Clinical and Microbiological Characteristics of Severe *Streptococcus pyogenes* Disease in Europe

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ABSTRACT

In an attempt to compare the epidemiology of severe *S. pyogenes* infection within Europe, prospective data were collected through the Strep-EURO programme. Surveillance of severe *S. pyogenes* infection diagnosed during 2003 and 2004 was undertaken in eleven countries across Europe using a standardised 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 analysed for presence of superantigen genes. Data were analysed to compare the clinical and microbiological patterns of infections across participating countries.

Totally 4353 isolates were collected from 5521 cases with severe *S. pyogenes* infection identified. It was wide diversity of M/*emm*-types (104) found among the S. pyogenes clinical isolates but M/*emm*-type distribution varied broadly between participating countries. The ten most predominant M/*emm*-types were 1, 28, 3, 89, 87, 12, 4, 83, 81, and 5 in descending order. A correlation was found between some specific disease manifestation, age of patients and *emm*-types. Streptococcal toxic shock syndrome and necrotizing fasciitis, although caused by a large number of types, were particularly associated with M/*emm*-types 1 and 3.

The *emm*-types included in the 26-valent vaccine under development, were generally well represented in the presentmaterial; 16 of the vaccine types accounted for 69% of isolates. The Strep-EURO collaborative programme has contributed to enhance the knowledge on the spread of invasive disease caused by *S. pyogenes* within Europe and encourage future surveillance with notification of cases and characterisation of strains, important for vaccine strategies and other health care issues.

INTRODUCTION

Streptococcus pyogenes (group A streptococcus, GAS), a major human pathogen (9) studied for decades may give rise to common throat and skin infections, but also to invasive diseases, such as arthritis, septicaemia, cellulitis, puerperal fever, necrotising fasciitis (NF) and streptococcal toxic shock syndrome (STSS) (14). Since the mid 1980´s there are increasing numbers of reports describing severe GAS manifestations, however the underlying factors of this pathogens worldwide resurgence remaining unknown (20).

The M-protein, encoded by the *emm*-gene, is an important virulence factor, and also an epidemiological marker that are used throughout the world to characterize GAS (5, 21-23). The type specificity of the M-protein, of which there are more than 100 different types known, is largely determined by the epitope located in 40 to 50 amino acid residues at the amino-terminal (4, 16, 27). These regions of M-proteins have been shown to evoke antibodies with great bactericidal activity, not likely cross-reactive with human tissues (3, 16). Hence,, an approach in 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). Also other surface proteins, like the serum opacity factor (SOF) and the T-protein are used to characterize different GAS types. In addition to the known linkage between T-serotype, SOF production, and *emm*-type (25, 26), several studies also indicated correlations between *emm*-types, disease manifestations, and also other virulence factors, especially the superantigens (SAg) (7, 10, 40, 42).

Epidemiological studies, providing the type distributions in the communities, are of basic importance for identification and control of streptococcal infections. Furthermore, by tracing selected virulence features of isolates causing disease, the understanding of pathogenic

mechanisms of the various disease manifestations would be enhanced. In order to improve knowledge on severe GAS infections, the Strep-EURO programme was implemented during 2003-2004. Overall epidemiological findings of the programme were reported recently (29). In the present paper, type characteristics and SAg repertoire of the streptococcal isolates are described and also possible associations with clinical findings.

MATERIAL AND METHODS

Clinical data and isolates. Through collaboration between eleven European countries (Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Romania, Sweden and the UK) on the epidemiology of invasive GAS disease, enhanced surveillance was undertaken between January 1st 2003 and December 31st 2004. Methods employed to identify cases varied by country, but mostly implied invited submission of isolates from local microbiology laboratories to the national streptococcal reference centre. Demographic and clinical data, as well as risk factor information were collected through a standardized questionnaire, with the exception of Denmark and Sweden, where surveillance with earlier designed questionnaires 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 (45). Identification of GAS isolates was confirmed using morphological and growth characteristics, bacitracin susceptibility, or pyrolidonyl-arylamidase testing and latex agglutination with group A antisera commercially available.

Typing of isolates. Isolates were T-typed using commercial poly- and monospecific T-antisera, according to the manufacturer's recommendation (Sevapharma, Prague, Czech Republic) (Moody *et al.*, 1965). M/*emm*-typing was performed using somewhat different methods between countries,

thus evaluated and further described in an external quality assurance (EQA) study (33). Although both serological and/or genotypical methods were used to determine the M/*emm*-types, the results are hereafter referred to as *emm*-types. The *emm*-sequences obtained by sequence based methods were identified by comparisons to available sequences in the CDC database (ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/tsemm/). Unusual type-combinations between T- and *emm*-types (rare or previously not reported) were verified blindly by another participating reference center.

SAg gene detection. Presence of SAg genes was tested in Lund, Sweden for 1127 isolates from five countries (Czech Republic, Denmark, Finland, France, and Romania) and included *spe*A, *spe*B, *spe*C, *spe*F, *spe*G, *spe*H, *spe*I, *spe*J, *ssa*, and *sme*Z (30). Isolates from remaining countries were tested in the respective national centres: Swedish isolates were tested for all the above mentioned SAg genes but *spe*I (18), Greek and Italian isolates for *spe*A, *spe*B, and *spe*C (13), and German isolates for *spe*A, *spe*C, and *ssa* (47). A fraction consisting of 256 UK isolates (18% of the total *emm*-typed strains) were tested locally for the presence of *spe*A, *spe*B, and *spe*C. In addition, 193 isolates (covering 38 out of the 74 different *emm*-types identified in the UK) were tested in Lund as described above.

Statistical analysis. Data were analyzed using GraphPad Prism, version 4 (GraphPad Software) and SAS, version 9.1.3, proc logistic (SAS Institute). For nominal data, χ^2 test or Fisher's exact test were used when appropriate. Logistic regression was performed using *emm*-type as outcome and clinical conditions or risk factors as predictors. The analyses were performed separately for each of the 10 most prevalent *emm*-types, and compared to 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 where the significant level was set at 5%. In the logistic regression analyses, only cases with age, gender and clinical/ risk factor information available were included.

RESULTS

From a total of 5521 patients with invasive streptococcal disease, 4354 (79%) bacterial isolates were submitted to the reference centers in the participating countries. Clinical information was available for 3404 isolates (62% of all cases).

T-types. In total 4171 isolates were subjected to T-typing, 408 (10%) of these being non typeable (NT). Fifty different T-types, or type profiles, were recognized, the most prevalent were T1 (19%), T28 (18%), T3/13/B3264 (23%), T12 (8%), T4 (5%), T5 (3%), T6, T11, and T8/25/Imp19 (2% each) (table 1).

***emm*-types.** Among 4353 *emm*-typed isolates one hundred and four different types were identified, of which the most prevalent (≥ 2%) were *emm*1 (19%), 28 (12%), 3 (10%), 89 (8%), 87 (6%), 12 (5%), 4 (5%), 83 (3%), 81 (3%) and 5, 77, 6, 22, and 18 (2% each) (table 1). The type distribution varied significantly between the eleven countries, but the overall prevalence was strongly influenced by the large proportion of isolates originating from the UK (figure 1), and also from Sweden. Although *emm*87 and *emm*83 were the fifth and eighth overall most common types, majority of these isolates were from the UK (93% and 90% of isolates respectively). In total 34 different *emm*-types encompassed the ten most prevalent types in the eleven countries. Importantly, *emm*1 was the most abundant type in the majority of countries, with a proportion ranging between 15% and 33% of isolates. In contrast, within Denmark, Finland, and Sweden *emm*28 was the most prevalent type, ranging from 16% to 45% of isolates. As shown in figure1B, certain types among the overall ten most prevalent *emm*-types were absent in some of the countries; *e.g* in Romania only three of the overall ten most prevalent types were found. Type *emm*3 was infrequent in the Czech Republic, Finland, Greece, and Sweden with prevalence ranging from 1% to 5%, and absent in Romania. Type *emm*43 was found exclusively in the UK.

Other types almost confined to the UK were *emm*82 (93% from the UK), *emm*5 (91%), *emm*83 (90%), and *emm*68 (81%). Type *emm*53 was found only in the Czech Republic, Greece and the UK. All the *emm*118 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 unfrequented, other previously not reported (underlined in the table). The most prevalent T-type was 3/13/B3264 (or combinations thereof *e.g.* 3/13, 13/B3264, 3/B3264) and associated to no less than 40 different *emm*-types. In general, *emm*1 was limited to T1 (98%) but a small number of these isolates expressed T-types 3, 3/13/B3264 or 4.

Correlation between age, gender and *emm*-types. Among 600 isolates collected from children (0-17 years), the most frequent *emm*-types were, in descending order 1 (26%), 12 (11%), 4, 3 (10% each), and 28 (7%). In patients aged 18 and older, the most prevalent type was also *emm*1 (19%), but followed by *emm*28 (13%), *emm*3 (10%) and *emm*89 (9%).

A significant female predominance for *emm*87 and *emm*28 (58%, $p<0.001$ for both) was found. Type *emm*28 was also more prevalent in age groups 30-39 years (17%) and 70-79 years (19%), in the younger group strongly associated to females (80%, $p<0.001$). Types *emm*81 and *emm*83 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 (figure 2). Overall, 59% of cases were reported in the 6 winter months (January to April + November and December) in both years. In contrast, tendencies towards higher frequencies of *emm*12 was noted during the warmer months (May-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 *emm*1 was the most prevalent, accounting for 37% and 31% of cases respectively (table 2); in addition, a considerable proportion were caused by *emm*3 isolates (17% and 14%, respectively). In the statistical regression model, when comparing each of the 10 most prevalent types versus the other types combined, STSS was statistically more often caused by *emm*1 or *emm*3 ($p<0.001$ for each).

Patients without focal symptoms were less often infected by *emm*1 (17%, $p<0.05$), in contrast to types *emm*81 (45%), *emm*77 (47%) ($p<0.001$ for each), *emm*83 (34%), and *emm*87 (26%) ($p<0.05$ for each), that were more common among these patients (table 2.). Furthermore, patients with arthritis were less prone to be infected by *emm*28 isolates (5%, $p<0.05$), and cellulitis was more often caused by either *emm*87 (32%, $p<0.0001$) or *emm*83 (30%, $p<0.05$), as compared to infections caused by types other than the 10 most prevalent. Though puerperal sepsis was caused by 16 different types and only 8% of *emm*28 were patients with puerperal sepsis a clear correlation with *emm*28 was noted (31% of cases, $p<0.001$). Other *emm* types significantly involved in causing puerperal sepsis were *emm*1, *emm*89 and *emm*87 (4% each, $p<0.001$ and <0.05 respectively.

Data regarding risk factors, as well as *emm*-type, were available for 2796 patients (table 3). Patients with diabetes were statistically more prone to an infection caused by either *emm*81 ($p<0.001$) or *emm*12 ($p<0.05$), as compared to "other types" in the logistic regression analysis.

Information on *emm*-type distribution among patients who were injecting drug users (IDU) was available for 359 of 471 (76%) cases, a majority of these (93%)was identified in the UK. The ten most prevalent types among these patients were, in descending order: *emm*83, 87, 82,

89, 81, 43, 33, 101, 1 and 53, accounting for 70% of these infections. Conversely, as many as 70% of *emm*33, *emm*82 and *emm*83, and 54% of the *emm*43 infections were IDU related.

Among 242 health care associated infections (HAI), the same types as the over all ten most prevalent ones caused the majority of infections (71%). However, *emm*1 and *emm*3 infections were less commonly related to surgery before disease onset, as determined by the regression model ($p>0.05$ for each).

Among patients with chicken pox, the probabilities for *emm*1 and *emm*12 were high ($p<0.001$ each), which is in concordance with the high frequency of both types among children.

Case fatality rates and *emm*-types. Overall, the CFR over 7 days among cases with typed isolates was 19% and highest among infections caused by *emm*3 (36%), followed by *emm*5, 1, 43, and 77 (table 2). Furthermore, the highest CFRs were, as expected, noted among cases with STSS (44%) and NF (31%), and as already mentioned correlated to *emm*1 and 3 infections. For patients with cellulitis, the overall CFR was 18%, but considerably higher for infectious caused by *emm*77, *emm*3 (33% $p<0.001$ each), or *emm*1 (25%, $p<0.05$) isolates. Among infections without focus the overall CFR was 15%, and the deaths predominantly caused by *emm*3 (32%), *emm*83 (19%), *emm*87 (17%), *emm*1 (16%), and *emm* 28 (15%) infections (table 2).

SAg genes patterns and *emm*-types. As expected, *spe*B, *spe*F, and *spe*G, were detected in the vast majority of strains, though *spe*G was lacking among *emm*4 and *emm*77 isolates from several countries.

Data regarding *spe*A and *spe*C was available for 2321 isolates. Overall, 30% and 54 % were positive for *spe*A and *spe*C, respectively. As shown in table 4, *spe*A was primarily associated with *emm*1 and *emm*3 ($p<0.001$ for both), whereas *spe*C was common in several other types such as *emm*4, 5, 6, 28 and 77 ($p<0.001$ for each), *emm*18 ($p< 0.01$). Both *emm*1 and *emm*3 harboured

*spe*C to a lesser extent ($p<0.001$ for both) and the same was true for *emm* 81and 12 isolates ($p<0.05$ for both). The *spe*A gene was less prevalent among Finnish and Swedish strains (10% and 13%, respectively), ascribable to the *emm*-type distribution in these countries where both *emm*1 and 3 isolates were less common than in the other countries (figure 1). However, among *emm*1 and *emm*3 isolates from the Czech Republic, Denmark and Finland frequencies of *spe*A were lower, about 70% and 50%, for each type respectively, as compared to more than 90% among these isolates from remaining countries (data not shown). Conversely, the high proportion of *emm*28 in Finland was reflected in an overall higher prevalence of *spe*C positive isolates (80%).

The presence of *spe*I was investigated in more than 800 isolates from five countries, and only one percent of these isolates harboured the gene. The gene *spe*H was detected in 10% of 1667 isolates tested, most notably in *emm*12 (65%; $p<0.001$) and *emm*81 (19%; $p<0.01$) (table 4). The highest prevalence of *spe*H among *emm*12 isolates was noted for Swedish (97%) and UK (91%) isolates, but surprisingly, *spe*H was not detected among *emm*12 isolates from either Denmark or Finland. The gene *ssa* was detected in 31% of tested isolates, primarily among *emm*3 and *emm*4 ($p<0.001$ for both) but also among *emm*87 isolates ($p<0.05$). However, *ssa* was less frequently found among *emm*1, *emm*81, *emm*89 ($p<0.001$ each) and *emm*6 ($p<0.05$) isolates (table 4).

DISCUSSION

In the present paper clinical and microbiological data obtained from patients with severe GAS infections from the eleven Strep-EURO participating countries are presented. The number of characterized isolates (4353) exceeds any previous European study. Strikingly, the overall distribution of the most prevalent *emm*-types agreed closely with recent data reported from the US where *emm*-types 1, 3, 28, 12, and 89 accounted for 55% of invasive isolates collected over a period of four years (2000-2004) (35). However, the country-specific *emm*-type distributions differed markedly, as exemplified by. *emm*87 though overall highly represented, essentially confined to the UK (figure 1). Differences in type proportions were also noted between neighbouring countries, like Denmark, Finland, and Sweden. In Sweden, high rates of *emm*81 and *emm*89 was seen, accounting for 30% of isolates, whereas *emm*28 was the most prevalent type in Denmark (26%), and *emm*89 only accounted for 7% of cases (30). In Finland, 45% of all isolates were *emm*28, being the only country with such a large proportion of a single type. Isolates of *emm*3, in addition to *emm*1, have previously been shown to be of major role in invasive GAS disease (19, 46, 48). However, in Finland, the number of *emm*3 isolates was negligible (3 cases), and a low prevalence of this type was also noted in Greece, the Czech Republic and Sweden (3-4%). As shown in the Swedish study (18) *emm*-types of invasive cases essentially agreed with those recorded among cases with non-invasive GAS disease. Though non-invasive isolates were not studied in other participating countries, the country-specific type distributions may to a large extent reflect ongoing epidemic waves, herd immunity (39) or population mobility (11), as previously seen for streptococcal disease (39).

There were significant differences between genders regarding some particular types. For example, *emm*28 and *emm*87 were overrepresented among female cases. The role of *emm*28

isolates in puerperal fever has already been recognized (2, 32), as 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 (region of difference – RD2) similar in content and organization to a region described in group B streptococci, apparently acquired by horizontal gene transfer and enabling *emm*28 strains to often cause puerperal sepsis (24, 49). Since *emm*87 was not among those types carrying RD2 (*e.g.* M2, 4, 48, 77, 124), it is of interest to investigate whether *emm*87 isolates may harbor similar pathogenic factors. In contrast, *emm*-types 83, 81, and 43, were associated with intravenous drug use and found preferentially among male patients (68%, 62%, and 61% respectively). Interestingly, also a predominance of *emm*81 isolates among male patients with skin involvement were found in Sweden (18).

It is known that no *emm*-type can be uniquely associated to a particular disease, though there is evidence correlating certain types, *e.g. emm*1 and *emm*3 with the most severe GAS diseases NF and STSS (12, 31, 43, 44), or *emm*28 with puerperal sepsis (36). However, in our material 50% of all STSS cases and 55% of NF cases were caused by types other than *emm*1 and *emm*3 respectively, and in Sweden no *emm*3 strain was involved in STSS, indicating that most types of GAS may have the potential to give rise to these severe manifestations. However, the mortality associated with either *emm*1 or *emm*3, whether causing STSS, NF or puerperal sepsis, clearly exceed that of remaining types which, in agreement with previous studies, demonstrates these two types as particularly virulent.

Over the years, the number of GAS SAgs identified have increased, and also the knowledge on their role in disease pathogenesis (8, 10, 14).The disease severity is also determined by many other GAS virulence factors (41) and is clearly host dependent (28, 34). In the present

study, a high occurrence of *spe*A was found for isolates of *emm*-types 1 and 3, types that were often involved in severe infections, and also for the less frequent type *emm*43; these *emm*-types were associated with high CFRs (29%, 36%, and 21%, respectively). However, *emm*5 and *emm*18 cases had high CFRs (30%, and 21%, respectively), though these types lacked *spe*A but harboured *spe*C at high proportions (both 91%). In addition, the presence of *spe*C was common in several prevalent types such as *emm*4, 6, 28, 77, 18, 81 and 12.

The *emm*-types included in the 26-valent vaccine now in clinical trial (17) were generally well represented in the present study (figure 3A). Within Strep-EURO, 16 of the vaccine types accounted for 69% of isolates, though proportion of coverage varied among participating countries (figure 3B), and the prevalence of some *emm*-types changed temporally, which could be at least partly related to epidemic waves (6), type substitution due to herd immunity, or population mobility (11). Nevertheless, the total number of *emm*-types detected exceeded one hundred, and expansion of non-vaccine types (1) and higher risk of infection by non-vaccine types(37), as is the case for recent pneumococcal experience after introduction of vaccination posing an obvious challenge to attempts of type-specific vaccine development.

In conclusion, among 104 GAS *emm*-types identified during the present project, 45 were involved in causing STSS and/or NF. A major role of *emm*1 and *emm*3 isolates in these severe entities, also found in previous studies was confirmed; however, a number of other types also caused high mortality rates, suggesting similar pathogenic potential. In general, the SAg gene repertoire of isolates appeared to correlate with *emm*-type in a complex pattern, precluding definite conclusions on the role of individual SAg for severe disease. The data here presented, demonstrating high mortality and devastating consequences of the invasive manifestations in

particular, should be of value for preventive work, including ongoing attempts at creating vaccine prophylaxis against GAS disease.

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Table 1. Type distribution among invasive GAS isolates collected within eleven Strep-EURO participating countries during 2003-2004

emm-type	No. of isolates	No. of T/*emm*-type combinations	T-type (no. of isolates)*
1	819	5	1 (802); 3 (1), 3/13/B3264 (1); 4 (2); NT (8); NA (5)
2	35	5	2 (6); 2/28 (13); 12 (1); 28 (1); NT (4)
3	431	8	3 (175), 3/13 (1), 3/13/B3264, (180); 3/B3264, (5); B3264 (1), 13/B3264 (1),13 (1); NT (52); NA (15)
4	199	3	4 (181); 2/4 (1); NT (13); NA (4)
5	98	5	5 (67), 5/27 (1), 5/27/44 (4); 11 (1); NT (15); NA (10)
6	91	3	6 (77); 12 (1); NT (9); NA (4)
8	2	2	8 (1); NT (1)
9	21	3	9 (17); 5/12 (1); NT (3)
11	48	3	11 (33); 8/11 (1); NT (11); NA (3)
12	227	3	12 (216); 11/12/27 (1); NT (9); NA (1)
13	1	1	13 (1)
15	1	1	15/17/19/23/47 (1)
18	66	3	8/25/Imp19 (2); 3/B3264 (1); NT (23); NA (40)
22	72	6	12 (57); 12/3/13/B3264 (1); 3/13 (1); 4 (1); 5/12 (1); NT (7); NA (4)
24	1	1	NT
25	8	3	25 (2); 14 (2); 1 (4)
26	3	1	NT (3)
27G	10	4	5/27/44 (4), 5/27 (1), 5 (3); NT (2)
28	505	9	28 (458); 2/28 (1); 28/11 (1); 28/11/12 (2); 3/13/B3264 (1), 3/B3264 (2); 12 (2); 14 (1); NT (31); NA (6)
29	8	1	4 (1); NT (6); NA (1)
30	1	1	NT
32	5	1	23 (5)
33	35	4	3/13/B3264 (20), 3 (4), 13 (6); NT (5)
36	1	1	NA
41	4	3	3/13/B3264 (2); 6 (1); NT (1)
43	51	5	3/13/B3264 (17), 3 (10); 28 (1); 15/17/19/23/47 (1); NT (9); NA (13)
44/61	30	7	5 (17), 5/27/44 (2), 27/44 (1); 12/27/44 (1); 11 (3); 8/11 (1); NT (2); NA (3)
48	1	1	4/28
49	16	4	14 (12); 3/B3264 (1); 8/25/Imp19 (1); NT (1); NA (1)
50/62	4	2	12 (3); NT (1)
52	3	2	3/13/B3264 (1); NT (2)
53	25	7	3/13/B3264 (5), 3 (2); 3/13/B3264/28/8 (1); 8/25 (1), 8/25/Imp19 (1); 28 (3); NT (12)
55	1	1	8/25/Imp19 (1)
57	1	1	NT
58	22	5	8/25/Imp19 (11), 8/25 (1), 8 (2); 2 (1); NT (4); NA (3)
59	5	4	11 (2); 8/25/Imp19 (1); 14/25 (1); NT (1)
60	5	2	4 (4); B3264 (1)
63	3	1	4 (3)
64	5	2	3 (1); NT (4)
65	3	2	3/B3264 (2); 8/25/Imp19 (1)
66	8	2	12 (5); NT (3)
67	1	1	3 (1)
68	16	6	3/13/B3264 (11), 3/B3264 (1), B3264 (1); 8 (1); 12 (1); 8/25/Imp19 (1)
69	2	2	3/13/B3264 (1), 3/B3264 (1)
70	1	1	5/11/27 (1)
71	1	1	8/25/imp19 (1)

73	37	6	3/13/B3264 (18), 13/B3264 (1), B3264 (5), 3 (2), 13 (8); NT (2); NA (1)
74	3	1	NT (3)
75	64	6	8/25/Imp19 (33), 8/25 (10), 8 (1), 25 (15); 12 (1); NT (3); NA (1)
76	15	4	12 (8); 22 (2); 23 (1); 8 (1); NA (3)
77	97	7	11 (4); 13 (29), 3/13/B3264 (4), B3264 (2); 13/28 (20); 28 (30);; NT (5); NA (3)
78	39	5	11 (32); 11/12/27 (1); 3/B3264 (1); 5/27/44 (1); NT (2); NA (2)
79	21	8	1 (1); 11 (3); 12 (1); 13 (1); 8 (5); 8/25/Imp19 (1); B3264 (1); NT (6); NA (2)
80	5	3	3/13/B32647 (2); 14/8/25 (1); NT (2)
81	143	11	12 (20); 3/13/B3264 (44), 13/B3264 (1), B3264 (24), 3/13 (1), 3 (4); 5/17/19/23/47 (2); 3/12/B3264 (2); 4 (1); 8 (4); NT (32); NA (8)
82	58	5	12 (1); 5 (42), 5/27 (1), 5/27/44 (1)
83	150	7	13 (7), 3 (20), 3/13 (1), 3/13/B3264 (78), 8 (1); 8/25 (2); NT (22); NA (19)
84	3	2	8/25/Imp19 (1); NT (2)
85	10	5	12 (1); 3 (5), 3/13 (1); 3/9 (2); NT (1)
87	256	6	11 (1); 28 (242); 3/13/B3264 (1); 4 (1); 8/25/Imp19 (1); NT (4); NA (6)
88	11	3	8/25/Imp19 (6), 8 (4); NT (1)
89	343	9	11 (2); 13 (3); 28 (1); 3 (15), 3/13/B3264 (262), 3/B3264 (12), B3264 (21); 8 (1); NT (16); NA (10)
90	4	2	3/13/B3264 (2); NT (1); NA (1)
91	12	5	3 (1), 13 (1); 25/Imp19 (1); 5 (2); NT (4); NA (3)
92	4	3	8/25/Imp19 (2), Imp19 (2); NT (1)
93	6	3	14 (2); 3/13/B3264 (1); NT (3)
94	11	4	3/13/B3264 (9), 3/B3264 (1); NT (1)
95	10	1	6 (3); 8/25 (1), 8/25/Imp19 (1); NT (1); NA (4)
96	2	1	13 (2)
97	1	3	14
100	3	5	3/13 (1); 8/25/Imp19 (1); NT (1)
101	17	6	13 (1); 28 (1); 3 (3); 3/13/B3264 (6); NT (3); NA (3)
102	13	1	12 (1); 13/B3264 (1), 3 (2), 3/13/B3264 (7); 4 (1); NT (1)
103	3	3	NT (2); NA (1)
104	5	2	11 (1); 8/11 (1); NT (3)
106	2	3	5 (1); NT (1)
108	9	1	5/12/27 (1); 6 (3); NT (5)
109	1	3	6 (1)
110	6	1	12 (2); 8 (1); NT (2); NA (1)
111	2	2	NT (1); NA (1)
112	4	1	8 (1); 13 (1); NA (2)
113	1	1	12
115	1	2	NT
117	5	8	28 (1); NT (4)
118	34	1	3/13/B3264 (23), 3/13 (3), B3264 (2), 3 (1), 13 (1); 6 (1); 11 (1); NT (2)
119	2	1	NT (2)
120	2	2	3 (2)
122	2	1	3/13/B3264 (1); NT (1)
124	2	1	NT (2)
*emm*st369	1	1	14
*emm*st1389	2	1	3/13/B3264 (2)
*emm*st2037	1	1	8/25/Imp19
*emm*st2147	1	1	5/27/44
*emm*st2460	1	1	3/13/B3264
*emm*st2904	2	1	NT
*emm*st3757	2	1	NT
*emm*st6735	1	1	5/27/44

*emm*st11014	2	1	4 (1); NA (1)
*emm*stD633	1	1	3/13/B3264
*emm*stNS1033	20	3	28 (6); 8/25/Imp19 (1); NT (2); NA (11)
*emm*stG6	1	1	8/25/Imp19
*emm*stG1750	1	1	NT
*emm*stG62647	1	1	12
*emm*st221	1	1	NT
*emm*st4986	1	1	4
NA	1168		3/8/B3264 (2), 3/B3264 (2), B3264 (5); 3/9/13 (1); 5 (1); 8 (2); 8/25/Imp19 (2); NT (6); NA (1147)
Total	5521	314	4171

Note. Underlined are uncommon T/*emm*-type combinations, according to the CDC homepage and previous publication (26).

T-types belonging to the same pool are separated by comma (,) and distinct patterns by semicolon (;)

NA, not available; NT, not typable; *emm*st, *emm* sequence type not yet assigned (as described at the CDC homepage).

* The number of isolates of each specific T/*emm*-type combination is described within brackets.

Table 2. Disease manifestations and case fatality rates (CFR) for 15 most prevalent *emm*-types.

	emm-typed	*emm*-type no/% (CFR %)															
		1	28	3	89	87	12	4	83	81	5	77	6	18	75	43	Other
Total with clin. info.	3458	694	356	333	276	217	191	161	113	121	74	77	75	51	43	38	584
CFR (%)	(19)	(29)	(14)	(36)	(13)	(19)	(17)	(10)	(8)	(10)	(30)	(20)	(18)	(21)	(9)	(21)	(11)
No focus	859	117/17	109/31	70/21	88/32	57/26	42/22	40/25	38/34	55/45	16/22	36/47	16/21	8/16	13/30	7/18	147/25
	(15)	(16)	(15)	(32)	(14)	(17)	(13)	(9)	(19)	(2)	(40)	(12)	(13)	(0)	(8)	(33)	(10)
STSS	476	174/25	32/9	83/25	18/7	12/6	27/14	20/12	6/5	14/12	4/5	7/9	9/12	8/16	3/7	1/<1	58/10
	(44)	(47)	(40)	(49)	(47)	(42)	(50)	(33)	(33)	(30)	(0)	(57)	(60)	(63)	(0)	(0)	(3)
NF	296	92/13	18/5	40/12	16/6	5/2	13/7	19/12	5/4	19/16	7/9	8/10	4/5	5/10	2/5	1/<1	42/7
	(31)	(36)	(13)	(51)	(36)	(50)	(39)	(13)	(0)	(21)	(43)	(25)	(0)	(75)	(0)	(0)	(13)
Cellulitis	865	177/26	74/21	95/29	72/26	71/33	49/26	36/22	34/30	20/17	21/28	14/18	15/20	12/24	8/19	16/42	151/26
	(18)	(25)	(18)	(33)	(17)	(14)	(17)	(9)	(0)	(6)	(14)	(33)	(0)	(20)	(14)	(18)	(10)
Arthritis	302	65/9	18/5	38/11	27/10	12/6	22/12	16/10	10/9	11/9	4/5	9/12	4/5	3/9	6/14	2/5	55/9
	(10)	(17)	(18)	(17)	(8)	(0)	(0)	(0)	(0)	(0)	(0)	(22)	(0)	(0)	(0)	(0)	(9)
Puerperal sepsis	96	15/2	30/8	7/2	11/4	9/4	4/2	3/2	-/-	-/-	-/-	1/1	2/3	1/1	-/-	-/-	13/2
	(4)	(18)	(0)	(17)	(0)	(0)	(0)	(0)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Meningitis	59	16/2	8/2	11/19	2/1	2/1	3/2	3/1	-/-	-/-	1/1	1/1	2/2	-/-	-/-	-/-	10/2
	(24)	(36)	(0)	(56)	(0)	(0)	(0)	(33)	(-)	(-)	(0)	(0)	(0)	(-)	(-)	(-)	(22)
Other	835	189/27	86/24	71/21	57/21	48/22	51/27	33/20	23/20	14/12	24/32	9/12	32/43	20/39	14/32	11/29	153/26
	(26)	(39)	(22)	(53)	(4)	(28)	(20)	(8)	(5)	(31)	(36)	(60)	(35)	(24)	(9)	(22)	(12)

Note. STSS, streptococcal toxic shock syndrome; NF, necrotizing fasciitis.

Table 3. Risk factor data of cases caused by 15 most prevalent *emm*-types.

		emm-type (no/%)														
	Total	1	28	3	89	87	12	4	83	81	5	77	6	18	75	43
Cases with risk factor information	2796	525/18.8	287/10.3	278/9.9	238/8.5	190/6.8	154/5.5	137/4.9	109/3.9	106/3.8	61/2.2	65/2.3	64/2.3	40/1.4	27/1.0	37/1.3
Diabetes	232	38/16.4	27/11.6	31/13.4	21/9.1	14/6.0	16/6.9	10/4.3	6/2.6	23/9.9	5/2.2	8/3.4	4/1.7	3/1.3	0/0	5/2.2
IDU	359	10/2.8	4/1.1	7/1.9	24/6.7	37/10.3	8/2.2	5/1.4	74/20.6	20/5.6	2/0.6	2/0.6	1/0.3	1/0.3	3/0.8	20/5.6
Chicken pox	72	29/40.3	3/4.2	6/8.3	1/1.4	4/5.6	13/18.1	4/5.6	0/0	0/0	0/0	1/1.4	3/4.2	0/0	1/1.4	0/0
Immunocomprom.	478	92/19.2	45/9.4	50/10.5	44/9.2	31/6.5	31/6.5	20/4.2	12/2.5	20/4.2	18/3.8	16/3.3	10/2.1	10/2.1	7/1.5	6/1.3
Skin lesions	593	128/21.6	54/9.1	57/9.6	46/7.8	49/8.3	35/5.9	25/4.2	24/4.0	17/2.9	11/1.9	16/2.7	6/1.0	5/0.8	5/0.8	10/1.7
Surgery	151	24/15.9	21/13.9	10/6.6	12/7.9	14/9.3	10/6.6	9/6.0	2/1.3	4/2.6	6/4.0	2/1.3	6/4.0	5/3.3	1/0.7	3/2.0
HAI*	242	37/15.3	39/16.1	16/6.6	22/9.1	18/7.4	17/7.0	15/6.2	2/0.8	5/2.1	9/3.7	2/0.8	7/2.9	5/2.1	3/1.2	4/1.7
Other	782	148/18.9	112/14.3	70/9.0	75/9.6	24/3.1	39/5.0	35/4.5	11/1.4	53/6.8	11/1.4	35/4.5	23/2.9	8/1.0	11/1.4	1/0.1
None reported	613	137/22.3	73/11.9	85//13.9	54/8.8	44/7.2	31/5.1	49/8.0	5/0.8	9/1.5	17/2.8	8/1.3	16/2.6	14/2.3	3/0.5	2/0.3

Note. HAI, health care associated infections; IDU, injecting drug users.

Table 4. Presence of superantigens as related to clinical presentation and *emm*-type

	*spe*A		*spe*C		*spe*F		*spe*G		*spe*H		*spe*I		*spe*J		*sme*Z		*ssa*	
	a	%	a	%	a	%	a	%	a	%	a	%	a	%	a	%	a	%
No focus	87/475	18	295/475	62	415/422	98	402/436	92	61/437	14	0/76	0	74/404	18	316/403	78	72/443	16
STSS	187/363	52	138/363	38	240/265	91	218/238	92	18/238	8	0/74	0	15/123	12	59/133	52	66/294	22
NF	93/224	42	83/224	37	134/145	92	130/146	89	9/145	6	0/47	0	15/99	15	56/105	53	37/172	22
Cellulitis	128/371	35	168/371	45	179/205	87	194/198	98	13/198	7	3/151	2	9/153	6	17/162	11	38/225	17
Arthritis	57/175	33	91/175	52	122/130	94	122/137	89	19/137	14	0/39	0	15/112	13	75/114	66	28/142	20
Puerperal sepsis	15/61	25	41/61	67	48/54	89	50/52	96	3/52	8	1/18	6	3/31	10	15/33	46	8/58	14
Meningitis	23/43	53	20/43	47	31/33	94	24/25	96	2/25	8	1/15	7	3/15	20	5/16	31	12/40	30
Other clin. pres.	135/347	39	174/347	50	244/258	95	184/191	96	15/191	8	2/146	1	10/149	7	25/163	15	28/281	10
M/*emm*1	400/456	88	88/456	19	285/329	87	280/289	97	5/289	2	1/143	1	35/203	17	67/208	32	79/362	22
M/*emm*28	31/387	8	343/387	89	343/355	97	311/322	97	15/322	5	1/199	1	72/289	25	106/291	36	114/370	31
M/*emm*3	153/182	84	34/182	19	123/128	96	107/109	98	7/109	6	1/66	2	1/86	1	25/86	29	114/133	86
M/*emm*89	9/169	5	88/169	52	140/146	96	136/138	99	5/138	4	0/47	0	2/131	2	86/131	66	24/148	16
M/*emm*87	4/52	8	38/52	73	20/34	59	34/34	100	1/34	3	0/24	0	8/33	24	10/33	30	18/35	51
M/*emm*12	11/129	9	83/129	64	89/90	99	82/83	99	54/83	65	2/35	6	4/72	6	47/75	63	25/95	26
M/*emm*4	8/120	7	111/120	93	82/92	89	52/88	59	3/88	3	0/26	0	3/66	5	44/68	65	81/96	84
M/*emm*83	7/34	21	16/34	47	25/27	93	26/26	100	0/26	0	0/17	0	0/25	0	9/26	35	4/27	15
M/*emm*81	2/112	2	49/112	44	91/95	96	102/105	97	20/104	19	0/30	0	6/99	6	48/102	47	6/105	6
M/*emm*5	4/23	17	21/23	91	6/9	67	8/8	100	0/8	0	0/6	0	0/8	0	2/8	25	0/10	0
M/*emm*77	3/76	4	57/76	75	60/62	97	27/59	46	4/60	7	0/20	0	0/55	0	35/55	64	13/67	19
M/*emm*6	8/44	18	39/44	89	28/34	82	27/28	96	2/28	7	1/11	9	4/20	20	10/20	50	8/34	24
M/*emm*18	11/22	50	20/22	91	5/11	46	11/13	85	1/13	8	0/7	0	0/11	0	3/11	27	1/14	7
M/*emm*75	3/43	7	23/43	53	30/37	81	28/34	82	0/34	0	0/19	0	0/24	0	8/26	31	7/38	18

NOTE. STSS, streptococcal toxic shock syndrome; NF, necrotizing fasciitis.
a – positive isolates/no. of tested isolates

Fig. 1A

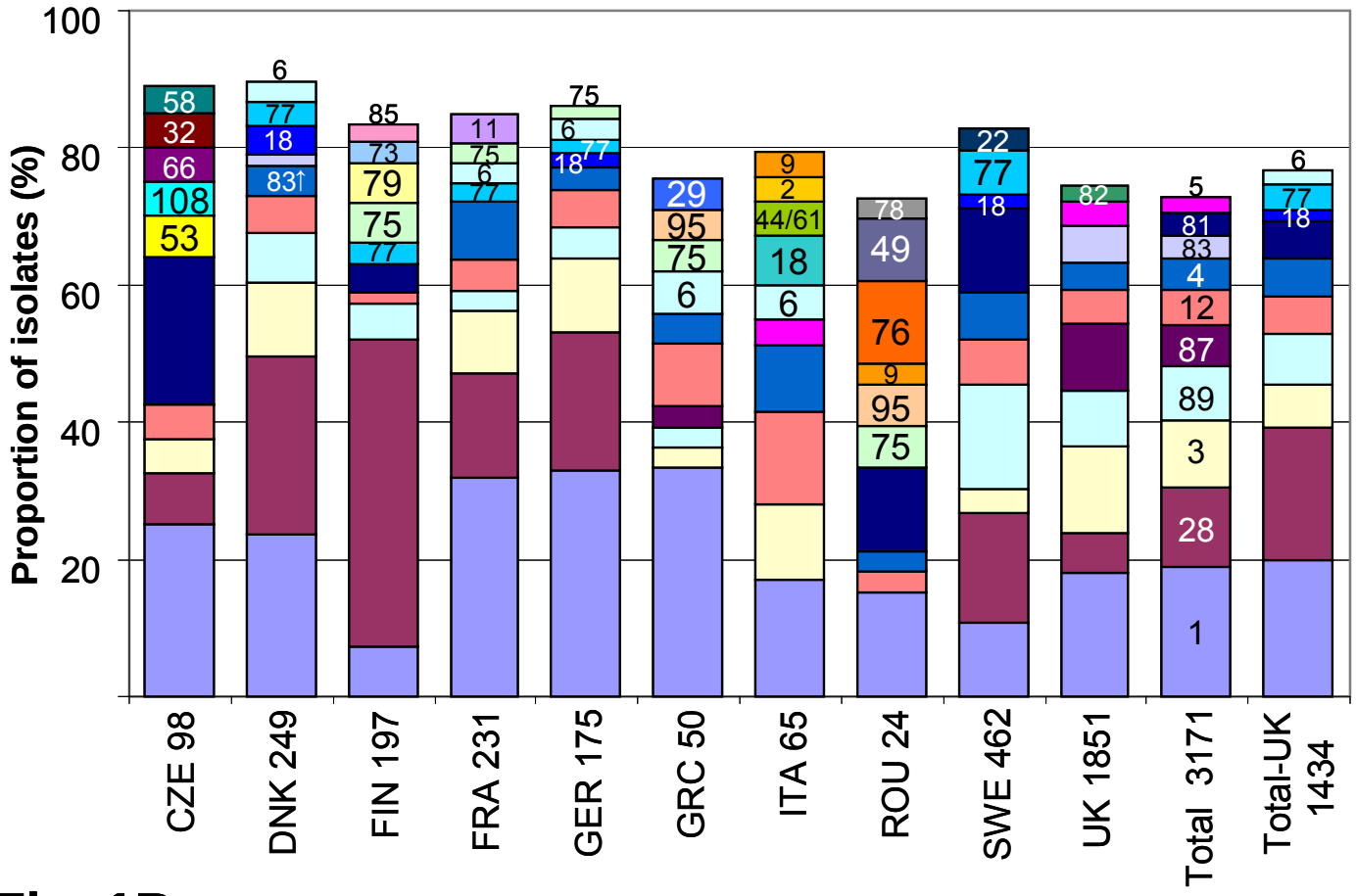

Proportion of isolates (%)
100
80
60
40
20
CZE 98
DNK 249
FIN 197
FRA 231
GER 175
GRC 50
ITA 65
ROU 24
SWE 462
UK 1851
Total 3171
Total-UK 1434

Fig. 1B

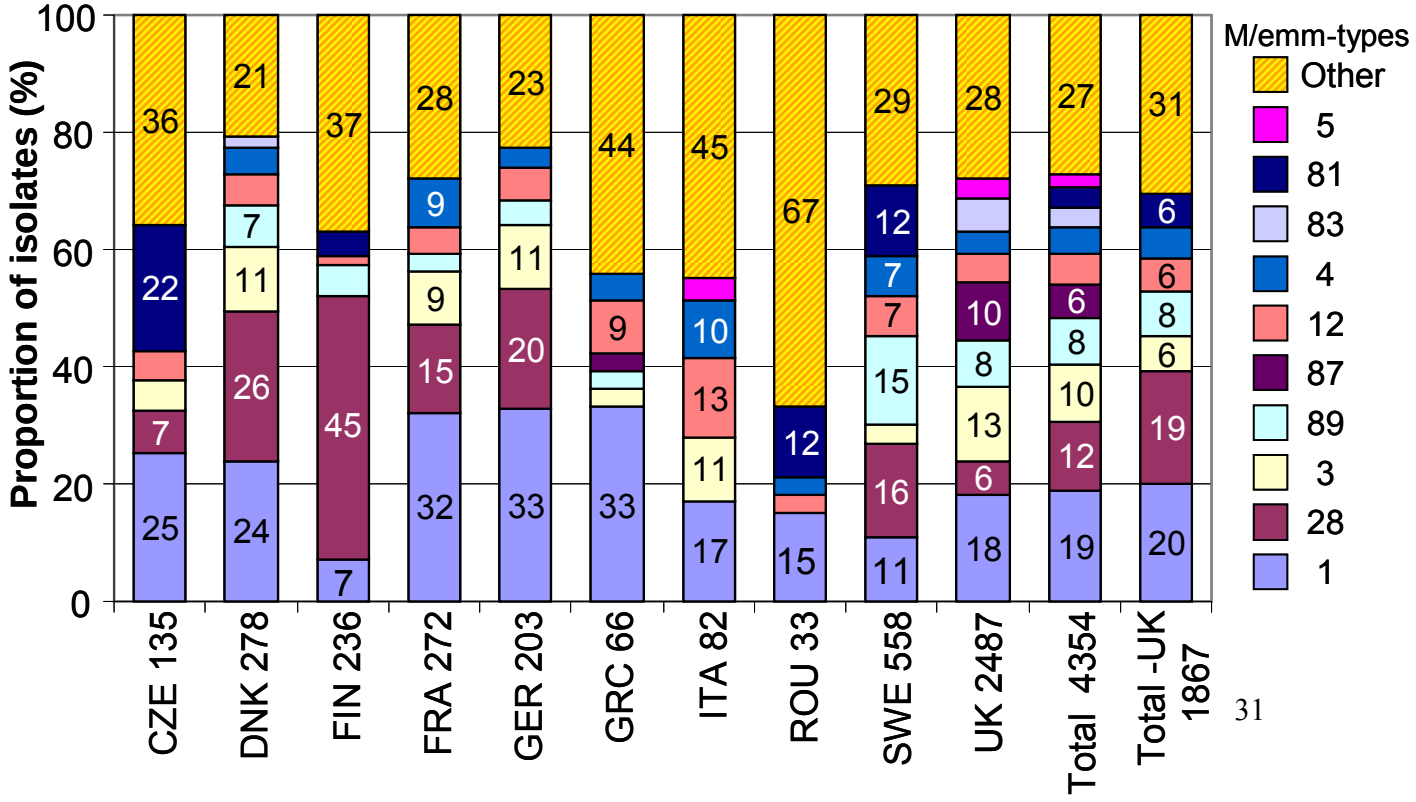

Proportion of isolates (%)
100
80
60
40
20
0
CZE 135
DNK 278
FIN 236
FRA 272
GER 203
GRC 66
ITA 82
ROU 33
SWE 558
UK 2487
Total 4354
Total -UK 1867
M/emm-types
Other
5
81
83
4
12
87
89
3
28
1

Fig. 2

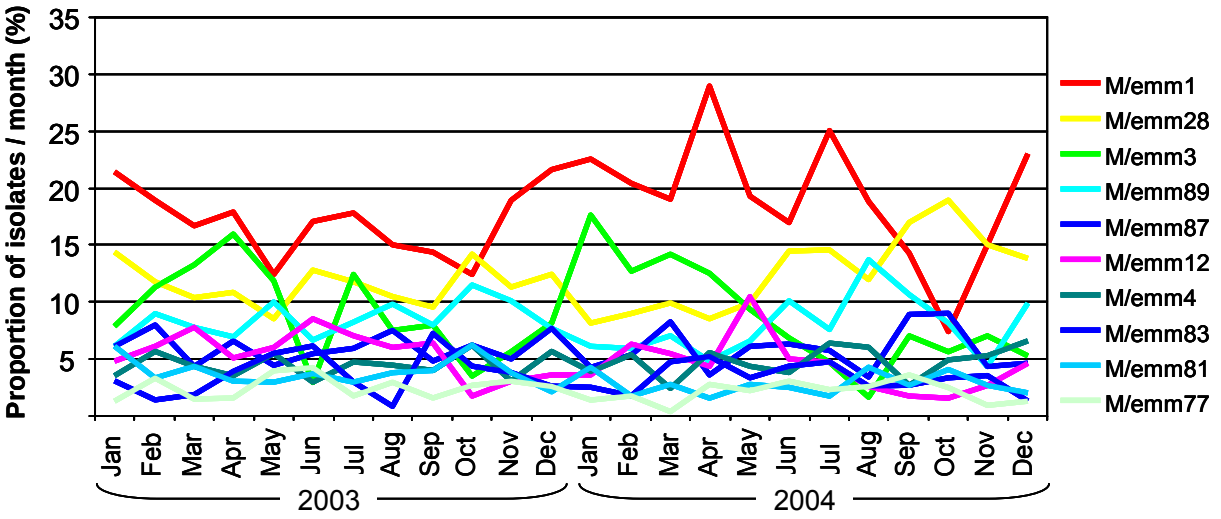

Proportion of isolates / month (%)
35
30
25
20
15
10
5
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
2003
2004
M/emm1
M/emm28
M/emm3
M/emm89
M/emm87
M/emm12
M/emm4
M/emm83
M/emm81
M/emm77

Fig. 3A

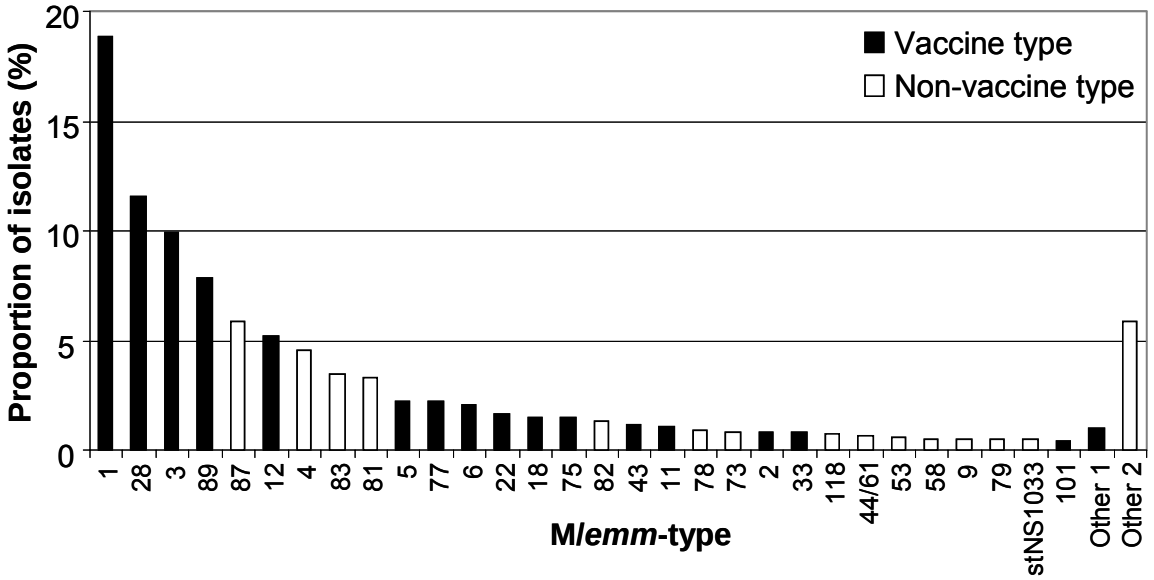
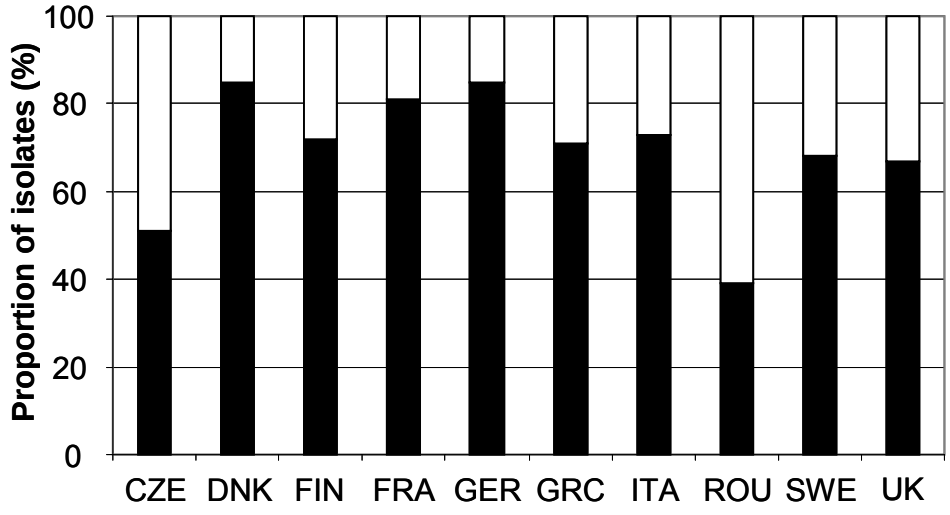


Fig. 3B



	CZE	DNK	FIN	FRA	GER	GRC	ITA	ROU	SWE	UK
Vaccine *emm*-types	14	15	10	14	13	12	15	5	14	21
Non- vaccine *emm*-types	20	14	20	22	19	11	8	14	32	53

Figure 1. *emm*-type distribution among the ten participating Strep-EURO countries in years 2003-2004. Numbers of isolates presented for each country are indicated beneath each bar. A) The distribution of the ten most prevalent *emm*-types within each country and overall, with and without UK isolates. The 10 overall prevalent types are indicated in the figure, within the bar for "Total", and other types indicated for each country. B) The distribution of the ten overall most prevalent *emm*- types. Types of more than 5% of all types within a country are indicated by numbers in the figure. Abbreviations for countries: CZE, the Check Republic; DNK, Denmark; FIN, Finland; FRA, France; GER, Germay; GRC, Greece; ITA, Italy; ROU, Roumania; SWE; Sweden; UK, the United Kingdom.

Figure 2. Overall seasonal fluctuation of the ten most prevalent *emm*-types among the Strep-EURO participating countries. Presentages are calculated by number of each major type divided to total number of isolates per month.

Figure 3. Distribution of *emm*-types among Strep-EURO invasive GAS cases, with special regard to coverage by a 26-valent candidate vaccine. Types hypothetically covered or not by the vaccine candidate are indicated.

A) Prevalence of 30 most common *emm*-types. Among these, 16 (accounting for 69% of reported cases) are included in the 26-valent vaccine (since subtypes were not assessed, vaccine subtype *emm*1.2 is not considered in the present discussion). Vaccine types *emm*14, *emm*19, and *emm*114 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) Country-specific *emm*-type proportions based on potential coverage by the 26-valent vaccine. Numbers of *emm*-types potentially covered or not are indicated below the graph for each country.

1 Abbreviations for countries: CZE, the Check Republic; DNK, Denmark; FIN, Finland; FRA,
2 France; GER, Germay; GRC, Greece; ITA, Italy; ROU, Roumania; SWE; Sweden; UK, the
3 United Kingdom.