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ARTICLE

Streptococcus pyogenes bacteraemia, *emm* types and superantigen profiles

S. Rantala · S. Vähäkuopus · T. Siljander · J. Vuopio · H. Huhtala · R. Vuento · J. Syrjänen

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Abstract The aim of this study was to investigate the *emm* types and superantigen profiles of bacteraemic group A streptococcal (GAS; *Streptococcus pyogenes*) isolates and to detect possible associations between the molecular characteristics of isolates and the clinical presentations of disease. In this population-based study, 87 bacteraemic GAS isolates from adult patients in Pirkanmaa Health District (HD), Finland, during the period 1995–2004 were *emm* typed and genotyped for superantigen (SAg) profiles. The epidemiological and clinical data of the patients were analysed with the microbiological characterisation data. Among the 87 isolates, 18 different *emm* types were found. *emm*1, *emm*28 and *emm*81 were the three most common types, covering 52% of isolates. The prevalence of specific *emm* types showed high variability during the 10-year study period. We could not find any association between the *emm* type and clinical features of bacteraemic infection, such as underlying diseases, disease manifestations or case fatality. Of nine superantigen genes examined, *spe*A and *spe*C were identified in 20 and 30% of the strains, respectively. No association was found between disease manifestation and the presence of single superantigen genes. The 26-valent GAS vaccine would have covered only 62% of isolates causing invasive disease in Pirkanmaa HD during the study period.

S. Rantala (✉) · J. Syrjänen
Department of Internal Medicine, Tampere University Hospital,
P.O. Box 2000, 33521 Tampere, Finland
e-mail: sari.rantala@uta.fi

S. Vähäkuopus · T. Siljander · J. Vuopio
Department of Infectious Disease Surveillance and Control,
National Institute for Health and Welfare,
Helsinki, Finland

H. Huhtala
Tampere School of Public Health, University of Tampere,
Tampere, Finland

R. Vuento
Centre for Laboratory Medicine, Tampere University Hospital,
Tampere, Finland

J. Syrjänen
Medical School, University of Tampere,
Tampere, Finland

Introduction

Group A streptococcus (GAS; *Streptococcus pyogenes*) is an important human pathogen causing a wide variety of disease manifestations, with bacteraemic infections representing the most severe. Advanced age, cardiac and vascular disease, diabetes, skin breakdown, corticosteroid use and malignancy are associated with increased risk for invasive GAS infection among adults [1–3].

The M protein is a major GAS virulence factor. Based on the variability of the N-terminal end of the *emm* gene encoding the M protein, as many as 150 defined *emm* types are recognised (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). *emm* typing can be used to analyse the molecular epidemiology of GAS infections. The prevalence of specific GAS types tends to vary over time and within a geographic area [4, 5]. M1 and M3 commonly dominate among invasive isolates, whereas M types 12, 4 and 28 have been shown to be more prevalent among non-invasive isolates [6–8].

A 26-valent GAS vaccine approach, which has shown promising results in phase II testing in adults, has been to combine small amino-terminal M-protein peptides of epidemiologically important GAS serotypes causing both invasive and non-invasive disease [9, 10]. As an invasive infection may also cause a fatal disease, the coverage of invasive serotypes

is of utmost importance. In earlier studies, the coverage of serotypes which have caused invasive infections has varied from 69 to 94% in Europe, Japan and the USA [4, 11, 12]. By contrast, this putative vaccine would provide only limited coverage in Africa, Asia, the Middle East and the Pacific region, because of a higher diversity of *emm* types [13].

The GAS superantigens (SAgs) are also important for virulence, participating in the induction of the systemic toxicity associated with severe infections [14–16]. Currently, at least 11 distinct streptococcal SAgs are known, including the streptococcal pyrogenic exotoxins (Spe) A, C, G, H, I, J, K, L and M, the streptococcal superantigen (SSA) and the streptococcal mitogenic exotoxin Z (SmeZ) [15]. Most GAS strains express several different SAgs, and the repertoire of genes varies between strains. Several SAgs, among these the pyrogenic exotoxins A and C (SpeA, and SpeC, respectively), have received particular attention in the context of invasive disease.

We have earlier reported the epidemiology of A, B, C and G beta-haemolytic streptococcal bacteraemia during the study period [17], where the incidence of group A streptococcal bacteraemia was relatively stable at 2.0 cases per 100,000 population [17]. As reported earlier [18], alcoholism and cardiovascular diseases were the two most prominent underlying conditions in group A streptococcal bacteraemia. The most frequent presenting clinical manifestations of GAS bacteraemia were skin and soft-tissue infection, followed by pneumonia and deep abscess [17]. The overall case–fatality was 15% [18]. Among the patients, 14% developed streptococcal toxic shock syndrome (STSS), 16% developed multiorgan failure and 8% developed necrotising fasciitis [18].

Not much is known about the associations between the clinical and molecular characteristics of GAS bacteraemia in Finland, as the surveillance of invasive GAS disease does not routinely include clinical data. Furthermore, the superantigen profiles of Finnish GAS isolates have not been studied in more detail. The aim of the present study was to study the *emm* types and superantigen profiles of all bacteraemic GAS isolates in adults during the period 1995–2004 in Pirkanmaa Health District (HD) and combine these data with the previously collected patient data. We aimed at describing the possible associations between the *emm* types, SAgs and the clinical presentations, and at estimating the potential coverage for the 26-valent putative vaccine in this region based on the *emm* typing data.

Materials and methods

Patients

Our case definition included all adult (over 16 years of age) patients in Pirkanmaa HD with a positive blood culture of group A beta-haemolytic streptococcus (*S. pyogenes*) combined with a clinical picture compatible with septicaemia during a 10-year period from January 1995 to December 2004. Pirkanmaa HD, with approximately 460,000 inhabitants representing approximately one-tenth of the population of Finland, is located in western Finland; there is one tertiary care hospital (Tampere University Hospital) and four other hospitals, including three district hospitals. The medical records of all patients with one or more blood cultures positive for GAS were retrospectively reviewed.

Blood culture methods

All blood cultures were cultivated and studied in the Centre for Laboratory Medicine at Tampere University Hospital. Routine blood samples were drawn into aerobic and anaerobic bottles. During the study period, the BACTEC NR 730 (only in 1995) and the BACTEC 9240 (during the period 1996–2004) (BD Diagnostic Systems, Sparks, MD, USA) blood culture systems were used. In the district hospitals, the Signal blood culture system (Oxoid, Cambridge, UK) was used until 2003 and the BACTEC 9240 bloody culture system thereafter. The Lancefield serogroups were defined by latex agglutination using the Streptex latex test system (Remel Europe Ltd., Dartford, UK). All isolates were also biochemically identified with the rapid ID 32 STREP system (bioMérieux SA, Marcy-l'Etoile, France). All group A streptococcal isolates were *emm* sequenced and further analysed by SAg genotyping.

emm typing

The *emm* gene was amplified using primer 1 and primer 2 and sequencing was performed using primer emmseq 2 (http://www.cdc.gov/ncidod/biotech/strep/protocol_emm-type.htm). *emm* types were assigned according to the guidelines of the Centers for Disease Control and Prevention (CDC) *emm* database (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm).

SAg genotyping

All isolates were genotyped for their SAg profile. Multiplex polymerase chain reaction (PCR) was used to detect the streptococcal superantigen *ssa* gene, protease gene *spe*B, deoxyribonuclease gene *spe*F and six pyrogenic exotoxin genes *spe*A, *spe*C, *spe*F, *spe*G, *spe*H and *spe*J [19]. Streptococcal mitogenic exotoxin *sme*Z was detected using single PCR as previously described [8].

Statistical methods

SPSS software version 7.5 (SPSS, Chicago, IL, USA) was used for the statistical analyses and a two-sided *p*-value

<0.05 was regarded as the level for statistical significance. Categorical data were analysed by the χ^2 test or Fisher's exact test as appropriate. Non-parametric data were analysed by the Kruskal–Wallis *H*-test.

Results

emm type distribution

A total of 92 cases with a GAS isolate were found. Five of the isolates were not available for *emm* typing but the remaining 87 GAS isolates were sequenced to identify the *emm* gene. The mean number of isolates during the period 1995–2004 was 9.2 (range; 4–17). Eighteen of the cases included in this study were part of the EU-wide Strep-EURO study, which covered years 2003 and 2004 and included all invasive GAS cases in Finland [11].

Among these isolates, 18 different *emm* types were found; five isolates remained non-typable (NT). The most abundant *emm* types were, in descending order, 1, 28, 81, 53, 12, 68 and 89 (Table 1). The three most common *emm* types covered 52% of all isolates. During the study period, the prevalence of common types *emm*1, *emm*28 and *emm*81 fluctuated considerably, with the amount of *emm*1 strains peaking in 1998–1999 and that of *emm*28 in 2002–2003 (Fig. 1). *emm* types included in the putative 26-valent GAS vaccine [10] accounted for 62% of isolates overall, but this proportion fluctuated considerably by year (range; 21% [year 2000]–100% [year 1998]).

Table 1 The *emm* types of group A streptococcal bacteraemic isolates during the period 1995–2004, Pirkanmaa Health District, Finland

emm types	No. of isolates	%
*emm*1*	17	19.5
*emm*28*	16	18.4
*emm*81	12	13.8
*emm*53	7	8.0
*emm*12*	6	6.9
*emm*68	4	4.6
*emm*89*	4	4.6
*emm*77*	3	3.4
*emm*2*	3	3.4
*emm*11*	2	2.3
*emm*44	2	2.3
*emm*75*	2	2.3
*emm*4	1	1.1
*emm*43*	1	1.1
*emm*78	1	1.1
*emm*83	1	1.1
Non-typable	5	5.7
Total	87	100

*Type included in the putative GAS vaccine

Seasonal fluctuations

Overall, 55% of cases occurred during the winter months (October–March). Higher frequencies of *emm*28 were noted during the winter months (October–March) (25% vs. 10%, $p=0.08$). Also, higher frequencies of *emm*53 were noted during the winter months (10% vs. 5%), but this did not reach statistical significance. Higher frequencies of *emm*12 were noted during the warmer months (April–September) (10%) as compared to the winter months (October–March) (4%), but this did not reach statistical significance either.

SAg gene profiles as markers

The multiplex PCR revealed the presence of three to six SAg genes in the GAS isolates (Table 2). Of the nine superantigen genes examined, *spe*A and *spe*C, which are known to be associated with severe disease, were identified in 20 and 30% of the strains, respectively. The *spe*A gene was detected among all *emm*1 isolates (100%) but was not found among other types. *spe*A was detected in two and *spe*C in five out of 13 strains causing STSS, whereas the combination of both genes was not found among the isolates. *spe*H was particularly linked to *emm*12 isolates, whereas *spe*J was linked to *emm* types 1, 28, 68 and 89. The *spe*C gene was not found among *emm* types 1, 81 and 53, but it was common among other isolates (62% total positive isolates among the other types). The majority of isolates harboured *spe*B, *spe*F, *spe*G and *sme*Z genes.

emm types or SAg gene profiles and clinical characteristics of GAS bacteraemia

No association was found between *emm* type and patient age or underlying disease (alcoholism, malignancies, diabetes, cardiovascular diseases, immunosuppressive treatment, liver diseases). There was a statistically significant difference between *emm* type and sex ($p=0.013$). *emm* types 28 and 12 were over-represented in females compared to males (25% vs. 13% and 10% vs. 4%, respectively), whereas *emm* types 81, 53 and 68 were more common in males (17% vs. 10%, 11% vs. 5%, and 6% vs. 3%, respectively). Nor was the presenting clinical manifestation associated with *emm* type (Table 3). Skin or soft-tissue infection was a common presenting clinical manifestation among *emm* types 1, 28 and 81. No association was found between *emm* type and either necrotising fasciitis or STSS. We also found a variety of *emm* types causing puerperal infections. No association was found between single SAg genes and presenting clinical manifestation (data not

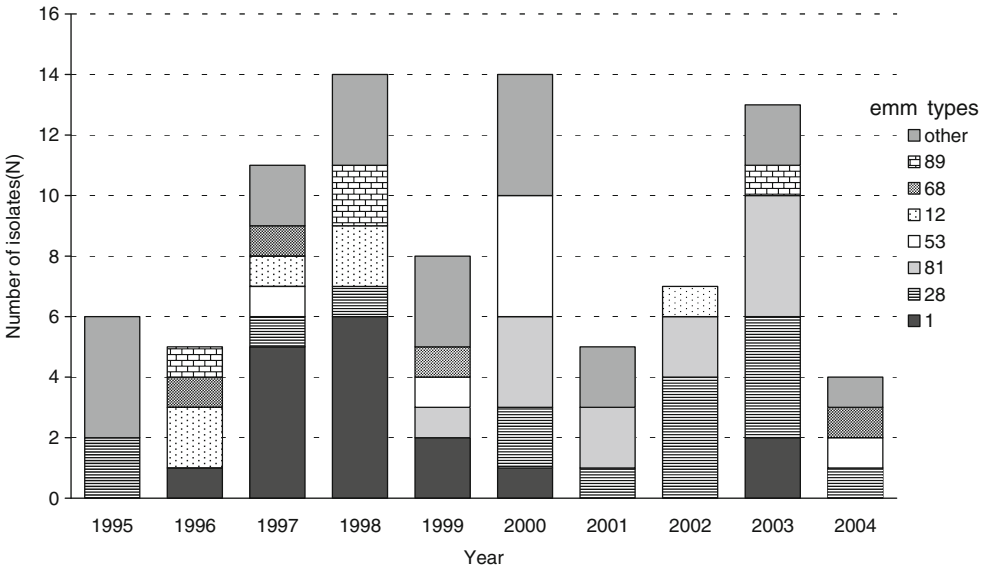

Fig. 1 Distribution of the seven most prevalent *emm* types among group A streptococcal bacteraemic isolates in relation to the year of the diagnosis of bacteraemic infection in Pirkanmaa Health District, Finland

shown). Neither *emm* types nor the presence of single SAg genes were associated with fatal outcome (data not shown).

Discussion

This is the first study to describe the *emm* types in relation to SAg profiles and clinical characteristics of GAS bacteraemia in Finland. We identified 92 episodes of group A streptococcal bacteraemia in a well-defined population in Finland during a 10-year period. Eighty-seven of the GAS isolates were *emm* typed and genotyped for superantigen (SAg) profiles. Five isolates remained non-typable (5.7%), which is within the frame of previous typing results in Finland and other regions [13, 20]. In our study, *emm*1, *emm*28 and *emm*81 were the three most common *emm* types, covering 52% of all isolates. Three recent studies describing the nationwide *emm* distribution in Finland also report *emm*1 and *emm*28 among the most common types in 2003–2007 [11, 20, 21]. Darenberg et al. identified *emm*89, *emm*81 and *emm*28 as the three most common *emm* types, covering 44% of 746 Swedish isolates [8]. Luca-Harari et al. reported *emm*28, *emm*1 and *emm*3 as the three most common *emm* types, covering 62% of 278 Danish isolates [22]. Lamagni et al. identified *emm*1, *emm*3 and *emm*87 as the three most common *emm* types, which accounted for 41% of isolates in the United Kingdom [23]. O'Loughlin et al. found that *emm* types 1, 3, 28, 12 and 89 were the most common genotypes, which cumulatively accounted for 55% of isolates in the United States [4].

The considerable fluctuations in the prevalence of *emm* types, also seen in this study, may be explained by the lack of immunity to specific GAS strains, which would allow the emergence of certain strains within the susceptible

Table 2 Superantigen (SAg) gene distribution within the most prevalent *emm* types of group A streptococcal bacteraemia isolates, Pirkanmaa Health District, Finland

emm type	No. of isolates	%	Mean no. of SAgs[a]	Percentage of positive isolates								
				*spe*A	*spe*B	*spe*C	*spe*F	*spe*G	*spe*H	*spe*J	*ssa*	*sme*Z
1	17	20	6.0	100	100	0	100	100	0	100	0	100
28	16	18	5.6	0	100	63	94	100	0	100	0	100
81	12	14	3.0	0	100	0	100	100	0	0	0	0
53	7	8	4.0	0	100	0	100	100	0	0	0	100
12	6	7	5.5	0	100	67	100	100	83	0	0	100
68	4	5	6.0	0	100	100	100	100	0	100	0	100
89	4	5	5.0	0	100	50	100	100	0	50	0	100
Other[b]	21	24	4.2	0	100	29	100	90	14	4	0	81

[a] Mean number of SAg genes for all bacteraemia isolates

[b] Includes also the five non-typable isolates

Table 3 *emm* type distribution in relation to the three most important infection focuses in patients with GAS bacteraemia

emm type	No. of isolates	%	Infection focuses					
			Skin and soft-tissue infections, *N*=62		Deep abscess, *N*=9		Pneumonia, *N*=16	
			n	%[a]	*n*	%[a]	*n*	%[a]
1	17	20	10	16	1	11	2	13
28	16	18	8	13	1	11	5	31
81	12	14	10	16	2	22	4	25
53	7	8	7	11	0	0	0	0
12	6	7	6	10	1	11	0	0
68	4	5	3	5	1	10	0	0
89	4	5	4	7	0	0	0	0
Other[b]	21	24	14	23	4	44	5	24

[a] Percentage of *emm* types in infection focus

[b] Includes also the five non-typable isolates

population. Variations may also reflect differences in the prevalence of risk factors for invasive GAS infection, such as chronic diseases and substance abuse [2, 24, 25].

The putative 26-valent vaccine would have covered 62% of the isolates (of nine *emm* types) of this study, which is a lower percentage than has been reported earlier in the United States (79%) and Japan (82%) [4, 10, 12]. There is a possibility for the expansion of non-vaccine *emm* types and a higher risk of infection by non-vaccine *emm* types. This phenomenon has been noticed in the population after pneumococcal vaccination, where invasive infections of serotypes included in the vaccine decreased, while serotypes not included in the vaccine became more common [26, 27]. In order to ensure the vaccine coverage, more *emm* types may need to be included in the GAS vaccine in the future. Another choice is that the *emm* types included in the vaccine would need to be tailored for different regions. This is a problem especially for regions such as Africa and the Pacific, because of a higher variability in the *emm* type distribution, possibly due to the high prevalence of impetigo [13].

Although our study covers 10 years and is population-based, the number of episodes was only 92. This may explain why we could not find significant associations between *emm* types and disease manifestations. In earlier studies with a larger number of patients, associations have been found between *emm* types and disease manifestations. Luca-Harari et al. *emm* identified 104 different *emm* types among 4,353 European GAS isolates [11]. They found that cellulitis was more often caused by either *emm*87 or *emm* 83, and they found a correlation with *emm*28 and puerperal sepsis. They also found that the most severe manifestations, STSS and necrotising fasciitis, were caused by 45 different types, of which *emm*1 was the most prevalent, accounting for 37 and 31% of cases, respectively. Our study was underpowered to observe these types of complex associations. In the present study, skin and soft-tissue infections were a common presenting clinical manifestation in bacteraemic infection among *emm* types 1, 28 and 81. In the study of Darenberg et al., skin or soft-tissue infections were most commonly caused by *emm* type 81 [8]. We studied only invasive infections and, therefore, we could not analyse the differences of *emm* types between invasive and non-invasive infections. We found a variety of *emm* types causing puerperal infections, indicating that we did not have any puerperal sepsis outbreaks during the study period.

Over the years, our knowledge of the role of GAS SAgs in disease pathogenesis has increased [15, 28–30]. Superantigen profiling might be a useful tool to identify subclones of particular invasive potential [15]. In a recent study by Lintges et al., SAgs were found to be more important for the invasiveness of GAS than the *emm* type, with *spe*A, *spe*J and *spe*Z having the most invasive potential [15]. However, as also previously reported [8], we found no correlation between clinical manifestations in invasive GAS infection and the presence of single SAg genes. The *spe*A gene has been commonly detected among *emm*1 isolates but rarely found among other types [8, 15]. Luca-Harari et al. found that *spe*A was primarily associated with *emm*1 and *emm*3 [11]; we also found the *spe*A gene in all *emm*1 isolates. Isolates of type *emm*3 were absent in our study. Several studies have described the potential involvement of streptococcal pyrogenic exotoxin SpeA in severe streptococcal disease [31–34], while others have reported an association with SpeC [35, 36]. Nevertheless, some cases of STSS have been found not to be associated with either of these SAgs [37]. In the present study, *spe*A was detected in

2 and *spe*C in 5 out of 13 strains causing STSS, whereas the combination of both SAgs was not found in any of those isolates.

This report provides a long-term, population-based analysis of group A streptococcal bacteraemia, their *emm* types and their SAg gene profiles. The characterisation of GAS by different typing methods helps to improve the current understanding of the epidemiology of invasive disease, with an impact on disease control, detection of outbreaks, changes in population immunity and vaccine development.

In conclusion, our population-based longitudinal design provided a unique opportunity to characterise the distribution of invasive GAS serotypes in a defined population over time. In view of the present results, the current formulation of GAS vaccine would provide only limited coverage of GAS *emm* types in Finland. Associations between *emm* types, SAg genes and particular disease manifestations were not observed.

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Conflict of interest The authors do not declare any conflicts of interest.

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