SARI RANTALA

A Population-based Study of Beta-hemolytic Streptococcal Bacteremia

Epidemiological, clinical and molecular characteristics

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ACADEMIC DISSERTATION
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University of Tampere, School of Medicine
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Finland

Supervised by
Docent Jaana Syrjänen
University of Tampere
Finland
Professor Jaana Vuopio
University of Turku
Finland

Reviewed by
Docent Markku Koskela
University of Oulu
Finland
Docent Jarmo Oksi
University of Turku
Finland

Distribution
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P.O. Box 617
33014 University of Tampere
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Tel. +358 40 190 9800
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To my family

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Rantala S, Vuopio-Varkila J, Vuento R, Huhtala H, Syrjänen J: Clinical presentations and epidemiology of beta-hemolytic streptococcal bacteremia: a population-based study. Clin Microbiol Infect 2009; 15: 286-288.

II. Rantala S, Vuopio-Varkila J, Vuento R, Huhtala H, Syrjänen J: Predictors of mortality in beta-hemolytic streptococcal bacteremia: a population-based study. J Infect 2009; 58: 266-272.

III. Rantala S, Vähäkuopus S, Vuopio-Varkila J, Vuento R, Syrjänen J: *Streptococcus dysgalactiae* subsp. *equisimilis* Bacteremia, Finland, 1995-2004. Emerg Inf Dis 2010; 16: 843-846.

IV. Rantala S, Vähäkuopus S, Siljander T, Vuopio J, Huhtala H, Vuento R, Syrjänen J: *Streptococcus pyogenes* bacteremia, *emm* types and superantigen profiles. Eur J Clin Microbiol Infect Dis: In Press.

In addition, some unpublished results are included.

ABBREVIATIONS

CDC	Centers for Disease Control and Prevention (USA)
CI	confidence interval
CLSI	Clinical and Laboratory Standards Institute (USA)
CRP	C-reactive protein
DIC	disseminated intravascular coagulation
Emm	*emm* gene; M protein gene
GAS	group A streptococcus
GBS	group B streptococcus
GCS	group C streptococcus
GGS	group G streptococcus
ICU	intensive care unit
KTL	National Public Health Institute (Kansanterveyslaitos)
MOF	multiorgan failure
NA	not available
NSAID	non-steroidal anti-inflammatory drug
NF	necrotizing fasciitis
NT	nontypable
OR	odds ratio
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
S.	*Streptococcus*
SAg (SAgs)	superantigen(s)
smeZ (*smeZ*)	streptococcal mitogenic exotoxin Z (gene)
spe (*spe*)	streptococcal pyrogenic exotoxin (gene)
S. agalactiae	*Streptococcus agalactiae*
S. pyogenes	*Streptococcus pyogenes*
SSA (ssa)	streptococcal superantigen (gene)
STSS	streptococcal toxic shock syndrome
subsp.	subspecies
THL	National Institute for Health and Welfare (Terveyden ja hyvinvoinnin laitos)

ABSTRACT

Background and aims. The serogroups A (*Streptococcus pyogenes*, GAS), B (*Streptococcus agalactiae*, GBS), C and G (GCS and GGS) are generally defined as beta-hemolytic streptococci. Human large colony-forming groups C and G streptococci are now classified as *S. dysgalactiae* subsp. *equisimilis*. Beta-hemolytic streptococci cause a variety of infections ranging from mild pharyngitis and skin and soft-tissue infections to severe life-threatening infections such as bacteremia, streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis (NF). The most common presenting clinical manifestations are infections of skin and soft-tissue, respiratory, urinary, intestinal or genital tract. *S. pyogenes* and *S. dysgalactiae* subsp. *equisimilis* share virulence factors. The M protein is a major virulence factor in that it confers resistance to phagocytosis. The classical M-protein serological typing was largely replaced in the late 1990s by sequence-based typing of the *emm*-gene encoding for the M protein. Based on the variability of the N-terminal end of the *emm* gene, as many as 150 defined *emm* types are recognized among *S pyogenes* isolates, and more than 50 sequence types presently described among *S. dysgalactiae* subsp. *equisimilis* isolates, respectively. The first aim of the present study was to determine the predisposing factors, clinical presentations and outcome in beta-hemolytic streptococcal bacteremia in the Pirkanmaa area, Finland. The second aim was to determine *emm* types of *S. dysgalactiae* subsp. *equisimilis* and *S. pyogenes* bacteremic isolates and the relation of *emm* types to the severity of bacteremia.

Methods. This study was based on population-wide surveillance for beta-hemolytic streptococcal bacteremia in adults. The predisposing factors, clinical presentations and outcome in beta-hemolytic streptococcal bacteremia during the 10-year observation period (1995-2004) in Pirkanmaa, Finland, was studied retrospectively. A case was defined as a positive blood culture for group A, B, C or G beta-hemolytic streptococci. The number of patients was 309 and the number of bacteremia episodes was 314. All risk factors associated with severe disease and mortality were analyzed. *S. pyogenes* and *S. dysgalactiae* subsp. *equisimilis* isolates were *emm*-typed. Non-typable *S. dysgalactiae* subsp. e*quisimilis* strains and strains isolated from patients with recurrent GGS bacteremia were characterized using pulsed field gel electrophoresis (PFGE). Isolates of *S. pyogenes* were superantigen profiled.

Results. The incidence of GGS bacteremia increased statistically significantly during the study period (p=0.013). Skin infections as the presenting manifestation were particularly common in patients with GAS and GGS bacteremia. A history of previous cellulitis seemed to be a protecting factor against death (p=0.014). Fever was associated with a good prognosis. Alcoholism, ultimately or rapidly

fatal underlying disease, high plasma C-reactive protein (CRP) level and leukopenia on admission predicted a poor outcome. StG480, stG6, stG485, stG643, stC6979, stG166b and stC74a were the seven most common *emm* types, covering 75 % of *S. dysgalactiae* subsp. *equisimilis* isolates. The mortality (p=0.01) and severity of the disease (p=0.001) were higher in *S. dysgalactiae* subsp. *equisimilis* bacteremia caused by rare *emm* types than those caused by common types. Skin and soft-tissue infections such as cellulitis were significantly more frequent clinical manifestations in episodes caused by common than rare types (p=0.007). No association was found between *emm* type, underlying diseases and disease manifestations of *S. pyogenes* bacteremia. No association was found between single SAg genes and presenting clinical manifestations of *S. pyogenes* infections. The putative 26-valent GAS vaccine could have covered 62% of the isolates causing invasive disease in the Pirkanmaa Health District during the study period.

Conclusions. The incidence of GGS bacteremia was increasing in Pirkanmaa, Finland, during 1995-2004. A similar trend in incidence has also been noted in Denmark. Disruption of the cutaneous barrier as a predisposing factor and skin infections as a presenting infection focus were particularly common in patients with GAS and GGS bacteremia.

A history of previous cellulitis seemed to be a protecting factor against death (p=0.014). Also cellulitis as the presenting clinical manifestation predicted a favourable outcome. Confusion, a lowered level of consciousness or dyspnea as the first sign or symptom were markers of a poor prognosis, while fever seemed to be a protecting factor against death. Alcoholism, ultimately or rapidly fatal underlying disease, high plasma CRP level and leukopenia on admission implied a poor prognosis. It is important to identify factors associated with a poor prognosis in order to find patients most likely to benefit from possible preventive measures as well as needing more intensive therapeutic regimens.

The mortality (p=0.01) and severity of the disease (p=0.001) were higher in *S. dysgalactiae* subsp. *equisimilis* bacteremia caused by rare *emm* types as against common types. Skin and soft-tissue infections such as cellulitis were significantly more frequent clinical manifestations among common than among rare *emm* types (p=0.007).

No associations between GAS *emm* types, SAg genes and disease manifestations were observed. The current formulation of GAS vaccine would provide only limited coverage of GAS *emm* types in Finland.

TIIVISTELMÄ

Tausta ja tavoitteet. Tautia aiheuttavat beetahemolyyttiset streptokokit ovat A-ryhmän beetahemolyyttinen streptokokki (GAS) eli *Streptococcus pyogenes*, B-ryhmän beetahemolyyttinen streptokokki (GBS) eli *Streptococcus agalactiae*, C-ryhmän beetahemolyyttinen streptokokki (GCS) ja G-ryhmän beetahemolyyttinen streptokokki (GGS). Viimeisten tutkimusten mukaan isopesäkkeiset C- ja G ryhmän streptokokit on luokiteltu *S. dysgalactiae* subsp. *equisimilis*-nimiseksi bakteeriksi. Beetahemolyyttiset streptokokit aiheuttavat tavallisia nielu- ja iho-infektioita, mutta myös hengenvaarallisia infektioita, kuten verenmyrkytyksiä, toksista sokkia ja nekrotisoivaa faskiittia. Tavallisia infektion lähtökohtia ovat iho-ja pehmytkudos, hengitystiet, virtsaelimet, suolisto tai sukuelimet. *S. pyogenes* ja *S. dysgalactiae* subsp. *equisimilis* jakavat virulenssitekijöitään. Molemmissa esiintyvä M-proteiini on solun tärkein virulenssitekijä, koska se estää fagosytoosin. 1990 luvun lopulla serotyypityksen perustuva tyypitysmenetelmä korvattiin genotyypitykseen perustuvalla tyypitysmenetelmällä (*emm*-tyypitys). Perustuen *emm*-geenin hybervariaabelin osan muuntuvuuteen, GAS:sta on tunnistettu noin 150 *emm* tyyppiä ja GGS:sta noin 50 *emm* tyyppiä. Tutkimuksen päätavoite oli selvittää beeta-hemolyyttisten streptokokkien aiheuttamien bakteremioiden riskitekijöitä, taudinkuvaa ja ennustetta Pirkanmaan sairaanhoitopiirin alueella Suomessa. Toinen tutkimuksen tavoite oli tehdä *emm* tyypitys *S. dysgalactiae* subsp. *equisimilis* ja *S. pyogenes* veriviljelykannoille ja tutkia eri *emm* tyyppien merkitystä bakteremian vaikeusasteeseen.

Menetelmät. Tutkimus perustui väestöpohjaiseen beetahemolyyttisten streptokokkien aiheuttamien bakteremioiden seurantaan aikuisilla. Tutkimuksessa selvitettiin retrospektiivisesti sairauskertomuksista 10 vuoden ajalta (v.1995-2004) beetahemolyyttisten streptokokkien aiheuttamien bakteremioiden riskitekijöitä, taudinkuvaa ja ennustetta Pirkanmaalla Suomessa. Tapausmääritelmänä oli veren positiivinen A-, B-, C- tai G- ryhmän beetahemolyyttinen streptokokkiviljelylöydös. Potilaita oli yhteensä 309 ja bakteremiaepisodeja 314. Tilastollisin menetelmin etsittiin vaikeaan taudinkuvaan ja kuolemaan liittyviä tekijöitä. Kaikki *S. pyogenes* ja *S. dysgalactiae* subsp. *equisimilis* veriviljelykannat *emm*-tyypitettiin. Tyypittymättömille *S. dysgalactiae* subsp. *equisimilis* kannoille ja niiden potilaiden kannoille, joilla oli toistuva GGS-bakteremia tehtiin pulssikenttä-geelielektroforeesi (PFGE)- tyypitys. *S. pyogenes* kannoille tehtiin superantigeenien määritys.

Tulokset. Tutkimuksessa havaittiin G-streptokokin aiheuttamien bakteremioiden ilmaantuvuuden suureneminen tilastollisesti merkitsevästi ($p=0.013$). Tutkimuksessa todettiin ihon olevan yleisin infektion lähtökohta GAS ja GGS

bakteremioissa. Tutkimuksessa todettiin myös, että aiempi ruusuinfektio näytti suojaavan kuolemalta beetahemolyyttisten streptokokkien aiheuttamissa bakteremioissa (p=0.014). Sairaalaan tullessa esiintyvän kuumeen todettiin olevan hyvän ennusteen merkki. Alkoholismi, vaikea perustauti, matala leukosyyttitaso ja korkea plasman C-reaktiivinen proteiini (CRP)- taso lisäsivät kuoleman riskiä. StG480, stG6, stG485, stG643, stC6979, stG166b, and stC74a olivat seitsemän yleisintä *emm*-tyyppiä *S. dysgalactiae* subsp. *equisimilis* bakteremiassa kattaen yhteensä 75% kaikista veriviljelykannoista. *S. dysgalactiae* subsp. *equisimilis* (C- ja G-streptokokki) bakteremian yhteydessä todettiin harvinaisten *emm*-tyyppien liittyvän suurempaan tapauskuolevuuteen (p=0.01) ja vaikeampaan taudinkuvaan (p=0.001). *S. dysgalactiae* subsp. *equisimilis* (C- ja G-streptokokki) bakteremiassa iho-ja pehmytkudosinfektioita kuten esim. selluliittia esiintyi tilastollisesti enemmän yleisten *emm*-tyyppien aiheuttamissa infektioissa (p=0.007). *S. pyogenes* bakteremian yhteydessä ei löydetty yhteyttä *emm*-tyypin, perustautien tai infektion lähtökohdan välillä. Myöskään yhteyttä infektiolähteen ja superantigeenien välillä ei havaittu. Nykyinen kehitteillä oleva GAS rokote olisi kattanut vain 62% baktereemisista GAS *emm*-tyypeistä Pirkanmaan alueella.

Yhteenveto. Tilastollisesti merkitsevä (p=0.013) G-streptokokin aiheuttamien bakteremioiden ilmaantuvuuden suureneminen todettiin Pirkanmaalla Suomessa vuosina 1995-2004, kuten myös on havaittu Tanskassa. GGS on ohittanut GAS:n ilmaantuvuudessa ja sen merkitys kliinisenä taudinaiheuttajana on kasvanut. Ihorikot altistavat GAS:n ja GGS:n aiheuttamille ihoinfektioille ja bakteremioille.

Aiempi ruusuinfektio näyttää suojaavan kuolemalta beetahemolyyttisissä streptokokkibakteremioissa (p=0.014). Selluliitti infektion lähtökohtana liittyy hyvään ennusteeseen. Sekavuus, tajuttomuus ja hengenahdistus ovat huonon ennusteen merkkejä, kun taas kuumeen esiintyminen parantaa ennustetta. Alkoholismi, vaikea perustauti, matala leukosyyttitaso ja korkea plasman CRP taso lisäsivät kuolemanvaaraa. On tärkeää havaita huonoon ennusteeseen liittyvät tekijät, jotta löydämme potilaat, jotka tarvitsevat erityisen tehokasta hoitoa.

Tutkimuksessa havaittiin harvinaisten *emm*-tyyppien liittyvän suurempaan kuolevuuteen (p=0.01) ja vaikeampaan taudinkuvaan (p=0.001) *S. dysgalactiae* subsp. *equisimilis* bakteremiassa. Iho-ja pehmytkudosinfektioita kuten esim. selluliittia esiintyi tilastollisesti enemmän yleisten *emm*-tyyppien joukossa (p=0.007).

GAS *emm*-tyyppien, superantigeenien ja infektion lähtökohdan välillä ei todettu yhteyttä. Nykyinen GAS rokote kattaa vain osan bakteremioita aiheuttavista *emm*-tyypeistä Pirkanmaalla.

1. INTRODUCTION

The beta-hemolytic streptococci of Lancefield groups A, B, C, and G (GAS, GBS, GCS and GGS) can cause a variety of invasive and non-invasive infections. The large colony-forming groups C and G streptococci, which infect humans, are now classified as *S. dysgalactiae* subspecies *equisimilis* (Facklam 2002). Disease manifestations can range in severity from mild throat and skin infections to severe invasive conditions such as bacteremia, pneumonia, necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS). *S. pyogenes* (group A streptococci) and *S. dysgalactiae* subsp. *equisimilis* (GCS and GGS) share many clinical features: skin and soft-tissue infections such as cellulitis are the most common clinical manifestations (Cohen-Poradosu et al. 2004; O'Loughlin et al. 2007; Lamagni et al. 2008b; Broyles et al. 2009). In *S. agalactiae* (GBS) bacteremia patients, primary bacteremia is the most common manifestation, accounting for 20 to 50% of cases (Skoff et al. 2009).

Underlying diseases are common in beta-hemolytic streptococcal bacteremia. In a previous study 67% of GAS patients had an underlying disease, the most common being heart disease (20%), diabetes mellitus (20%), and skin condition (17%) (O'Loughlin et al. 2007). A more recent study showed 96% of *S. dysgalactiae* subsp. *equisimilis* patients to be suffering from an underlying condition, the three most common being cardiovascular disease (44%), diabetes mellitus (42%) and chronic skin condition (30%) (Broyles et al. 2009).

Spread of beta-hemolytic streptococcus to the blood can precede a very rapid progression of the disease and severe complications. Globally, at least 163 000 deaths annually are associated with invasive GAS disease (Carapetis et al. 2005). The majority of GBS cases involve adults over 65 years of age and more than 50% of all deaths now occur in elderly patients (O'Loughlin et al. 2007; Thigpen et al. 2007). The global burden of diseases caused by *S. dysgalactiae* subsp. *equisimilis* (group C and G) is not known.

The incidence of GAS disease typically varies over time and by geographic region (O'Brien et al. 2002; O'Loughlin et al. 2007). Recent epidemiological studies have shown increasing numbers of invasive GGS infections in Denmark (Ekelund et al. 2005). In addition to GAS and GBS, *S. dysgalactiae* subsp. *equisimilis* is now also recognized as an important bacterial pathogen. However, the population-based data on *S. dysgalactiae* subsp. *equisimilis* infections are limited (Ekelund et al. 2005; Broyles et al. 2009).

A wide range of factors associated with increased case fatality rates in GAS bacteremia have been described, including pneumonia (O'Brien et al. 2002; Hollm-Delgado et al. 2005; Mehta et al. 2006; O'Loughlin et al. 2007), alcoholism (Davies et al. 1996; Ekelund et al. 2005), age (O'Brien et al. 2002; Sharkawy et al. 2002; Ekelund et al. 2005; O'Loughlin et al. 2007), immunosuppression (Nielsen et al. 2002; Ekelund et al. 2005), cancer (Sharkawy et al. 2002; Hollm-Delgado et al. 2005), chronic heart or lung disease (O'Brien et al. 2002; Ekelund et al. 2005), working or living in a hospital (Hollm-Delgado et al. 2005), liver dysfunction (Mehta et al. 2006), use of non-steroidal anti-inflammatory drug (NSAID) (Stevens 1995), disseminated intravascular coagulation (DIC) (Mehta et al. 2006) and STSS (O'Brien et al. 2002; Mehta et al. 2006; O'Loughlin et al. 2007). In contrast, the number of population-based studies describing predictors of case-fatality in GBS, GCS or GGS bacteremia is limited (Ekelund et al. 2005).

The major virulence factor in GAS is the M protein, as it confers resistance to phagocytosis (Fischetti 1989). The most commonly used typing method for GAS is based on sequencing the hypervariable part of the *emm* gene (Fischetti 1989; Beall et al. 1996). Currently, over 150 *emm* sequence types have been identified among GAS strains and 50 among *S. dysgalactiae* subsp. *equisimilis* strains (CDC 2011d; CDC 2011c). *Emm* typing can be used to analyze the molecular epidemiology of GAS and *S. dysgalactiae* subsp. *equisimilis* infections. Population-based data on *S. dysgalactiae* subsp. *equisimilis* bacteremia and their *emm* types are scant (Broyles et al. 2009). The GAS superantigens (SAgs) are also important for virulence, participating in the induction of the systemic toxicity associated with severe infections (Kotb 1995; Norrby-Teglund et al. 2000; Lintges et al. 2010). Most GAS strains express several different superantigens, and the pyrogenic exotoxins A and C (SpeA, and SpeC, respectively) have received particular attention in the context of invasive disease.

2. REVIEW OF THE LITERATURE

2.1 General aspects of sepsis

Sepsis ranges in severity from mild systemic inflammation to multiorgan failure (MOF) in septic shock, which is associated with high mortality rates. The overall case fatality rate in sepsis has been estimated to be around 30% (Angus et al. 2001; Karlsson et al. 2007; Lever et al. 2007) and it increases with severity, with rates of 40 to 80% in patients with septic shock (Friedman et al. 1998; Martin et al. 2003; Lever et al. 2007). Although medical care has improved, the incidence of sepsis is still increasing in developing countries (Abraham et al. 2000; Alberti et al. 2002; Martin et al. 2003; Danai et al. 2005). The number of gram-positive bacteria identified as causative pathogens has increased (Martin et al. 2003). This change may be associated with improved medical technology, expanded use of invasive devices, and increased numbers of patients at risk of developing sepsis, for example immunocompromised patients and the elderly (Angus et al. 2001; Lever et al. 2007).

The most severe manifestation of sepsis is STSS, caused by GAS. STSS is characterized by hypotension and multiorgan failure, and it may also affect young healthy individuals without underlying diseases. The case fatality rate in STSS frequently exceeds 50% (Davies et al. 1996).

2.2 Classification of the groups A, B, C and G beta-hemolytic streptococci

The classification of streptococci is based on their hemolysis pattern on blood agar plates. There are three groups of these patterns; alpha, beta and gamma, which correspond to partial, complete or no lysis of the red blood cells, respectively. The streptococci were further classified into different serogroups (from A to V) by Rebecca Lancefield in 1933, based on the cell-wall carbohydrates. Beta-hemolytic

streptococci generally include serogroups A (GAS ie. *S. pyogenes*), B (GBS ie. *S. agalactiae*), C (GCS) and G (GGS). The taxonomy of these organisms has been changed in recent years. The Lancefield group G carbohydrate may be noted in several beta-hemolytic streptococcal species, including *S. anginosus* and *S. canis*, but mainly in *S. dysgalactiae* subsp. *equisimilis.* This species also hosts variants with Lancefield group A, C and L carbohydrates. The species was determined by gene sequencing of the group C species, formerly named *Streptococcus equisimilis*, which showed it to be indistinguishable from group G *S. dysgalactiae.* This finding resulted in the new taxonomy, *S. dysgalactiae* subsp. *equisimilis*. According to the current taxonomy, all large colony-forming organisms previously classified within group C and G are included within *S. dysgalactiae* subsp. *equisimilis*. (Facklam 2002)

2.3 Clinical manifestations and epidemiology of groups A, B, C and G beta-hemolytic streptococcal diseases

2.3.1 Group A streptococcal diseases

GAS constitute an important human pathogen commonly causing throat infections such as tonsillitis and pharyngitis, as well as skin and soft-tissue infections such as impetigo, erysipelas and cellulitis and more uncommonly severe invasive diseases such as bacteremia, NF and STSS (Davies et al. 1996; O'Brien et al. 2002; O'Loughlin et al. 2007). GAS is the most common cause of bacterial pharyngitis or tonsillitis. Scarlet fever is a diffuse red rash on neck and chest occurring together with tonsillitis. Diffuse erythematous eruption of scarlet fever occurs as a result of delayed-type skin reactivity to streptococcal pyrogenic exotoxin. However, bacteremia is a very rare manifestation in tonsillitis or scarlet fever.

The most conspicuous presenting clinical manifestation in conditions caused by GAS or GGS is skin infection (Cohen-Poradosu et al. 2004; Ekelund et al. 2005; O'Loughlin et al. 2007). Impetigo is a superficial and purulent infection of the dermis. Erysipelas and cellulitis are non-necrotizing infections of the skin and underlying tissue. Classically, erysipelas is fiery red, tender and painful, with well-

demarcated edges, while cellulitis is a more deeply situated skin infection with less clearly demarcated inflammation of the skin. However, the distinction between erysipelas and cellulitis is not clear-cut (Bisno et al. 1996). Cellulitis is a very common disease, the incidence of lower-extremity cellulitis is 199 per 100,000 person-years (McNamara et al. 2007). Bacteremia occurs under 5% of cellulitis cases (Bisno et al. 1996; Perl et al. 1999). It has recently been shown that skin and soft-tissue infections are the most common presenting clinical manifestations (42%) among GAS patients, followed by respiratory tract infections (17%) (Lamagni et al. 2008b). NF is a severe infection of the deeper subcutaneous tissue and fascia, where local pain and rapid tissue destruction are typical.

Invasive GAS infections include NF, STSS, meningitis, peritonitis, pneumonia and sepsis. The definition of STSS includes identification of beta-hemolytic streptococci at a normally sterile site, septic shock and MOF (The Working Group on severe Streptococcal Infections 1993). It is mediated by streptococcal toxins which act as superantigens. Puerperal sepsis, also known as "childbed fever", was formerly a common cause of death in young women (Areschoug et al. 2004). Puerperal sepsis is very rare in Western countries. In a recent European multi-center study (Strep-EURO), 3% of invasive GAS patients had puerperal sepsis (Lamagni et al. 2008a). GAS can also cause severe fatal infections in previously healthy young individuals (Stevens et al. 1989). GAS infection can be followed by nonsuppurative complications such as acute rheumatic fever and acute post-streptococcal glomerulonephritis (O'Brien et al. 2002).

The global burden of invasive GAS disease is very high, with at least 663 000 new cases and 163 000 deaths each year worldwide (Carapetis et al. 2005). At least 517 000 deaths occur globally each year due to severe GAS disease (eg. acute rheumatic fever, rheumatic heart disease, post-streptococcal glomerulonephritis and invasive infections) (Carapetis et al. 2005). The greatest burden is due to rheumatic heart disease, with a prevalence of at least 15.6 million cases, 280 000 new cases per year and 233 000 deaths each year (Carapetis et al. 2005). The incidence of acute rheumatic fever is not available, because almost all of these cases occur in less developed countries (Carapetis et al. 2005). The incidence of post-streptococcal glomerulonephritis in adults is 0.3/100 000/year in more developed countries and 2/100 000/year in less developed countries (Carapetis et al. 2005).

2.3.2 Group B streptococcal diseases

GBS-disease is an important cause of illness in neonates, pregnant women and the elderly with underlying diseases (Schuchat et al. 1994; Hussain et al. 1995; Schuchat 1998). In the neonate, infection can lead to meningitis (Farley et al. 1993). Bacteremia without a defined focus, and skin and soft–tissue infections are the most common disease manifestations among non-pregnant patients with GBS infection (Trivalle et al. 1998; Tyrrell et al. 2000; Edwards et al. 2005; Phares et al. 2008; Skoff et al. 2009).

In several reports, primary bacteremia without a defined focus is the most common manifestation among GBS bacteremia patients, accounting for 20-50% of cases, and this manifestation carries a high case fatality rate. Cellulitis, foot ulcers, abscess and infection of decubitus ulcers are common skin infections (Edwards et al. 2005). Urinary tract infection and pneumonia are more common in elderly persons than in younger adults (Trivalle et al. 1998; Tyrrell et al. 2000). GBS also causes peritonitis, meningitis, septic arthritis and endocarditis, and very rarely NF or STSS (Trivalle et al. 1998; Tyrrell et al. 2000; Edwards et al. 2005). In a recent study from the United States bacteremia without focus (48%), bacteremic cellulitis (22%) and pneumonia (11%) were the three most common clinical manifestations, followed by osteomyelitis (9%) and arthritis (9%) (Phares et al. 2008). Infections in the elderly include severe invasive disease manifestations, and high mortality (Farley et al. 1993; Schuchat 1998; Trivalle et al. 1998). About 4% of sufferers have recurrent infections, recurrence being possibly associated with a focus of infection such as endocarditis or osteomyelitis.

In the United States in 2005, GBS caused 21 500 cases of invasive disease and 1700 deaths (Phares et al. 2008; CDC 2011a). The majority of GBS cases are recorded among adults over 65 years of age, and more than 50% of all deaths now occur in elderly patients (Edwards et al. 2005; CDC 2011a). GBS is also a leading cause of illness in neonates (Phares et al. 2008). The GBS incidence increases with age and is quadrupled in older adults in nursing homes, and nursing home residents have significantly higher case-fatality rates (Henning et al. 2001; Edwards et al. 2005). Infections in the elderly include severe invasive disease manifestations and high mortality (Farley et al. 1993; Schuchat 1998; Trivalle et al. 1998).

2.3.3 Group C and G streptococcal diseases

S. dysgalactiae subsp. *equisimilis* constitutes a major cause of illness in older adults with underlying diseases (Ekelund et al. 2005; Broyles et al. 2009). It is an important bacterial pathogen and the clinical spectrum of diseases it causes is the same as that of *S. pyogenes*, including the occurrence of poststreptococcal sequelae (Reid et al. 1985; Haidan et al. 2000; Brandt et al. 2009). The spectrum of *S. dysgalactiae* subsp. *equisimilis* diseases ranges from pharyngitis and skin and soft-tissue infections such as impetigo, wound infections, erysipelas, and cellulitis to life-threatening NF and STSS (Broyles et al. 2009). Cellulitis was found to be the most common presenting clinical manifestation (61%) among GGS bacteremia (Cohen-Poradosu et al. 2004). Cellulitis was also the most common presenting clinical manifestation (41%) among *S. dysgalactiae* subsp. *equisimilis* bacteremia (Broyles et al. 2009). This pathogen can also cause bacteremia without focus, pneumonia, septic arthritis, osteomyelitis, meningitis, endocarditis and puerperal sepsis. A high rate of recurrent GGS bacteremia has been reported (Cohen-Poradosu et al. 2004). Pediatric *S. dysgalactiae* subsp. *equisimilis* infection is very rare (Ekelund et al. 2005; Broyles et al. 2009).

Infections due to *S. dysgalactiae* subsp. *equisimilis* are transmitted from person to person similarly to GAS infections (Davies et al. 2007; Broyles et al. 2009). Most severe infections occur sporadically. Many of the virulence factors present in *S. pyogenes* can also be found in *S. dysgalactiae* subsp. *equisimilis* strains (Davies et al. 2007). Only a few laboratories identify GCS and GGS at species level and the exact numbers of infections caused by them is thus not known. The global burden of diseases caused by *S. dysgalactiae* subsp. *equisimilis* is likewise unknown.

2.3.4 Seasonal patterns of beta-hemolytic streptococcal infection

Invasive GAS disease has typically a higher frequency between late winter and early spring, and a substantially lower frequency in the summer/autumn, a finding fairly ubiquitous across Europe (Martin et al. 1990; Andersen et al. 1995). Invasive pneumococcal disease shows a very similar seasonal pattern (Dowell 2001).

Seasonal variation in the incidence of GBS, GCS and GGS would not appear to have been reported.

2.4 Incidence of groups A, B, C and G beta-hemolytic streptococcal bacteremic infections

GAS bacteremia is most common in very young children and in patients over 60 years of age (Davies et al. 1996; O'Brien et al. 2002). The annual incidence of invasive GAS disease in the USA has remained stable being 3.5 cases per 100000 persons during 1995-2004 (O'Brien et al. 2002; O'Loughlin et al. 2007). This is consistent with rates 3.5-3.6 cases per 100000 persons/year found in the United Kingdom during 2002-2003 (Lamagni et al. 2005). In Finland, Sweden and Denmark, the GAS incidence was 2.2 to 2.3/100 000/year during 2003-2004 in Strep-EURO study (Lamagni et al. 2008a). In Denmark, no increase in invasive GAS infections was observed during the study period 1999-2002 (Ekelund et al. 2005).

A three-fold increase in invasive GBS infections was found in Denmark during 1999-2002 (Ekelund et al. 2005). A two to four fold increase in the incidence of invasive GBS disease has occurred in adults, and more than two-thirds of the cases in the United States involve adults (Farley et al. 1993; Blumberg et al. 1996; Farley 2001; Skoff et al. 2009). The majority of GBS cases occur in adults over 65 years of age and more than 50% of all deaths now occur among elderly patients (Edwards et al. 2005; CDC 2011a). The GBS incidence increases with age and is quadrupled in older adults in nursing homes (Henning et al. 2001; Edwards et al. 2005). The incidence of GBS bacteremia increased from 3.0 to 4.7 cases per 100 000 population in Finland from year 2000 to 2009 (THL 2011). This increase is statistically significant ($p<0.001$, unpublished data). In 2009 41 per cent of GBS bacteremia cases in Finland occurred in adults over 65 years of age (THL 2011).

GGS bacteremia is most common in patients over 65 years of age (Ekelund et al. 2005). In Denmark, the incidence of invasive GGS infection was on an average 1.9/100 000 per year during 1999-2002, and a three-fold increase in GGS infections was noted during 1999-2002 (Ekelund et al. 2005). In the United States the

incidence of beta-hemolytic streptococcal bacteremia other than GAS or GBS was 3.2 cases per 100000 persons during 2002-2004 (Broyles et al. 2009). The authors found the burden of invasive *S. dysgalactiae* subsp. *equisimilis* infections to approximate that of invasive *S. pyogenes* infections (Broyles et al. 2009). *S. dysgalactiae* subsp. *equisimilis*, *S. anginosus* and *S. canis* were included in the study (Broyles et al. 2009), and hence its findings cannot be directly compared with the *S. dysgalactiae* subsp. *equisimilis* incidence.

2.5 Predisposing factors in beta-hemolytic streptococcal bacteremias

2.5.1 Contacts and human carriage of beta-hemolytic streptococci and breakdowns of skin and mucous membranes

Transmission of group A, B, C and G streptococci is usually through direct contact with droplets of saliva or nasal secretions, or through skin contact, especially contact with infected skin lesions. Both infected persons and carriers can transmit the disease. Mothers colonized with group B streptococci transmit bacteria to their infants in utero or during delivery (Schuchat 1998).

Carriage of GAS has been found asymptomatic on the skin, pharynx, genital tract and perianal area (Barth 1987; Stevens 2000). GAS frequently colonize the throats of school-aged children; carriage rates of 15 to 20% have noted in several studies (Martin et al. 2004; Tanz et al. 2007). Adults have low pharyngeal carrier rates (<5%). In a Finnish study about acute bacterial cellulitis they found 90 control subjects, who had not suffered from cellulitis and found that two out of 90 carried GAS (2%) in their throat swab (Siljander et al. 2008). Carriage can be transient or persistent (Martin et al. 2004; Tanz et al. 2007). GBS is common as a colonizer of the gastrointestinal and female genital tracts (10-40%) and rare as a colonizer of the pharynx (5%). In same Finnish study GBS was carried in the pharynx by 1% of control subjects. (Siljander et al. 2008). GCS and GGS are known to be commensals and pathogens in domestic animals. They also occur as a part of the normal flora of

the pharynx, skin, intestinal tract and vagina (Auckenthaler et al. 1983; Vartian et al. 1985). Sites of colonization are the most likely reservoirs for transmission.

Breakdowns of the skin such as skin erosions, eczemas, psoriasis, chronic ulcers, traumatic ulcers, or operative wounds are common bacterial portals of entry. (Davies et al. 1996; O'Brien et al. 2002; Factor et al. 2003; O'Loughlin et al. 2007; Lamagni et al. 2008b).

2.5.2 Group A streptococcal bacteremia

A number of host factors have been identified as being associated with an increased risk of severe *S. pyogenes* infection. These include underlying diseases which affect immune function, for example diabetes, malignancy and alcoholism. The elderly and young children have a higher risk of infection than young adults (Davies et al. 1996; O'Brien et al. 2002). In several studies, males have had a higher incidence of invasive GAS disease as compared to females (O'Loughlin et al. 2007; Lamagni et al. 2008a). GAS patients are generally younger and associated less frequently with predisposing factors as compared to GBS, GCS and GGS patients (Farley et al. 1993; Tyrrell et al. 2000; Lewthwaite et al. 2002; Sylvetsky et al. 2002; Ekelund et al. 2004). In a study conducted in the United States, 67% of GAS patients had at least one underlying disease. The most common of them were heart disease (20%), diabetes mellitus (20%) and skin conditions (17%) (O'Loughlin et al. 2007). Alcoholism is associated especially with GAS infections (Skogberg et al. 1988; Davies et al. 1996). Many countries worldwide have reported a higher incidence of severe GAS disease in particular ethnic groups. Studies from North America have found higher rates of GAS infections in black Americans (O'Brien et al. 2002), native Americans (Hoge et al. 1993) and aboriginals in the arctic region of Canada compared to white European settlers. Among risk factors among women, childbirth is one of the most prominent as a result of bacteria colonizing the vagina contaminating traumatic wounds incurred during the birthing process (Maharaj 2007).

2.5.3 Group B streptococcal bacteremia

A number of factors inherent in patients have also been identified as imposing an increased risk of severe GBS infections. These include cardiac disease, diabetes mellitus, malignancy, cirrhosis, neurologic impairment (dementia, cerebrovascular disease and paraplegia), decubitus ulcers, bedridden state, residence in a nursing home and immune senescence (Farley et al. 1993; Jackson et al. 1995; Trivalle et al. 1998; Tyrrell et al. 2000; Henning et al. 2001). Older patients have proved to have more often cardiovascular disease, to be bedridden or to be residents in a nursing home as compared to younger patients (Trivalle et al. 1998; Tyrrell et al. 2000). In contrast, younger patients have more often had diabetes mellitus and malignancy as compared to older patients (Trivalle et al. 1998; Tyrrell et al. 2000). A recent study in United States found diabetes (44%) to be the most common underlying condition among GBS patients, followed by cardiovascular disease (21%), obesity (17%) and cancer (15%) (Skoff et al. 2009). Overall, 88% of GBS patients had some underlying disease (Skoff et al. 2009). GBS, as well as GCS, and GGS are common among elderly patients, often in association with chronic underlying diseases and predisposing factors (Sylvetsky et al. 2002; Cohen-Poradosu et al. 2004; Edwards et al. 2005). The incidence of GBS is twice as high in the black population as in the white in the USA (Farley et al. 1993; Phares et al. 2008). Among risk factors for women, childbirth is one of the most prominent due to bacteria colonizing the vagina (Maharaj 2007). Mothers colonized with GBS also increase the burden of neonatal GBS disease (Schuchat 1998).

2.5.4 Group C and G streptococcal bacteremias

GCS and GGS bacteremias are linked to diabetes mellitus, cardiovascular disease, malignancy, immunosuppression or breakdown of the skin (Auckenthaler et al. 1983; Vartian et al. 1985; Salata et al. 1989; Woo et al. 2001; Sylvetsky et al. 2002; Cohen-Poradosu et al. 2004). In the last mentioned study 92% of GGS bacteremia patients had an underlying disease and the most common of them were malignancy (35%) and diabetes mellitus (35%) (Cohen-Poradosu et al. 2004). In an American study cardiovascular disease (44%) and diabetes mellitus (42%) were the most common underlying diseases among patients with *S. dysgalactiae* subsp. *equisimilis*

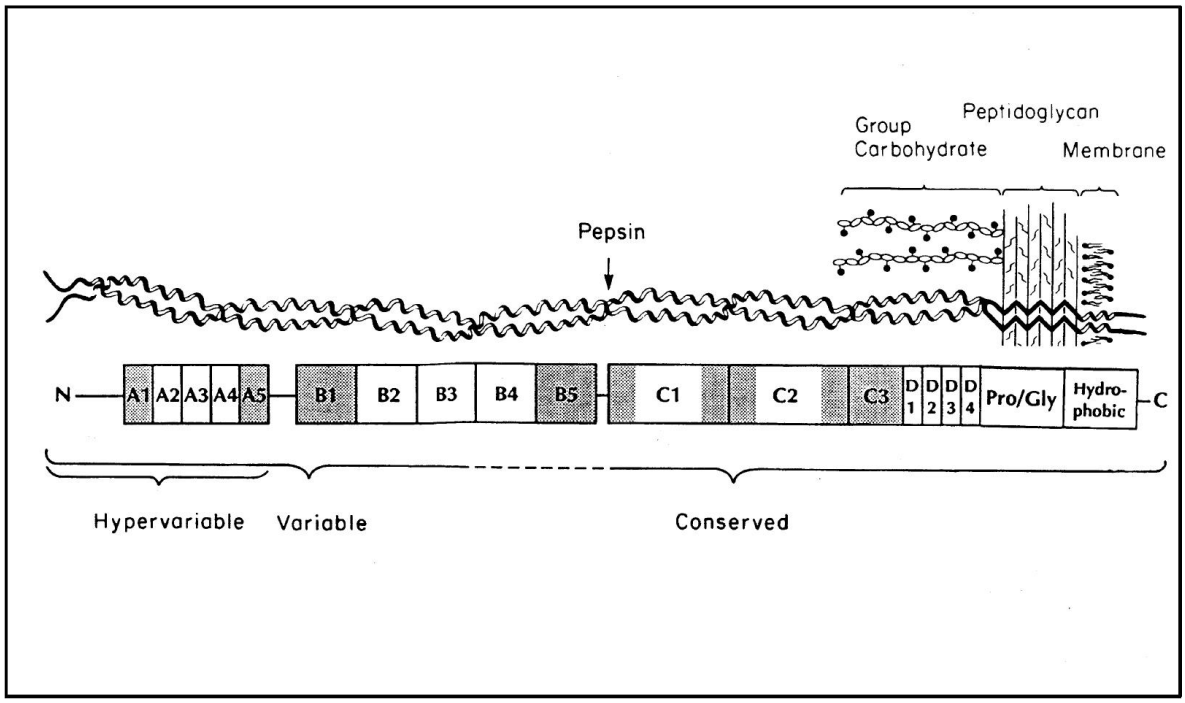
bacteremia, followed by obesity (30%) and chronic skin disease (30%) (Broyles et al. 2009).

2.6 Virulence factors in group A streptococci and *S. dysgalactiae* subsp. *equisimilis* (group C and G streptococci)

2.6.1 *emm* types of group A streptococci

The major virulence factor in GAS is the M protein as this confers resistance to phagocytosis. GAS can be classified into about 80 different serotypes, based on serological reactivity with the hypervariable N-terminus of the M protein. The coverage of antisera available against M proteins involves limitations, and there have been problems with strains remaining non-typable by serotyping (Moses et al. 2003). Partly for this reason the classic M protein serological typing was largely replaced in the late 1990s by sequence typing of the 5'portion of the emm-gene (Facklam et al. 1999). Based on the variability of the N-terminal end of the *emm* gene (encoding the M protein), about 150 defined *emm* types are recognized (CDC 2011d). *Emm* typing can be used for epidemiological analysis of GAS isolates. GAS serotypes causing invasive infections vary over time and within given geographic areas (O'Brien et al. 2002; O'Loughlin et al. 2007). No *emm* type can be uniquely associated with a particular disease, although there is evidence correlating to certain types. Several studies have found a correlation of *emm*1 and *emm*3 with severe invasive disease, NF and STSS (Colman et al. 1993; Darenberg et al. 2007; Luca-Harari et al. 2009; Lintges et al. 2010). Several studies have also found an association between *emm*28 and puerperal sepsis (Raymond et al. 2005; Darenberg et al. 2007; Luca-Harari et al. 2009). An association between *emm* 81 and skin and tissue involvement has also been reported (Colman et al. 1993; Darenberg et al. 2007). In addition to GAS, GCS and GGS also harbor M proteins.

Figure 1. A representation of the streptococcal M protein. Adapted from (Fischetti 1989)



2.6.2 *emm* types of *S. dysgalactiae* subsp. *equisimilis* (group C and G streptococci)

Serologic M typing was developed years ago for GAS typing, but it has also been used for *S. dysgalactiae* subsp. *equisimilis* (Stanley et al. 1995). The M protein encoded by *emm* is a virulence factor in *S. dysgalactiae* subsp. *equisimilis* similar to the GAS surface protein. Hitherto approximately 50 *emm* types of *S. dysgalactiae* subsp. *equisimilis* have been identified (CDC 2011c). Many of the virulence factors present in *S. pyogenes* can also be found in *S. dysgalactiae* subsp. *equisimilis* strains (Davies et al. 2007). A history of lateral gene transfer (from GAS to GCS/GGS) has recently been suggested by an Australian group: virulence factors which are among all GAS isolates are present in only a smaller proportion of *S. dysgalactiae* subsp. *equisimilis* (GCS, GGS) isolates (Davies et al. 2007). No association was found between *emm* type of GCS or GGS and disease manifestations. However, *emm* type

stG6792 proved to be associated with poor outcome in a recent study from Japan (Takahashi et al. 2010).

2.6.3 The streptococcal superantigens (SAgs)

The streptococcal superantigens are bacterial toxins and able to induce potent inflammatory responses (Kotb 1995). During the few last years, our knowledge of the role of GAS SAgs in disease pathogenesis has increased (Chatellier et al. 2000; Cunningham 2000; Bisno et al. 2003; Lintges et al. 2010). The GAS SAgs are significant for virulence as they participate in the induction of the systemic toxicity associated with severe infections (Kotb 1995; Norrby-Teglund et al. 2000; Lintges et al. 2010). There are at least 11 known streptococcal SAgs produced by corresponding GAS genes, including for example the streptococcal SAg *ssa* gene, the protease gene *speB*, the deoxyribonuclease gene *speF* and six pyrogenic exotoxin genes *speA, speC, speF, speG, speH* and *speJ* (Lintges et al. 2010). GAS carrying *speA* and *speC* genes are known to be associated with severe disease (Musser et al. 1991). Most GAS strains express several different SAgs, although the distribution of SAg genes varies among the strains. In a recent study published in the United States, SAgs were found to be more important for the invasiveness of GAS than the *emm* type (Lintges et al. 2010). *SpeA*, *speJ* and *smeZ* genes were more often present in isolates from invasive infections (Lintges et al. 2010). The SAgs genes *ssa*, *speC* and *speJ*, again, were more often present in isolates from noninvasive infections (Lintges et al. 2010). Profiling of the SAg genes, combined with *emm* typing and PFGE, may be useful in epidemiological investigations.

2.7 Mortality

2.7.1 Overall mortality

Several factors associated with an increased case fatality rate in GAS bacteremia have been described in population-based surveillance studies, whereas in the case of GBS, GCS, and GGS such reports are scant (Ekelund et al. 2005; Broyles et al. 2009). According to recent reports the case fatality rate in GAS bacteremia is 14 to 15% (Darenberg et al. 2007; O'Loughlin et al. 2007). The case fatality rate due to bacteremias caused by GBS has varied from 8 to 19 % in different studies (Ekelund et al. 2005; Skoff et al. 2009). The rate attributable to GGS bacteremias is 15-18% (Ekelund et al. 2005; Broyles et al. 2009).

2.7.2 Risk factors for mortality

Increased case fatality rate in GAS bacteremia has been associated with pneumonia (O'Brien et al. 2002; Hollm-Delgado et al. 2005; Mehta et al. 2006; O'Loughlin et al. 2007), alcoholism (Davies et al. 1996; Ekelund et al. 2005), age (O'Brien et al. 2002; Sharkawy et al. 2002; Ekelund et al. 2005; O'Loughlin et al. 2007), immunosuppression (Nielsen et al. 2002; Ekelund et al. 2005), cancer (Sharkawy et al. 2002; Hollm-Delgado et al. 2005), chronic heart or lung disease (O'Brien et al. 2002; Ekelund et al. 2005), working or living in hospital (Hollm-Delgado et al. 2005), liver dysfunction (Mehta et al. 2006), use of NSAID medication (Stevens 1995), DIC (Mehta et al. 2006) and STSS (O'Brien et al. 2002; Mehta et al. 2006; O'Loughlin et al. 2007). A recent study involving 5400 patients found increasing age, STSS, meningitis, fasciitis, pneumonia, or bacteremia without a source and *emm* types 1, 3, or 12 as independent factors associated with death in GAS bacteremia (O'Loughlin et al. 2007). Factors associated with increased case fatality rates in GBS bacteremia have been diabetes mellitus, liver disease, malignancy, age, alcoholism, shock at diagnosis and nursing home residence (Farley et al. 1993; Trivalle et al. 1998; Tyrrell et al. 2000; Farley 2001; Edwards et al. 2005). In contrast, the number of population-based studies describing predictors of case

fatality in GCS or GGS bacteremia is limited (Ekelund et al. 2005). In one Danish study chronic heart or lung disease, alcohol abuse and immune incompetence were associated with increased case fatality (Ekelund et al. 2005). In addition, the authors noticed that low case fatality was associated with GBS and GGS bacteremia as compared to GAS bacteremia (Ekelund et al. 2005).

2.8 Prevention and treatment strategies

2.8.1 Group A and B streptococcal vaccines under development

Only one 26-valent M-protein-based GAS vaccine has recently reached clinical trials. This vaccine covers M/emm-types 1, 1.2, 2, 3, 5, 6, 11, 12, 14, 18, 19, 22, 24, 28, 29, 33, 43, 59, 75, 76, 77, 89, 92, 94, 101 and 114 (Kotloff et al. 2004; McNeil et al. 2005; Cohen-Poradosu et al. 2007). Phase II testing of the 26-valent vaccine in adults has yielded promising results (McNeil et al. 2005). This vaccine has proved to be safe and immunogenic in human beings. The current formulation of this proposed multivalent GAS vaccine would provide good coverage in developed countries, particularly the USA, Canada, and Europe, but poor coverage in Africa and the Pacific, and only average coverage in Asia and the Middle East (Steer et al. 2009). It is estimated, that it could prevent 40-50% of cases and 50-60% of deaths due to invasive GAS infections (O'Loughlin et al. 2007).

Vaccine development for GBS has proved particularly challenging. A pentavalent conjugate GBS vaccine which includes types 1a, 1b, II, III, and V could prevent 96% of neonatal disease and 88% of pediatric, adult and pregnancy-associated GBS disease (Harrison et al. 1998; Davies et al. 2001). Both serotype prevalence and virulence are important considerations for vaccine formulation. The GBS vaccines being developed for the prevention of neonatal disease should be given to adults who are at risk. To the author’s knowledge there is no ongoing vaccine development in the field of group C or G streptococcal disease.

2.8.2 Antimicrobial therapy

Beta-hemolytic streptococcus has to date remained sensitive to penicillin, and this antibiotic group remains the first-line treatment of choice once beta-hemolytic streptococcus has been identified as the cause of sepsis. The use of clindamycin in combination with penicillin G (benzylpenicillin) has been shown to inhibit the activity of virulence factors in *S. pyogenes*, thus lowering the risk of STSS (Zimbelman et al. 1999). When penicillin allergy is reported, cephalosporins or vancomycin can be used as an alternative. Rapid initiation of intravenous antibiotic therapy is essential.

2.8.3 Surgery

When tissue necrosis or gangrene is suspected, surgical exploration is essential along with immediate debridement of affected and surrounding tissue. Abscess drainage is essential when loculated fluid is present. Amputation of a limb may be necessary in the treatment of severe necrotizing infections. Debridement or removal of prosthetic joint implants is usually required when treating prosthetic joint infection.

3. THE AIMS OF THE STUDY

The purpose of the present study was to investigate the epidemiology of groups A, B, C and G beta-hemolytic streptococcal bacteremia, and predictors of its outcome. The specific aims were:

1. to estimate the incidence of bacteremia caused by GAS, GBS, GCS and GGS during a 10-year period from 1995 to 2004 in the Pirkanmaa Health District, Finland.

2. to determine the predisposing factors, underlying diseases, presenting clinical manifestations, outcome and predictors of death in beta-hemolytic streptococcal bacteremia.

3. to determine the *emm* types of *S. dysgalactiae* subsp. *equisimilis* bacteremic isolates during the 10-year observation period and the relation of *emm* types to disease severity in bacteremia.

4. to determine the *emm* types and superantigens in GAS bacteremic isolates during the 10-year observation period and the relation of *emm* types and superantigens to the clinical presentations.

5. to analyze epidemiological information on GAS *emm* types in order to estimate the possible vaccine coverage during the 10-year period in a defined population.

4. PATIENTS AND METHODS

4.1 Patients

Studies I-IV were carried out in the Pirkanmaa Health District (Tampere University Hospital, Hatanpää City Hospital, and the District Hospitals in Valkeakoski, Vammala and Mänttä and the Centre for Laboratory Medicine, Tampere University Hospital) in collaboration with the Department of Infectious Disease Surveillance and Control in the National Institute for Health and Welfare (THL), Helsinki, Finland.

4.1.1 Patients with beta-hemolytic streptococcal bacteremia (I-II)

This population-based study was conducted in the Pirkanmaa area. Pirkanmaa, with about 460 000 inhabitants, is located in western Finland. The study material comprised 309 patients with beta-hemolytic streptococcal bacteremia hospitalized in Tampere University Hospital, Hatanpää City Hospital or the District Hospitals in Valkeakoski, Vammala and Mänttä, Finland, from January 1995 to December 2004. Six patients had recurrent beta-hemolytic bacteremia, two of them recurrent GBS and four GGS bacteremias, respectively. The number of bacteremia episodes was 314. Beta-hemolytic streptococci grew in 314 cultures, distributed as GAS 92 cases (29%), GBS 76 cases (24%), GCS 18 cases (6%) and GGS 128 cases (41%). The medical records of all adult (≥16 years-old) patients in the Pirkanmaa Health District with one or more blood cultures positive for GAS, GBS, GCS and GGS during the 10-year period from January 1995 to December 2004 were retrospectively reviewed. All blood cultures were analyzed in the Centre for Laboratory Medicine in Tampere University Hospital. The files of this laboratory were screened to identify all blood cultures positive for beta-hemolytic streptococci during the study period. The case definition included all patients with a positive blood culture for GAS, GBS, GCS or

GGS, combined with a clinical picture compatible with septicemia. All patients who had a positive blood culture also showed symptoms and signs of septicemia.

4.1.2 Patients with *S. dysgalactiae* subsp. *equisimilis* bacteremia (III)

This retrospective, population-based study cohort comprised a subgroup of patients (Studies I and II) who suffered from *S. dysgalactiae* subsp. *equisimilis* bacteremia between January 1995 and December 2004. The number of patients was 137 and the number of episodes 140. Two of the isolates (one GGS and one GCS) were not available for *emm* typing. Thus, 138 of the *S. dysgalactiae* subsp. *equisimilis* isolates were *emm* sequenced, involving 135 patients, and comprised the study material. Four of the patients had recurrent bacteremias. The *emm* typing of *S. dysgalactiae* subsp. *equisimilis* isolates was performed at the Department of Infectious Disease Surveillance and Control in THL, Helsinki. PFGE of non-typable *S. dysgalactiae* subsp. *equisimilis* strains and strains isolated from the patients with recurrent *S. dysgalactiae* subsp. *equisimilis* bacteremia was also conducted.

4.1.3 Patients with *S. pyogenes* bacteremia (IV)

This retrospective, population-based study cohort comprised a subgroup of patients (Studies I and II) who suffered from *S. pyogenes* bacteremia between January 1995 and December 2004. The number of patients and episodes was 92. Five of the isolates were not available for *emm* typing. Thus, the study comprised 87 patients and their *S. pyogenes* isolates, which were *emm* sequenced and genotyped for superantigen (SAg) profiles. The *emm* typing and SAg profiling of *S. pyogenes* isolates were carried out at the Department of Infectious Disease Surveillance and Control in THL, Helsinki. Eighteen of the cases included in this study were part of the EU-wide Strep-EURO study, which covered the years 2003 and 2004 and included all invasive GAS cases in the Pirkanmaa area, Finland (Luca-Harari et al. 2009).

4.2 Definitions

Alcoholism was defined here as an alcohol-related medical or social problem. Smoking habits were registered and patients were classed as current smokers, ex-smokers or non-smokers. Fever was defined as having an ear temperature > 37.5 °C and afebrility at an ear temperature ≤37.5 °C. A lowered level of consciousness was defined as unconscious or confused at least once during the first two days of hospitalization. A patient was classified as hypotensive if the systolic blood pressure was <90 mmHg at least once during the first two days after a positive blood culture. DIC was defined as a platelet count lower than 100 x 10^9 /l and MOF as three or more concomitant organ failures. Chronic diseases were registered. Previous corticosteroid and other immunosuppressive treatments were registered during one month before the bacteremia episode. The McCabe classification was used to assess the severity of the underlying medical condition (McCabe 1962). McCabe I was defined as nonfatal underlying disease. McCabe class II (ultimately fatal disease) was defined as the normal evolution of the disease which could lead to death in 1-5 years and McCabe class III (rapidly fatal disease) as life expectancy less than 12 months.

Cellulitis included non-necrotizing infections of the skin and underlying tissue (both erysipelas and deeper cellulitis). A history of previous cellulitis comprised cellulitis occurring more than one month prior to the current bacteremia episode. Prior episodes of cellulitis had been adequately treated at Tampere University Hospital, Hatanpää City Hospital or the District Hospitals in Valkeakoski, Vammala and Mänttä. Recurrent bacteremias were involved if episodes had occurred at least 3 weeks apart and the first episode had been treated adequately. NF was defined as a progressive, destructive subcutaneous infection with necrosis observed either directly or under surgery. Septic shock was involved if the patient had systolic blood pressure of <90 mmHg. The definition of STSS was based on a consensus, including identification of beta-hemolytic streptococci at a normally sterile site (typically blood or deep tissue), septic shock and MOF (The Working Group on severe Streptococcal Infections 1993). Nosocomial disease was defined as the isolation of beta-hemolytic streptococci from blood in a hospitalized case patient over two days after hospital admission. Infection was defined as community-acquired if a blood culture was taken before two days after admission to hospital. Laboratory test results

recorded were: plasma CRP, blood leukocytes, blood thrombocytes and plasma creatinine level. A plasma CRP value over the median of the study population (105 mg/ml) was rated high. Leukopenia was defined as a blood white cell count below 4.0 x 10^9 /l and thrombocytopenia as thrombocytes below 100 x 10^9 /l. An elevated creatinine value comprised a value over 120 µmol/l.

4.3 Clinical data collection

The collection of clinical data was retrospective. The author reviewed all patient records and filled in a structured data collection form. Underlying diseases, social status, alcohol and tobacco consumption were registered. The review covered the whole hospitalization period, and all complications were registered (treatment in ICU, need for mechanical ventilation, renal replacement therapy or surgery, occurrence of hypotension, lowered level of consciousness, DIC, MOF, or STSS). Mortality was recorded within 30 days from the positive blood culture (30-day mortality). First symptoms or signs of bacteremia were recorded, including fever, pain, confusion, lowered level of consciousness and dyspnea. Alterations in mental status were recorded. The need for renal replacement therapy (hemodialysis or hemodiafiltration), vasopressive support and possible surgical intervention were recorded. Laboratory tests were carried out on the day of blood culture, during the following five days after blood culture and ten days thereafter. The values of CRP, leukocytes, thrombocytes or creatinine on admission were used in defining high CRP, leukopenia, thrombocytopenia or elevated creatinine value. Antimicrobial treatment was registered during hospitalization.

4.4 Microbiological methods

4.4.1 Blood culture methods

Routine blood samples were drawn into aerobic and anaerobic bottles. During the study period the BACTEC NR 730 (only 1995) and BACTEC 9240 (BD Diagnostic

Systems, Sparks, MD, USA) (1996-2004) blood culture systems with standard culture media were used. In the district hospitals, the Signal blood culture system (Oxoid, Cambridge, UK) was used until 2003. The Lancefield serogroups were defined by latex agglutination using the Streptex latex test system (Remel Europe Ltd, Dartford, UK). All isolates were also strain identified by a commercial test (Rapid ID 32 STREP, bioMérieux SA, Marcy-1'Etoile, France).

4.4.2 Antimicrobial susceptibility testing

Susceptibility testing was performed by the disk diffusion method paralleling the guidelines of the Clinical Laboratory Standards Institute (CLSI, former NCCLS) (CLSI 2007).

4.4.3 *emm* typing (III-IV)

GAS *and S. dysgalactiae* subsp. *equisimilis* isolates were further analyzed by *emm* typing. This was carried out according to the Centers for Disease Control (CDC) and Prevention guidelines (CDC 2011d; CDC 2011c). The *emm* gene was amplified using primers 1 (TATT(C/G)GCTTAGAAAATTAA) and 2 (GCAAGTTCTTCAGCTTGTTT) and if the *emm* gene could not be amplified MF1 and MR1 were used (Siljander et al. 2006). Polymerase Chain reaction (PCR) products were purified using Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequencing was performed using primer emmseq2 (TATTCGCTTAGAAAATTAAAAACAGG) (CDC 2011e).

4.4.4 Superantigen genotyping (IV)

GAS isolates were genotyped for their SAg profile. Multiplex PCR was used to detect the streptococcal superantigen *ssa* gene, protease gene *spe*B, deoxyribonuclease gene *speF* and six pyrogenic exotoxin genes *speA*, *speC*, *speF*, *speG*, *speH* and *speJ* (Schmitz et al. 2003; Darenberg et al. 2007). A single PCR

was used to detect the streptococcal mitogenic exotoxin *sme*Z gene (Schmitz et al. 2003; Darenberg et al. 2007).

4.4.5 Pulsed field gel electrophoresis (PFGE) analysis (III)

Non-typable *S. dysgalactiae* subsp. *equisimilis* strains and strains isolated from patients with recurrent *S. dysgalactiae* subsp. *equisimilis* bacteremia were characterized using PFGE. This approach is a tool to investigate genetic relationships between isolates. PFGE was conducted as previously described (Stanley et al. 1995). The restriction enzyme *Sma*I was used in digestion and the fragments were separated using CHEF DR II ((Bio-Rad, Hercules, California, USA) with pulse times 10-35 s for 23 h. Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium) was used to analyze DNA profiles. Strains with >85 % similarity were considered to belong to the same cluster.

4.5 Statistical methods (I-IV)

The SPSS software version 7.5 (SPSS, Chicago, IL, USA) was used in statistical analyses and a two-sided p-value <0.05 was regarded as the level for statistical significance. Change in the incidence of GGS and *S. dysgalactiae* subsp. *equisimilis* (GCS and GGS) bacteremia during the study period and the following five years was analyzed by Poisson regression. Categorical data were analyzed by χ^2 test or Fisher`s exact test, as appropriate. Nonparametric data were analyzed by Mann-Whitney U-test and Kruskal-Wallis H-test, as appropriate. To identify factors which may be evaluated on admission to hospital (*i.e.* underlying diseases, first signs and symptoms, laboratory findings on admission) and may predict death, univariate and multivariate analyses were undertaken. As there were only 42 deaths during the 30 days' follow-up, the effect of different factors on case fatality was assessed in three separate models: 1) underlying diseases 2) first signs and symptoms of bacteremia and 3) laboratory findings on admission. In the final multivariate analysis the significant factors from the previous multivariate analyses, age and streptococcal group (A, B, C, or G) were included. Odds ratios (OR) were expressed with their 95% confidence intervals (CI).

5. RESULTS

5.1 Epidemiology of beta-hemolytic streptococcal bacteremia (Study I)

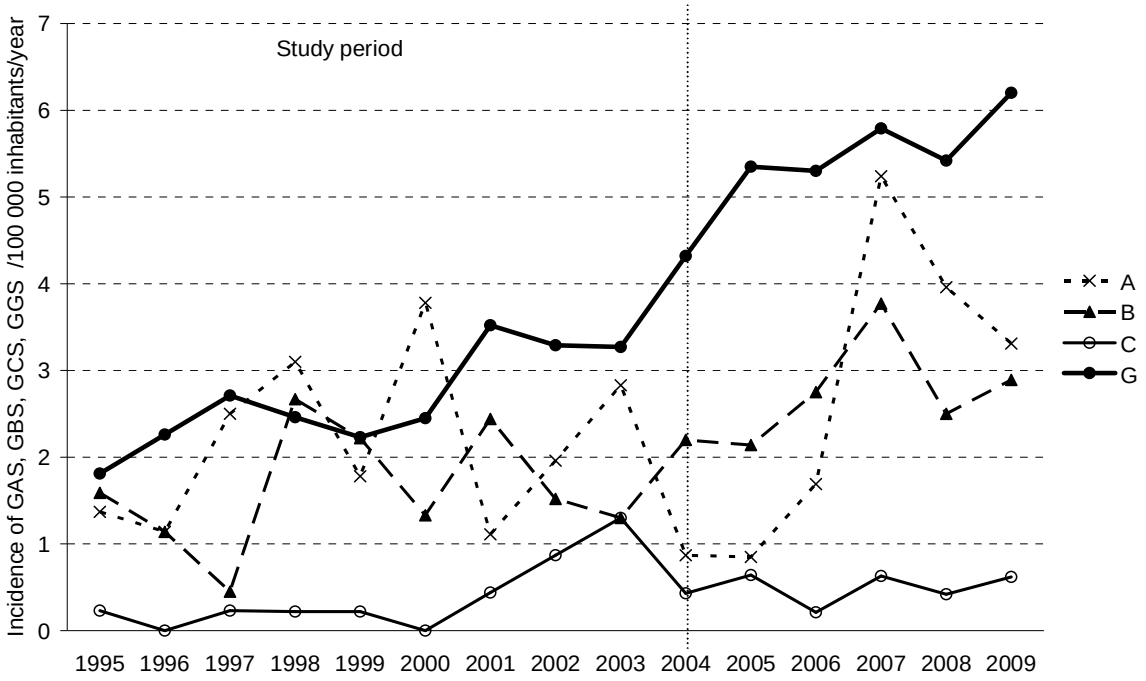
All episodes of beta-hemolytic streptococcal bacteremia occurring in adult (≥16 years-old) patients during the study period 1995-2004 were included in the study. Beta-hemolytic streptococci grew in 314 cultures, comprising GAS 92 cases (29%), GBS 76 cases (24%), GCS 18 cases (6%) and GGS 128 cases (41%). All GAS were *S. pyogenes*, all GBS were *S. agalactiae*, and all GGS were *S. dysgalactiae* subsp. *equisimilis*. Six of the GCS were identified as *S. equi* subsp. *zooepidemicus* and 12 were *S. dysgalactiae* subsp. *equisimilis*. These 314 episodes of beta-hemolytic streptococcal bacteremia (involving 309 patients) comprised the material for the present series. Six patients had recurrent beta-hemolytic bacteremia, two of them recurrent GBS and four GGS bacteremia. All episodes of beta-hemolytic bacteremia were included in the study and analyzed as separate episodes.

The incidence of beta-hemolytic streptococcal bacteremia caused by GAS, GBS, GCS and GGS in the Pirkanmaa Health District during the study period 1995-2004 is shown in Figure 2. Also data from the year 2004 until the year 2009 is added in Figure 2. Within the study period the number of cases due to GGS bacteremia increased, while the number of cases due to other groups was fairly stable, albeit fluctuating. The incidence of GGS bacteremia increased from 1.8 cases per 100 000 population in 1995 to 4.3 cases per 100 000 population in 2004 (Figure 2) ($p=0.013$).

The number of blood cultures taken in the Pirkanmaa area increased during the study period. The incidence of positive blood cultures (all bacterial findings) increased from 1995 to 2004 from 279/100.000 to 368/100.000 (1.3 fold), while that of GGS increased from 1.81/100.000 to 4.32/100.000 (2.4 fold). The laboratory methodology used was consistent throughout the study period. The incidence of *S. dysgalactiae* subsp. *equisimilis* bacteremia increased from 2.05 cases per 100.000 in

1995 to 4.75 cases per 100.000 in 2004 (p= 0.006). In contrast the incidence of GAS and GBS bacteremia did not increase significantly. There was a slight fluctuation in the incidence of GAS bacteremia, peaking in the years 1998, 2000 and 2003 (Figure 2).

Figure 2. Incidence/100.000 inhabitants/year of group A, B, C and G streptococcal bacteremias in the Pirkanmaa Health District 1995-2009.



Source of the data: The present study, the Centre for Laboratory Medicine (unpublished data) and Finnish Population Register Centre.

Since the study period the increase in the incidence of GGS bacteremia has continued. The incidence of GGS bacteremia increased from 1.8 cases per 100.000 population in 1995 to 6.2 cases per 100.000 population in 2009 ($p<0.001$) (unpublished data) (Figure 2). Since the study period, the incidence of invasive GAS disease has fluctuated considerably and peaked in 2007.

During the study period there was an outbreak (6 cases) caused by *S. equi* subsp. *zooepidemicus* associated with consumption of unpasteurized goat cheese in 2003 (Kuusi et al. 2006). Most GAS, GCS and GGS bacteremia cases were community-

acquired. The proportions of nosocomial infections were 14% in GAS, 32% in GBS, and 15% in GGS bacteremias.

5.2 Predisposing factors and clinical characteristics of beta-hemolytic streptococcal bacteremias (Study I-II)

5.2.1 Portals of entry

The occurrence of disruptions of the cutaneous barrier as possible or probable portals of entry of GAS, GBS, GCS or GGS bacteremia is presented in Table 1. Disruption of the cutaneous barrier was more common in patients with GAS (58%) or GGS (53%) bacteremias as compared to GBS (32%) bacteremia ($p=0.001$ and $p=0.003$, respectively).

Preceding trauma (during the previous month) was a common predisposing factor in GAS bacteremia (27%), occurring less frequently in other bacteremias (GBS 9%, GCS 17%, GGS 10%). A preceding operation (during the previous month) was uncommon in these bacteremic patients (GAS 5%, GBS 3%, and GGS 5%). A history of previous cellulitis was found more often in patients with GBS (20%) or GGS (29%) bacteremia as compared to those with GAS (4%) ($p=0.002$ and $p<0.001$, respectively).

Table 1. Probable or possible portal of entry and history of trauma or cellulitis in beta-hemolytic streptococcal bacteremia in relation to Lancefield group

	GAS N=92 (%)	**GBS N=76 (%)**	**GCS N=18 (%)**	**GGS N=128 (%)**	**All N=314 (%)**	**Overall p-value**
Disruption of cutaneous barrier[a]	53 (58)	24 (32)	8 (44)	68 (53)	153 (49)	0.005
-chronic eczema or skin erosion	29 (28)	11 (15)	5 (28)	41 (32)	83 (26)	0.050
-psoriasis	5 (5)	1 (1)	1 (6)	5 (4)	12 (4)	0.552
-chronic ulcer	12 (13)	9 (12)	1 (6)	18 (14)	40 (13)	0.777
-traumatic wound	15 (16)	3 (4)	2 (11)	10 (8)	30 (10)	0.044
-operation wound	5 (5)	2 (3)	0 (0)	1 (1)	8 (3)	0.160
Preceding trauma[b]	25 (27)	7 (9)	3 (17)	13 (10)	48 (15)	0.002
Previous history of erysipelas[c]	4 (4)	15 (20)	2 (11)	37 (29)	58 (19)	<0.001

GAS, GBS, GCS and GGS; Lancefield group A, B, C and G beta-hemolytic streptococci, respectively.
[a]whether infected or not
[b]during the previous month
[c]cellulitis occurring more than one month prior to the current bacteremia episode

5.2.2 Demographic factors and underlying diseases

The median age of bacteremic GAS patients was 53, as compared to 69, 70 or 67 years in GBS, GCS or GGS bacteremias ($p<0.001$ in all comparisons). One hundred and fifty seven of the patients were males and 152 females. Demographic factors and underlying diseases as well as presenting clinical manifestations are presented in Table 2. Underlying diseases were common, 84% having some underlying disorder. Furthermore, 90% of the patients with GGS bacteremia had an underlying disease. Alcoholism was significantly more common in patients with GAS than in those with GBS or GGS bacteremia ($p=0.001$ and $p=0.006$; respectively). Malignancies were most common in patients with GBS bacteremia (38%). The difference in occurrence of malignancies between GBS and GAS, GCS or GGS was significant ($p<0.001$, $p=0.028$, or $p=0.025$; respectively). In patients with GBS bacteremia especially metastatic malignancies and gynecologic cancers were common. Diabetes was significantly more frequently observed in patients with GBS and GGS as compared to GAS bacteremia ($p=0.002$ and $p=0.02$; respectively), while cardiovascular diseases were more common in patients with GBS, GCS and

GGS as compared to GAS bacteremia (p=0.02, p=0.028, and p=0.001; respectively). Corticosteroid or other immunosuppressive treatment were significantly more frequently in use with patients with GGS as compared to GAS bacteremia (p=0.016 and 0.018; respectively). Cardiovascular diseases (41%), diabetes (25%) and malignancies (23%) were the three most prominent underlying conditions in *S. dysgalactiae* subsp. *equisimilis* (group C and G) bacteremias. Older patients (>65years) had malignancies, diabetes and cardiovascular diseases more often than younger patients (≤65 years) (p=0.003, p=0.003 and p<0.001; respectively). In contrast, younger patients (≤65 years) were more often alcoholics as compared to older patients (p<0.001). A considerable proportion of GAS bacteremia patients (25%) had no underlying diseases. The occurrence of liver diseases, chronic lung diseases, neurological and renal diseases or breast cancer did not differ significantly between patients with bacteremia caused by the different Lancefield groups. There were only two patients who were injecting drug users and one with HIV infection.

Table2. Demographic factors, underlying diseases and presenting clinical manifestations of beta-hemolytic streptococcal bacteremia in relation to Lancefield group

Characteristic	GAS N=92 (%)	GBS N=76 (%)	GCS N=18 (%)	GGS N=128 (%)	All N=314 (%)	Overall p-value
Age						<0.001
17-40	21 (60)	9 (26)	0	5 (14)	35	
41-65	48 (36)	23 (17)	8 (6)	54 (41)	133	
66-75	12 (17)	21 (30)	6 (9)	30 (44)	69	
>75	11 (14)	23 (30)	4 (5)	39 (51)	77	
Median	53	69	79	67	64	
Sex (Male/Female)	48/44	32/44	9/9	68/60	157/152	0.463
Underlying disease						
Alcoholism	24 (26)	5 (7)	2 (11)	15 (12)	46 (15)	0.002
Malignancy	10 (11)	29(38)	2 (11)	30 (23)	71 (23)	<0.001
Diabetes	11 (12)	24(32)	5 (28)	31 (24)	71 (23)	0.019
Cardiac disease[a]	17 (19)	26(34)	8 (44)	51 (40)	102 (33)	0.005
Immunosuppression	4 (4)	8 (11)	2 (11)	18 (14)	32 (10)	0.135
No underlying disease	23 (25)	10(13)	4 (22)	13 (10)	50 (16)	0.002
Clinical manifestation[b]						
Skin/soft-tissue infection	65 (71)	34(45)	10 (56)	88 (69)	197 (63)	0.002
-Cellulitis	36 (39)	26(34)	8 (44)	73 (57)	143 (46)	0.006
-Fasciitis	7 (8)	0	0	1 (1)	8 (3)	0.008
-Infected wound or eczema	24 (26)	12(16)	1 (6)	23 (18)	60 (19)	0.15
Deep abscess	10 (11)	5 (7)	0	2 (2)	17 (5)	0.02
Pneumonia	16 (17)	3 (4)	2 (11)	12 (9)	33 (11)	0.04
Urinary tract	1 (1)	7 (9)	0	1 (1)	9 (3)	0.008
Puerperal sepsis	7 (8)	6 (8)	0	4 (3)	17 (5)	0.27
Arthritis	5 (6)	3 (4)	1 (6)	11 (9)	20 (6)	0.58
Osteomyelitis	4 (4)	5 (7)	0	6 (5)	15 (5)	0.83
Meningitis	3 (3)	0	0	1 (1)	4 (1)	0.34
Endocarditis	1 (1)	3 (4)	1 (6)	2 (2)	7 (2)	0.30
Unknown focus	10 (11)	21(28)	8 (44)	21 (16)	61 (19)	0.002

GAS, GBS, GCS and GGS; Lancefield group A, B, C and G beta-hemolytic streptococci, respectively

[a] coronary artery disease or heart failure

[b] one patient may have one or more clinical manifestations

5.2.3 The presenting clinical manifestations

The presenting clinical manifestations associated with bacteremias are given in Table 2. Skin infections were the most prominent clinical manifestations in GAS (71%) and in GGS (69%) bacteremias, the most common being cellulitis (46 %) and wound infection (19 %). Eight cases of NF were found, seven of them in patients with GAS bacteremia and one in a patient with GGS bacteremia. The next most common presenting clinical manifestation was respiratory tract infection (pneumonia) in GAS bacteremias (17%). There were more deep abscesses in GAS bacteremias than in bacteremias caused by other groups. Deep abscesses included empyemas (5 cases), intra-abdominal abscesses (3 cases), epidural abscesses (2 cases), gluteal abscesses (2 cases), psoas abscess, femoral abscess and abscess in an operation region. In about 28% of the bacteremias caused by GBS and in 44% of those caused by GCS the infection focus remained unknown. Presenting clinical manifestations did not differ significantly in relation to age (patients≤ or >65 years).

Of those six patients who had recurrent beta-hemolytic bacteremia, two had recurrent GBS and four GGS bacteremias, as against none with GAS or GCS. Five patients had recurrent cellulitis and one recurrent spondylitis as their presenting clinical manifestation.

5.2.4 Treatment of bacteremia

Antimicrobial susceptibility test results were analyzed. All strains were susceptible to penicillin, cephalosporins and vancomycin. Decreased susceptibility (intermediate and resistant) to tetracycline was common in GAS (39%), GBS (70%), GCS (72%), and GGS (44%). Decreased susceptibility to erythromycin was found in 20% of GAS, 1% of GBS, none of GCS and 10% of GGS. Single isolates resistant to clindamycin were found in GAS (1%) and GGS (1%). Decreased susceptibility to clindamycin was more common in GCS (33%). In all cases, the causative microbe was susceptible to the empiric antimicrobial therapy given. Antimicrobial therapy was initiated immediately after blood cultures were drawn. A total of 22% of GAS patients and 14% of all beta-hemolytic bacteremia patients required surgical intervention (Table 3). It was needed significantly more often in those with GAS as compared to GGS bacteremia (p=0.029). Altogether 27% of

GAS patients and 7% of GGS patients were admitted to the intensive care unit (Table 3), and 20% of GAS patients and 6% of GGS patients needed mechanical ventilation.

Table 3. Clinical markers of severe streptococcal bacteremia in relation to Lancefield group

Disease severity	GAS N=92 n (%)	GBS N=76 n (%)	GCS N=18 n (%)	GGS N=128 n (%)	All N=314 n (%)	Overall p-value*
30-day mortality	14 (15)	5 (7)	4 (22)	19 (15)	42 (13)	0.19
Needed ICU stay	25 (27)	8 (11)	2 (11)	9 (7)	44 (14)	0.001
Hypotension[a]	23 (25)	10 (13)	5 (28)	20 (16)	58 (19)	0.12
DIC[b]	27 (29)	5 (7)	2 (11)	6 (5)	40 (13)	<0.001
Multiorgan failure[c]	15 (16)	4 (5)	2 (11)	4 (3)	25 (8)	0.003
STSS[d]	13 (14)	4 (5)	2 (11)	4 (3)	23 (7)	0.02
Surgical intervention	20 (22)	13 (17)	1 (6)	14 (11)	48 (15)	0.096

*difference between groups of patients with GAS, GBS, GCS and GGS bacteremia
[a]Hypotensive (BP<90 mmhg) at least once 0-2 days after positive blood culture
[b]Thrombocytes lower than 100 x 10^9/l
[c]Three or more concomitant organ failures
[d]The definition of STSS (Streptococcal toxic shock syndrome) included identification of beta-hemolytic streptococci at a normally sterile site, septic shock and multi-organ failure

5.3 Predictors of mortality in beta-hemolytic streptococcal bacteremia (Study II)

The 30-day case fatality rate was on average 13%; in GAS it was 15%, in GBS 7%, in GCS 22% and in GGS 15% (Table 3). The overall case fatality rate in GAS bacteremia was 15%, compared with 69 % in STSS. Nine out of 33 (27%) septic pneumonia patients died. On admission to hospital the median ear temperature was lower in patients who died as compared to those who survived (38.3 vs 39.0; p=0.001). Admittance to the ICU was significantly more frequently required in patients with GAS bacteremia as compared to GBS or GGS bacteremia (p=0.008 and p<0.001; respectively). DIC was significantly more frequent in patients with GAS as compared to GBS or GGS bacteremia (p<0.001 and p<0.001; respectively) (Table 3). MOF was also significantly more commonly observed in patients with GAS as compared to GBS or GGS bacteremia (p=0.025 and p=0.001; respectively).

STSS was more often caused by GAS than by GBS or GGS (p=0.058 and p=0.003; respectively). Seventy per cent of patients with STSS died during the 30-day period after the positive blood culture, compared to 52% of patients with septic shock and 5 % of those who did not have septic shock. DIC, MOF or STSS occurred in 30 out of 92 patients (33 %) with GAS bacteremia. The case fatality rate was high (37%) in patients with GAS bacteremia who suffered from the aforementioned complications. Other patients with GAS bacteremia who did not have these severe manifestations had a case fatality rate of only 5%.

NF carried a high risk of death (38%), while patients with bacteremic cellulitis had a low risk (8%). A history of previous cellulitis seemed to be a factor protecting against death. Patients with a history of previous cellulitis had a case-fatality rate of only 3% as compared to 16% among those without such a history (p=0.014). In patients with a history of previous cellulitis the duration of symptoms before admission to hospital was shorter than in those without such a history (median 0 day vs 1 day; p= 0.002).

Cellulitis was the most frequent presenting clinical manifestation, occurring in 143 patients (46 %). Cellulitis as a presenting clinical manifestation was especially common in those patients who had a history of previous cellulitis (48 out of 58 patients, 83%). The subgroup of 143 patients who had cellulitis as the presenting clinical manifestation were studied separately. Also among these 143 cellulitis patients those with a history of previous cellulitis had a shorter duration of symptoms before admission to hospital as compared to those without such a history (median 0 day vs 1 day, p=0.01). Previous cellulitis had a significant effect on case fatality also in this subgroup of patients; none of the 48 patients with previous cellulitis died as compared to 12 out of 95 patients (13%) among those without such a history (p=0.009).

When analyzing the factors associated with death, all patients were studied as a group. The correlations of underlying diseases with mortality are shown in Table 4. The number of patients older than 65 years did not differ between those who survived or died. The median age was higher in GBS patients who died as compared to those who survived (79 year vs. 67 year; p=0.013). There was no association between age and survival in GAS, GCS or GGS bacteremia. Alcoholism was associated with poor outcome; 14 out of 46 (30%) died (Table 4). Liver disease was also associated with poor outcome; 10 out of 32 patients with liver disease

(31%) died (Table 4). In univariate analysis alcoholism, liver disease, having an ultimately or rapidly fatal underlying disease (McCabe class II or III) or at least one chronic disease were significantly associated with death (Table 4). In multivariate analysis including the aforementioned underlying diseases, factors significantly associated with death were alcoholism and McCabe class II or III (ultimately or rapidly fatal underlying disease) (Table 4).

Table 4. Underlying diseases in patients with beta-hemolytic streptococcal bacteremia in relation to mortality

Underlying disease	**Survivors N=272 (%)**	**Non-survivors N=42 (%)**	**Univariate analysis OR (95 % CI)**	**Multivariate analysis OR (95 % CI)**
Alcoholism	32 (12)	14 (33)	3.8 (1.8 to 7.9)	4.7 (2.1 to 10.4)
Diabetes	59 (22)	12 (29)	1.4 (0.7 to 3)	not entered
Malignancy	60 (22)	11 (26)	1.3 (0.6 to 2.6)	not entered
Liver disease	22 (8)	10 (24)	3.6 (1.5 to 8.2)	removed
Cardiovascular disease	85 (31)	17 (41)	1.5 (0.8 to 2.9)	not entered
Immunosuppressive treatment	27 (10)	5 (12)	1.2 (0.4 to 3.4)	not entered
Mc Cabe II or III[a]	94 (35)	25 (59)	2.8 (1.4 to 5.4)	3.4 (1.7 to 6.9)
Chronic disease[b]	224 (82)	40 (95)	4.3 (1.0 to 18.3)	removed

[a]McCabe class II or III: ultimately fatal disease (the normal evolution of the disease could lead to death in 1-5 years) or rapidly fatal disease (life expectancy less than 12 months)

[b]At least one chronic disease

In univariate analysis of first signs and symptoms, confusion (OR 3.3, 95% CI 1.4 to 6.8), unconsciousness (OR 6.0 (1.7 to 20.6)) or dyspnea (OR 4.4 (2.0 to 9.6)) were significantly associated with increased case fatality. In contrast, fever was associated with decreased case fatality (OR 0.2 (0.1 to 0.6). In multivariate analysis including these first signs and symptoms, likewise confusion (OR 3.8, 95% CI 1.6 to 9.0), unconsciousness (OR 6.4 (1.6 to 25.6)), and dyspnea (OR 6.4 (2.8 to 14.8))

were found to be independently associated with death, while fever (OR 0.2 (0.1 to 0.5) seemed to be a factor protecting from death.

In univariate analysis leukopenia (OR 4.4, 95% CI 1.7 – 11.4), thrombocytopenia (OR 3.4 (1.6 – 7.5)), high plasma CRP (OR 3.9 (1.9 – 8.4)) and elevated creatinine value (OR 3.1 (1.5 – 6.4)) on admission to hospital were significantly associated with death. Leukopenia (OR 3.8 (1.4 – 10.8)) and high plasma CRP (OR 4.0 (1.7 – 9.2)) were also found to be associated with case fatality in multivariate analysis including the aforementioned factors.

Finally, a multivariate analysis was undertaken where all significant factors from earlier models were included. The final model included alcoholism, McCabe class II or III, confusion, unconsciousness, fever, dyspnea, leukopenia, high CRP, age and also the streptococcal Lancefield group. In this final analysis alcoholism (OR 3.9, 95% CI 1.3 – 11.9), McCabe class II or III (OR 3.7 (1.4 – 9.5)), unconsciousness (OR 5.0 (1.1 – 22.4)), dyspnea (OR 9.2 (3.0 – 27.6)), high CRP (OR 6.1 (2.3 – 16.5)), leukopenia (OR 5.6 (1.6 – 19.7)) and having GCS bacteremia as compared to GBS (OR 7.1 (1.3 – 40.1)) proved significant.

Several markers of disease severity, hypotension (OR 21.8, 95% CI 10.1 – 47.3), lowered level of consciousness (OR 16.3 (7.1 – 37.2)), DIC (OR 7.4 (3.5 – 15.6)), MOF (OR 18.0 (7.2 – 44.7)) and afebrility (OR 3.8 (1.7 – 8.2)) were significantly associated with death during the first two days of hospitalization. The present study also analyzed the effect of these aforementioned factors on death in a multivariate model in patients who survived the first two days. Hypotension (OR 18.7 (7.0 – 49.7)), lowered level of consciousness (OR 17.8 (5.8 – 54.6)), DIC (OR 7.5 (2.9 – 19.4)), and MOF (OR 13.6 (4.5 – 41.6)) remained significant.

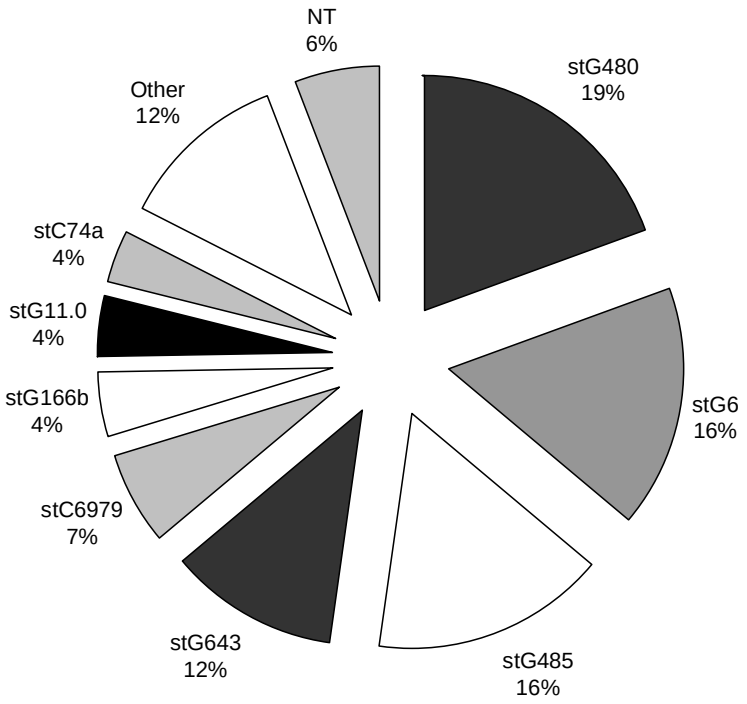
5.4 *S. dysgalactiae* subsp. *equisimilis* bacteremia (group C and G) in Finland (Study III)

One hundred and thirty eight isolates of *S. dysgalactiae* subsp. *equisimilis* were *emm*- typed, involving 135 patients. Four of the patients had recurrent bacteremias. Among these were two patients who suffered one episode of *S. dysgalactiae* subsp. *equisimilis* bacteremia on their vacation and were treated outside the Pirkanmaa Health District. These two episodes were thus not included in the study, as the

organisms were not cultivated in the Centre for Laboratory Medicine in Tampere University Hospital. Nevertheless, we received one of these isolates for molecular typing, and performed *emm* and PFGE- typing.

Among these isolates, 18 different *emm* types were found (including 4 subtypes of stG6: stG6.0, stG6.1, stG6.3 and stG6.4); eight isolates remained non-typable (NT). The three most common *emm* types, StG480 (27 isolates), stG6 (23 isolates) and stG485 (22 isolates) covered 51% of the total (Figure 3). PFGE analysis of the eight NT isolates showed two strains to be related (>85% similarity). The rest of the NT strains were sporadic (6 isolates).

Figure 3. Distribution of the eight most prevalent *emm* types causing *Streptococcus dysgalactiae* subsp. *equisimilis* bacteremia during the years 1995-2004 in the Pirkanmaa Health District, Finland.



The bacteremia episodes were divided into two groups: those caused by the five most common *emm* types and representing each at least over 5% of all episodes (97 episodes, *i.e.* common types) and those caused by the less common or NT *emm* types (41 episodes, *i.e.* rare types). Common *emm* types were StG480, stG6, stG485, stG643 and stC6979 and rare *emm* types were stG166b, stG11, stC74a,

stG10, stG245, stG507-1, stG840, stC9431, stG652, stG2078, stG62647, stC839.0, stCK401 and NT isolates. Clinical features such as age or underlying diseases in relation to common or rare *emm* types did not differ significantly. Severe disease was caused more often by rare *emm* types than by common (Table 5). Case fatality rates were higher in patients with *S. dysgalactiae* subsp. *equisimilis* bacteremia caused by rare *emm* types than among those with bacteremia caused by common *emm* types (Table 5). DIC was also a more common finding in patients suffering from bacteremia caused by rare as opposed to common types (Table 5).

Table 5. Disease severity among 138 episodes of *S. dysgalactiae* subsp. *equisimilis* bacteremia[a]

Disease severity	Common types N=97 (%)	Rare types N=41 (%)	OR (95% CI)	p-value*
30-day mortality	11 (11)	12 (29)	3.2 (1.3 to 8.1)	0.01
Death or ICU treatment	12 (12)	15 (37)	4.1 (1.7 to 9.8)	0.001
DIC[b]	2 (2)	6 (15)	8.1 (1.6 to 42.3)	0.009

*Chi-square test or Fisher's exact test as appropriate
[a]patients who had both clinical data and isolates available
[b]Thrombocyte count < 100 x 10^9/l

The present study identified four patients with recurrent bacteremia, three of whom had recurrent cellulitis and one recurrent spondylitis occurring several months apart (range; 3- 68 months): two patients had *emm* type stG 6 in all of their episodes, and one had *emm* type stG480 in both of his episodes. In the fourth case with two episodes, the second isolate was not available for molecular typing. PFGE profiles of the strains isolated from a single patient in recurring infections were identical. All the episodes were treated adequately.

Common *emm* types were more often manifested as skin and soft-tissue infections than rare *emm* types, 75% compared to 54%, respectively (p=0.012). Cellulitis was the most common presenting clinical manifestation (51%). A common *emm* type was associated with cellulitis; 64% of the patients with common *emm*

types had cellulitis as a presenting clinical manifestation while 39% of patients with rare *emm* types had such an infection (p=0.007). No other associations were found between *emm* type and presenting clinical manifestation.

5.5 Group A streptococcal bacteremia, *emm* types and Superantigen Profiles (Study IV)

5.5.1 Characteristics and distribution of *emm* types causing group A streptococcal bacteremia

Ninety-two cases of GAS bacteremia were identified, and 87/92 of the causative GAS isolates were available for *emm* sequencing to identify the *emm* gene. Eighteen of the cases included in this study were part of the EU-wide Strep-EURO study, which covered the years 2003 and 2004 and included all invasive GAS cases in the Pirkanmaa area, Finland (Luca-Harari et al. 2009).

The present study found 18 different *emm* types. The most abundant of them were, in descending order, *emm* types 1, 28, 81, 53, 12, 68 and 89, accounting for 75 % of the infections (Figure 4). Five of the GAS isolates remained non-typable (NT). The prevalence of *emm*1 strains peaked in 1998-9 and that of *emm*28 in 2002-3 (Figure 5). *Emm* types included in the putative 26-valent GAS vaccine (McNeil et al. 2005) accounted for 62% of isolates overall. The proportion of *emm* types included in the vaccine fluctuated considerably by year (range; 21%-100%, year 2000 and 1998). Overall, 55% of GAS bacteremia cases occurred during the winter (October – March). Higher frequencies of *emm*28 (25% vs. 10%) and *emm*53 (10% vs. 5%) were registered during winter months (October – March) as compared to warmer months (April-September), but the differences were not statistically significant.

Figure 4. Distribution of the seven most prevalent *emm* types causing group A streptococcal bacteremia during the years 1995-2004 in the Pirkanmaa Health District, Finland.

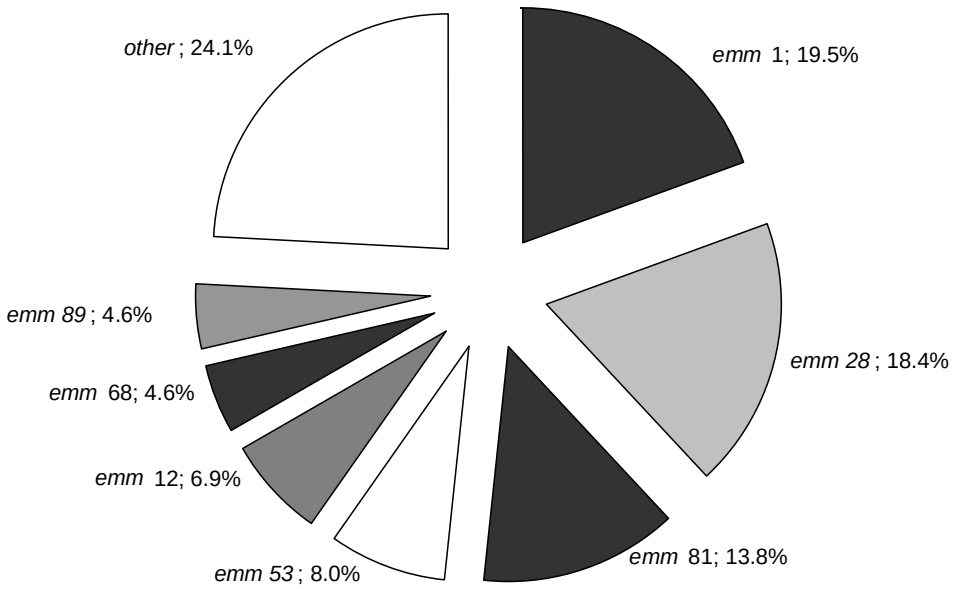
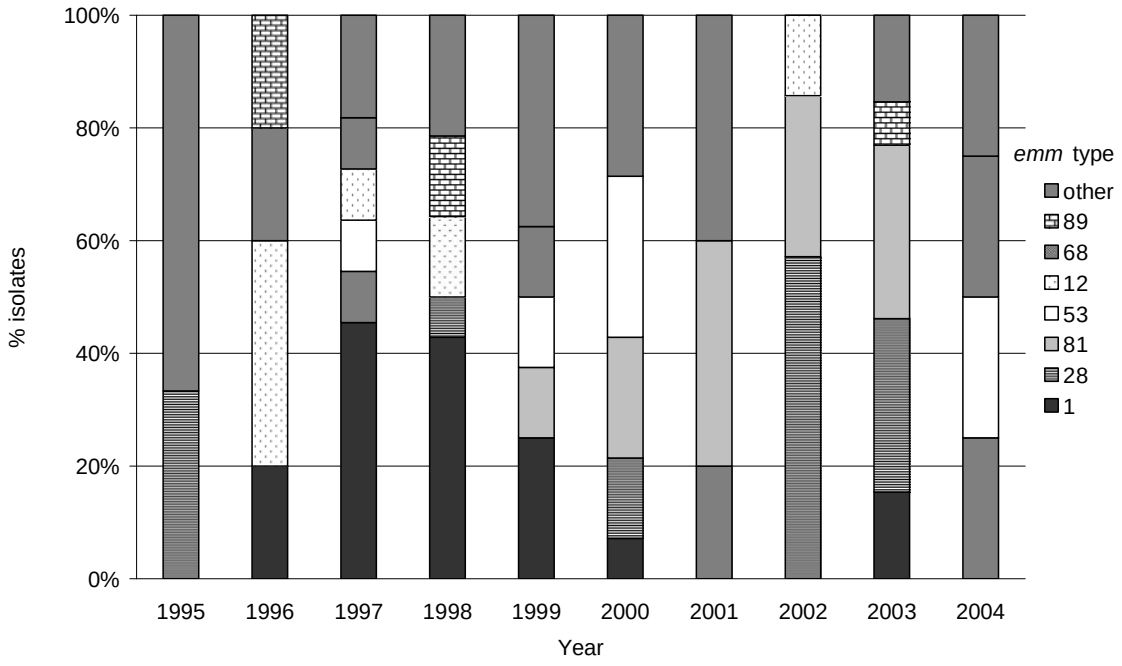


Figure 5. Distribution of the seven most prevalent *emm* types among group A streptococcal bacteremic isolates in relation to the year of diagnosis of bacteremic infection in the Pirkanmaa Health District, Finland.



5.5.2 SAg gene profiles of group A streptococci

The mean number of SAg genes present in invasive GAS isolates ranged from 3 to 6. The pyrogenic exotoxins A and C (*spe*A and *spe*C, respectively) were identified in 20% and 30% of the strains. The *speA* gene was always detected in invasive *emm* type 1 isolates but was absent in other types. *speA* was detected in two and *speC* in five out of 13 strains causing STSS, whereas the combination of both was not found in any of these isolates. *spe*H was markedly linked to *emm* type 12, *spe*J to *emm* types 1, 28, 68 and 89. The *speC* gene was absent in *emm* types 1, 81 and 53 but was frequently found in other isolates (62% total positive isolates among the other types) (data not shown). The *speB*, *speF*, *speG* and *smeZ* genes were found in the majority of isolates (data not shown).

5.5.3 *Emm* types, superantigen profiles and clinical characteristics of group A streptococci

There was no association between *emm* type and patient age, underlying diseases (alcoholism, malignancies, diabetes, cardiovascular diseases, immunosuppressive treatment, liver diseases), presenting clinical manifestations or fatal outcome. There was a statistically significant difference only between *emm* type and sex (p=0.013). *Emm* types 28 and 12 were more common in females (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). Skin or soft-tissue infection was the most common presenting clinical manifestation (71%) and was a common presenting clinical manifestations among *emm* types 1, 81 and 28 (16%, 16% and 13%). There was no association between *emm* type and NF or STSS. A considerable range of *emm* types caused puerperal infections. There was no association between single SAg genes and presenting clinical manifestation or fatal outcome (data not shown).

6. DISCUSSION

6.1 Advantages and weaknesses of the study design

The main asset in the present study is that it was a population-based analysis and all beta-hemolytic streptococcal bacteremias (A, B, C and G) in the Pirkanmaa Health District were included. Another advantage was that the study was longitudinal (10-year period) and the clinical data on the patients were thoroughly reviewed by one infection diseases specialist doctor. This design provided unique opportunity to characterize *emm* types of *S. dysgalactiae* subsp. *equisimilis* and *S. pyogenes* in a defined population over time. Most of the *S. dysgalactiae* subsp. *equisimilis* bacteremia strains (138/140) were available for *emm* typing and also most of the GAS bacteremia strains (87/92) were available for both *emm* typing and SAg genotyping. However, the study also had certain limitations. It was retrospective and did not include pediatric patients. In addition the study covered only invasive infections and therefore could not analyze differences in *emm* types between invasive and non-invasive infections.

6.2 Incidence of beta-hemolytic streptococcal bacteremia

The number of invasive GAS infections did not increase during the study period. The average annual incidence and the fluctuating pattern of invasive GAS infections were similar to those in earlier reports (Davies et al. 1996; O'Brien et al. 2002; Ekelund et al. 2005; Lamagni et al. 2005; Siljander et al. 2006). In contrast to recent GBS studies, no increase in the incidence of GBS during the study period was found (Phares et al. 2008; Skoff et al. 2009). The incidence of GCS bacteremia did not increase significantly during the study period. Only a few population-based studies of the incidence of GGS bacteremia have been published (Ekelund et al. 2005;

Broyles et al. 2009). The novel finding here was that the incidence of GGS increased significantly during the study period (1995-2004) and also thereafter (2005-2009). The incidence of GGS has also increased in some other geographic areas like in Denmark (Ekelund et al. 2005). The explanation for the increase in GGS since 1995 remains unclear. Prolonged survival of adults with underlying chronic diseases such as diabetes mellitus, cancer and heart disease may be one contributing factor (Sylvetsky et al. 2002). There must also be other reasons, since no significant difference in age, diabetes or cardiovascular diseases among patients with GGS bacteremias as compared to those with GBS or GCS bacteremias was found. Diabetes was significantly more frequently observed in patients with GGS as compared to GAS bacteremia ($p=0.02$), cardiovascular disease was significantly more common in patients with GGS as compared to GAS bacteremia ($p=0.001$). The prevalence of diabetes and obesity has more than doubled among US adults since the 1980s, which can be a factor contributing to the increasing GGS (CDC 2011b). In contrast, the incidences of cancer and cardiovascular disease have not increased in recent years (Arciero et al. 2004; Jemal et al. 2008). Patients with GAS bacteremia were younger as compared to GBS, GCS and GGS patients, which is in accord with an earlier report (Ekelund et al. 2005). In addition, host factors and GGS virulence factors may have a role (Kalia et al. 2001). Conceivable there are also seasonal or environmental factors affecting the occurrence of skin and soft-tissue infections.

In summary, the incidence of GGS bacteremia is increasing in the Pirkanmaa Health District of Finland and the same trend has continued since the study period (5-year period) in Pirkanmaa.

6.3 Predisposing factors and clinical manifestations of beta-hemolytic streptococcal bacteremias

The present series confirmed that patients with GAS bacteremia have underlying diseases less frequently as compared to GBS, GCS and GGS patients (Sylvetsky et al. 2002). It was also established that GBS bacteremia is more frequently associated with malignancies than GAS, GCS and GGS bacteremia. The frequency of

malignancies (38%) in GBS patients was clearly higher than observed in previous studies (Farley et al. 1993; Farley 2001; Skoff et al. 2009). Metastatic malignancies and gynecological cancers were the usual malignancies. Alcoholism was significantly more common as an underlying disease in patients with GAS than in those with GBS, GCS or GGS bacteremias, as also previously reported (Skogberg et al. 1988; Schugk et al. 1997). Diabetes mellitus was common in patients with GBS, GCS and GGS bacteremia, again as previously noted (Broyles et al. 2009; Skoff et al. 2009). Malignancies, cardiovascular diseases and diabetes were the three most prominent underlying conditions in GBS and GGS bacteremia. In a recent prospective questionnaire-based study of beta-hemolytic bacteremia the data showed no significant differences between the Lancefield groups in the prevalence of diabetes mellitus, chronic heart diseases, or alcohol abuse (Ekelund et al. 2005). This is in contrast to the present findings where diabetes and chronic heart diseases were more common in GBS, GCS and GGS bacteremia and alcoholism was common in GAS bacteremia. The difference in methodology between the two studies may be one explanation for the discrepancy. In the present study all the patient records could be reviewed and underlying diseases were based on patient records and diagnoses were made by a doctor.

Diabetes predisposes individuals to skin and soft-tissue infections, and prevention of diabetes and obesity could also reduce the risk of invasive GBS and GGS disease. Preventive interventions aimed at reducing alcoholism could lower the risk of invasive GAS disease and could reduce the mortality related to alcoholism and also to alcoholism-related liver disease. Alcohol consumption also predisposes individuals to trauma and traumatic wounds and a reduction in alcohol consumption could reduce these factors predisposing to GAS. Treating chronic eczemas or skin erosions carefully could reduce the risk of invasive GAS and GGS disease in cases where disruptions of the cutaneous barrier are most common. In the present study, disruption of the cutaneous barrier was a very common predisposing factor for GAS and GGS bacteremias, and more frequent than previously reported (Skogberg et al. 1988; Schugk et al. 1997; Ekelund et al. 2005). An important new finding here was that about 49% of all patients had a disruption of the cutaneous barrier as a probable or possible portal of entry. Chronic eczema or skin erosion, chronic ulcer or traumatic wound were the three most common disruptions of the cutaneous barrier. No earlier data are available concerning breaches of cutaneous barrier and beta-

hemolytic bacteremia. Preceding trauma has been reported to be a common predisposing factor in GAS bacteremias (Bisno et al. 1996; Sharkawy et al. 2002). However, a preceding operation was uncommon in these patients, which is in contrast to earlier findings (Daneman et al. 2005). A history of previous cellulitis was over-represented in GBS and GGS bacteremias (20% and 29%, respectively) as compared to those with GAS (4%). This adds to our knowledge of recurrent GBS and GGS cellulitis.

In line with previous reports, skin disorders were the most common presenting clinical manifestation in all groups (Skogberg et al. 1988; Farley et al. 1993; Schugk et al. 1997; Efstratiou 2000; Lewthwaite et al. 2002; Sylvetsky et al. 2002; Ekelund et al. 2005). Bacteremia in association with skin and soft-tissue infections as well as bacteremia without focal symptoms were the most prevalent clinical presentations of invasive beta-hemolytic streptococcal infections in the present cohort. Two-thirds of GAS and GGS bacteremias comprised skin infections. GAS and GGS share many virulence factors (Kalia et al. 2001), which may be one explanation for their similar disease spectra. Cellulitis was recorded more frequently for patients with GCS and GGS than for those with GAS and GBS. Likewise, cellulitis occurred in 60% of all patients with invasive GGS infections in a study from Israel (Cohen-Poradosu et al. 2004). This may reflect a change in the microbiology of this disease group, since cellulitis has been traditionally regarded as a skin and subcutaneous infection caused predominantly by GAS (Eriksson et al. 1996; Swartz 2004). In a recent Finnish study, the most common etiology of cellulitis was GGS (Siljander et al. 2008). It will be interesting to see whether the spectrum of beta-hemolytic streptococcal bacteremias will change as the number of patients with underlying diseases grows. It seems likely that in the near future the number of GGS bacteremias, which affect particularly patients with chronic illness and advanced age, will increase. The burden of *S. dysgalactiae* subsp. *equisimilis* (GCS/GGS) disease has previously been under-recognized (Ekelund et al. 2005; Broyles et al. 2009). To improve the understanding of the changing epidemiology of beta-hemolytic streptococcal infections, all invasive beta-hemolytic streptococci should be identified to species level and tested for antimicrobial susceptibility.

In summary, the present results confirm earlier findings in that skin infections as a presenting infection focus are particularly common in patients with GAS and GGS bacteremia. In light of the present and previous reports of predisposing factors,

disruption of the cutaneous barrier was a more common predisposing factor in GAS and GGS bacteremias than previously reported. This study also yielded more detailed information on forms of disruptions of the cutaneous barrier than has previously been reported.

6.4 The outcome and the severity of disease in beta-hemolytic streptococcal bacteremias

The case fatality rate due to bacteremias caused by GAS was 15%, a value similar to the 14-15% reported by others (Darenberg et al. 2007; O'Loughlin et al. 2007). The case fatality rate in the case of GBS bacteremia was lower (7%) than in other studies (8-19%) (Ekelund et al. 2005; Skoff et al. 2009). One explanation for this can be our population-based approach, including pregnant women who had low rates of death. The case fatality rate in the case of GGS bacteremia was 15%, a value similar to the 15-18% reported by others (Ekelund et al. 2005; Brandt et al. 2009). Most *S. dysgalactiae* subsp. *equisimilis* patients are elderly and have more underlying diseases than GAS patients, but the case fatality rates are almost the same (Ekelund et al. 2005; O'Loughlin et al. 2007; Broyles et al. 2009). This may mean that GAS is more virulent than *S. dysgalactiae* subsp. *equisimilis*, but this issue needs further investigation. Advanced age was a factor predisposing to death in GBS bacteremias, a finding in line with those in a Danish study (Ekelund et al. 2005). In contrast, the present study showed that increasing age was not associated with death in GAS, GCS or GGS bacteremias, which is contrary to other studies (O'Brien et al. 2002; Sharkawy et al. 2002; Ekelund et al. 2005; O'Loughlin et al. 2007). One explanation for this might be that GAS patients were younger and the elderly had low rates of infection. According to one recent study the age distribution of patients in Finland is unusual in consequence of the low numbers of elderly subjects (Lamagni et al. 2008a). The elderly have the highest rates of GAS death (O'Loughlin et al. 2007).

STSS was more often caused by GAS or GCS than by GBS or GGS, and 70% of patients with STSS died, the figure being higher than those previously reported (Stevens 1995b; Davies et al. 1996; Stevens 1996; Lamagni et al. 2008b). The development of STSS caused by GBS, GCS or GGS has previously been reported in case series studies (Schlievert et al. 1993; Natoli et al. 1996; Thomas et

al. 1996; Tang et al. 2000; Jankowski et al. 2002), but data from population-based studies are limited in comparison with GAS STSS. As reported in a Danish study, ICU treatment was significantly more common in cases with GAS and GCS bacteremias as compared to GBS or GGS bacteremias (Ekelund et al. 2005). In line with the study in question, invasive *S. dysgalactiae* subsp. *equisimilis* infections were associated with older age, a higher frequency of underlying diseases and a lower frequency of NF and STSS than invasive GAS disease (Ekelund et al. 2005).

In summary, GAS and GGS patients have the same case fatality rates, although GAS patients are more often treated in the ICU. Hypotension, DIC, MOF and STSS as markers of disease severity are more frequently observed in patients with GAS as compared to GGS bacteremia. GGS patients have more underlying diseases and are clearly older than GAS patients, and these factors contribute to their outcome.

6.5 Predisposing factors of death in beta-hemolytic streptococcal bacteremia

The following factors predisposing to death in GAS bacteremia have previously been reported: alcoholism (Davies et al. 1996; Ekelund et al. 2005), liver disease (Mehta et al. 2006), pneumonia (O'Brien et al. 2002; Hollm-Delgado et al. 2005; Mehta et al. 2006; O'Loughlin et al. 2007) and chronic disease (O'Brien et al. 2002; Sharkawy et al. 2002; Ekelund et al. 2005; O'Loughlin et al. 2007). The present study confirmed the earlier finding in GAS bacteremia that alcoholism and having an ultimately or rapidly fatal underlying disease are factors significantly associated with increased case fatality. This study yielded new information to indicate that these conditions are factors significantly associated with increased case fatality also in GBS, GCS and GGS bacteremia. According to one previous report, the outcome is significantly influenced by the patient`s underlying disease (McCabe 1962). In the present series, 90% of the patients with GGS bacteremia had an underlying disease. The number of papers analyzing the association between underlying diseases and case fatality in GCS or GGS bacteremia is limited (Ekelund et al. 2005; Broyles et al. 2009). Fever as a first sign and symptom was shown to imply a good prognosis and confusion, unconsciousness or dyspnea were markers of a poor prognosis in

beta-hemolytic streptococcal bacteremia. This is in keeping with several earlier sepsis studies, which have found that patients with severe sepsis who are hypothermic (<35.5 °C) have almost twice the risk of dying in comparison to those with normal or elevated body temperature (Weinstein et al. 1983; Pittet et al. 1996). Central nervous system symptoms of patients with bacteremia have also been associated with poor prognosis (Syrjanen 1989).

The severity of disease has not been widely studied in population-based GBS, GCS or GGS studies. Hypotension and the presence of an underlying disease are reported to predict death in GAS bacteremia (Sharkawy et al. 2002; O'Loughlin et al. 2007). In the present study high plasma CRP and leukopenia on admission were associated with poor prognosis. The number of studies describing laboratory parameters as predictors of death in beta-hemolytic bacteremia is limited. Thrombocytopenia, leukopenia and anemia have been described to be associated with death in beta-hemolytic streptococcal bacteremia in a univariate analysis (Bernaldo de Quiros et al. 1997). In bacteremia the results regarding plasma CRP as a possible predictor of death are controversial. One recent review concludes that the likelihood of plasma CRP level reflecting the severity of sepsis may be limited (Mitaka 2005). No information was available concerning high plasma CRP and increased case fatality rates in beta-hemolytic bacteremia in the review in question.

In summary, the present study confirms earlier findings in GAS that alcoholism and the presence of an ultimately or rapidly fatal underlying disease are factors associated with increased case fatality. These present series brought out new information indicating that these conditions are factors significantly associated with increased case fatality also in GBS, GCS and GGS bacteremia. Another new finding was that fever was associated with a good prognosis in beta-hemolytic streptococcal bacteremia. The present study adds to current knowledge the recognition that among beta-hemolytic bacteremia patients high plasma CRP and leukopenia constitute a poor prognosis. These particular laboratory tests have not been included in other population-based GCS or GGS studies.

6.6 Skin and soft-tissue infections, a history of previous cellulitis and outcome

NF was associated with high case fatality (38%) an observation in keeping with earlier studies (Stevens 1992; Sharkawy et al. 2002). In accord with the present findings non-necrotizing cellulitis has been associated with a good prognosis in beta-hemolytic bacteremia (Ekelund et al. 2005).

The novel present finding was that a history of previous cellulitis seemed to be a factor protective against death. One explanation for this might be that patients with previous cellulitis had a shorter duration of symptoms before admission to hospital, as they recognized their condition more rapidly and received earlier treatment. This may not, however, be the only explanation; also prior antigen challenge and development of antibodies may play a role. In the subgroup of patients with cellulitis as a presenting clinical manifestation none who had a history of previous cellulitis died, but the fatality rate was 13% among those without such a history. Therefore, it is possible that it is not only the disease manifestation which plays a role but possibly the previous cellulitis reduces mortality by some other mechanism, too.

In summary, the present study brought out new information suggesting that previous cellulitis seemed to be a factor protecting against death. This unique finding highlights the possible role of prior antigen challenge and the possibility of the development of antibodies among patients with cellulitis.

6.7 Molecular characteristics of *S. dysgalactiae* subsp. *equisimilis* **(**Group C or G streptococcal) bacteremic isolates

The present study revealed that case fatality was higher in *S. dysgalactiae* subsp. *equisimilis* bacteremia caused by rare *emm* types than that caused by common *emm* types. This interesting epidemiologic finding has not previously been reported. The reason for the difference is not known. One explanation might be that patients contact certain prevailing bacterial strains (*i.e.* so-called common *emm* types) more

often and during years prior antigen challenge and development of antibodies may play a role in protecting against infection with these strains. Severe disease, defined as leading to death or admittance to ICU, was also caused more frequently by rare than common *emm* types. This aspect has not previously been studied in *S. dysgalactiae* subsp. *equisimilis* bacteremia. However, we were not able to study other possible virulence properties in our GCS/GGS isolates. Data of other virulence factors among GCS/GGS isolates are limited (Igwe et al. 2003). The present study reported a new association between a common *emm* type and cellulitis as the presenting clinical manifestation, which has not previously been reported. This 10-year retrospective survey identified 140 episodes of *S. dysgalactiae* subsp. *equisimilis* bacteremia in the Pirkanmaa area. This is one of the largest series of *S. dysgalactiae* subsp. *equisimilis* bacteremia described in the literature to date. Some similarities were found in the epidemiologic characteristics of *S. dysgalactiae* subsp. *equisimilis* bacteremia between the present and other GGS bacteremia findings. Most patients (90 %) had underlying diseases, as reported elsewhere (Auckenthaler et al. 1983; Vartian et al. 1985; Woo et al. 2001; Sylvetsky et al. 2002; Cohen-Poradosu et al. 2004). Cardiovascular diseases, diabetes and malignancies were the three most prominent underlying conditions in *S. dysgalactiae* subsp. *equisimilis* bacteremias, as has previously been noted (Auckenthaler et al. 1983; Vartian et al. 1985; Woo et al. 2001; Lewthwaite et al. 2002; Cohen-Poradosu et al. 2004; Ekelund et al. 2005; Broyles et al. 2009). Several studies have shown cellulitis to be the most common presenting clinical manifestation (Cohen-Poradosu et al. 2004; Broyles et al. 2009).

In the present series with molecular typing data for 138 invasive *S. dysgalactiae* subsp. *equisimilis* isolates from human infections, 18 different *emm* types were found. These *emm* types have also been found earlier in *S. dysgalactiae* subsp. *equisimilis* isolates (Cohen-Poradosu et al. 2004; Pinho et al. 2006). *S. dysgalactiae* subsp. *equisimilis* bacteremia is caused by a variety of *emm* types, as previously reported (Kalia et al. 2001; Cohen-Poradosu et al. 2004; Liao et al. 2008; Broyles et al. 2009). In the present material StG480, stG6 and stG485 were the three most common *emm* types, covering 51% of all isolates. In a previous study, 18 GGS isolates were *emm*-typed from human infections (Kalia et al. 2001). In the small study in question 13 *emm* types were found, the most common being stG480.0; the most common type of the present study was likewise stG480.0. In a retrospective

study in Israel, 56 GGS isolates were *emm* typed (Cohen-Poradosu et al. 2004). The authors found 13 *emm* types, the most common of which was stG485.0. None of their isolates was *emm* non-typable. In a recent study in the United States, 212 *S. dysgalactiae* subsp. *equisimilis* isolates were *emm*-typed and the three most common *emm* types were stG6, stG245 and stG2078, representing 39% of isolates (Broyles et al. 2009). In a Norwegian study, stG643 was the most prevalent type (Kittang et al. 2011). Kittang's results illustrate that predominant *emm* types vary with geographical location. Thus, *emm* typing can be used to analyze the molecular epidemiology of *S. dysgalactiae* subsp. *equisimilis* isolates and the prevalence of specific *emm* types tends to vary within a geographic region. A similar aspect has reported elsewhere with GAS (O'Brien et al. 2002; O'Loughlin et al. 2007). The present study showed that certain *emm* types may prevail among human infections. No obvious time shifts were noted in the occurrence of certain *emm* types. The data on other virulence factors among GCS/GGS isolates are limited (Igwe et al. 2003).

The present findings reflected a relatively high frequency of recurrent group G *S. dysgalactiae* subsp. *equisimilis* bacteremia, which is in line with earlier findings (Cohen-Poradosu et al. 2004; Liao et al. 2008). Cohen-Poradosu and group found 14% of patients to have recurrent GGS bacteremia and lymphatic drainage disorders to be a highly significant risk factor for recurrence (Cohen-Poradosu et al. 2004). In the present series the PFGE pattern showed that strains isolated from the same patient in recurring infections were identical. Recurrent bacteremia has previously been described for GBS and GGS (Harrison et al. 1995; Cohen-Poradosu et al. 2004). A recent study of GGS bacteremia showed that a high percentage of recurrence was caused by identical strains (Liao et al. 2008). In contrast, recurrent bacteremia has not been described for GAS.

The dynamics of interspecies horizontal transfer of genetic material of virulence factors between GAS, GGS, and GCS are unclear (Igwe et al. 2003; Kalia et al. 2003). Characterization of the *S. dysgalactiae* subsp. *equisimilis* strains by multilocus sequence typing would be of interest (Ahmad et al. 2009). No scheme for this precedure has yet been developed for *S. dysgalactiae* subsp. *equisimilis.*

The true burden of disease caused by *S. dysgalactiae* subsp. *equisimilis* (group C and G) is unknown. We need more population-based analyses to elucidate the epidemiology, clinical and molecular characteristics and outcome *S. dysgalactiae* subsp. *equisimilis* infections.

In summary, this study yielded new information indicating that the severity of disease and case fatality were higher in *S. dysgalactiae* subsp. *equisimilis* bacteremia caused by rare *emm* types than in that caused by common types. Patients may contact prevailing bacterial strains more often, and a prior antigen challenge and also humoral response may play a role. Of particular note is that cellulitis was a more frequent presenting clinical manifestation among patients with common *emm* types than rare types, and patients with cellulitis had a good prognosis. This is the first report of associations between common *S. dysgalactiae* subsp. *equisimilis emm* types and skin and soft-tissue infections.

6.8 Molecular characteristics of Group A streptococcal bacteremic isolates

The present population-based longitudinal design provided a unique opportunity to characterize the distribution of invasive GAS serotypes in a defined population in Finland over a 10-year period. The survey identified 92 episodes of GAS bacteremia in the Pirkanmaa area, Finland. In the molecular typing of 87 GAS bacteremia isolates we found 18 *emm* types. *Emm* 1, *emm* 28 and *emm* 81 were the three most frequent, accounting for 52 % of isolates. In a recent study in the United States, *emm* types 1 (22%), 3 (9%), 28 (9%), 12 (9%), and 89 (6%) were the most common, cumulatively accounting for 55% of isolates (O'Loughlin et al. 2007). In a Swedish study, 746 isolates were *emm*-typed and *emm* 89, *emm* 81 and *emm* 28 emerged as the three most common *emm* types, covering 44% of all isolates (Darenberg et al. 2007). In a Danish study, again, *emm*28, *emm*1 and *emm*3 were identified as the three most frequent *emm* types, covering 62% of 278 isolates (Luca-Harari et al. 2008). In Britain *emm*1, *emm*3 and *emm*87 have been reported as the three most common *emm* types, that accounting for 41% of isolates (Lamagni et al. 2008b). Thus, *emm* typing provides a useful tool for the epidemiological and molecular analysis of GAS isolates from various geographical regions. The prevalence of specific GAS types tends to vary over time and within a geographic area (O'Brien et al. 2002; O'Loughlin et al. 2007). One theory to explain this phenomenon is the lack of herd immunity to specific GAS isolates due to the low prevalence of these types in the population during previous years, which would allow the emergence of these

clones within the susceptible population. Variations may also be due to differences in the prevalence of risk factors for invasive GAS infection, for example chronic diseases (diabetes, cancer and substance abuse), race, and number of persons living in the home (Factor et al. 2003; Factor et al. 2005).

The *emm* types included in the 26-valent vaccine under development (Dale et al. 2005), were only limitedly represented in the present material (Figure 2); 9 of the vaccine types accounted for only 62% of isolates. In contrast, the vaccine coverage in the United States was good; the proposed 26-valent vaccine accounted for 79% of all isolates and 79% of isolates in patients who died (O'Loughlin et al. 2007). In the present study the vaccine coverage also varied considerably, from 100% to 21% in different years. In a previous Japanese study, the coverage of the putative 26-valent GAS vaccine fell from 94% in 1996-2000 to 82% in 2001-2005 (Ikebe et al. 2007). In order to ensure the vaccine coverage, alternate formulations with different *emm* type profiles would be needed for different regions and more *emm* types may need to be included in the vaccine in regions such as Africa and the Pacific in view of higher variability in the *emm* type distribution (Steer et al. 2009).

In disagreement with previous reports, no associations between *emm* types and particular disease manifestations were observed in the present study. E*mm* type 1 was not found to be associated with STSS nor *emm* 81 with skin or soft-tissue involvement. *Emm* 81 has been associated with skin or soft-tissue infection in Sweden (Darenberg et al. 2007). In the present series skin and soft-tissue infection were common presenting clinical manifestations in *emm* type 81 but also in *emm* types 1 and 28. In the present study puerperal sepsis was slightly associated (without statistical significancy) with both *emm* types 28 and 81. In a recent European multi-center study, covering 11 countries, 4253 GAS isolates were *emm* typed and 104 different types were identified (Luca-Harari et al. 2009). The most severe manifestations, STSS and NF, were caused by 45 different types, of which *emm* 1 was the most prevalent, accounting for 37% and 31% of cases, respectively (Luca-Harari et al. 2009). According to other recent reports *emm*1 was the strongest predictor for invasive GAS infection among different *emm* types (Luca-Harari et al. 2008; Lintges et al. 2010). In the European multi-center study mentioned above, cellulitis was more often caused by either *emm*87 or *emm* 83, and a correlation with *emm* 28 and puerperal sepsis was also found (Luca-Harari et al. 2009). The present study was underpowered to observe such associations. According to *emm* typing, we

identified a variety of *emm* types causing puerperal sepsis, suggesting that no puerperal sepsis outbreak occurred during the study period.

During the last few years, the number of GAS SAgs identified has increased, likewise knowledge of their role in disease pathogenesis (Chatellier et al. 2000; Cunningham 2000; Bisno et al. 2003). In one recent study SAgs proved more important for the invasiveness of GAS than *emm* type (Lintges et al. 2010). The SAg genes *speA, speJ* and *speZ* are present more often in isolates from invasive infections and *ssa*, *speC* and *speJ* are present more often in isolates from noninvasive infections (Lintges et al. 2010). In a Swedish study no correlation between invasiveness and the presence of single SAg genes was found, but certain SAg profiles within clonal clusters were more common in invasive cases (Darenberg et al. 2007). The authors found that the *speA* gene was commonly detected among *emm* 1 isolates, both invasive and non-invasive, but rarely among other types (Darenberg et al. 2007). *SpeA* is primarily associated with *emm* 1 and *emm* 3 (Luca-Harari et al. 2008). These earlier findings parallel those in the present series, where the *speA* gene was always detected among *emm* type 1 isolates. No *emm* type 3 isolates were found there. The differences in invasiveness of GAS isolates could not be studied in this material as there were no isolates from non-invasive infections.

Studies of GAS isolate SAg profiles may represent a useful strategy to identify subclones which are particularly prone to cause invasive disease. Several previous studies have described the potential involvement of streptococcal pyrogenic exotoxin speA in severe streptococcal disease (Stevens et al. 1989; Musser et al. 1991; Talkington et al. 1993; Eriksson et al. 1999). An association has also been reported between *spe*C and severe streptococcal disease (Holm et al. 1992; Demers et al. 1993). Some cases of STSS are reported not to be associated with either *spe*A or *spe*C (Hsueh et al. 1998). Here *spe*A was detected in 2 and *spe*C in 5 out of 13 strains causing STSS, whereas a combination of both was not found in any of these isolates.

In view of the present results, the current formulation of the GAS vaccine would provide only limited coverage of GAS *emm* types in Finland. Relative stability of serotype distribution among GAS patients is required for a vaccine strategy to be a good means for prevention of GAS disease. Although the present study covered 10 years and was population-based, it was underpowered to observe associations between *emm* types and disease manifestations. In contrast, earlier

studies with a larger number of patients (4353 patients), have found associations between *emm* types and disease manifestations, for example associations between *emm* 28 and puerperal sepsis, and *emm*87 or *emm* 83 and cellulitis (Luca-Harari et al. 2009). In the Swedish study already referred to skin or soft-tissue infections were most commonly caused by *emm* type 81 (Darenberg et al. 2007). In conclusion, in light of the present and previous reports of SAgs, no correlation was found between invasiveness and the presence of single SAg genes. Characterization of GAS by different typing methods will help to improve our understanding of the epidemiology of invasive disease such as circulating *emm* types of GAS and populations' susceptibility to a particular *emm* type, detection of outbreaks, and vaccine development.

6.9 Future considerations

The present study brought out an increasing incidence of GGS bacteremia. The reason for this increase calls for further research.

A novel association was found between a history of cellulitis and lower case fatality in streptococcal bacteremia. Immunological studies of patients suffering only from cellulitis, of those who have suffered previous cellulitis and have developed streptococcal bacteremia, and of those with streptococcal bacteremia without any previous cellulitis are needed to understand the possible mechanisms underlying this association.

Large, population-based studies should be undertaken to investigate predictors for mortality in beta-hemolytic streptococcal bacteremias. Especially elucidation of the effect of underlying diseases on case fatality in GCS and GGS is limited. Also the effect of disease severity (for example hypotension, lowered level of consciousness, DIC, MOF and afebrility) on the outcome should be investigated in detail in GBS, GCS or GGS bacteremia. The present paper reports that high CRP and leukopenia on admission predicted a poor prognosis. Population-based studies investigating several biomarkers as predictors of death in streptococcal bacteremia would warrant research.

The present study revealed a novel association wherein case fatality was higher in *S. dysgalactiae* subsp. *equisimilis* bacteremia caused by rare *emm* types than that caused by common types. An association between common *emm* type and cellulitis as a clinical manifestation in *S. dysgalactiae* subsp. *equisimilis* bacteremia was also found. Larger population-based studies investigating associations between *emm* type and mortality and *emm* type and clinical manifestation in *S. dysgalactiae* subsp. *equisimilis* bacteremia should be initiated to confirm these findings. Other virulence properties among *S. dysgalactiae* subsp. *equisimilis* isolates also warrant further research, for example SAg profiling.

Emm type surveillance remains important for the rapid detection of changes in type distribution which might lead to an increase in incidence and mortality. To document changes in *emm* distribution over time and in different regions is essential in vaccine development for GAS. Epidemiological studies are essential to identify target populations for vaccination for GAS, with a view to reducing mortality in risk groups in the future. Monitoring of antimicrobial resistance is also essential. Identification of isolate types will serve as a basis for studies of pathogenesis and vaccine development.

7. SUMMARY AND CONCLUSIONS

The present findings indicate that the incidence of GGS bacteremia is increasing in the Pirkanmaa Health District, Finland. Other epidemiological studies have also shown increasing numbers of invasive *S. dysgalactiae* subsp. *equisimilis* infections, and this species will have even greater clinical importance in the future. Doctors should consider the possibility of invasive *S. dysgalactiae* subsp. *equisimilis* infections when treating elderly patients with underlying diseases and breakdowns of the skin. The observations here highlight the fact that disruptions of the cutaneous barrier and skin infections are particularly common in patients with GAS and GGS bacteremia. Early recognition of invasive streptococcal infection, a search for deep-seated infection, rapid initiation of effective antibiotic therapy and surgery are important parts of therapy.

The case fatality rate in beta-hemolytic bacteremia is approximately 15%, but 30-70% in the case of pneumonia, NF or STSS. Alcoholism and the presence of an ultimately or rapidly fatal underlying disease are factors significantly associated with increased case fatality in beta-hemolytic bacteremia. The present study added to current knowledge the observation that a previous history of cellulitis seems to be a factor protecting against death. Among first signs and symptoms confusion, unconsciousness or dyspnea are markers of poor prognosis, while fever seems to protect against death. Also cellulitis as a presenting clinical manifestation predicts a favorable outcome. High plasma CRP (>105 mg/ml) and leukopenia ($<4.0 \times 10^9$ /l) on admission indicate a poor prognosis. It is important to identify factors associated with a poor prognosis in order to identify patients who need special care, for example ICU treatment or surgery.

Among 138 *S. dysgalactiae* subsp. *equisimilis* isolates 18 different *emm* types were found. StG480, stG6, stG485, stG643, stC6979, stG166b, and stC74a were the seven most common *emm* types, covering 75 % of all isolates. It emerged here that the severity of disease and case fatality were higher in persons with *S. dysgalactiae* subsp. *equisimilis* bacteremia caused by rare *emm* types than that

caused by common *emm* types. This is the first report where an association between *S. dysgalactiae* subsp. *equisimilis* isolate and disease severity and case fatality has been observed. The present paper also reports that cellulitis was a significantly more common clinical manifestation among common than rare *emm* types. The true burden of disease caused by *S. dysgalactiae* subsp. *equisimilis* is not known and associations between *emm* types and clinical outcome warrant further population-based investigation. The prevalence of specific *S. dysgalactiae* subsp. *equisimilis* *emm* types tends to vary within a geographical area. Continued epidemiological and microbiological surveillance is important to assess the future trends in invasive *S. dysgalactiae* subsp. *equisimilis* infections.

This study series added to our understanding of the molecular epidemiology of *S. pyogenes* infection in the Pirkanmaa area. In the molecular typing of 87 GAS bacteremia isolates, 18 different *emm* types were found. Types *emm* 1, *emm* 28 and *emm* 81 were the three most common *emm* types, covering 52 % of all isolates. The putative 26-valent GAS vaccine would have covered 62% of the isolates of this study, which is a lower percentage than hitherto reported in Western countries. These results suggest that the current formulation of the GAS vaccine would provide only limited coverage of GAS *emm* types in Finland. Continued surveillance is particularly essential to document changes in *emm* distribution.

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Clinical presentations and epidemiology of β-haemolytic streptococcal bacteraemia: a population-based study

S. Rantala[1], J. Vuopio-Varkila[2], R. Vuento[3], H. Huhtala[4] and J. Syrjänen[1,5]

1) *Department of Internal Medicine, Tampere University Hospital, Tampere,* 2) *National Public Health Institute, Helsinki,* 3) *Centre for Laboratory Medicine, Tampere University Hospital,* 4) *Tampere School of Public Health, University of Tampere,* 5) *Medical School, University of Tampere, Tampere, Finland*

Abstract

In this population-based study, all 314 episodes of β-haemolytic streptococcal bacteraemia in adult patients in the Pirkanmaa area, Finland, during the 10-year period 1995–2004 were retrospectively reviewed. Altogether, 92 cases of bacteraemia caused by Lancefield group A β-haemolytic streptococci (GAS), 76 caused by group B β-haemolytic streptococci (GBS), 18 caused by group C β-haemolytic streptococci (GCS) and 128 caused by group G β-haemolytic streptococci (GGS) were identified. The most important finding was that the incidence of GGS increased during the study period. Disruption of the cutaneous barrier was a very common predisposing factor in GAS and GGS bacteraemias. Skin infections were the presenting clinical manifestations in two-thirds of GAS and GGS bacteraemias.

Keywords: Bacteraemia, β-haemolytic, clinical presentations, epidemiology, streptococci

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Corresponding author and reprint requests: S. Rantala, Department of Internal Medicine, Tampere University Hospital, PO Box 2000, FIN-33521 Tampere, Finland
E-mail: sari.rantala@pshp.fi

Lancefield group A streptococci (GAS), group B streptococci (GBS), group C streptococci (GCS) and group G streptococci (GGS) are part of the normal flora of the pharynx, skin, intestinal tract and vagina [1] but also cause a variety of invasive and non-invasive infections. This study focuses on the estimation of the incidence of bacteraemia caused by GAS, GBS, GCS and GGS and the determination of the predisposing factors, underlying diseases, presenting clinical manifestations and outcome of these diseases during a 10-year period.

The medical records of all adult (over 16 years of age) patients in the Pirkanmaa Health District (HD), Finland with one or more blood cultures positive for GAS, GBS, GCS or GGS from January 1995 to December 2004 were retrospectively reviewed. Pirkanmaa HD (460 000 inhabitants) harbours one tertiary-care hospital (Tampere University Hospital) and four other hospitals, including Hatanpää City Hospital and the district hospitals in Valkeakoski, Vammala and Mänttä. Laboratory records were screened to identify all blood cultures positive for β-haemolytic streptococci during the study period. The case definition included all patients with a positive blood culture for GAS, GBS, GCS or GGS combined with a clinical picture compatible with septicaemia.

An infectious disease specialist (SR) reviewed all of the patient records. Cellulitis included infections of skin and underlying tissue (erysipelas and deeper non-necrotizing soft tissue infections).

The routine blood cultures were drawn into aerobic and anaerobic bottles. During the study period, the BACTEC NR 730 (only in 1995) and BACTEC 9240 (BD Diagnostic Systems, Sparks, MD, USA) (1996–2004) blood culture systems were used. In the district hospitals, the signal blood culture system (Oxoid, Cambridge, UK) was used until 2003. The Lancefied serogroups were determined by latex agglutination using the Streptex latex test system (Remel Europe Ltd, Dartford, UK). All isolates were also identified biochemically using the Rapid ID 32 STREP system (bioMérieux SA, Marcy-l'Etoile, France).

The categorical data were analysed using the chi-square test or Fisher's exact test as appropriate. The non-parametric data were analysed by Mann–Whitney *U*-test. Change in incidence was analysed by Poisson regression. A two-sided p-value <0.05 was regarded as statistically significant.

β-Haemolytic streptococci grew in 314 cultures, distributed as follows: GAS, 92 cases (29%); GBS, 76 cases (24%); GCS, 18 cases (6%); and GGS, 128 cases (41%). All GAS were identified as *Streptococcus pyogenes*, all GBS as *Streptococcus agalactiae*, and all GGS as *Streptococcus dysgalactiae* subsp. *equisimilis*. Six of the GCS were confirmed to be

Streptococcus equi subsp. *zooepidemicus* and 12 to be *S. dysgalactiae* subsp. *equisimilis*. Positive blood cultures were obtained from 309 patients. Six patients had recurrent β-haemolytic bacteraemia within the study period (two GBS and four GGS).

The presenting clinical manifestations associated with bacteraemia are given in Table 1. In all cases, the causative microbe was susceptible to the empirical antibiotic therapy given. The 30-day mortality was 13%, being highest in patients with GCS (22%); mortality was 15% in patients with GAS, 7% in those with GBS, and 15% in those with GGS.

The incidence of GGS bacteraemia increased from 1.8 cases per 100 000 population in 1995 to 4.3 cases per 100 000 population in 2004 (Fig. 1); this was a statistically significant increase (p 0.013). The incidence of GAS or GBS bacteraemia fluctuated over time (Fig. 1). The number of blood cultures taken increased during the study period. However, the incidence of positive blood cultures (all cases of bacteria included) increased from 279/100 000 in 1995 to 368/100 000 in 2004 (1.3-fold), and the incidence of GGS increased from 1.81/100 000 to 4.32/100 000 during the same period (2.4-fold).

There were no changes in laboratory methodology during the study period, which could explain the increase in the incidence of GGS. An outbreak caused by *S. equi* subsp. *zooepidemicus* associated with consumption of unpasteurized goat cheese occurred in 2003 (six cases), and has been reported elsewhere [2].

Alcoholism (an alcohol-related medical or social problem) was common in patients with GAS bacteraemia (26%), and diabetes was common in patients with GBS (32%) and GGS

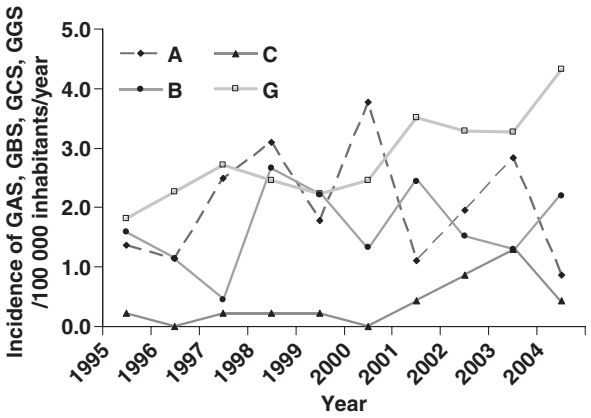

FIG. 1. Incidence/100 000 inhabitants/year of bacteraemia caused by Lancefield group A streptococci (GAS), Lancefield group B streptococci (GBS), Lancefield group C streptococci (GCS) and Lancefield group G streptococci (GGS) in Pirkanmaa Health District 1995–2004.

(23%) bacteraemia. Malignancies were common in patients with GBS being higher than in previous studies [3,4] (38%) bacteraemia.

Disruption of the cutaneous barrier was more common in patients with GAS (58%) or GGS (53%) bacteraemia than in those with GBS (32%) bacteraemia (p 0.001 and p 0.003, respectively). Twenty-six per cent of all patients had chronic eczema or skin erosion, 4% psoriasis, 13% chronic ulcer, 10% traumatic wounds and 3% operation wounds as a probable or possible portal of entry. A trauma during the previous month was a common predisposing factor in cases of GAS bacteraemia (27%). A history of previous cellulitis that was treated in a hospital or in an outpatient clinic at least 1 month prior to the current episode of bacteraemia was

TABLE 1. Comparison of the presenting clinical manifestations of β-haemolytic streptococcal bacteraemias by Lancefield group

Presenting clinical manifestation[a]	GAS *n* = 92 *n* (%)	GBS *n* = 76 *n* (%)	GCS *n* = 18 *n* (%)	GGS *n* = 128 *n* (%)	All *n* = 314 *n* (%)	Overall p-value
Skin/soft tissue infection	65 (71)	34 (45)	10 (56)	88 (69)	197 (63)	0.002
Cellulitis	36 (39)	26 (34)	8 (44)	73 (57)	143 (46)	0.006
Fasciitis	7 (8)	0	0	1 (1)	8 (3)	0.008
Infected wound or eczema	24 (26)	12 (16)	1 (6)	23 (18)	60 (19)	0.15
Deep abscess[b]	10 (11)	5 (7)	0	2 (2)	17 (5)	0.02
Pneumonia	16 (17)	3 (4)	2 (11)	12 (9)	33 (11)	0.04
Urinary tract infection	1 (1)	7 (9)	0	1 (1)	9 (3)	0.008
Puerperal sepsis	7 (8)	6 (8)	0	4 (3)	17 (5)	0.27
Arthritis (all)	5 (6)	3 (4)	1 (6)	11 (9)	20 (6)	0.58
Prosthetic joint infection	0	3 (4)	0	6 (5)	9 (3)	0.14
Osteomyelitis	4 (4)	5 (7)	0	6 (5)	15 (5)	0.83
Meningitis	3 (3)	0	0	1 (1)	4 (1)	0.34
Endocarditis	1 (1)	3 (4)	1 (6)	2 (2)	7 (2)	0.30
Bacteraemia without defined focus	10 (11)	21 (28)	8 (44)	21 (16)	61 (19)	0.002

GAS, Lancefield group A β-haemolytic streptococci; GBS, Lancefield group B β-haemolytic streptococci; GCS, Lancefield group C β-haemolytic streptococci; GGS, Lancefield group G β-haemolytic streptococci.
[a]One patient may have one or more clinical manifestations.
[b]Included empyema in five patients, intra-abdominal abscess in three, epidural abscess in two, psoas abscess in one, femoral abscess in one, abscess of operation region in one.

found more often in patients with GBS (20%) or GGS (29%) bacteraemia than in those with GAS (4%) bacteraemia (p 0.002 and p <0.001, respectively).

The most important finding here was the increasing incidence of GGS bacteraemia during the study period. A similar trend in the incidence of GGS bacteraemia has also been noted in some other geographical areas [5]. However, the population-based data concerning the incidence of GGS bacteraemia are limited [5–7]. The reason for the continuous increase in GGS bacteraemia remains unclear, although prolonged survival of adults with underlying diseases (e.g. diabetes mellitus, cancer and heart disease) may be one contributing factor [8]. However, this cannot be the only explanation, as no significant difference in age, presence of diabetes or cardiovascular diseases was found among patients with GGS bacteraemia as compared with those with GBS or GCS bacteraemia (data not shown). In addition to a change in the host factors, GGS virulence factors may have a role [9]. The average annual incidence and the fluctuating pattern of invasive GAS infections were in concordance with those in previous reports [5,10–13].

The important new finding was that a disruption of the cutaneous barrier was a very common predisposing factor in GAS and GGS bacteraemia, occurring more frequently than previous reports would imply [5,11,14–16]. As described elsewhere, skin infections were the most common clinical manifestation in all groups [3,5,8,10,15–20]. Skin infections were the presenting clinical manifestations in two-thirds of GAS and GGS bacteraemias. It is known that GAS and GGS share virulence factors. This may be one explanation for the similar spectrum of disease that they cause. A new finding was that a history of previous cellulitis was very common in GBS and GGS bacteraemia.

It is concluded that the incidence of GGS bacteraemia is increasing in Pirkanmaa HD, Finland. Disruption of the cutaneous barrier as a predisposing factor and skin infections as a presenting focus of infection were found very commonly in patients with GAS and GGS bacteraemia.

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Transparency Declaration

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Predictors of mortality in beta-hemolytic streptococcal bacteremia: A population-based study

Sari Rantala [a,*], Jaana Vuopio-Varkila [b], Risto Vuento [c], Heini Huhtala [d], Jaana Syrjänen [a,e]

[a] *Department of Internal Medicine, Tampere University Hospital, P.O. Box 2000, FIN-33521 Tampere, Finland*
[b] *National Public Health Institute, Helsinki, Finland*
[c] *Centre for Laboratory Medicine, Tampere University Hospital, Tampere, Finland*
[d] *Tampere School of Public Health, University of Tampere, Tampere, Finland*
[e] *Medical School, University of Tampere, Tampere, Finland*

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Cellulitis

Summary *Objectives:* Several factors associated with mortality in Lancefield group A beta-hemolytic streptococcal bacteremia have been described in population-based surveillance studies, whereas such reports on group B, C, and G streptococcal are scant.
Methods: In this population-based study all 314 episodes of beta-hemolytic streptococcal bacteremia in adult patients in the Pirkanmaa area, Finland, during the 10-year period 1995–2004 were retrospectively reviewed.
Results: The 30-day case-fatality rate was 13%, being highest in group C (22%); in group A it was 15%, in group B 7%, and in group G 15%. Confusion, unconsciousness and dyspnea as the first sign or symptom were associated with increased case-fatality, while fever seemed to be a protecting factor for death. Alcoholism and ultimately or rapidly fatal underlying disease were significantly associated with increased case-fatality. Among infections of the skin and soft-tissues, necrotizing fasciitis had the highest risk of death (38%), while patients with cellulitis had a case-fatality of 8%. A history of previous cellulitis seemed to protect against death (case-fatality of 3% as compared to 16% among those without such a history ($p = 0.014$)).
Conclusion: A history of previous cellulitis seemed to be a protecting factor against death. Fever was also associated with a good prognosis.

Introduction

Lancefield groups A, B, C and G beta-hemolytic streptococci (GAS, GBS, GCS, and GGS) can cause a variety of invasive and non-invasive infections. GAS has been described since the mid-1980s as an emerging cause of

* Corresponding author. Tel.: +358 400 857413; fax: +358 3 35516164.
E-mail address: sari.rantala@pshp.fi (S. Rantala).

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potentially fatal infections such as necrotizing fasciitis (NF), necrotizing myositis and streptococcal toxic shock syndrome (STSS), originally described in healthy young individuals.[1,2]

Various factors associated with increased case-fatality rate in GAS bacteremia have been described, including pneumonia,[3–7] alcoholism,[1,8] age,[4,5,8,9] immunosuppression,[8,10] cancer,[7,9] chronic heart or lung disease,[5,8] working or living in hospital,[7] liver dysfunction,[6] use of NSAID medication,[11] disseminated intravascular coagulation (DIC)[6] and STSS.[3–6,12] In contrast, the number of population-based studies describing predictors of case-fatality in GBS, GCS or GGS bacteremia is limited.[8]

We here report a retrospective analysis of all patients yielding a blood culture positive for beta-hemolytic streptococci in Pirkanmaa Health District (HD), Finland, during 1995–2004. The aim of our study was to determine the clinical characteristics, outcome and predictors of death in beta-hemolytic streptococcal bacteremia in a defined population during the 10-year observation period.

Materials and methods

Patients

The medical records of all adult (over 16 years of age) patients in Pirkanmaa Health District (HD) with one or more blood cultures positive for a beta-hemolytic group A, B, C or G streptococcus during the 10-year period from January 1995 to December 2004 were retrospectively reviewed. In the Pirkanmaa HD there is one tertiary care hospital (Tampere University Hospital) and four other hospitals, Hatanpää City Hospital and the District Hospitals in Valkeakoski, Vammala and Mänttä. Pirkanmaa, with about 460,000 inhabitants, is located in western Finland. All blood cultures were cultivated and studied in the Centre for Laboratory Medicine in Tampere University Hospital, which is the only laboratory in the Pirkanmaa HD, where blood cultures are cultivated and studied. Laboratory records were screened to identify all blood cultures positive for beta-hemolytic streptococci during the study period. Our case definition included all patients with a positive blood culture for a group A, group B, group C or group G beta-hemolytic streptococcus, combined with a clinical picture compatible with septicemia.

An infectious disease specialist (SR) reviewed all the patient records and filled in a structured data collection form. Mc Cabe classification was used to assess the severity of the underlying medical condition.[13] Mc Cabe class II (ultimately fatal disease) was defined as the normal evolution of the disease could lead to death in 1–5 years and Mc Cabe III (rapidly fatal disease) was defined as life expectancy less than 12 months. Alcoholism was defined as a known social or medical problem due to alcohol. Cellulitis included infections of the skin and underlying tissues (erysipelas and deeper non-necrotizing soft-tissue infections). A history of previous cellulitis was defined as a cellulitis occurring more than one month prior to the current bacteremia episode. The prior episodes of cellulitis had been adequately treated at Tampere University Hospital, Hatanpää City Hospital or the District Hospitals in Valkeakoski, Vammala and Mänttä. Recurrent bacteremias were defined as bacteremias occurring at least 3 weeks apart and the first episode had been treated adequately. Necrotizing fasciitis was defined as a progressive, destructive subcutaneous streptococcal infection with necrosis observed either directly or under surgery. The definition of STSS (Streptococcal toxic shock syndrome) was based on a consensus definition, including identification of beta-hemolytic streptococci from a normally sterile site, a septic shock and a multiorgan failure.[14] Hypotension was defined as a patient with a systolic blood pressure of <90 mmHg. A lowered level of consciousness was defined as being unconscious or confused at least once during the first 2 days of hospitalization. DIC was defined as a platelet count lower than 100×10^9/l. Multiorgan failure (MOF) was defined as three or more concomitant organ failures.

Chronic diseases were registered, as well as treatments (antibiotics, intensive care unit (ICU) treatment and surgical intervention). Laboratory tests included plasma C-reactive protein (CRP), blood leucocytes, blood thrombocytes and plasma creatinine level. We used the values on admission of CRP, leucocytes, thrombocytes or creatinine in defining high CRP, leucopenia, thrombopenia or elevated creatinine value. High CRP was defined as a CRP value over the median value of the whole study population (105 mg/ml). Leucopenia was defined as a blood white cell count below 4.0×10^9/l and thrombopenia as thrombocytes below 100×10^9/l. An elevated creatinine value comprised a figure over 120 μmol/l. Afebrility was defined as having an ear temperature $\leq 37.5\,^{\circ}$C and fever was defined as having an ear temperature $>37.5\,^{\circ}$C. Mortality was recorded within 30 days from the positive blood culture (30-day mortality).

Blood culture methods

Routine blood samples were drawn into aerobic and anaerobic bottles. During the study period the BACTEC NR 730 (only 1995) and BACTEC 9240 (BD Diagnostic Systems, Sparks, MD, USA) (1996–2004) blood culture systems with standard culture media were used. In the district hospitals the Signal blood culture system (Oxoid, Cambridge, UK) was used until 2003. The Lancefield serogroups were defined by latex agglutination using the Streptex latex test system (Remel Europe Ltd, Dartford, UK). All isolates were also identified biochemically using the rapid ID 32 STREP system (bioMérieux SA, Marcy-1'Etoile, France). Susceptibility testing was performed by the disk diffusion method paralleling the guidelines of the Clinical Laboratory Standards Institute (CLSI, former NCCLS).

Statistical methods and multivariable models

The SPSS package (version 7.5) 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 analyzed by χ^2 test or Fisher's exact test, as appropriate. Nonparametric data were analyzed by Mann–Whitney U-test. To find out factors that can be evaluated on admission to the hospital (i.e. underlying diseases, first signs and symptoms, laboratory findings on admission) and may predict death, we performed univariate and multivariate

analyses. As there were only 42 deaths during the 30 days follow-up, we evaluated the effect of different factors on case-fatality in three separate models: 1) underlying diseases 2) first signs and symptoms of bacteremia and 3) laboratory findings on admission. In the final multivariate analysis we included the significant factors from the previous multivariate analyses, age and streptococcal group (A, B, C, or G). Odds ratios (OR) were expressed with their 95% confidence intervals (CI).

Results

Beta-hemolytic streptococci grew in 314 cultures, distributed as group A 92 cases (29%), group B 76 cases (24%), group C 18 cases (6%) and group G 128 cases (41%). All the patients with positive blood culture had also clinical signs and symptoms of septicemia. During 10-year period GAS was fluctuating annually from 4 cases to 17, GBS from 2 cases to 12, and GGS from 8 cases to 20. All GAS were identified as *Streptococcus pyogenes*, all GBS were *Streptococcus agalactiae*, and all GGS were *Streptococcus dysgalactiae* subspecies *equisimilis*. Six of the GCS were confirmed to be *Streptococcus equi* subspecies *zooepidemicus* and 12 were *S. dysgalactiae* subspecies *equisimilis*. During the study period there was an outbreak caused by *S. equi* subspecies *zooepidemicus* associated with consumption of unpasteurized goat cheese in 2003 (6 cases).[15] Positive blood cultures were obtained from 309 patients. Six patients had a recurrent beta-hemolytic bacteremia, two of them recurrent GBS and four GGS bacteremias. The antibiotic therapy was started immediately after the blood cultures were drawn. The causative microbe was susceptible to the empiric antibiotic therapy given in all cases.

The patients with GAS bacteremias were significantly younger than those with group B, C or G streptococcal bacteremias (median 53 years vs. 69, 70 or 67 years, respectively; $p < 0.001$ in all comparisons). One hundred and fifty seven of the patients were males and 152 females. The overall gender distribution did not differ significantly between the four serogroups. Table 1 gives details of the underlying diseases in relation to Lancefield group. Table 2 gives details of the clinical characteristics of bacteremia. The outcome of patients with septic pneumonia was poor; 9 out of 33 of them (27%) died. Of severe infections, STSS was more often caused by GAS than by GBS or GGS ($p = 0.058$ and $p = 0.003$; respectively). Overall, 70% of patients with STSS died within the 30-day period following a positive blood culture, compared to 52% of patients with septic shock, and 5% of those who did not have septic shock. ICU treatment was significantly more frequently required in patients with GAS bacteremia as compared to GBS or GGS bacteremia ($p = 0.008$ and $p < 0.001$; respectively). DIC was significantly more frequent in patients with GAS as compared to GBS or GGS bacteremia ($p < 0.001$ and $p < 0.001$;respectively) (Table 2). Multiorgan failure was also significantly more frequently observed in patients with GAS as compared to GBS or GGS bacteremia ($p = 0.025$ and $p = 0.001$; respectively), and surgical intervention was significantly more frequently needed in those with GAS as compared to GGS bacteremia ($p = 0.029$). DIC, MOF or STSS occurred in 30 out of 92 patients (33%) with GAS bacteremia.

The 30-day case-fatality rate was 13%, being highest in group C (22%); in group A it was 15%, in group B 7%, and in group G 15% (Table 2). Case-fatality rate was high (37%) in patients with GAS bacteremia who suffered from DIC, MOF or STSS. However, those patients with GAS bacteremia without any of these severe manifestations had case-fatality rate of only 5 percent. On admission the median ear temperature was lower in patients who died as compared to those who survived (38.3 vs. 39.0; $p = 0.001$) likewise the systolic blood pressure was lower in those who died (85 vs. 130; $p = 0.001$).

Among infections of the skin and soft-tissues, necrotizing fasciitis carried the highest risk for death (38%), while patients with cellulitis had a case-fatality rate of 8%. A history of previous cellulitis seemed to constitute a factor protecting from death. Patients with a history of previous cellulitis had a case-fatality rate of only 3% as compared to 16% among those without such a history ($p = 0.014$). The

Table 1 Underlying diseases in relation to Lancefield group.

Underlying disease	GAS, $N = 92$ (%)	GBS, $N = 76$ (%)	GCS, $N = 18$ (%)	GGS, $N = 128$ (%)	All $N = 314$ (%)	Overall p-value
Alcoholism	24 (26)	5 (7)	2 (11)	15 (12)	46 (15)	0.002
Malignancy	10 (11)	29 (38)	2 (11)	30 (23)	71 (23)	< 0.001
Diabetes	11 (12)	24 (32)	5 (28)	31 (24)	71 (23)	0.019
Cardiovascular diseases	17 (19)	26 (34)	8 (44)	51 (40)	102 (33)	0.005
Immunosuppressive treatment	4 (4)	8 (11)	2 (11)	18 (14)	32 (10)	0.135
Corticosteroidal treatment[a]	3 (3)	5 (7)	2 (11)	16 (13)	26 (8)	0.088
Liver diseases	11 (12)	5 (7)	2 (11)	14 (11)	32 (10)	0.684
COPD	3 (3)	2 (3)		7 (6)	12 (4)	0.566
Neurological disorders	12 (13)	13 (17)	3 (17)	22 (17)	50 (16)	0.840
Renal diseases	7 (8)	8 (11)	2 (11)	11 (9)	28 (9)	0.906
No underlying disease	23 (25)	10 (13)	4 (22)	13 (10)	50 (16)	0.002

[a] Corticosteroids used in a dose equivalent to over 5 mg of prednisolone per day during one month prior to the episode of bacteremia.

Table 2 Clinical characteristics of streptococcal bacteremia in relation to Lancefield group.

Disease severity	GAS, $N = 92$, n (%)	GBS, $N = 76$, n (%)	GCS, $N = 18$, n (%)	GGS, $N = 128$, n (%)	All $N = 314$, n (%)	Overall p-value[a]
Case-fatality	14 (15)	5 (7)	4 (22)	19 (15)	42 (13)	0.19
Admitted to intensive care unit	25 (27)	8 (11)	2 (11)	9 (7)	44 (14)	0.001
Needed mechanical ventilation	18 (20)	3 (4)	1 (6)	7 (6)	29 (9)	0.002
Needed continuous renal replacement therapy	5 (5)	0 (0)	0 (0)	2 (2)	7 (2)	0.11
Needed hemodialysis	5 (5)	2 (3)	0 (0)	4 (3)	11(4)	0.73
Lowered level of consciousness[b]	31 (34)	16 (21)	5 (28)	36 (28)	88 (28)	0.35
Hypotension[c]	23 (25)	10 (13)	5 (28)	20 (16)	58 (19)	0.12
DIC[d]	27 (29)	5 (7)	2 (11)	6 (5)	40 (13)	<0.001
Multiorgan failure[e]	15 (16)	4 (5)	2 (11)	4 (3)	25 (8)	0.003
STSS[f]	13 (14)	4 (5)	2 (11)	4 (3)	23 (7)	0.02
Underwent surgical intervention	20 (22)	13 (17)	1 (6)	14 (11)	48 (15)	0.096

[a] The difference between groups of patients with GAS, GBS, GCS and GGS bacteremia.
[b] Lowered level of consciousness (unconscious or confusion) at least once during the first 2 days after positive blood culture.
[c] Hypotensive (BP < 90 mmHg) at least once 0–2 days after positive blood culture.
[d] Thrombocytes lower than 100×10^9/l.
[e] Three or more concomitant organ failures.
[f] The definition of STSS (Streptococcal toxic shock syndrome) included identification of beta-hemolytic streptococci from a normally sterile site, a septic shock and a multiorgan failure.

duration of symptoms before admission to hospital was shorter in patients with a history of previous cellulitis as compared to those without such a history (median 0 day vs. 1 day; $p = 0.002$).

Cellulitis was the presenting clinical manifestation in 143 patients (46%). Cellulitis as the presenting clinical manifestation was especially common in patients with a history of previous cellulitis (48 of 58 patients (83%)). We studied separately the subgroup of 143 patients who had cellulitis as the presenting clinical manifestation. Also in this subgroup of patients those with a history of previous cellulitis had a shorter duration of symptoms before admission to hospital as compared to those without such a history (median 0 day vs. 1 day, $p = 0.01$). In addition, the history of previous cellulitis had a significant effect on case-fatality in this subgroup of patients, whilst none of the 48 patients with a history of previous cellulitis died as compared to 12 out of 95 patients (13%) of those without such a history ($p = 0.009$).

We first studied all patients as one group when analyzing the factors associated with death. The underlying factors of case-fatality are given in Table 3. The number of patients older than 65 years did not differ between those who survived or died. However, the median age was higher in GBS patients who died as compared to those who survived (79 year vs. 67 year; $p = 0.013$), but not in the case of GAS, GCS and GGS bacteremias. Alcoholism as an underlying disease was associated with poor outcome; 14 out of 46 (30%) died, as well as 10 out of 32 (31%) with liver disease, mostly alcoholic in origin (Table 3). Alcoholism, liver disease, having ultimately or rapidly fatal underlying disease (McCabe class II or III) or at least one chronic disease were significantly associated with death in univariate analysis and were included in multivariate model. Alcoholism and ultimately or rapidly fatal underlying disease remained in that model (Table 3).

Table 4 shows the first signs and symptoms associated with case-fatality in univariate and multivariate analysis. In multivariate analysis including the first signs and symptoms, confusion, unconsciousness or dyspnea were found to be independently associated with death, while fever as the first sign seemed to be a factor protecting from death (Table 4).

Of laboratory parameters leucopenia (OR 4.4, 95% CI 1.7–11.4), thrombopenia (OR 3.4 (1.6–7.5)), high CRP (OR 3.9 (1.9–8.4)) and elevated creatinine value (OR 3.1 (1.5–6.4)) on admission were significantly associated with death. Leucopenia (OR 3.8 (1.4–10.8)) and high CRP (OR 4.0 (1.7–9.2)) were found to be associated with case-fatality also in multivariate analysis including the aforementioned factors.

The final model included alcoholism, McCabe class II or III, confusion, unconsciousness, fever, dyspnea, leucopenia, high CRP, age, and also streptococcal group. In this model alcoholism (OR 3.9, 95% CI 1.3–11.9), McCabe class II or III (OR 3.7 (1.4–9.5)), unconsciousness (OR 5.0 (1.1–22.4)), dyspnea (OR 9.2 (3.0–27.6)), high CRP (OR 6.1 (2.3–16.5)), leucopenia (OR 5.6 (1.6–19.7)) and having group C streptococcal bacteremia as compared to having group B streptococcal bacteremia (OR 7.1 (1.3–40.1)) were significant.

The following markers of clinical severity during the first two days of hospitalization were significantly associated with an increased risk of death: hypotension (OR 21.8, 95% CI 10.1–47.3), lowered level of consciousness (OR 16.3 (7.1–37.2)), DIC (OR 7.4 (3.5–15.6)), MOF (OR 18 (7.2–44.7)) and afebrility (OR 3.8 (1.7–8.2)). As the aforementioned factors may also be characteristics of death itself, we also studied the effect of these factors

Table 3 Underlying factors in patients with β-hemolytic streptococcal bacteremia in relation to mortality.

Underlying factor	Survivors $N = 272$ (%)	Non-survivors $N = 42$ (%)	Univariate analysis Odds ratio (95% confidence interval)	Multivariate analysis Odds ratio (95% confidence interval)
Age >65 years	123 (45)	23 (55)	1.5 (0.8–2.8)	not entered
Alcoholism[a]	32 (12)	14 (33)	3.8 (1.8–7.9)	4.7 (2.1–10.4)
Current smoker or ex-smoker[b]	84 (36)	13 (45)	1.5 (0.7–3.2)	not entered
Diabetes	59 (22)	12 (29)	1.4 (0.7–3)	not entered
Malignancy	60 (22)	11 (26)	1.3 (0.6–2.6)	not entered
Liver disease	22 (8)	10 (24)	3.6 (1.5–8.2)	removed
Cardiovascular disease[c]	85 (31)	17 (41)	1.5 (0.8–2.9)	not entered
Immunosuppressive treatment	27 (10)	5 (12)	1.2 (0.4–3.4)	not entered
Corticosteroidal treatment[d]	21 (8)	5 (12)	1.6 (0.6–4.5)	not entered
Mc Cabe II or III[e]	94 (35)	25 (59)	2.8 (1.4–5.4)	3.4 (1.7–6.9)
Chronic disease[f]	224 (82)	40 (95)	4.3 (1.0–18.3)	removed

[a] Social or medical problem due to alcohol.
[b] Data available on 265 patients.
[c] Coronary artery disease or heart failure.
[d] Corticosteroids used in a dose equivalent to over 5 mg of prednisolone per day during one month prior to the episode of bacteremia.
[e] Mc Cabe class II or III: ultimately fatal disease (the normal evolution of the disease could lead to death in 1–5 years) or rapidly fatal disease (life expectancy less than 12 months).
[f] At least one chronic disease.

on death in a subgroup of patients who survived the first two days. In these analyses hypotension (OR18.7 (7.0–49.7)), lowered level of consciousness (OR 17.8 (5.8–54.6)), DIC (OR 7.5 (2.9–19.4)), and MOF (OR 13.6 (4.5–41.6)) were still significant.

Discussion

The present study showed that a history of previous cellulitis seemed to be a protective factor against death. This interesting finding has not previously been reported. One reason for this might be that the patients with a previous cellulitis had a shorter duration of symptoms before admission as they recognized their symptoms more rapidly. Therefore, they received earlier treatment. Also prior antigen challenge and development of antibodies may play a role. In the subgroup of patients with cellulitis as a presenting clinical manifestation none of the patients who had a history of previous cellulitis died as compared to 13% of those without such a history thus strengthening the finding that a history of previous cellulitis decreases mortality. Also cellulitis as the presenting clinical manifestation, *per se*, predicts a favourable prognosis. Fever as a first sign and symptom was associated with a good prognosis, which aspect has not been studied in beta-hemolytic streptococcal bacteremia earlier. This is, however, in line with several earlier sepsis studies, which have found that patients with severe sepsis who are hypotermic (<35.5 °C) have almost twice the risk of dying as patients with normal or elevated body temperature.[16,17]

The case-fatality rate in the case of GAS bacteremia was 15%, a value similar to the 14–15% reported by others.[4,18] The case-fatality rate due to bacteremias caused by GBS was lower (7%) than in other studies 14–28%.[8,19–21] The case-fatality rate attributable to GGS bacteremias was 15%, a value similar to the 13–22% reported by others.[8,22–24] Advanced age was a factor predisposing to death in GBS bacteremias, which is in line with earlier findings.[8] We found that increasing age was not associated with death in GAS, GCS or GGS bacteremias, which is in contrast with

Table 4 First signs and symptoms predisposing to mortality during one month's follow-up in β-hemolytic streptococcal bacteremia.

First sign or symptom	Survivors $N = 272$ (%)	Non-survivors $N = 42$ (%)	Univariate analysis Odds ratio (95% confidence interval)	Multivariate analysis Odds ratio (95% confidence interval)
confusion	28 (10)	11 (26)	3.3 (1.4–6.8)	3.8 (1.6–9.0)
pain	120 (44)	12 (29)	0.5 (0.2–1.0)	removed
unconsciousness	6 (2)	5 (12)	6 (1.7–20.6)	6.4 (1.6–25.6)
fever	253 (93)	32 (76)	0.2 (0.1–0.6)	0.2 (0.1–0.5)
dyspnea	25 (9)	13 (31)	4.4 (2.0–9.6)	6.4 (2.8–14.8)

other studies, where age has been found to be a predisposing factor for death in beta-hemolytic bacteremias.[4,5,8,9] One reason for this might be that our GAS patients were younger and rates of infection were relative low in the elderly. Also Theresa Lamagni et al. found that age distribution of patients in Finland was unusual in having low rates in the elderly.[25] O'Loughlin et al. found that the elderly have highest rates of GAS death.[4]

STSS was more often caused by GAS and GCS than by GBS and GGS, and 70% of patients with STSS died, a figure somewhat higher than that reported elsewhere.[1,12,26,27] The occurrence of STSS caused by GBS, GCS or GGS has been reported primarily in case series studies,[28–32] but data from population-based studies have not often been presented in comparison with GAS STSS. ICU treatment was significantly more common in patients with GAS and GCS bacteremias as compared to GBS or GGS bacteremias, as also previously reported.[8]

Alcoholism,[1,8] liver disease,[6] pneumonia[4–7] and chronic disease[4,5,8,9] have previously been reported as predisposing factors for death in GAS bacteremia. We identified alcoholism and having an ultimately or rapidly fatal underlying disease as factors significantly associated with increased case-fatality. As initially noted by McCabe and Jackson,[13] outcome is significantly influenced by the patient's underlying disease. Furthermore 90% of the patients with GGS bacteremia had an underlying disease. The number of studies describing the effect of underlying diseases on case-fatality in GCS or GGS bacteremia is limited.[8] The markers of disease severity have not been hitherto widely studied in population-based GBS, GCS or GGS studies. Hypotension and having an underlying disease have previously been reported to predict death in GAS bacteremias.[4,9] In the present study also high CRP and leucopenia on admission predicted a poor prognosis. The number of studies describing laboratory parameters as predictors of death in beta-hemolytic bacteremia is limited. De Quiros et al. reported trombopenia, leucopenia and anemia to be associated with death in a univariate analysis.[33] In bacteremia the results of CRP as a predictor of death are controversial. A recent review concludes that the ability of CRP level to reflect the severity of sepsis may be limited.[34] To our knowledge there is no study about beta-hemolytic bacteremia concerning high CRP and increased case-fatality rate.

This report provides a long-term, population-based analysis of the clinical characteristics and the predictors of mortality in beta-hemolytic bacteremia. This study has certain limitations. It is retrospective and does not include pediatric patients. The strengths of the study are that all records of adult patients during a 10-year period in a defined population were thoroughly reviewed by the same infectious disease specialist (SR) and all beta-hemolytic streptococcal bacteremias (A, B, C and G) in Pirkanmaa HD were included to study.

Conclusion

A history of previous cellulitis seemed to be a protecting factor against death. Among first signs and symptoms confusion, unconsciousness or dyspnea were markers of a poor prognosis while fever seemed to be a protecting factor against death. Also cellulitis as the presenting clinical manifestation, predicts a favourable prognosis. High CRP and leucopenia on admission predicts a poor prognosis. It is important to identify factors associated with a poor prognosis in order to find patients most likely to benefit from possible preventive measures.

Conflict of interest

The authors do not declare any conflicts of interest.

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Streptococcus dysgalactiae subsp. *equisimilis* Bacteremia, Finland, 1995–2004

Sari Rantala, Susanna Vähäkuopus, Jaana Vuopio-Varkila, Risto Vuento, and Jaana Syrjänen

We conducted a retrospective population-based study of 140 episodes of *Streptococcus dysgalactiae* subsp. *equisimilis* bacteremia occurring in Finland during 1995–2004. Rare *emm* types were associated with more severe disease and increased mortality rates. Skin and soft tissue infections were more frequent clinical signs among cases caused by common *emm* types.

Lancefield groups C and G β-hemolytic streptococci (GCS and GGS) may colonize the pharynx, skin, gastrointestinal tract, and female genitourinary tracts (*1*). According to recent taxonomic studies, large colony-forming groups C and G streptococci that infect humans are classified as *Streptococcus dysgalactiae* subsp. *equisimilis* (*2*). *S. dysgalactiae* subsp. *equisimilis* and *S. pyogenes* share virulence factors (*3*,*4*). The M protein is an important virulence factor because it confers resistance to phagocytosis (*5*). As with *emm* genes of *S. pyogenes*, the *emm* homologs of groups C and G *S. dysgalactiae* subsp. *equisimilis* are used for sequence-based typing (*4*,*6*,*7*), with >50 sequence types currently described (www.cdc.gov/ncidod/ biotech/strep/emmtypes.htm). The aim of our study was to determine the clinical signs, epidemiologic characteristics, and *emm* types of *S. dysgalactiae* subsp. *equisimilis* bacteremia during the 10-year observation period in Finland.

The Study

We retrospectively reviewed the medical records of all adult patients (>16 years of age) in Pirkanmaa Health District, Finland, with ≥1 blood cultures positive for group C or group G *S. dysgalactiae* subsp. *equisimilis* from January 1995 through December 2004. The Pirkanmaa Health District (460,000 inhabitants) has 1 tertiary care hospital (Tampere University Hospital) and 4 other hospitals (Hatanpää City Hospital and the District Hospitals in Valkeakoski, Vammala, and Mänttä). Laboratory records were screened to identify all blood cultures positive for group C or group G *S. dysgalactiae* subsp. *equisimilis* during the study period. Our case definition included all patients who had a positive blood culture for *S. dysgalactiae* subsp. *equisimilis* and clinical signs compatible with septicemia. A severe disease was defined as a septicemia leading to death or needing intensive care unit treatment. All 128 GGS isolates and 12 of 18 GCS isolates were confirmed to be *S. dysgalactiae* subsp. *equisimilis*. Thus, these 140 episodes of *S. dysgalactiae* subsp. *equisimilis* septicemia (involving 137 patients) comprised the present study. Two of the isolates (1 GGS and 1 GCS) were not available for *emm* typing, and 138 of the *S. dysgalactiae* subsp. *equisimilis* isolates (from 135 patients) were sequenced to identify the *emm* gene.

Routine blood samples were drawn into aerobic and anaerobic bottles and cultivated by standard methods as reported (*8*). *S. dysgalactiae* subsp. *equisimilis* isolates were further analyzed by *emm* typing. Nontypeable strains and strains isolated from patients with recurrent bacteremia were characterized by using pulsed-field gel electrophoresis (PFGE).

The *emm* typing was performed according to the protocol of the Centers for Disease Control and Prevention (www.cdc.gov/ncidod/biotech/strep/strepblast.htm). If the *emm* gene could not be amplified with primers 1 and 2, alternative primers MF1/MR1 were used (*9*). PFGE was performed as described (*10*). DNA profiles were analyzed by using Bionumerics software (Applied Maths, Kortrijk, Belgium) and interpreted according to the guidelines described (*11*). Strains with >85% similarity were considered to be related types.

SPSS software version 7.5 (SPSS, Chicago, IL, USA) was used for statistical analyses, and a 2-sided p value <0.05 was regarded as the level for significance. Categorical data were analyzed by χ^2 test or Fisher exact test as appropriate. Nonparametric data were analyzed by using the Mann-Whitney U test. Odds ratios were expressed with 95% confidence intervals.

The median age of patients (73 men, 62 women) was 67 years (range 17–90 years). Cardiovascular diseases (41%), diabetes (25%), and malignancies (23%) were the 3 most prominent underlying conditions. We found 18 *emm* types (including 4 subtypes of stG6: stG6.0, stG6.1, stG6.3, and stG6.4). StG480 (27 isolates), stG6 (23 isolates), and stG485 (22 isolates) were the 3 most common *emm* types and represented 51% of all isolates (Figure 1). Eight of group G *S. dysgalactiae* subsp. *equisimilis* isolates remained nontypeable. PFGE analysis showed 2 strains to be related (>85% similarity). The rest of the nontypeable strains were sporadic (6 isolates).

Author affiliations: Tampere University Hospital, Tampere, Finland (S. Rantala, R. Vuento, J. Syrjänen); National Institute for Health and Welfare, Helsinki, Finland (S. Vähäkuopus, J. Vuopio-Varkila); and Medical School, University of Tampere, Tampere (J. Syrjänen)

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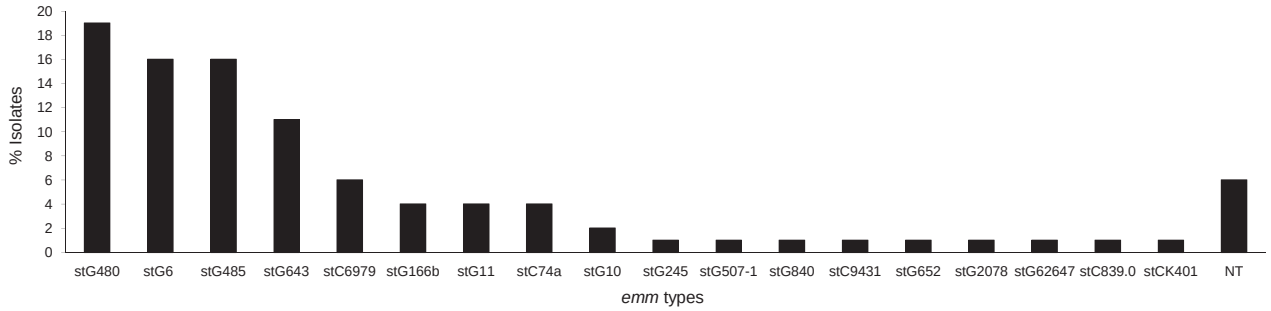

Figure 1. *emm* types of 138 *Streptococcus dysgalactiae* subsp. *equisimilis* bacteremic isolates obtained during 1995–2004, Finland. NT, nontypeable.

We divided bacteremia episodes into 2 groups: those caused by the 5 most common *emm* types and each representing >5% of all episodes (97 episodes, common *emm* types) and those caused by the less common or nontypable *emm* types (41 episodes, rare *emm* types). We could not find an association between *emm* type and clinical features such as age or underlying disease. Severe disease was caused more often by rare *emm* types than by common *emm* types. Mortality rates were higher in patients with bacteremia caused by rare types than that caused by common types (Table 1). Four patients had recurrent *S. dysgalactiae* subsp. *equisimilis* bacteremia (Table 2). PFGE profiles showed that strains isolated from the same patient in recurring infections were identical (Figure 2).

Common *emm* types were more frequently manifested as skin and soft tissue infections than were rare *emm* types, 75% vs. 54%, respectively (p = 0.012). The most frequent source of bacteremia was cellulitis (51%). We also found an association between a common *emm* type and cellulitis. Cellulitis was a more frequent clinical sign among patients with infections caused by common *emm* types than by rare *emm* types (p = 0.007); 64% of patients infected by common *emm* types had cellulitis as an initial clinical manifestation versus 39% of patients infected by rare *emm* types.

Conclusions

Our study showed that mortality rates were higher in patients with *S. dysgalactiae* subsp. *equisimilis* bacteremia caused by rare *emm* types than in those with bacteremia caused by common *emm* types. The reason for this finding is unclear. One explanation for this might be that patients contract certain prevailing bacterial strains (so-called common types) more often, and a prior antigen challenge and subsequent humoral response may play a role. Severe disease (death or intensive care unit treatment) was also caused more often by rare *emm* types than by common *emm* types. We found also an association between a common *emm* type and cellulitis as a clinical manifestation; the common *emm* types were also associated with skin and soft tissue infections.

In our comprehensive study with molecular typing data for 138 invasive *S. dysgalactiae* subsp. *equisimilis* isolates from human infections, we found 18 *emm* types, which is consistent with previous reports by Cohen-Poradosu et al. (*12*) and Broyles et al. (*13*) . These 2 studies reported stG485.0. or StG6, StG245, and StG2078 as the most common *emm* types, respectively. Thus, *emm* typing provides a useful tool for comparative epidemiologic analysis of GGS isolates from various geographic regions. Our results also suggest that certain *emm* types may prevail among bacteria

Table 1. Disease severity among 138 episodes of *Streptococcal dysgalactiae* subsp. *equisimilis* bacteremia, Finland, 1995–2004*

Disease severity	No. (%) common *emm* types, n = 97	No. (%) rare *emm* types, n = 41	Odds ratio (95% CI)	p value†
30-day mortality rate	11 (11)	12 (29)	3.2 (1.3–8.1)	0.01
Patient admitted to ICU	5 (5)	6 (15)	3.2 (0.9–11)	0.084
Patient death or ICU treatment	12 (12)	15 (37)	4.1 (1.7–9.8)	0.001
Hypotension‡	13 (13)	10 (24)	2.1 (0.8–5.2)	0.113
DIC§	2 (2)	6 (15)	8.1 (1.6–42.3)	0.009
Multiorgan failure	2 (2)	4 (10)	5.1 (0.9–29.2)	0.064
STSS¶	2 (2)	4 (10)	5.1 (0.9–29.2)	0.064

*CI, confidence interval; ICU, intensive care unit; DIC, disseminated intravascular coagulation; STSS, streptococcal toxic shock syndrome. Patients who had both clinical data and isolates available.
†χ^2 test or Fisher exact test as appropriate.
‡Hypotensive (BP <90 mm Hg) at least once 0–2 days after positive blood culture.
§Thrombocyte count <100 x 10^9/L.
¶The definition of STSS was based on a consensus definition, including identification of β-hemolytic streptococci from a normally sterile site, septic shock, and multiorgan failure.

Table 2. Characteristics of recurrent episodes of group G *Streptococcal dysgalactiae* subsp. *equisimilis* bacteremia, Finland, 1995–2004*

Patient no.	*emm* type			Time to recurrence, mo	Clinical signs	PFGE pattern
	Episode 1	Episode 2	Episode 3			
1	stG6	stG6	stG6	15; 3	Cellulitis	Unique, identical in episodes 1–3
2	stG6	stG6		68	Cellulitis	Unique, identical in episodes 1 and 2
3	stG480†	stG480		28	Spondylitis	Unique, identical in episodes 1 and 2
4	stG480	NA‡		21	Cellulitis	Unique

*PFGE, pulsed-field gel electrophoresis.
†Blood culture taken outside Pirkanmaa Health District, isolate available.
‡Blood culture taken outside Pirkanmaa Health District, no isolate available.

that cause human infections. Our study did not show any obvious time shifts in the occurrence of certain *emm* types.

A noteworthy finding in our series was the high frequency of recurrent group G *S. dysgalactiae* subsp. *equisimilis* bacteremia as reported earlier (*12*,*14*). Clinicians should be alert to this phenomenon, which seems to be more common than recurrent group A streptococcal bacteremia.

The dynamics of interspecies transfer of virulence loci between group A streptococci, GGS, and GCS (*3*), as well as potential genetic transfer or intragenomic events causing interconversion of group antigen types, remains to be resolved. Further characterization of the strains by multilocus sequence typing would be of interest (*15*).

We conclude that severity of disease and mortality rates were higher in persons with *S. dysgalactiae* subsp. *equisimilis* bacteremia caused by rare *emm* types than that caused by common *emm* types. Skin and soft tissue infections such as cellulitis were significantly more frequent clinical signs among episodes caused by common *emm* types.

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Dr Rantala is a specialist in infectious disease and internal medicine in the Department of Internal Medicine, Tampere University Hospital, and a consultant in infectious diseases at Tampere University Hospital. Her research interests include streptococcal infections.

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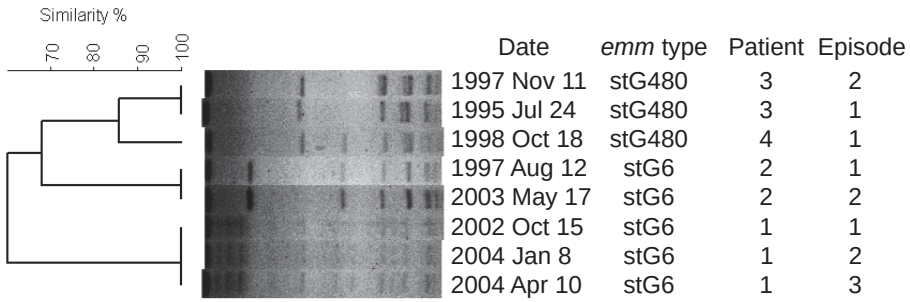

Figure 2. Dendrogram and pulsed-field gel electrophoresis (PFGE) profiles of the strains isolated from patients with recurrent group G *Streptococcus dysgalagtiae* subsp. *equisimilis* bacteremia, Finland. Dendogram was generated by using Bionumerics software (Applied Maths, Kortrijk, Belgium) with a 1.0% lane optimization and 1.5% band position tolerance.

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Address for correspondence: Sari Rantala, Department of Internal Medicine, Tampere University Hospital, PO Box 2000, FIN–33521, Tampere, Finland; email: sari.rantala@uta.fi