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

3
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15 **Key words:** *Streptococcus pyogenes*; Virulence factors; *emm*-type; Superantigens; Streptococcal
16 toxic shock syndrome; necrotizing fasciitis; Puerperal sepsis

1 **ABSTRACT**

2 In an attempt to compare the epidemiology of severe *S. pyogenes* infection within Europe,
3 prospective data were collected through the Strep-EURO programme. Surveillance of severe *S.*
4 *pyogenes* infection diagnosed during 2003 and 2004 was undertaken in eleven countries across
5 Europe using a standardised case definition and questionnaire. Patient data as well as bacterial
6 isolates were collected and characterized by T- and, M/*emm*-typing and selected strains were
7 analysed for presence of superantigen genes. Data were analysed to compare the clinical and
8 microbiological patterns of infections across participating countries.

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

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

22

1 INTRODUCTION

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

8 The M-protein, encoded by the *emm*-gene, is an important virulence factor, and also an
9 epidemiological marker that are used throughout the world to characterize GAS (5, 21-23). The
10 type specificity of the M-protein, of which there are more than 100 different types known, is
11 largely determined by the epitope located in 40 to 50 amino acid residues at the amino-terminal (4,
12 16, 27). These regions of M-proteins have been shown to evoke antibodies with great bactericidal
13 activity, not likely cross-reactive with human tissues (3, 16). Hence,, an approach in the
14 development of a GAS vaccine has been to combine small amino-terminal M-protein peptides to
15 make multivalent vaccines that would elicit opsonic antibodies against epidemiologically
16 important GAS serotypes (15). Also other surface proteins, like the serum opacity factor (SOF)
17 and the T-protein are used to characterize different GAS types. In addition to the known linkage
18 between T-serotype, SOF production, and *emm*-type (25, 26), several studies also indicated
19 correlations between *emm*-types, disease manifestations, and also other virulence factors,
20 especially the superantigens (SAg) (7, 10, 40, 42).

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

1 mechanisms of the various disease manifestations would be enhanced. In order to improve
2 knowledge on severe GAS infections, the Strep-EURO programme was implemented during 2003-
3 2004. Overall epidemiological findings of the programme were reported recently (29). In the
4 present paper, type characteristics and SAg repertoire of the streptococcal isolates are described
5 and also possible associations with clinical findings.

6

7 **MATERIAL AND METHODS**

8 **Clinical data and isolates.** Through collaboration between eleven European countries (Cyprus,
9 Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Romania, Sweden and the
10 UK) on the epidemiology of invasive GAS disease, enhanced surveillance was undertaken between
11 January 1st 2003 and December 31st 2004. Methods employed to identify cases varied by country,
12 but mostly implied invited submission of isolates from local microbiology laboratories to the
13 national streptococcal reference centre. Demographic and clinical data, as well as risk factor
14 information were collected through a standardized questionnaire, with the exception of Denmark
15 and Sweden, where surveillance with earlier designed questionnaires was already operational.

16 **Case definition and isolate identification.** For invasive GAS disease and STSS the consensus
17 definition proposed by the Working Group on Severe Streptococcal Infections in 1993 was used
18 (45). Identification of GAS isolates was confirmed using morphological and growth
19 characteristics, bacitracin susceptibility, or pyrolydonyl-arylamidase testing and latex agglutination
20 with group A antisera commercially available.

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

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

8 **SAg gene detection.** Presence of SAg genes was tested in Lund, Sweden for 1127 isolates from
9 five countries (Czech Republic, Denmark, Finland, France, and Romania) and included *speA*,
10 *speB*, *speC*, *speF*, *speG*, *speH*, *speI*, *speJ*, *ssa*, and *smeZ* (30). Isolates from remaining countries
11 were tested in the respective national centres: Swedish isolates were tested for all the above
12 mentioned SAg genes but *speI* (18), Greek and Italian isolates for *speA*, *speB*, and *speC* (13), and
13 German isolates for *speA*, *speC*, and *ssa* (47). A fraction consisting of 256 UK isolates (18% of the
14 total *emm*-typed strains) were tested locally for the presence of *speA*, *speB*, and *speC*. In addition,
15 193 isolates (covering 38 out of the 74 different *emm*-types identified in the UK) were tested in
16 Lund as described above.

17 **Statistical analysis.** Data were analyzed using GraphPad Prism, version 4 (GraphPad Software)
18 and SAS, version 9.1.3, proc logistic (SAS Institute). For nominal data, χ^2 test or Fisher's exact test
19 were used when appropriate. Logistic regression was performed using *emm*-type as outcome and
20 clinical conditions or risk factors as predictors. The analyses were performed separately for each of
21 the 10 most prevalent *emm*-types, and compared to the group consisting of cases caused by all
22 other types (i.e. except the 10 most prevalent ones). Each model was reduced by backward

- 1 elimination where the significant level was set at 5%. In the logistic regression analyses, only
- 2 cases with age, gender and clinical/ risk factor information available were included.

1 RESULTS

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

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

9 **emm-types.** Among 4353 *emm*-typed isolates one hundred and four different types were identified,
10 of which the most prevalent ($\geq 2\%$) were *emm1* (19%), 28 (12%), 3 (10%), 89 (8%), 87 (6%), 12
11 (5%), 4 (5%), 83 (3%), 81 (3%) and 5, 77, 6, 22, and 18 (2% each) (table 1). The type distribution
12 varied significantly between the eleven countries, but the overall prevalence was strongly
13 influenced by the large proportion of isolates originating from the UK (figure 1), and also from
14 Sweden. Although *emm87* and *emm83* were the fifth and eighth overall most common types,
15 majority of these isolates were from the UK (93% and 90% of isolates respectively). In total 34
16 different *emm*-types encompassed the ten most prevalent types in the eleven countries.
17 Importantly, *emm1* was the most abundant type in the majority of countries, with a proportion
18 ranging between 15% and 33% of isolates. In contrast, within Denmark, Finland, and Sweden
19 *emm28* was the most prevalent type, ranging from 16% to 45% of isolates. As shown in figure1B,
20 certain types among the overall ten most prevalent *emm*-types were absent in some of the
21 countries; *e.g* in Romania only three of the overall ten most prevalent types were found. Type
22 *emm3* was infrequent in the Czech Republic, Finland, Greece, and Sweden with prevalence
23 ranging from 1% to 5%, and absent in Romania. Type *emm43* was found exclusively in the UK.

1 Other types almost confined to the UK were *emm82* (93% from the UK), *emm5* (91%), *emm83*
2 (90%), and *emm68* (81%). Type *emm53* was found only in the Czech Republic, Greece and the
3 UK. All the *emm118* isolates (n=34) originated from either Denmark or Sweden.

4 **T/*emm*-type combinations.** As shown in table 1, the number of T/*emm*- type combinations was
5 high (N=314), some of these were unfrequented, other previously not reported (underlined in the
6 table). The most prevalent T-type was 3/13/B3264 (or combinations thereof *e.g.* 3/13, 13/B3264,
7 3/B3264) and associated to no less than 40 different *emm*-types. In general, *emm1* was limited to
8 T1 (98%) but a small number of these isolates expressed T-types 3, 3/13/B3264 or 4.

9 **Correlation between age, gender and *emm*-types.** Among 600 isolates collected from children
10 (0-17 years), the most frequent *emm*-types were, in descending order 1 (26%), 12 (11%), 4, 3 (10%
11 each), and 28 (7%). In patients aged 18 and older, the most prevalent type was also *emm1* (19%),
12 but followed by *emm28* (13%), *emm3* (10%) and *emm89* (9%).

13 A significant female predominance for *emm87* and *emm28* (58%, p<0.001 for both) was
14 found. Type *emm28* was also more prevalent in age groups 30-39 years (17%) and 70-79 years
15 (19%), in the younger group strongly associated to females (80%, p<0.001). Types *emm81* and
16 *emm83* were significantly overrepresented among males (62%, p<0.05, and 68%, p<0.001,
17 respectively).

18 **Seasonal fluctuations.** During the study period, several *emm*-types presented a steady seasonal
19 prevalence, whereas other showed fluctuations (figure 2). Overall, 59% of cases were reported in
20 the 6 winter months (January to April + November and December) in both years. In contrast,
21 tendencies towards higher frequencies of *emm12* was noted during the warmer months (May-
22 August; p<0.05).

1 **Disease manifestations, risk factors and *emm*-types.**

2 The most severe manifestations, STSS and NF, were caused by 45 different types, of which *emm1*
3 was the most prevalent, accounting for 37% and 31% of cases respectively (table 2); in addition, a
4 considerable proportion were caused by *emm3* isolates (17% and 14%, respectively). In the
5 statistical regression model, when comparing each of the 10 most prevalent types versus the other
6 types combined, STSS was statistically more often caused by *emm1* or *emm3* ($p < 0.001$ for each).

7 Patients without focal symptoms were less often infected by *emm1* (17%, $p < 0.05$), in
8 contrast to types *emm81* (45%), *emm77* (47%) ($p < 0.001$ for each), *emm83* (34%), and *emm87*
9 (26%) ($p < 0.05$ for each), that were more common among these patients (table 2.). Furthermore,
10 patients with arthritis were less prone to be infected by *emm28* isolates (5%, $p < 0.05$), and cellulitis
11 was more often caused by either *emm87* (32%, $p < 0.0001$) or *emm83* (30%, $p < 0.05$), as compared
12 to infections caused by types other than the 10 most prevalent. Though puerperal sepsis was
13 caused by 16 different types and only 8% of *emm28* were patients with puerperal sepsis a clear
14 correlation with *emm28* was noted (31% of cases, $p < 0.001$). Other *emm* types significantly
15 involved in causing puerperal sepsis were *emm1*, *emm89* and *emm87* (4% each, $p < 0.001$ and < 0.05
16 respectively).

17 Data regarding risk factors, as well as *emm*-type, were available for 2796 patients
18 (table 3). Patients with diabetes were statistically more prone to an infection caused by either
19 *emm81* ($p < 0.001$) or *emm12* ($p < 0.05$), as compared to “other types” in the logistic regression
20 analysis.

21 Information on *emm*-type distribution among patients who were injecting drug users
22 (IDU) was available for 359 of 471 (76%) cases, a majority of these (93%) was identified in the
23 UK. The ten most prevalent types among these patients were, in descending order: *emm83*, 87, 82,

1 89, 81, 43, 33, 101, 1 and 53, accounting for 70% of these infections. Conversely, as many as 70%
2 of *emm33*, *emm82* and *emm83*, and 54% of the *emm43* infections were IDU related.

3 Among 242 health care associated infections (HAI), the same types as the over all ten
4 most prevalent ones caused the majority of infections (71%). However, *emm1* and *emm3* infections
5 were less commonly related to surgery before disease onset, as determined by the regression model
6 ($p>0.05$ for each).

7 Among patients with chicken pox, the probabilities for *emm1* and *emm12* were high
8 ($p<0.001$ each), which is in concordance with the high frequency of both types among children.

9 **Case fatality rates and *emm*-types.** Overall, the CFR over 7 days among cases with typed isolates
10 was 19% and highest among infections caused by *emm3* (36%), followed by *emm5*, 1, 43, and 77
11 (table 2). Furthermore, the highest CFRs were, as expected, noted among cases with STSS (44%)
12 and NF (31%), and as already mentioned correlated to *emm1* and 3 infections. For patients with
13 cellulitis, the overall CFR was 18%, but considerably higher for infectious caused by *emm77*,
14 *emm3* (33% $p<0.001$ each), or *emm1* (25%, $p<0.05$) isolates. Among infections without focus the
15 overall CFR was 15%, and the deaths predominantly caused by *emm3* (32%), *emm83* (19%),
16 *emm87* (17%), *emm1* (16%), and *emm 28* (15%) infections (table 2).

17 **SAg genes patterns and *emm*-types.** As expected, *speB*, *speF*, and *speG*, were detected in the
18 vast majority of strains, though *speG* was lacking among *emm4* and *emm77* isolates from several
19 countries.

20 Data regarding *speA* and *speC* was available for 2321 isolates. Overall, 30% and 54 %
21 were positive for *speA* and *speC*, respectively. As shown in table 4, *speA* was primarily associated
22 with *emm1* and *emm3* ($p<0.001$ for both), whereas *speC* was common in several other types such
23 as *emm4*, 5, 6, 28 and 77 ($p<0.001$ for each), *emm18* ($p< 0.01$). Both *emm1* and *emm3* harboured

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

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

1 **DISCUSSION**

2 In the present paper clinical and microbiological data obtained from patients with severe GAS
3 infections from the eleven Strep-EURO participating countries are presented. The number of
4 characterized isolates (4353) exceeds any previous European study. Strikingly, the overall
5 distribution of the most prevalent *emm*-types agreed closely with recent data reported from the US
6 where *emm*-types 1, 3, 28, 12, and 89 accounted for 55% of invasive isolates collected over a
7 period of four years (2000-2004) (35). However, the country-specific *emm*-type distributions
8 differed markedly, as exemplified by. *emm87* though overall highly represented, essentially
9 confined to the UK (figure 1). Differences in type proportions were also noted between
10 neighbouring countries, like Denmark, Finland, and Sweden. In Sweden, high rates of *emm81* and
11 *emm89* was seen, accounting for 30% of isolates, whereas *emm28* was the most prevalent type in
12 Denmark (26%), and *emm89* only accounted for 7% of cases (30). In Finland, 45% of all isolates
13 were *emm28*, being the only country with such a large proportion of a single type. Isolates of
14 *emm3*, in addition to *emm1*, have previously been shown to be of major role in invasive GAS
15 disease (19, 46, 48). However, in Finland, the number of *emm3* isolates was negligible (3 cases),
16 and a low prevalence of this type was also noted in Greece, the Czech Republic and Sweden (3-
17 4%). As shown in the Swedish study (18) *emm*-types of invasive cases essentially agreed with
18 those recorded among cases with non-invasive GAS disease. Though non-invasive isolates were
19 not studied in other participating countries, the country-specific type distributions may to a large
20 extent reflect ongoing epidemic waves, herd immunity (39) or population mobility (11), as
21 previously seen for streptococcal disease (39).

22 There were significant differences between genders regarding some particular types. For
23 example, *emm28* and *emm87* were overrepresented among female cases. The role of *emm28*

1 isolates in puerperal fever has already been recognized (2, 32), as this type are known to express
2 R28, which is related to the Rib protein in group B streptococci, the major cause of neonatal
3 infections (38, 39). Recently it was shown that the gene encoding R28 is located on a 37.4-kb
4 region (region of difference – RD2) similar in content and organization to a region described in
5 group B streptococci, apparently acquired by horizontal gene transfer and enabling *emm28* strains
6 to often cause puerperal sepsis (24, 49). Since *emm87* was not among those types carrying RD2
7 (e.g. M2, 4, 48, 77, 124), it is of interest to investigate whether *emm87* isolates may harbor similar
8 pathogenic factors. In contrast, *emm*-types 83, 81, and 43, were associated with intravenous drug
9 use and found preferentially among male patients (68%, 62%, and 61% respectively).
10 Interestingly, also a predominance of *emm81* isolates among male patients with skin involvement
11 were found in Sweden (18).

12 It is known that no *emm*-type can be uniquely associated to a particular disease, though
13 there is evidence correlating certain types, e.g. *emm1* and *emm3* with the most severe GAS
14 diseases NF and STSS (12, 31, 43, 44), or *emm28* with puerperal sepsis (36). However, in our
15 material 50% of all STSS cases and 55% of NF cases were caused by types other than *emm1* and
16 *emm3* respectively, and in Sweden no *emm3* strain was involved in STSS, indicating that most
17 types of GAS may have the potential to give rise to these severe manifestations. However, the
18 mortality associated with either *emm1* or *emm3*, whether causing STSS, NF or puerperal sepsis,
19 clearly exceed that of remaining types which, in agreement with previous studies, demonstrates
20 these two types as particularly virulent.

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

1 study, a high occurrence of *speA* was found for isolates of *emm*-types 1 and 3, types that were
2 often involved in severe infections, and also for the less frequent type *emm43*; these *emm*-types
3 were associated with high CFRs (29%, 36%, and 21%, respectively). However, *emm5* and *emm18*
4 cases had high CFRs (30%, and 21%, respectively), though these types lacked *speA* but harboured
5 *speC* at high proportions (both 91%). In addition, the presence of *speC* was common in several
6 prevalent types such as *emm4*, 6, 28, 77, 18, 81 and 12.

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

16 In conclusion, among 104 GAS *emm*-types identified during the present project, 45
17 were involved in causing STSS and/or NF. A major role of *emm1* and *emm3* isolates in these
18 severe entities, also found in previous studies was confirmed; however, a number of other types
19 also caused high mortality rates, suggesting similar pathogenic potential. In general, the SAg gene
20 repertoire of isolates appeared to correlate with *emm*-type in a complex pattern, precluding definite
21 conclusions on the role of individual SAg for severe disease. The data here presented,
22 demonstrating high mortality and devastating consequences of the invasive manifestations in

- 1 particular, should be of value for preventive work, including ongoing attempts at creating vaccine
- 2 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

<i>emm</i> -type	No. of isolates	No. of T/ <i>emm</i> -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)
emms1369	1	1	14
emms1389	2	1	3/13/B3264 (2)
emms2037	1	1	8/25/Imp19
emms2147	1	1	5/27/44
emms2460	1	1	3/13/B3264
emms2904	2	1	NT
emms3757	2	1	NT
emms16735	1	1	5/27/44

<i>emmst</i> 11014	2	1	4 (1); NA (1)
<i>emmst</i> D633	1	1	3/13/B3264
<i>emmst</i> NS1033	20	3	28 (6); 8/25/Imp19 (1); NT (2); NA (11)
<i>emmst</i> G6	1	1	8/25/Imp19
<i>emmst</i> G1750	1	1	NT
<i>emmst</i> G62647	1	1	12
<i>emmst</i> 221	1	1	NT
<i>emmst</i> 4986	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; *emmst*, *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.

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

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

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

Cases with risk factor information	<i>emm</i> -type (no/%)															
	1	28	3	89	87	12	4	83	81	5	77	6	18	75	43	
Total	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

	<i>speA</i>		<i>speC</i>		<i>speF</i>		<i>speG</i>		<i>speH</i>		<i>speI</i>		<i>speJ</i>		<i>smeZ</i>		<i>ssa</i>	
	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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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/ <i>emm</i> 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

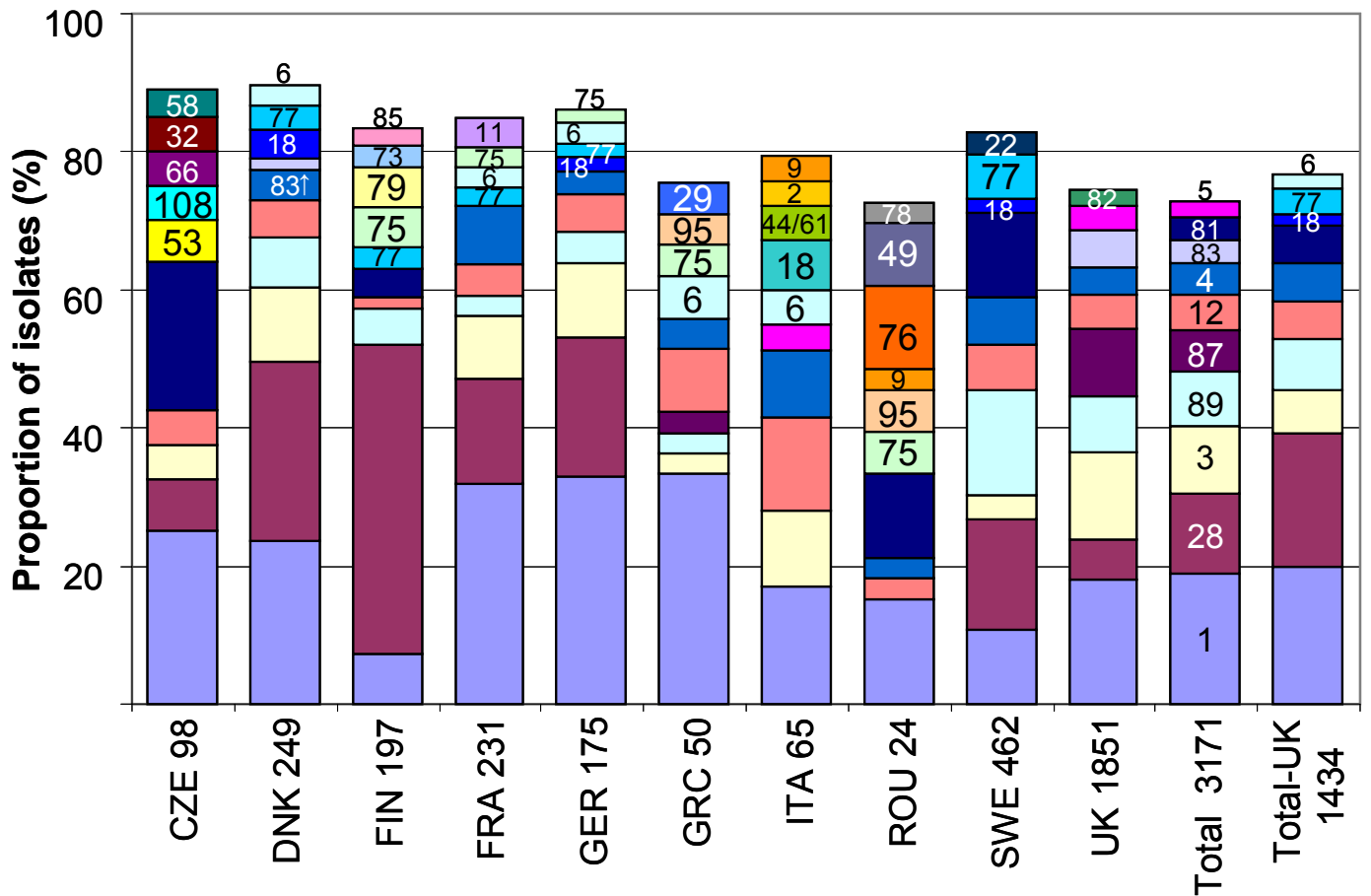


Fig. 1B

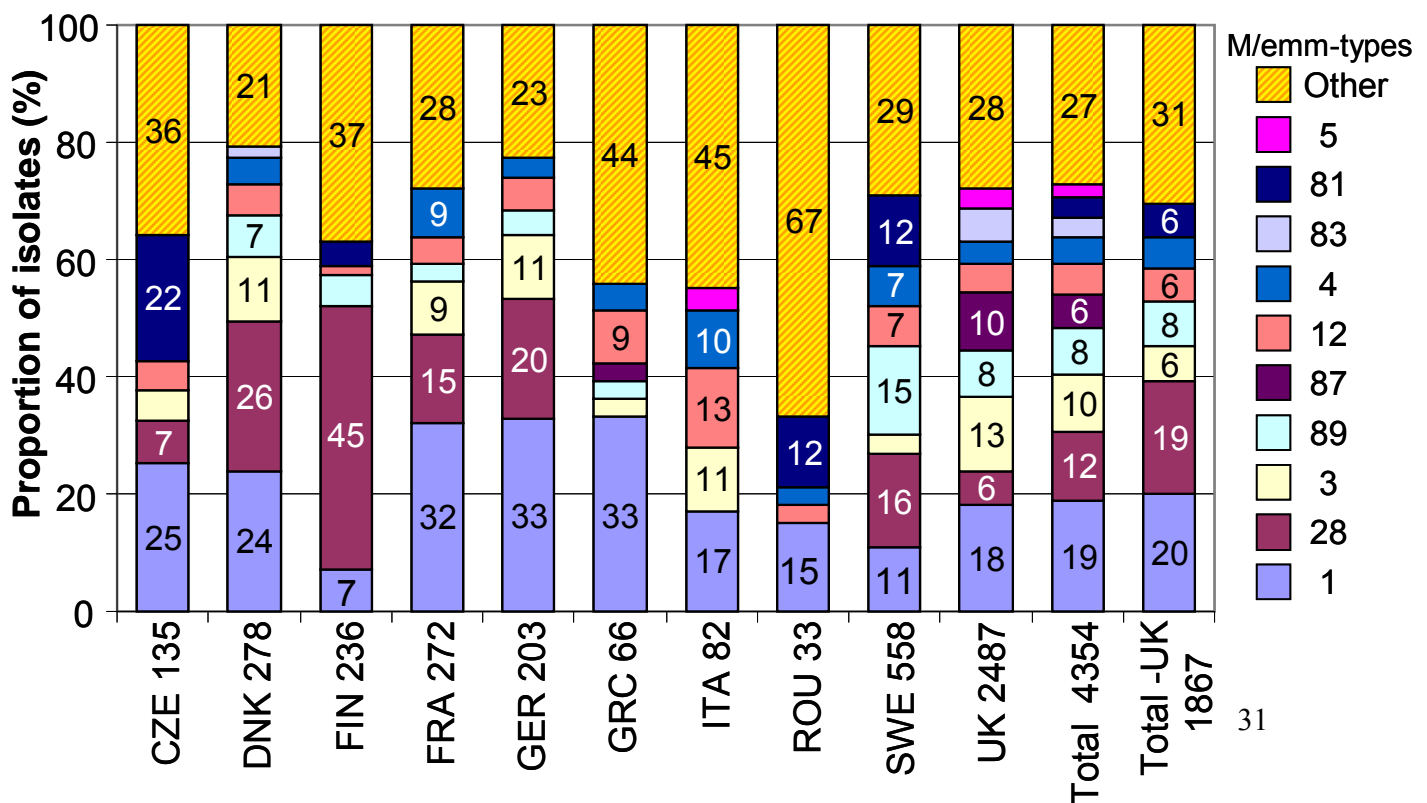


Fig. 2

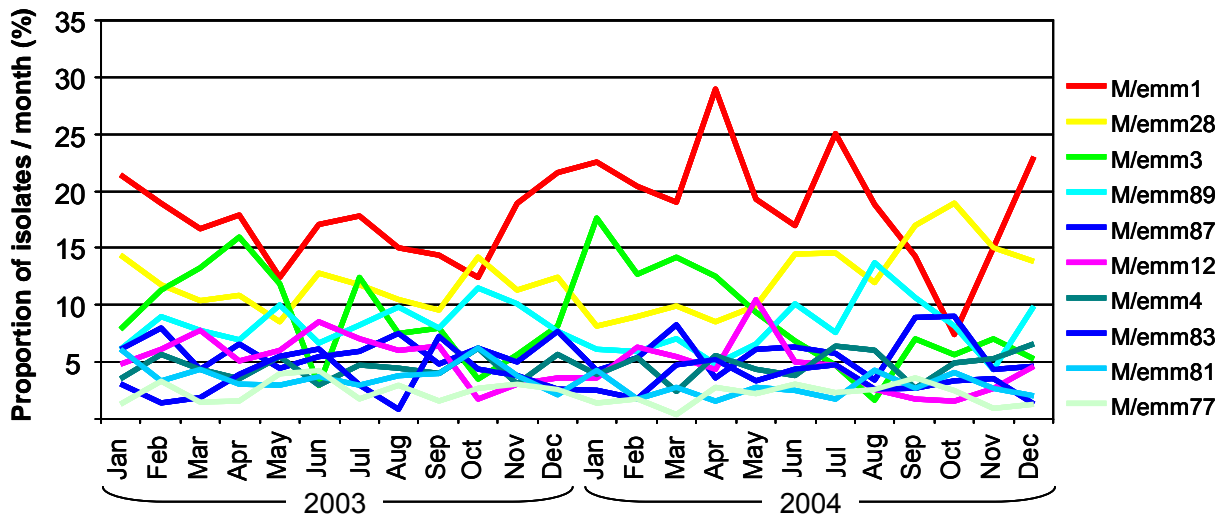


Fig. 3A

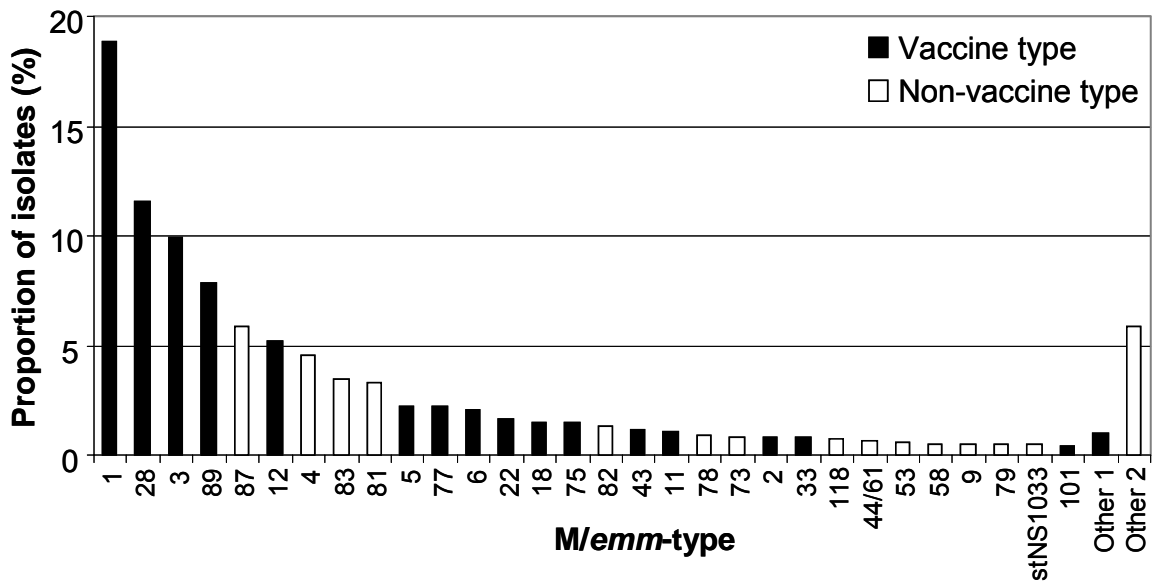
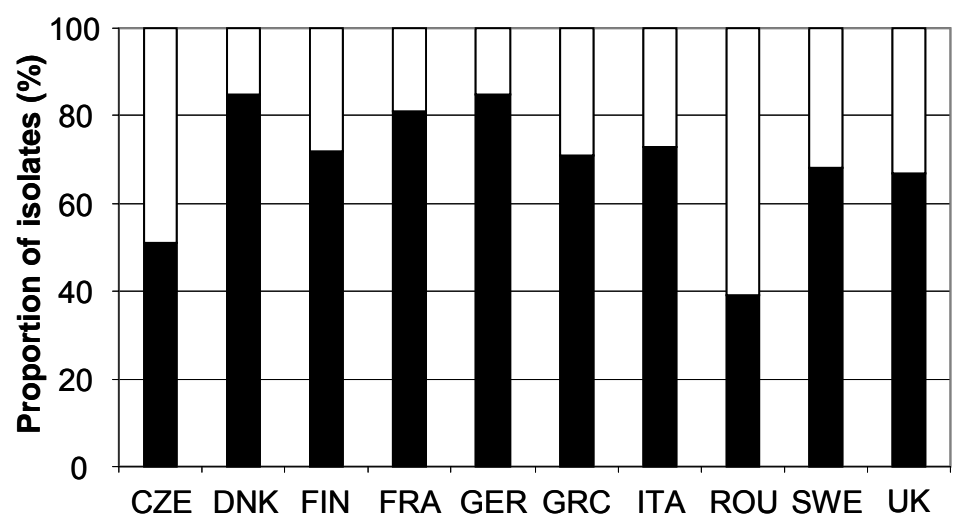


Fig. 3B



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

1 **Figure 1.** *emm*-type distribution among the ten participating Strep-EURO countries in years
2 2003-2004. Numbers of isolates presented for each country are indicated beneath each bar. A)
3 The distribution of the ten most prevalent *emm*-types within each country and overall, with and
4 without UK isolates. The 10 overall prevalent types are indicated in the figure, within the bar for
5 “Total”, and other types indicated for each country. B) The distribution of the ten overall most
6 prevalent *emm*- types. Types of more than 5% of all types within a country are indicated by
7 numbers in the figure. Abbreviations for countries: CZE, the Check Republic; DNK, Denmark;
8 FIN, Finland; FRA, France; GER, Germa; GRC, Greece; ITA, Italy; ROU, Roumania; SWE;
9 Sweden; UK, the United Kingdom.

10
11 **Figure 2.** Overall seasonal fluctuation of the ten most prevalent *emm*-types among the Strep-
12 EURO participating countries. Percentages are calculated by number of each major type divided
13 to total number of isolates per month.

14
15 **Figure 3.** Distribution of *emm*-types among Strep-EURO invasive GAS cases, with special
16 regard to coverage by a 26-valent candidate vaccine. Types hypothetically covered or not by the
17 vaccine candidate are indicated.

18 A) Prevalence of 30 most common *emm*-types. Among these, 16 (accounting for 69% of reported
19 cases) are included in the 26-valent vaccine (since subtypes were not assessed, vaccine subtype
20 *emm*1.2 is not considered in the present discussion). Vaccine types *emm*14, *emm*19, and *emm*114
21 were not encountered. Other 1= other types included in the vaccine (6 *emm*-types); Other 2=
22 remaining types not covered by the vaccine (70 different *emm*-types)

23 B) Country-specific *emm*-type proportions based on potential coverage by the 26-valent vaccine.
24 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.