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Invasive group A, C and G streptococcal disease in western Norway: virulence gene profiles, clinical features and outcomes

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Abstract

Invasive group A streptococcal (iGAS) disease is endemic in Norway, but data on invasive group C and group G streptococcal (iGCS/GGS) disease are lacking. We investigated the characteristics of iGAS and iGCS/GGS infections in western Norway from March 2006 to February 2009. Clinical information was retrospectively obtained from medical records. GAS and GCS/GGS isolates were *emm* typed and screened for the presence of 11 superantigen (SAg) genes and the gene encoding streptococcal phospholipase A_2 (*SlaA*). GCS/GGS isolates were also subjected to PCR with primers targeting *speG*^{dys}. Sixty iGAS and 50 iGCS/GGS cases were identified, corresponding to mean annual incidence rates of 5.0 per 100 000 and 4.1 per 100 000 inhabitants, respectively. Skin and soft tissue infections were the most frequent clinical manifestations of both iGAS and iGCS/GGS disease, and 14 iGAS patients (23%) developed necrotizing fasciitis. The 30-day case fatality rates of iGAS and iGCS/GGS disease were 10% and 2%, respectively. *emm1*, *emm3* and *emm28* accounted for 53% of the GAS isolates, and these types were associated with severe clinical outcome. SAg gene and *SlaA* profiles were conserved within most of the GAS *emm* types, although five profiles were obtained within isolates of *emm28*. *stG643* was the most prevalent GCS/GGS *emm* type, and *speG*^{dys} was identified in 73% of the GCS/GGS isolates. Neither GAS SAg genes nor *SlaA* were detected in GCS/GGS. Our findings indicate a considerable burden of both iGAS and iGCS/GGS disease and a high frequency of necrotizing fasciitis caused by GAS in our community.

Keywords: *emm* gene, GAS, GCS, GGS, invasive infections, streptococcal phospholipase A2 gene, superantigen genes
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Introduction

Subsequent to the resurgence of invasive group A streptococcal (iGAS) disease in western countries from the mid 1980s, the epidemiology and pathogenesis of iGAS disease has been extensively investigated [1,2]. Group C streptococci (GCS) and group G streptococci (GGS) causing human disease are phylogenetically and clinically related to GAS and are almost always of the species *Streptococcus dysgalactiae* subsp. *equisimilis*. Traditionally, this species has rarely been the cause of severe human infections. However, during the last two decades, the incidence of invasive GCS/GGS (iGCS/GGS) disease appears to have increased, and has been comparable to that of iGAS disease in different countries [3–6].

GAS and GCS/GGS share virulence factors, and among them is the M protein. This multifunctional protein is encoded by the *emm* gene. *emm* typing, based on the nucleotide sequence in the hypervariable 5′ end of the *emm* gene, is a powerful epidemiological tool in surveys on streptococcal disease. Streptococcal superantigens (SAgs) and streptococcal phospholipase A_2 (SlaA) are also important GAS virulence factors [7,8]. *SlaA* and the majority of SAg genes are found on phages, and both phage- and chromosomally-encoded SAg genes have been involved in lateral genetic transfers among GAS and GCS/GGS [9]. The relationship between GAS virulence gene profiles and clinical syndromes has previously been investigated, and *emm* types, such as *emm1*/*emm3*, and SAg genes, such as *speA*/*speC*, have been associated with severe clinical syndromes, such as necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS) [2,8,10]. However, data on comprehensive virulence gene

profiling of iGCS/GGS isolates are relatively scarce [9,11–13].

The incidence of iGAS disease has been high in Norway during the last two decades [14,15], but data on iGCS/GGS disease are lacking. In the present study, we have analyzed the clinical and molecular characteristics of invasive β-haemolytic streptococcal disease in western Norway from March 2006 to February 2009.

Materials and Methods

Study population and case definitions

All cases of iGAS and iGCS/GGS disease within the catchment area of the Department of Bacteriology, Haukeland University Hospital, from March 2006 to February 2009 were retrospectively registered. This laboratory receives bacterial isolates from Haukeland University Hospital, Haraldsplass Deaconal Hospital and Voss Hospital. The population in the catchment area increased from 397 000 to 411 500 inhabitants during the study period. Cases were defined by the isolation of GAS, GCS or GGS from a normally sterile site, or from a nonsterile site in association with NF or STSS. NF was defined as described previously [16], and STSS was defined using criteria originally meant for GAS [17]. Severe sepsis was defined as sepsis accompanied by one or more signs of organ dysfunction [18]. Skin or soft tissue infection (SSTI) included wound infection, erysipelas, cellulitis and deep-seated abscesses. Patients were categorized as having primary bacteremia if blood cultures were positive and no source of infection was identified. Infections were classified as nosocomial in patients hospitalized at least 48 h before their positive cultures were drawn.

Clinical information was obtained from medical records. Demographics, diagnoses, comorbidities and information about treatment and outcome were recorded, along with clinical chemistry data and microbiological results. Informed consent was obtained from all the patients. The study was approved by the Regional Committee of Medical Research Ethics.

Bacterial isolates

The isolates were identified in the Department of Bacteriology at Haukeland University Hospital. Group carbohydrate was ascertained using the Streptococcal Grouping kit (Oxoid Ltd, Basingstoke, UK).

emm typing

emm typing was carried out as described previously [19], with previously reported primers [20]. The 5′ end of each sequence was compared with sequences in the *emm* type database, available on the CDC website (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). When a correct subtype assignment could not be determined, the sequence trace was submitted to the CDC for assignment of a new subtype.

Detection of SAg genes and *SlaA*

We used multiplex PCR with specific primers for the 11 GAS exotoxin genes *speA*, *speC*, *speG*, *speH*, *speI*, *speJ*, *speK*, *speL*, *speM*, *ssa* and *smeZ* as described previously [21]. To cover allelic variations of *smeZ*, a simplex PCR with previously reported primers was also used [22]. Simplex PCR amplifications of $speG^{dys}$, a *speG* orthologue found in *Streptococcus dysgalactiae* subsp. *equisimilis*, and *SlaA* were performed with primers described previously [11]. The $speG^{dys}$ primers amplified segments of equal size in both $speG^{dys}$ and *speG*, whereas the *speG* primers only amplified *speG*. Hence, all the GAS and GCS/GGS were screened for the presence of the 11 GAS SAg genes and *SlaA*, whereas only the GCS/GGS were subjected to PCR with the $speG^{dys}$ primers.

Statistical analysis

Categorical data were analyzed using Fisher's exact test or the chi-square test. Nonparametric data were analyzed using the Mann–Whitney test. A two-sided p value ≤ 0.05 was considered statistically significant.

Results

Epidemiology and clinical characteristics

A total of 110 cases were included. GAS, GCS and GGS accounted for 60, four and 46 of these, respectively. Clinical information was available for 109 patients. A total of 107 isolates were available for analysis, and consisted of 59, three and 45 of GAS, GCS and GGS, respectively. Among GAS, 48 isolates were from blood and 11 were from other sterile sites (synovial/pleural/cerebrospinal fluid or tissue biopsy). Forty-two GGS were from blood and the remaining three isolates were from bone ($n = 2$) and synovial fluid ($n = 1$). All three GCS were from blood.

The mean annual incidence of iGAS and iGCS/GGS disease was 5.0 per 100 000 and 4.1 per 100 000 population, respectively. Year-to-year variations were slight because the annual incidence of iGAS disease was in the range 4.5–5.3 per 100 000 and that of iGCS/GGS infections in the range 3.6–4.8 per 100 000. Seasonal variations were observed, with 24% of the cases occurring during March to May, 18% during June to August, 31% during September to November and 27% during December to February. Clinical characteristics of

TABLE 1. Clinical characteristics of invasive group A streptococcal (iGAS) and invasive group C and group G streptococcal (iGCS/GGS) disease

	Number of GAS (%), *n* = 60[a]	Number of GCS/GGS, (%) *n* = 50	p-value
Clinical manifestations			
STSS	6 (10)	1 (2)	0.122
Severe sepsis	29 (48)	18 (36)	0.180
Primary bacteremia	5 (8)	12 (24)	0.034[b]
SSTI	16 (27)	30 (60)	0.001[c]
NF	14 (23)	1 (2)	0.001[b]
Pneumonia	5 (8)	0	0.061
Puerperal sepsis	7 (12)	2 (4)	0.175
Other[d]	14 (23)	7 (14)	0.235
Treatment and outcome			
Mechanical ventilation	6 (10)	0	0.030[b]
Vasopressor treatment	9 (15)	4 (8)	0.375
Surgery	25 (42)	9 (18)	0.007[b]
30 day CFR	6 (10)	1 (2)	0.122

STSS, streptococcal toxic shock syndrome; NF, necrotizing fasciitis; SSTI, skin or soft tissue infection; CFR, case-fatality rate.
[a]Clinical information unavailable for one patient.
[b]Statistically significant (Fisher's exact test).
[c]Statistically significant (chi-square test).
[d]Tonsillitis and bacteraemia (GAS, *n* = 4), pyomyositis (GAS, *n* = 1), arthritis (GAS, *n* = 3, GGS, *n* = 1), osteomyelitis (GCS, *n* = 1; GGS, *n* = 2), endocarditis (GAS, *n* = 1; GGS, *n* = 2), peritonitis (GAS, *n* = 1; GGS, *n* = 1), salpingitis (GAS, *n* = 1), meningitis (GAS, *n* = 2), unknown source of infection (GAS, *n* = 1).

TABLE 2. Demographic variables and risk factors associated with invasive group A streptococcal (iGAS) and invasive group C and group G streptococcal (iGCS/GGS) disease

	Number of GAS (%), *n* = 60	Number of GCS/GGS (%), *n* = 50	p-value
Demographics			
Female sex	35 (58)	20 (40)	0.056
Age < 15 years	2 (3)	0	0.500
Age 15–40 years	16 (27)	4 (8)	0.013[b]
Age 41–65 years	25 (42)	17 (34)	0.410
Age > 65 years	17 (29)	29 (58)	0.002[c]
Risk factors[a]			
One or more risk factors	42 (71)	47 (94)	0.002[c]
Skin lesion	32 (54)	31 (62)	0.442
Hospital acquired infections	5 (8)	5 (10)	1.000
Diabetes	5 (8)	6 (12)	0.751
Chronic heart disease	4 (7)	19 (38)	<0.001[b]
Chronic lung disease	6 (10)	4 (8)	0.751
Chronic renal failure	2 (3)	2 (4)	1.000
Chronic liver disease	5 (8)	3 (6)	0.724
Alcoholism	2 (3)	2 (4)	1.000
Intravenous drug use	6 (10)	2 (4)	0.285
Cancer	11 (19)	13 (26)	0.366
Immunosuppression	7 (12)	5 (10)	1.000

[a]Data on risk factors available for 59 out of 60 iGAS patients.
[b]Statistically significant (Fisher's exact test).
[c]Statistically significant (chi-square test).

iGAS and iGCS/GGS disease are summarized in Tables 1 and 2. A high proportion of NF was observed in the iGAS group. Four of the NF cases occurred during a 1-month period, whereas the remaining 11 cases were distributed throughout the rest of the study period. iGCS/GGS disease was associated with higher age, a larger proportion of male patients, a higher prevalence of chronic heart disease and a higher frequency of SSTI than iGAS disease. Although STSS (*n* = 7) or severe sepsis (*n* = 47) developed in 50% of the patients, the case-fatality rates of iGAS and iGCS/GGS disease at day 30 were only 10% and 2%, respectively. Table 3 shows initial laboratory findings and clinical parameters of patients with NF and SSTI. The six laboratory tests included are the same as those on which a diagnostic scoring system for identification of NF patients [laboratory risk indicator for necrotizing fasciitis (LRINEC) score] is based [23]. NF was associated with higher values for initial pulse rate/C-reactive protein/leucocytes/glucose/LRINEC score than the SSTI, but the only significant differences between the groups were a lower systolic blood pressure and higher haemoglobin values in the NF group compared to the SSTI group.

emm type, SAg gene profile and presence of *SlaA* in relation to clinical disease and outcome

Virulence gene profile in relation to clinical iGAS and iGCS/GGS disease are shown in Tables 4 and 5, respectively. *emm1*, *emm3*, *emm12*, *emm28*, *emm82* and *emm89* accounted for 81% of the GAS isolates. *emm3* was associated with three cases of NF that all developed STSS. *emm1* was associated with three cases of NF and two cases of STSS without NF. *emm3* (cellulitis, *n* = 1, NF + STSS, *n* = 1), *emm12* (pneumonia, *n* = 1) and *emm28* (primary bactaeremia, *n* = 1, pneumonia, *n* = 2) accounted for the six patients with iGAS disease who died within 30 days after admission. The age of these patients was in the range 60–86 years, and five of them had a predisposing disease. SAg gene and *SlaA* profiles were conserved within most of the GAS *emm* types, although five

TABLE 3. Initial clinical findings and laboratory values associated with necrotizing fasciitis (NF) and skin or soft tissue infection (SSTI)

Clinical parameters and initial laboratory values	NF (*n* = 15)	SSTI (*n* = 46)	p-value
Systolic blood pressure (mmHg)	95 (76–125)	115 (70–205)	0.0169[c]
Pulse rate (per min)	105 (84–160)	97 (70–160)	0.0797
Temperature (°C)	38.3 (36.8–41.0)	38.9 (35.4–41.0)	0.3657
C-reactive protein (mg/L)	188 (9–544)	80 (1–526)	0.1007
Leukocyte count ($\times 10^9$/L)	15.9 (2.2–36.1)	13.1 (0.6–30.0)	0.7376
Haemoglobin (g/dL)	13.5 (10.6–16.7)	12.2 (9.6–16.6)	0.0471[c]
Creatinine (μmol/L)	83 (37–510)	83 (49–378)	0.3837
Sodium (mmol/L)	135 (128–145)	137 (128–143)	0.0772
Glucose (mmol/L)[a]	7.3 (4.2–14.2)	6.4 (4.4–15.4)	0.9189
LRINEC score[b]	5 (0–9)	3 (0–10)	0.1732

Values are expressed as the median (range). Pulse rate, temperature and laboratory values recorded on admission. Systolic blood pressure; the lowest value recorded during the first 48 h after admission. LRINEC, laboratory risk index for necrotizing fasciitis.
[a]Results unavailable for five patients with SSTI.
[b]Based on values for C-reactive protein, leukocyte count, haemoglobin, creatinine, glucose and sodium [23]. Seven patients with NF (47%) and 11 patients with SSTI (24%) had a LRINEC score ≥6.
[c]Statistically significant (Mann–Whitney test).

TABLE 4. Virulence gene profile in relation to clinical manifestations of invasive group A streptococcal (iGAS) disease

emm type	Number of isolates	*speA*	*speC*	*speG*	*speH*	*speI*	*speJ*	*speK*	*speL*	*speM*	*ssa*	*smeZ*	*SlaA*	Clinical disease
emm1.0	9	+	–	+	–	–	+	–	–	–	–	+	–	NF (3), pneumonia + STSS (1), pneumonia (1), meningitis (1), puerperal sepsis + STSS (1) puerperal sepsis (1), tonsillitis and bacteraemia (1)
emm3.1	9	+	–	+	–	–	–	+	–	–	+	+	+	NF + STSS (3), SSTI (1), salpingitis (1), arthritis (1), pyomyositis (1), puerperal sepsis (2)
	1	+	–	+	–	–	–	–	–	–	+	+	–	Primary bacteraemia (1)
emm4.0	2	–	+	–	–	–	–	–	–	–	+	+	–	SSTI (1), tonsillitis and bacteraemia (1)
emm9.0	1	–	–	+	–	–	–	+	–	–	–	+	+	Tonsillitis and bacteraemia (1)
emm12.0	5	–	+	+	+	+	–	–	–	–	–	+	–	NF (1), SSTI (1), pneumonia (1), meningitis + SSTI (1), arthritis (1)
	1	–	–	+	+	+	–	–	–	–	+	+	–	SSTI (1)
emm28.0	6	–	+	+	–	–	+	–	–	–	–	+	–	SSTI (3), puerperal sepsis (1), pneumonia (1), primary bacteraemia (1)
	4	–	+	+	–	–	+	+	–	–	–	+	+	Primary bacteraemia (1), pneumonia (1), peritonitis (1), unknown[a] (1)
	1	+	+	+	–	–	+	+	–	–	–	+	+	NF + STSS (1)
	1	–	–	+	–	–	+	+	–	–	–	+	+	SSTI (1)
	1	–	–	+	–	–	+	–	–	–	–	+	–	SSTI (1)
emm75.0	3	–	+	+	–	–	–	+	+	+	–	+	+	NF (1), tonsillitis and bacteraemia (1), puerperal sepsis (1)
emm77.0	1	–	+	–	–	–	–	–	–	–	–	+	–	NF (1)
emm82.0	6	–	+	+	+	+	–	–	–	–	–	+	–	NF (2), SSTI (2), endocarditis (1), primary bacteraemia (1)
emm89.0	4	–	–	+	–	–	–	–	–	–	–	+	–	SSTI (2), arthritis (1), puerperal sepsis (1)
emm89.0	1	–	+	+	–	–	–	–	–	–	–	+	–	SSTI (1)
emm102.0b	1	–	+	+	–	–	+	–	–	–	–	+	–	NF (1)
emm104.0	1	–	–	–	–	–	–	–	+	+	–	+	–	Primary bactaeremia (1)
emm118.0	1	–	+	+	–	–	–	–	–	–	–	+	–	SSTI (1)
Total	59	20	31	55	12	12	23	19	4	4	13	59	19	

One GAS isolate associated with necrotizing fasciitis (NF) was not available for analysis. STSS, streptococcal toxic shock syndrome; SSTI, skin or soft tissue infection.
[a]Clinical information unavailable.

different profiles were detected among *emm28* isolates. All GAS associated with NF and/or STSS possessed *speA*, *speC* or both of these genes. *speA* was significantly associated with GAS NF compared to GAS SSTI (p 0.001). Virulence gene pairs *speH-speI*, *speL-speM* and *speK-SlaA*, encoded on three different phages, were detected exclusively in pairs.

The most prevalent GCS/GGS *emm* type was *stG643* (n = 11), and this type, together with *stC74a*, *stG10*, *stG485*, *stG6* and *stG652*, accounted for 77% of the GCS/GGS. Three novel GGS *emm* subtypes were identified. Two of these types were associated with particularly severe clinical disease because one GGS of *stC74a.2* caused cellulitis and STSS and another GGS of *stG485.2* caused vertebral osteomyelitis in a terminal cancer patient, who died 20 days after admission. *speG*^{dys} was detected in 35 out of 48 GCS/GGS and was restricted to certain *emm* types. Neither GAS SAg genes, nor *SlaA* were detected in GCS/GGS.

Discussion

The data obtained in the present study indicate high rates of both iGAS and iGCS/GGS disease in our community throughout the study period. The rates of iGAS disease were comparable to rates of 2.5–5.7 cases per 100 000 per year that were recently observed in other western countries [4,10,22,24,25]. The incidence of iGAS STSS was in line with those reported from Canada, Denmark and Sweden [10,22,25]. A considerable proportion of our iGAS patients developed NF (23%; 3.5 per 100 000). In Alberta, Canada, 12% (1.7 per 100 000) of the patients with iGAS disease developed NF during 2000–2002 [25] and, in large samples from the USA and several European countries, NF accounted for <10% of iGAS infections [2,3,24]. It is also noteworthy that we found a considerably higher frequency of GAS NF in our region compared to that reported from the total Norwegian population during 2006–2007 [15]. The limited temporal and geographical diversity and a high degree of clinical awareness of NF at the hospitals in our community might have lead to collection biases in the present study compared to larger population-based studies. However, the GAS NF cases were distributed throughout the study period, and high rates of this clinical syndrome have previously been reported in western Norway [26,27]. Therefore, we might speculate that the incidence of NF caused by GAS is particularly high in our community.

Severe local pain and signs of systemic toxicity are clinical hallmarks of NF, although the paucity of early signs from skin and soft tissue can make the clinical diagnosis difficult. Recently, the LRINEC score, based on selected laboratory

TABLE 5. Virulence gene profile in relation to clinical manifestations of invasive group C and group G streptococcal (iGCS/GGS) disease

emm type	Number of isolates	*speG*^{dys}	Clinical disease
stC5345.1	1	–	SSTI (1)
stC74a.0	4	+	SSTI (2), peritonitis (1), endocarditis (1)
stC74a.2[a]	1	+	SSTI + STSS
stG10.0	5	+	SSTI (3), primary bacteraemia (2)
stG245.0	1	–	SSTI (1)
stG2078.0	2	–	SSTI (2)
stG2574.1[a]	1	+	Puerperal sepsis (1)
stG480.0	2	+	SSTI (1), primary bacteraemia (1)
stG485.0	5	+	SSTI (3), puerperal sepsis (1), primary bacteraemia (1)
stG485.2[a]	1	+	SSTI + vertebral osteomyelitis
stG5420.0	1	–	Primary bacteraemia
stG6.0	4	+	SSTI (2), endocarditis (1), primary bacteraemia (1)
stG6.1	2	–	SSTI (2)
stG643.0	6	–	SSTI (4), primary bacteraemia (2)
	1	+	SSTI (1)
stG643.1	4	+	SSTI (2), primary bacteraemia (1), arthritis (1)
stG652.0	4	+	SSTI (3), primary bacteraemia (1)
stG62647.0	2	+	SSTI + vertebral osteomyelitis (1), primary bacteraemia (1)
stG840.0	1	+	Primary bacteraemia (1)
Total	48	35	

Two GCS/GGS isolates associated with osteomyelitis or necrotizing fasciitis were not available for analysis. STSS, streptococcal toxic shock syndrome; SSTI, skin or soft tissue infection.
[a]New *emm* subtype.

values, was proposed as a robust tool for distinguishing NF from SSTI [23]. The results from that study indicated that a LRINEC score ≥6 was highly suggestive of NF. Only seven out of 15 NF cases in our material reached this cut-off value of 6. Hence, we recommend a meticulous clinical approach and a low threshold for early surgical exploration when soft tissue necrosis is suspected.

Results from recent studies have indicated that the burden of iGCS/GGS disease previously has been under-recognized [3–6]. In line with these reports, we found a high incidence of iGCS/GGS disease. iGCS/GGS infections were associated with older patients, a higher frequency of comorbidities and SSTI and a lower incidence of NF than iGAS disease, similar to the findings obtained in a Danish study [3]. However, in a survey on streptococcal disease from Japan during 2006–2007, the rates of NF and cellulitis in the iGCS/GGS group were equal to those among iGAS cases [6]. Our iGAS patients needed surgery and mechanical ventilation more often than the iGCS/GGS patients, probably as a result of the frequent occurrence of severe manifestations among patients with iGAS disease. Yet, the case-fatality rate was relatively low, even among iGAS cases. We consider that this is partly explained by the early recognition and treatment of the most severe clinical syndromes, including surgical debridement of necrotic tissue.

We used *emm* typing and SAg gene and *SlaA* profiling to characterize our isolates. Multilocus sequence typing (MLST) has gained popularity in epidemiological surveys on β-haemolytic streptococci, and results from recent studies indicate a frequent concordance between GAS *emm* type and MLST profile [14,15,28]. Such a correlation also appears to exist among isolates belonging to certain GCS/GGS *emm* types [29]. Given these observations and our focus on virulence associated genes, MLST was not applied on our streptococcal sample.

Relatively few *emm* types dominated among both iGAS and iGCS/GGS isolates in our material. The most prevalent GAS *emm* types (*emm1*, *emm3*, *emm28*) were linked to severe disease or fatal outcome. These types have been dominant in previous studies on iGAS disease, and both *emm1*/M1 and *emm3*/M3 have been significantly associated with NF, STSS or death [2,24,25]. *emm1* and *emm28* were highly prevalent types in Norway during 2006–2007 [15]. *emm3* was associated with only 6% of iGAS cases in that material, indicating an over-representation of this type in our community compared to the total Norwegian population. Six *emm* types accounted for almost 80% of the iGCS/GGS isolates. The most prevalent type, *stG643*, was frequently reported in a survey from the USA during 2002–2005, but absent from a sample collected in Japan [6,29], illustrating that predominant types vary with geographical location.

Phages are probably the primary means of lateral genetic exchange between β-haemolytic streptococci and contribute to strain diversification and enhancement of virulence. In the present study, GAS isolates belonging to *emm28* were associated with five distinct SAg gene profiles. Among the other GAS isolates, we found a close linkage between *emm* type and the SAg gene profile. Similar results have been reported previously [15,30], and it has been speculated that a biological interaction between M protein and phages might partly explain the differences in phage receptiveness among isolates belonging to different *emm* types [30]. Hence, the over-representation of *speA* in GAS causing NF compared to GAS associated with SSTI merely appeared to reflect the genetic armament of isolates belonging to *emm1* and *emm3*. The presence of *SlaA* was also, in line with a previous report, restricted to certain *emm* types, including *emm3*, *emm28* and *emm75* [31]. We detected *speG*^{dys} in the majority (but not all) of the GCS/GGS isolates, and its presence was confined to certain *emm* types. *speG*^{dys} shares a high degree of similarity with *speG* in GAS, and was detected in only two out of 46 iGCS/GGS isolates in a recent study [32]. This might indicate that *speG*^{dys} is situated on an unstable chromosomal location or that some alleles of this gene are not detected by PCR as a result of mutations at primer annealing sites.

The pathogenetic role of *speG*^{dys} is uncertain because previous results do not indicate that severe human disease caused by GCS/GGS is associated with superantigen mitogenicity [32].

In summary, the burden of invasive β-haemolytic streptococcal disease was significant in our community during the study period. The high incidence of skin and soft tissue infections prompts the need for investigations of host and microbe interactions in NF and SSTI.

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Transparency Declaration

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References

1. Beres SB, Sylva GL, Barbian KD *et al.* Genome sequence of a serotype M3 strain of group A *Streptococcus*: phage-encoded toxins, the high-virulence phenotype, and clone emergence. *Proc Natl Acad Sci USA* 2002; 99: 10078–10083.
2. Luca-Harari B, Darenberg J, Neal S *et al.* Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol* 2009; 47: 1155–1165.
3. Ekelund K, Skinhoj P, Madsen J, Konradsen HB. Invasive group A, B, C and G streptococcal infections in Denmark 1999–2002: epidemiological and clinical aspects. *Clin Microbiol Infect* 2005; 11: 569–576.
4. Rantala S, Vuopio-Varkila J, Vuento R, Huhtala H, Syrjanen J. Clinical presentations and epidemiology of beta-haemolytic streptococcal bacteraemia: a population-based study. *Clin Microbiol Infect* 2009; 15: 286–288.
5. Broyles LN, van Beneden C, Beall B *et al.* Population-based study of invasive disease due to beta-hemolytic streptococci of groups other than A and B. *Clin Infect Dis* 2009; 48: 706–712.
6. Takahashi T, Sunaoshi K, Sunakawa K, Fujishima S, Watanabe H, Ubukata K. Clinical aspects of invasive infections with *Streptococcus dysgalactiae* ssp. *equisimilis* in Japan: differences with respect to *Streptococcus pyogenes* and *Streptococcus agalactiae* infections. *Clin Microbiol Infect* 2009. [Epub ahead of print].
7. Sitkiewicz I, Nagiec MJ, Sumby P, Butler SD, Cywes-Bentley C, Musser JM. Emergence of a bacterial clone with enhanced virulence by acquisition of a phage encoding a secreted phospholipase A2. *Proc Natl Acad Sci USA* 2006; 103: 16009–16014.
8. Hauser AR, Stevens DL, Kaplan EL, Schlievert PM. Molecular analysis of pyrogenic exotoxins from *Streptococcus pyogenes* isolates associated with toxic shock-like syndrome. *J Clin Microbiol* 1991; 29: 1562–1567.
9. Igwe EI, Shewmaker PL, Facklam RR, Farley MM, van Beneden C, Beall B. Identification of superantigen genes speM, ssa, and smeZ in invasive strains of beta-hemolytic group C and G streptococci recovered from humans. *FEMS Microbiol Lett* 2003; 229: 259–264.
10. Luca-Harari B, Ekelund K, van der Linden M, Staum-Kaltoft M, Hammerum AM, Jasir A. Clinical and epidemiological aspects of invasive *Streptococcus pyogenes* infections in Denmark during 2003 and 2004. *J Clin Microbiol* 2008; 46: 79–86.
11. Ikebe T, Murayama S, Saitoh K *et al.* Surveillance of severe invasive group-G streptococcal infections and molecular typing of the isolates in Japan. *Epidemiol Infect* 2004; 132: 145–149.
12. Davies MR, McMillan DJ, Beiko RG *et al.* Virulence profiling of *Streptococcus dysgalactiae* subspecies *equisimilis* isolated from infected humans reveals 2 distinct genetic lineages that do not segregate with their phenotypes or propensity to cause diseases. *Clin Infect Dis* 2007; 44: 1442–1454.
13. Hashikawa S, Iinuma Y, Furushita M *et al.* Characterization of group C and G streptococcal strains that cause streptococcal toxic shock syndrome. *J Clin Microbiol* 2004; 42: 186–192.
14. Meisal R, Hoiby EA, Aaberge IS, Caugant DA. Sequence type and emm type diversity in *Streptococcus pyogenes* isolates causing invasive disease in Norway between 1988 and 2003. *J Clin Microbiol* 2008; 46: 2102–2105.
15. Meisal R, Andreasson IK, Hoiby EA, Aaberge IS, Michaelsen TE, Caugant DA. *Streptococcus pyogenes* isolates causing severe infections in Norway, 2006–2007: emm types, multilocus sequence types and superantigen profiles. *J Clin Microbiol* 2010; 48: 842–851.
16. Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Crit Care Med* 2005; 33: 1538–1548.
17. Breiman RF, Davis JP, Facklam RR *et al.* Defining the group A streptococcal toxic shock syndrome: rationale and consensus definition. *JAMA* 1993; 269: 390–391.
18. Levy MM, Fink MP, Marshall JC *et al.* 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 2003; 31: 1250–1256.
19. Kittang BR, Langeland N, Mylvaganam H. Distribution of emm types and subtypes among noninvasive group A, C and G streptococcal isolates in western Norway. *APMIS* 2008; 116: 457–464.
20. Musser JM, Kapur V, Szeto J, Pan X, Swanson DS, Martin DR. Genetic diversity and relationships among *Streptococcus pyogenes* strains expressing serotype M1 protein: recent intercontinental spread of a subclone causing episodes of invasive disease. *Infect Immun* 1995; 63: 994–1003.
21. Lintges M, Arlt S, Uciechowski P *et al.* A new closed-tube multiplex real-time PCR to detect eleven superantigens of *Streptococcus pyogenes* identifies a strain without superantigen activity. *Int J Med Microbiol* 2007; 297: 471–478.
22. Darenberg J, Luca-Harari B, Jasir A *et al.* Molecular and clinical characteristics of invasive group A streptococcal infection in Sweden. *Clin Infect Dis* 2007; 45: 450–458.
23. Wong CH, Khin LW, Heng KS, Tan KC, Low CO. The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. *Crit Care Med* 2004; 32: 1535–1541.
24. O'Loughlin RE, Roberson A, Cieslak PR *et al.* The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000–2004. *Clin Infect Dis* 2007; 45: 853–862.
25. Tyrrell GJ, Lovgren M, Kress B, Grimsrud K. Invasive group A streptococcal disease in Alberta, Canada (2000 to 2002). *J Clin Microbiol* 2005; 43: 1678–1683.

26. Chelsom J, Halstensen A, Haga T, Hoiby EA. Necrotising fasciitis due to group A streptococci in western Norway: incidence and clinical features. *Lancet* 1994; 344: 1111–1115.
27. Mylvaganam H, Bruun T, Vindenes HA, Langeland N, Skrede S. Molecular epidemiological investigation of an outbreak of invasive beta-haemolytic streptococcal infection in western Norway. *Clin Microbiol Infect* 2009; 15: 245–252.
28. Enright MC, Spratt BG, Kalia A, Cross JH, Bessen DE. Multilocus sequence typing of *Streptococcus pyogenes* and the relationships between emm type and clone. *Infect Immun* 2001; 69: 2416–2427.
29. Ahmad Y, Gertz RE Jr, Li Z *et al.* Genetic relationships deduced from emm and multilocus sequence typing of invasive *Streptococcus dysgalactiae* subsp. *equisimilis* and *S. canis* recovered from isolates collected in the United States. *J Clin Microbiol* 2009; 47: 2046–2054.
30. Schmitz FJ, Beyer A, Charpentier E *et al.* Toxin-gene profile heterogeneity among endemic invasive European group A streptococcal isolates. *J Infect Dis* 2003; 188: 1578–1586.
31. Nagiec MJ, Lei B, Parker SK *et al.* Analysis of a novel prophage-encoded group A *Streptococcus* extracellular phospholipase A(2). *J Biol Chem* 2004; 279: 45909–45918.
32. Brandt CM, Schweizer KG, Holland R, Lutticken R, Freyaldenhoven BS. Lack of mitogenic activity of speG- and speGdys-positive *Streptococcus dysgalactiae* subspecies *equisimilis* isolates from patients with invasive infections. *Int J Med Microbiol* 2005; 295: 539–546.