each type) and among emm6 isolates (P < 0.05) (Table 4).

**DISCUSSION**

In the present paper, clinical and microbiological data obtained from patients with severe GAS infections from the 11 countries participating in the Strep-EURO program are presented. The number of isolates characterized (a total of 4,353) exceeds the number characterized in any previous European study. Strikingly, the overall distribution of the most prevalent emm types agreed closely with recent data reported from the United States, where emm1, emm3, emm28, emm12, and emm89 accounted for 55% of invasive isolates collected over a period of 4 years (2000 to 2004) (35). However, the country-specific emm type distributions differed markedly, as exemplified by emm87, which, although it was highly represented overall, it was essentially confined to the United Kingdom (Fig. 1). Differences in the proportions of the different types between neighboring countries, like Denmark, Finland, and Sweden, were also noted. In Sweden, high rates of emm81 and emm89 were seen, and these types accounted for 30% of isolates, whereas emm28 was the most prevalent type in Denmark (26%), and emm89 accounted for only 7% of cases (30). In Finland, 45% of all isolates were emm28, and Finland was the only country with such a large proportion of a single type. Isolates of emm3, in addition to those of emm1, have previously been shown to play a major role in invasive GAS disease (19, 45, 46). However, in Finland, the number of emm3 isolates was negligible (three cases); and a low prevalence of this type was also noted in Greece, the Czech Republic, and Sweden (3 to 4%). As shown in the Swedish study (18), the emm types of isolates causing invasive disease essentially agreed with those recorded for isolates causing noninvasive cases of GAS disease. Although noninvasive isolates were not studied in other participating countries, the country-specific type distributions may reflect, to a large extent, ongoing epidemic waves, herd immunity (39), or population mobility (11), as was previously seen for streptococcal disease (39).

There were significant differences between genders regarding their infection with some particular types. For example, emm28 and emm87 were overrepresented among females. The role of emm28 isolates in puerperal sepsis has already been recognized (2, 32), as isolates of this type are known to express R28, which is related to the Rib protein in group B streptococci, the major cause of neonatal infections (38, 39). Recently, it was shown that the gene encoding R28 is located on a 37.4-kb region (range of difference 2 [RD2]) that is similar in

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**TABLE 2—Continued**

<table>
<thead>
<tr>
<th>No. of patients/No. of patients infected with the following emm type (% CFR):</th>
</tr>
</thead>
<tbody>
<tr>
<td>emm83</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>113 (8)</td>
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<td>38/34 (19)</td>
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<tr>
<td>6/5 (33)</td>
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<td>10/9 (0)</td>
</tr>
<tr>
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</tr>
<tr>
<td>23/20 (5)</td>
</tr>
</tbody>
</table>

Downloaded from http://jcm.asm.org on September 22, 2017 by guest
content and organization to a region described in group B streptococci and that is apparently acquired by horizontal gene transfer; RD2 enables emm28 strains to often cause puerperal sepsis (24, 49). Since emm87 was not among those types carrying RD2 (e.g., emm2, emm4, emm48, emm77, and emm124), it is of interest to investigate whether emm87 isolates may harbor similar pathogenic factors. In contrast, emm83, emm81, and emm43 were associated with intravenous drug use and were preferentially found among male patients (68%, 62%, and 61%, respectively). Interestingly, a predominance of emm81 isolates among male patients with skin involvement was also found in Sweden (18).

It is known that no emm type can be uniquely associated with a particular disease, although there is evidence correlating certain types, e.g., emm1 and emm3, with the most severe GAS diseases, NF and STSS (12, 31, 43, 44), or emm28 with puerperal sepsis (36). However, in our material, 50% of all STSS cases and 55% of NF cases were caused by types other than emm1 and emm3, respectively, and in Sweden, no emm3 strain was involved with STSS, indicating that most types of GAS may have the potential to give rise to these severe manifestations. However, the rate of mortality associated with either an emm1 or an emm3 isolate, whether it causes STSS, NF, or puerperal sepsis, clearly exceeds that associated with the remaining types, which, in agreement with previous studies, demonstrates that these two types are particularly virulent.

Over the years, the number of GAS SAGs identified has increased, as has knowledge of their role in disease pathogenesis (8, 10, 14). The severity of disease is also determined by many other GAS virulence factors (41) and is clearly host dependent (28, 34). In the present study, a high rate of occurrence of speA was found among isolates of emm1 and emm3, types that were often involved in severe infections, and also for the less frequent type emm43; these emm types were associated

<table>
<thead>
<tr>
<th>TABLE 4. Presence of SAGs as related to clinical presentation and emm type</th>
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<tbody>
<tr>
<td>Disease manifestation or emm type</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>No focus</td>
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<td>STSS</td>
</tr>
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<td>Meningitis</td>
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</table>

* HAI, health care institution-associated infections.
with high CFRs (29%, 36%, and 21%, respectively). However, cases infected with isolates of emm5 and emm18 had high CFRs (30% and 21%, respectively), although these types lacked speA but harbored speC in high proportions (91% for both types). In addition, the presence of speC was common in several prevalent types, such as emm4, emm6, emm28, emm77, emm18, emm81, and emm12.

The emm types included in the 26-valent vaccine now in clinical trials (17) were generally well represented in the present study (Fig. 3A). Within the Strep-EURO program, 16 of the vaccine types accounted for 69% of the isolates, although the proportion of coverage varied among the participating countries (Fig. 3B) and the prevalence of some emm types changed temporally, which could at least partly be related to epidemic waves (6), the type substitution due to herd immunity, or the mobility of the population (11). Nevertheless, the total number of emm types detected exceeded 100, and the expansion of nonvaccine types (1) and a higher risk of infection by nonvaccine types (37), as recently experienced after the introduction of pneumococcal vaccination, pose obvious challenges to attempts to develop a type-specific vaccine.

In conclusion, among the 104 GAS emm types identified during the present project, 45 were the cause of STSS and/or NF. The major role that emm1 and emm3 isolates play in these severe disease entities, as has also been found in previous studies, was confirmed; however, a number of other types also caused high rates of mortality, suggesting that they have similar pathogenic potentials. In general, the SAg gene repertoire of the isolates appeared to correlate with the emm type in a complex pattern, precluding the formation of definite conclusions on the role of individual SAgS in severe disease. The data presented here, which demonstrated high rates of mortality and the devastating consequences of the manifestations of invasive disease, in particular, should be of value for preventive work, including ongoing attempts at creating vaccine prophylaxis against GAS disease.

ACKNOWLEDGMENTS

We direct our sincere thanks to all the clinicians and microbiologists across the 11 participating countries who took the time to report cases to their respective country leads. We also thank Helena Petterson at the Department of Epidemiology, Swedish Institute for Infectious Disease Control, for statistical analysis and advice.

The following additional members of the country teams also contributed significantly to the project: in Cyprus, Maria Alexandrou, Yiannis Ioannou, and Eleni Konteatou; in the Czech Republic, Radmila Dousova and Ivetu Mouchova; in Finland, Sari Rantala and Petri Ruutu; in France, Gislene Collobert; in Germany, Claudia Brandt; in Greece, Angeliki Stathi and Anastasia Pangalis; in Italy, Marco Patakacchia and Simona Recchia; in Sweden, Hans Tapper, Ulrich von Pawel-Rammingen, Madeleine Kais, Christina Johansson, Gunnel

TABLE 3—Continued

<table>
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<td>2 (0.3)</td>
<td>66 (10.8)</td>
</tr>
</tbody>
</table>

FIG. 3. Distribution of emm types among invasive GAS cases the countries participating in the Strep-EURO program with special regard to coverage by a 26-valent candidate vaccine. Types hypothesically covered or not covered by the vaccine candidate are indicated. (A) Prevalence of the 30 most common emm types. Among these, 16 (accounting for 69% of the reported cases) are included in the 26-valent vaccine (since subtypes were not assessed, vaccine subtype emm1.2 is not considered in the present discussion). Vaccine types emm14, emm19, and emm114 were not encountered. Other 1, other types included in the vaccine (6 emm types); Other 2, remaining types not covered by the vaccine (70 different emm types). (B) Proportions of country-specific emm types determined on the basis of potential coverage by the 26-valent vaccine. The numbers of emm types potentially covered or not covered are indicated below the graph for each country. Country abbreviations: CZE, the Czech Republic; DNK, Denmark; FIN, Finland; FRA, France; GER, Germany; GRC, Greece; ITA, Italy; ROU, Romania; SWE, Sweden; UK, the United Kingdom.
Möllerberg, and Ingrid Andersson; and in the United Kingdom, Chensh-Chal Dhami.

Financial support was provided by the EU Fifth Framework Research Programme (QLK2-CT-2002-013).

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