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Clinical and molecular studies of β -haemolytic streptococci

Trell, Kristina

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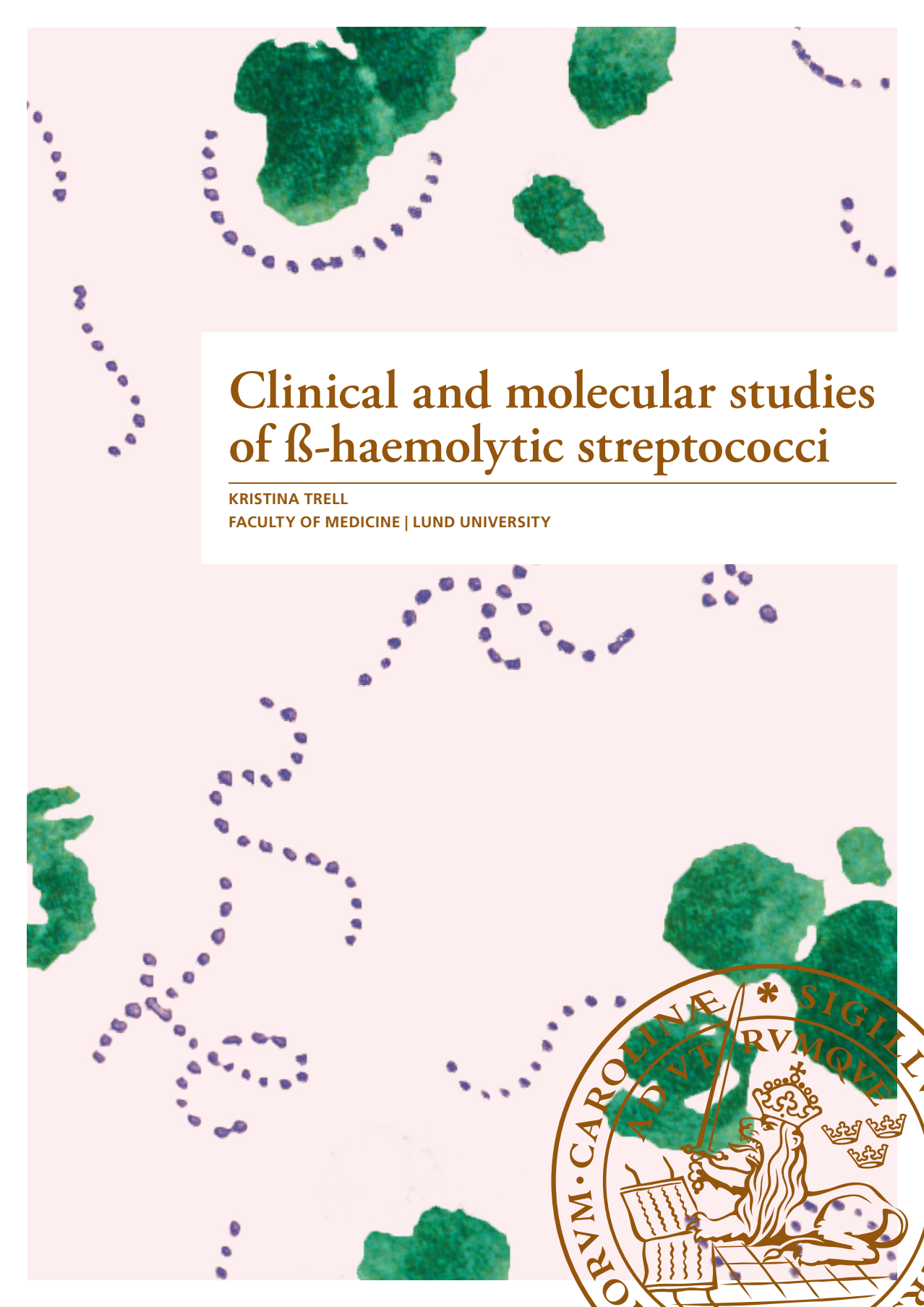
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Clinical and molecular studies of β -haemolytic streptococci

KRISTINA TRELL

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Department of Clinical Sciences
Division of Infection Medicine

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Clinical and molecular studies of β -haemolytic streptococci

Kristina Trell



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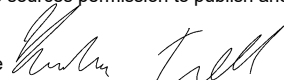
DOCTORAL DISSERTATION

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University of Bergen, Norway

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Title and subtitle Clinical and molecular studies of β -haemolytic streptococci	
Abstract <p>Beta-haemolytic streptococci (BHS) are important causes of human infections and they have traditionally been grouped according to Lancefield antigens. The spectrum of infections caused by BHS includes pharyngitis, skin infections, bacteraemia/sepsis, endocarditis, septic arthritis and meningitis. The most studied BHS is the group A <i>Streptococcus</i> (GAS), which concurs with the species <i>Streptococcus pyogenes</i> (SP). In the last decades, several studies have shown an increase of invasive infections caused by beta-haemolytic streptococci group C (GCS) and group G (GGS). GCS and GGS cause clinical disease similar to GAS. The vast majority of clinical isolates associated with human infections, that are identified as GCS and GGS belong to the species <i>Streptococcus dysgalactiae</i> (SD). With the introduction of Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) in routine diagnostics, it became possible to determine species of GGS and GCS. GCS and GGS exhibit the M-protein, a known virulence factor in GAS infections. The M-protein is encoded by the <i>emm</i>-gene and sequencing of this gene can be used for typing purposes.</p> <p>In the first study, isolates of GCS and GGS from different sites of isolation (throat, wound, and blood) were species determined and <i>emm</i>-typed to investigate if certain types have a predilection to cause particular infections. We found that GCS and GGS express different <i>emm</i>-types and that GCS and GGS from different sites of isolation were similar, suggesting that <i>emm</i>-types are not associated to certain disease presentations.</p> <p>In the second study, subjects with recurrent bacteraemia with GCS and GGS were identified and compared to controls with only one episode of bacteraemia to detect risk factors for recurrence. In the 23 patients with recurrent episodes of SD bacteraemia, most recurrences were caused by the same <i>emm</i>-type suggesting a host-specific colonization. In addition, no specific <i>emm</i>-types or other clinical factors were significantly associated with recurrences.</p> <p>Among GCS causing human infections, isolates of <i>Streptococcus equi</i> (SE) have been found. In the third study, the clinical course of patients with bacteraemia with SE was described and the isolates were typed through sequencing of the gene encoding the M-like protein SzP. Eighteen cases of SE were found during a thirteen-year period, which confirms that SE is a rare cause of infection in humans. No temporal or geographical clustering was found in our material. Our study also indicated that sporadic cases of SE bacteraemia have a favourable prognosis.</p> <p>In the fourth work, we investigated the possibility of using cultures for diagnostic purposes by determining the perianal colonization with BHS in patients with erysipelas compared with a control group. In the group with erysipelas, 44% of the patients were colonized with BHS compared with 4 % of the patients in the control group. The BHS found were most commonly GGS and when subjected to MALDI-TOF MS, these were found to be SD. We concluded that SD colonizes the perianal area in a substantial proportion of patients with erysipelas.</p> <p>SP is a well-known cause of postpartum infections and is still causing significant morbidity and mortality worldwide. In the final study, we described the use of whole genome sequencing (WGS) to investigate an outbreak of postpartum SP <i>emm75</i> infections related to an asymptomatic carrier working in a maternity ward. Source identification and WGS proved to be vital for outbreak control.</p>	
Key words Beta-haemolytic streptococci, <i>Streptococcus dysgalactiae</i> , MALDI-TOF MS, <i>Streptococcus equi</i> , <i>Streptococcus pyogenes</i> , Erysipelas, Endometritis	
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Clinical and molecular studies of β -haemolytic streptococci

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To my family

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- I. **Trell K**, Nilson B, Rasmussen M. Species and *emm*-type distribution of group C and G streptococci from different sites of isolation. *Diagnostic Microbiology and Infectious Diseases*. 2016; 86(4):467-9.
- II. **Trell K**, Sendi P, Rasmussen M. Recurrent bacteremia with *Streptococcus dysgalactiae*: a case-control study. *Diagnostic Microbiology and Infectious Diseases*. 2016; 85(1):121-4.
- III. **Trell K**, Nilson B, Petersson AC, Rasmussen M. Clinical and microbiological features of bacteraemia with *Streptococcus equi*. *Diagnostic Microbiology and Infectious Diseases*. 2017; 87(2):196-8.
- IV. **Trell K**, Rigner S, Wierzbicka M, Nilson B, Rasmussen M. Colonization of β -hemolytic streptococci in patients with erysipelas-a prospective study. *European Journal of Clinical Microbiology and Infectious Diseases*. 2019.
- V. **Trell K**, Senneby E, Jørgenssen J, Rasmussen M. Management of an outbreak of postpartum *Streptococcus pyogenes emm-75* infections. *Manuscript* 2019.

Abbreviations

BHS	Beta-haemolytic streptococci
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
GAS	Group A streptococcus
GBS	Group B streptococcus
GCS	Group C streptococcus
GGs	Group G streptococcus
IVIG	Intravenous immunoglobulins
MALDI-TOF MS	Matrix assisted laser desorption/ionization time of flight mass spectrometry
MLST	Multilocus sequence typing
NSTI	Necrotizing soft tissue infection
PBP _s	Penicillin binding proteins
PCR	Polymerase chain reaction
PYR	L-pyrrolidonyl arylamidase
rRNA	Ribosomal ribonucleic acid
SD	<i>Streptococcus dysgalactiae</i>
SDSD	<i>Streptococcus dysgalactiae</i> subspecies <i>dysgalactiae</i>
SDSE	<i>Streptococcus dysgalactiae</i> subspecies <i>equisimilis</i>
SE	<i>Streptococcus equi</i>
SESZ	<i>Streptococcus equi</i> subspecies <i>zooepidemicus</i>
SLO	Streptolysin O
SLS	Streptolysin S
SNP	Single nucleotide polymorphism
SP	<i>Streptococcus pyogenes</i>
Spes	Streptococcal pyrogenic exotoxins
STSS	Streptococcal toxic shock syndrome
VP	Voges-Proskauer
WGS	Whole genome sequencing

Abstract

Beta-haemolytic streptococci (BHS) are important causes of human infections and they have traditionally been grouped according to Lancefield antigens. The spectrum of infections caused by BHS includes pharyngitis, skin and soft tissue infections, bacteremia/sepsis, endocarditis, septic arthritis and meningitis. The most studied BHS is the group A *Streptococcus* (GAS), which concurs with the species *Streptococcus pyogenes* (SP). In the last decades, several studies have shown an increase of invasive infections caused by beta-haemolytic streptococci group C (GCS) and group G (GGS). GCS and GGS cause clinical disease similar to GAS. The vast majority of clinical isolates associated with human infections, that are identified as GCS and GGS belong to the species *Streptococcus dysgalactiae* (SD). With the introduction of Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) in routine diagnostics, it became possible to determine species of GGS and GCS. GCS and GGS exhibit the M-protein, a known virulence factor in GAS infections. The M-protein is encoded by the *emm*-gene and sequencing of this gene can be used for typing purposes.

In the first study, isolates of GCS and GGS from different sites of isolation (throat, wound and blood) were species determined and *emm*-typed to investigate if certain types have a predilection to cause particular infections. We found that GCS and GGS express different *emm*-types and that GCS and GGS from different sites of isolation were similar, suggesting that *emm*-types are not associated to certain disease presentations.

In the second study, subjects with recurrent bacteraemia with GCS and GGS were identified and compared to controls with only one episode of bacteraemia to detect risk factors for recurrence. In the 23 patients with recurrent episodes of SD bacteraemia, most recurrences were caused by the same *emm*-type suggesting a host-specific colonization. In addition, no specific *emm*-types or other clinical factors were significantly associated with recurrences.

Among GCS causing human infections, isolates of *Streptococcus equi* (SE) have been found. In the third study, the clinical course of patients with bacteraemia with SE was described and the isolates were typed through sequencing of the gene encoding the M-like protein SzP. Eighteen cases of SE were found during a thirteen-year period, which confirms that SE is a rare cause of infection in humans. No

temporal or geographical clustering was found in our material. Our study also indicated that sporadic cases of SE bacteraemia have a favorable prognosis.

In the fourth work, we investigated the possibility of using cultures for diagnostic purposes by determining the perianal colonization with BHS in patients with erysipelas compared to a control group. In the group with erysipelas 44% of the patients were colonized with BHS compared with 4 % of the patients in the control group. The BHS found were most commonly GGS and when subjected to MALDI-TOF MS, these were found to be SD. We concluded that SD colonizes the perianal area in a substantial proportion of patients with erysipelas.

SP is a well-known cause of postpartum infections and is still causing significant morbidity and mortality worldwide. In the final study, we described the use of WGS to investigate an outbreak of postpartum SP *emm75* infections related to an asymptomatic carrier working in a maternity ward. Source identification and WGS proved to be vital for outbreak control.

Introduction

Brief history of β -haemolytic streptococci

Beta-haemolytic streptococci (BHS) are a genus of Gram-positive and facultative anaerobic bacteria. Streptococcus comes from the Greek words; *Streptos* meaning “chains” and *kokkus* denoting “berry”. Beta-haemolytic refers to the haemolysis of red blood cells on blood agar, as proposed by Hugo Schottmüller in 1903 (1). In 1919, a system for grouping streptococci according to their haemolysis was described by James Brown (2). The classification of BHS according to carbohydrate antigens present on the surface suggested by Rebecca Lancefield in 1933 is still in use today (3). In 1937, James Sherman placed the streptococci into four categories, the pyogenic division, the viridians division, the lactic division and the enterococci. The pyogenic division included the beta-haemolytic isolates with defined group carbohydrate antigens (4). These different classification systems were previously used to define BHS, but with new diagnostic methods identification of BHS to the species and subspecies level has become possible (5).

BHS with Lancefield group A (GAS) concurs largely with the species *Streptococcus pyogenes* and BHS with Lancefield group B (GBS) with the species *Streptococcus agalactiae*. BHS with Lancefield group C (GCS) and group G (GGG) can belong to several species, of which the predominant in humans is *Streptococcus dysgalactiae* (SD) (5), which can be further subdivided into the subspecies *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) and *Streptococcus dysgalactiae* subspecies *dysgalactiae* (SDSD) (6). Among GCS causing human infections are isolates of *Streptococcus equi* and some isolates of GGG are *Streptococcus canis*, both with animal origin (7, 8).

The definition of the species SD has been the object of much uncertainty. *Streptococcus dysgalactiae* was first used to describe bovine streptococci and the name was revived in 1983 (9). *Streptococcus equisimilis* was previously used for human beta-haemolytic streptococci of Lancefield group C (10). In 1984 DNA-DNA hybridization data indicated that *Streptococcus dysgalactiae* and *Streptococcus equisimilis* were a single species, namely SD (11). The division into the two subspecies SDSE and SDSD was suggested in 1996 by Vandamme *et al* (12). A definition of SDSD as an alpha-haemolytic phenotype expressing Lancefield

group C of animal origin and SDSE as beta-haemolytic SD of both human and animal origin was proposed by Vieira (13).

Sequencing of the 16S rRNA gene has been used in clinical bacteriology to determine species in infections caused by BHS, but is time-consuming and cannot with certainty differentiate to a subspecies level (14, 15). With the introduction of matrix-assisted laser desorption/ionization time of flight spectrometry (MALDI-TOF MS) in routine practice, it has become possible to get rapid identification down to the species level (16, 17). Studies performing genome sequencing have shown that identification of SD and its subspecies is problematic and that additional taxonomic changes might be needed (6).

Methods for identification and typing of β -haemolytic streptococci

Phenotypic methods for identification

Phenotypic methods used for the differentiation of streptococci are based on differences in their observable properties. Historically, identification of bacteria relied on visual inspection of bacterial colonies and even smell. Characterization of bacteria according to colony size, form and colour remains fundamental for the discrimination between bacteria.

Gram-staining is to this day used for the separation of Gram-positive bacteria from Gram-negative (18). Streptococci are stained purple in this procedure and are denoted Gram-positive bacteria, which reflects the thickness of their cell walls. With Gram-staining it is also possible to characterize the shape of bacteria and their pattern of growth in the microscope.

Streptococci can be further classified according to haemolytic patterns, Lancefield grouping and biochemical properties. Streptococci can display complete haemolysis (β -haemolysis), incomplete (α -haemolysis) and no haemolysis (γ -haemolysis) (19). The system used for grouping streptococci according to the presence of a unique cell wall carbohydrate on the surface was introduced by Rebecca Lancefield. Detection of these antigens by immunological assays providing rapid identification is still in use today for BHS (3, 19).

Biochemical properties that have been used for further differentiation of bacteria are carbon source utilization, enzymatic activities and antibiotic susceptibility. Streptococci are for example catalase-negative in contrast to staphylococci, i.e. do not possess the enzyme catalase (19). Commercial kits examining a wide range of

these properties are used in many clinical laboratories for species identification of streptococci, primarily of α -haemolytic and γ -haemolytic streptococci (20, 21). These kits can also be used for species identification of BHS, but usually need to be combined with other methods for species confirmation (22).

16S rRNA

16S ribosomal ribonucleic acid (rRNA) is a bacterial gene that encodes the 30S, the RNA-part of the small subunit of a bacterial ribosome. This gene contains both highly conserved sites providing suitable targets for primer binding, as well as hypervariable regions that can be sequenced to either identify species of bacteria or to perform taxonomic studies (23). The DNA sequence of the 16S rRNA gene of multiple bacterial species can be used to construct phylogenetic trees (24).

Sequencing of the 16S rRNA gene can determine species of BHS, but the resolution is insufficient for identification at the subspecies level (14, 15, 25).

MALDI-TOF MS

Matrix-assisted laser desorption/ionization time of flight spectrometry (MALDI-TOF MS) is a method based on mass spectrometry. By employing the fact that microbes have an exclusive protein content, it can provide a so-called mass finger print. Using mass spectrometry as a method for microbiological identification was suggested in 1975 (26). During the following decades the method of MALDI-TOF MS was developed and proved to be valuable for rapid species identification of bacteria (27, 28). Further studies demonstrated that MALDI-TOF MS was precise in bacterial identification compared to conventional phenotypic methods and 16S rRNA (29). Commercial systems combining MALDI-TOF MS with software for microbiological identification were introduced in routine practice in the 2010s (30-32).

At present, MALDI-TOF MS is used for species identification of BHS (16, 17), but the method cannot separate SD or SE at the subspecies level (17).

Emm- and *SzP*-typing

Rebecca Lancefield presented a typing system for GAS based on the presence of different serotypes of a surface protein, the M protein. The Lancefield M-typing system is based on an antigen-antibody reaction (33). T-typing, an agglutination reaction to the T-protein was previously also used for typing purposes (34).

GCS and GGS also express the M-protein (35, 36). Today typing of GAS, GCS and GGS is made by sequencing of the *emm*-gene encoding the M-protein (37, 38). The

5'-terminal end of the *emm*-genes is highly heterogeneous and the typing is based on the sequence of this hypervariable region. Two isolates are regarded as sharing the same *emm*-type if they are > 95% identical over their 5' end. A database of *emm*-types has been set up by the Centers for Disease Control and Prevention (CDC) (<https://www2a.cdc.gov/ncidod/biotech/strepblast.asp>).

The M-like SzP proteins of SE are the basis for a typing system that can be used in this zoonotic infection for examining epidemiological connections (39).

MLST and WGS

Multilocus sequence typing (MLST) is a method based on sequencing of different housekeeping genes with low mutation rates. For each housekeeping gene, the different sequences present are designated as distinct alleles and for each isolate, these combinations of alleles determine a sequence type (ST-profiles). The advantage of MLST is that the sequence data are unambiguous and that the allelic profiles of isolates can easily be compared to others in a MLST database (40, 41). MLST is a method of typing that allows additional discrimination than *emm*-typing, but it can also provide insights into phylogenetic ancestry (6).

MLST was first used for the characterization of SP (42, 43), but the method is now also available for SD (44). For SP, there is a concurrence between *emm*-types and ST-profiles and a majority of ST-profiles are found in association with a single *emm*-type (42, 43). With SD, identical or closely similar STs can exhibit multiple unrelated *emm*-types, but the associations need to be further studied (45).

The latest addition to bacterial typing methods is whole genome sequencing (WGS), which makes highly sensitive sequence comparison at the single nucleotide level possible (46). Genomic fingerprinting provides a basis for understanding the clonal relatedness of bacterial strains (47). But it also provides a method for genomic analysis of changes in bacterial populations responsible for emerging pathogenic strains (48, 49).

Streptococcus dysgalactiae subspecies *equisimilis* and *Streptococcus pyogenes*

Epidemiology

SP is an important human pathogen and the majority of infections are seen in children and young adults, including pregnant women in resource-limited environments (50-52). The importance of crowding to promote transmission of SP infections is well documented and outbreaks have been described (53, 54). Historically, as living conditions improved the incidence of SP infection began to fall and decreased even more with the introduction of antibiotics (55, 56). However, SP remains an important cause of morbidity and mortality in high income countries and since the 1980s, increasing rates have been reported from northern Europe and the US (57-61). The rising rates have been connected to the emergence of especially virulent *emm*-types (62), to changes in the expression of virulence factors and the presence of immunity to circulating strains (57, 63).

The annual incidence of invasive SP disease in high income countries ranges from 2,8 to 3,8 per 100 000 inhabitants (50, 51, 64). In 2005, it was estimated that 18.1 million people were suffering from a serious SP infection causing 517,000 deaths annually (65).

An increase in the incidence of invasive SDSE disease has been recognized with incidence ranging from 2,2 to 4,3 per 100 000 inhabitants in high income countries (64, 66-68). Invasive forms of SDSE infections are most commonly found in elderly patients with chronic conditions. The increase has been suggested to be caused by prolonged survival of adults with underlying chronic conditions (69, 70), but also changes in expression of virulence factors have been implicated (71). Invasive infections with SDSE approximate those of invasive SP disease in several reports (68, 72) and the predominant *emm*-types fluctuate both temporally and geographically (51, 73, 74).

Microbiological characteristics

SDSE and SP are Gram-positive, facultative anaerobic cocci of the genus *Streptococcus*, belonging to the phylum Firmicutes. They grow in chains. The typical phenotype of SDSE and SP on blood agar plates is white-greyish colonies of 1-2 mm in diameter and they display β -haemolysis (1) (Figure 1). SDSE can be separated from other β -haemolytic streptococci by being VP (Voges-Proskauer) and PYR (pyrrolidonyl arylamidase) negative (19). SP can be separated from SDSE by being susceptible to bacitracin and PYR positive (19).



Figure 1: SDSE displaying β -haemolysis on a blood agar plate. Courtesy of Anna Bläckberg

SDSE is a subspecies of SD. As previously outlined the overlap of classical methods for species identification of streptococci has led to taxonomic difficulties (5, 6). The present definition of SDSE (12, 13) is that it consists of all β -haemolytic SD isolates of both human and animal origin.

Species identification and typing

SP and SDSE were traditionally typed according to the Lancefield classification. SP expresses the group antigen A (5). Among SDSE, the group antigens C and G are most frequently found (72). SDSE of animal origin can express Lancefield group C and L (75, 76). Finally, SDSE can express Lancefield group A (77). Because of these shortcomings of the Lancefield grouping system, MALDI-TOF MS is used for species identification of BHS in routine practice today (16, 17). At present this method cannot separate SD to a subspecies level (Figure 2) (17), but considering the definition proposed by Vieira (13), assigning all β -haemolytic SD isolates as SDSE, β -haemolytic isolates identified by MALDI-TOF MS as SD are most probably SDSE.

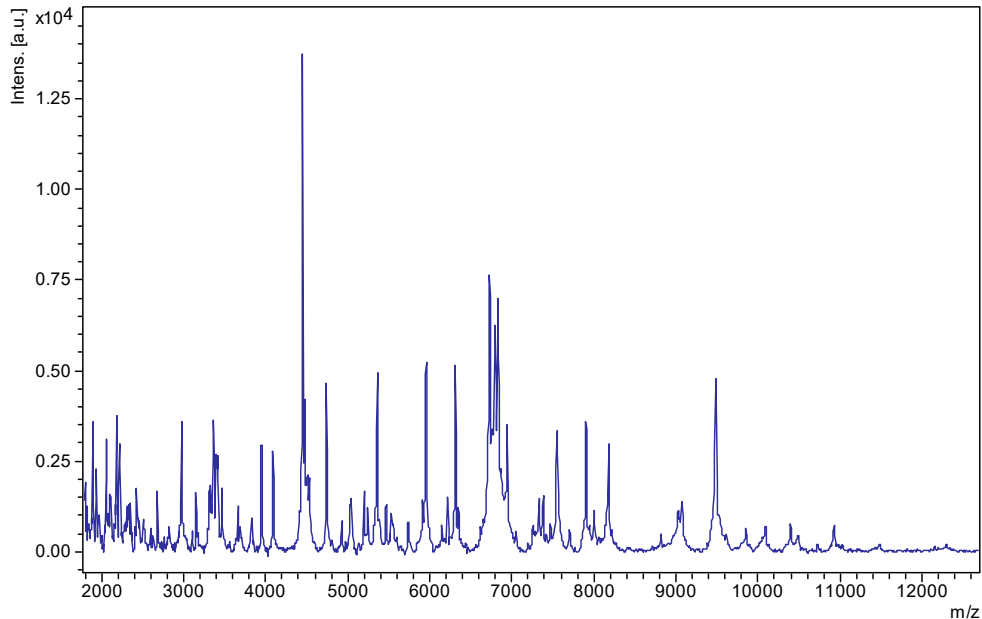


Figure 2: MALDI-TOF MS mass spectrum of SD. Courtesy of Bo Nilson.

To investigate epidemiological connections and trends in disease caused by SP and SDSE, *emm*-typing can be used (37, 68, 78-80). To date there are more than 250 *emm*-types known for SP and 90 *emm*-types known for SDSE (Streptococcus Lab | StrepLab | Blast-*emm* GAS Subtyping Request Form | CDC (<https://www2a.cdc.gov/ncidod/biotech/strepblast.asp>)). *Emm*-typing of SP is performed for invasive isolates in Sweden as part of a national surveillance, but not for SDSE.

Clinical manifestations

SDSE are colonizers of the upper respiratory tract, the gastrointestinal tract, the female genital tract and the skin and were previously considered as commensals (81-84). SDSE were earlier denoted beta-haemolytic streptococci of Lancefield group C and G and were not recognized as important human pathogens until the 1980s (81). Partly because of these taxonomic confusions, the burden of invasive SDSE infections has been uncertain. More recent figures of the rates of invasive SDSE disease are similar to those of invasive SP infections (64, 69, 79, 85). The asymptomatic carriage of SP has been discussed, in a study of patients attending general practice in Denmark the carriage rate was 11% in patients <14 years old, 2.3% in patients between 15-44 years old and 0.6% in patients > 44 years old (86).

Furthermore, transient colonization of the gut and perianal area may develop after throat infections (87).

SP give rise to a wide spectrum of clinical manifestations ranging from skin and pharyngeal infection to invasive infections, toxin-mediated diseases like streptococcal toxic shock syndrome (STSS) and necrotizing soft tissue infection (NSTI). Invasive infections are often defined as the isolation of SP from blood and other sterile sites. Finally, SP cause disability because of the autoimmune sequelae of rheumatic fever and glomerulonephritis (88). Mortality rates at around 15 % in invasive infections have been reported, rising in patients with STSS (59, 89, 90).

SDSE is now also recognized as an important human pathogen that can cause both non-invasive and invasive disease. The spectrum of diseases closely resembles that of SP infections, including the occurrence of post-streptococcal sequelae (91, 92) pharyngitis (83, 93), erysipelas (94, 95), bacteraemia (68, 74, 79, 96), endocarditis (97-99), septic arthritis (100-102), postpartum endometritis (103, 104), NSTI (74, 105) and meningitis (106). STSS has also been increasingly linked with SDSE (107-109).

Infections in humans due to SDSE are usually transmitted person to person, but animal reservoirs have been described (76). Sites of colonization and focal infections serve as reservoirs for transmission. Several modes of transmission have been demonstrated for SP like direct transmission by air droplets, by direct skin contact and indirect through contaminated objects and surfaces (110-112). The presence of outbreaks in the community and hospitals indicates similar modes of transmission for SDSE (113, 114).

SDSE cause disease primarily in the elderly (64, 68, 74, 79) and in patients with comorbidities like diabetes, chronic renal failure and immunosuppression (70, 72, 73). The mortality in invasive SDSE disease has been shown to be dependent on clinical presentation, underlying comorbidities and age (74, 79, 96, 115, 116) and ranges from 10% to 20% (74, 79, 96). Some studies have shown a higher mortality in connection with invasive infections with SDSE with a rare *emm*-type (68, 79).

As outlined above, SDSE can cause a similar spectrum of diseases in humans as SP and also has the M-protein, expressed by the *emm*-gene (68, 78-80). In recent years several studies have investigated what *emm*-types are present in infections caused by SDSE and their correlation to invasive disease (74, 79, 117, 118).

SDSE has a propensity to recur and recurrence rates of between 3 and 9 % have been reported (69, 78, 85, 119, 120). Some studies have tried to link the tendency of SDSE to recur to certain *emm*-types (78, 119, 120). Furthermore, underlying clinical conditions predisposing for reappearance of SDSE have been examined (78, 119).

Virulence mechanisms

The ability of bacteria to cause disease in the host is termed virulence and the bacterial components enabling this process are called virulence factors. Many virulence factors have been identified in SP (121). They promote adhesion, dissemination in human tissues and interference with the host immune responses. Virulence factors also include toxins as well as superantigens.

SDSE is closely related to SP, with approximately 70% sequence identity (122). Fewer studies have analysed particular virulence factors in SDSE than in SP, but several key virulence factors present in SP are lacking (123).

M-protein

The M-protein is a virulence factor on the cell surface of SP and SDSE. The M protein is a surface expressed coiled coil protein that extends up to 60 nm from the cell wall and is attached at its C-terminus with the more variable N-terminus extending into the extracellular media. The M-protein is encoded by the *emm*-gene and more than 90 different *emm*-types of SDSE and 250 different *emm*-types for SP have been recognized (36-38). The hypervariable region is localized to the N-terminal portion of the M protein and proximal to this hypervariable region are sequence-variable regions denoted the A and B repeats. Following these regions are the conserved C repeats and the D region, containing a motif for cell wall anchoring. The sequence diversity is the basis for the variety of virulence properties among M-proteins (124).

The M-protein confers resistance to phagocytosis (35, 38, 125). For SP, the anti-phagocytic properties have been shown to be linked to the binding of the M-protein to plasma proteins and immunoglobulin G (IgG), preventing the activation of the complement system (126, 127). It is also involved in the adhesion to host cells and internalization into human epithelial cells (35, 128-133).

Adhesion

In addition to the M-protein, other adhesive determinants have been described for SP (134). One more recently described determinant is the bacterial pilus. Pili are filamentous structures extending 1 to 3 μ m from the bacterial cell surface. The genes encoding pili are found in genomic loci denoted pilus islands known as the fibronectin-binding, collagen-binding, T antigen (FCT) region that forms part of the T-typing system in SP (135, 136). Pili have recently been described in SDSE (122).

Adhesins are bacterial proteins that are linked to the cell wall and promote adhesion to and internalization into host cells (134). Fibronectin-binding proteins have been described both for SP and SDSE (137-139).

Toxins

Streptolysin S (SLS) is the cytotoxin responsible for the beta-haemolytic phenotype of both SP and SDSE (140-142). SLS is a cytolytic toxin and damages the host cell membranes of many human cells, including erythrocytes, leukocytes and platelets. It has been implicated in soft tissue damage, phagocytosis and translocation across the epithelial barrier (143).

Another cytotoxin is streptolysin O (SLO) that damages host cell membranes by binding to cholesterol. SLO promotes resistance to phagocytic killing by disrupting the integrity of host cell membranes and contributes to tissue damage at the site of infection (121). In SDSE, increased cytotoxicity activity has been shown in strains causing invasive tissue infections (144).

Superantigens

Spe (streptococcal pyrogenic exotoxins), also called superantigens, are toxins that cause expansion and cytokine production in T cells. SpeA and SpeC were the first superantigens to be identified in SP. SpeB present in almost all isolates of SP was later found to be a cysteine protease rather than a superantigen and is chromosomally encoded. Most superantigens in SP are associated with bacteriophages (145, 146). Superantigens like speA have been implicated in the pathogenesis of streptococcal toxic shock syndrome (STSS) with the activation of large numbers of T cells leading to extensive immune activation with release of proinflammatory cytokines causing shock and multi-organ failure, but the connection remains to be elucidated (147).

Genes for SpeB, SpeA and SpeC have not been found in the genome of SDSE (71, 148). SpeG has been found to be present in 50% of clinical SDSE isolates (149), but the involvement of SpeG in clinical diseases of SDSE remains unclear (150-152).

Evasion of the host immune system

The hyaluronic acid capsule produced by SP is presumed to be beneficial for the ability of the bacterium to evade the immune system (153, 154). Resistance to phagocytosis is thought to be the result of the capacity of the capsule to cover opsonizing complement components deposited on the surface (155). In SDSE, the presence of the capsule remains to be elucidated (71, 122).

The capacity to form biofilm has recently been described for SP and SDSE and is considered a protective mechanism that allows the bacteria to survive and proliferate in hostile environments (156, 157).

Resistance to phagocytosis in SP and SDSE is primarily mediated by the M-protein as previously outlined. Recruitment of phagocytes by the human chemotaxin C5a can be interfered by C5a peptidases present both in SP and SDSE (125). Streptococcal inhibitor of complement (SIC) is an extracellular protein produced by

a few specific *emm*-types of SP that interferes with the immune system through several mechanisms, including inhibition of lysozyme and antimicrobial peptides (158). A *sic*-like gene has been identified in SDSE (*sicG*), but it seems that the distribution of *sicG* in SDSE is also restricted (122, 159).

Protein G is present in SDSE, but not found in SP. Protein G binds to IgG, albumin and α_2 -macroglobulin. The function of this protein needs to be further examined (160, 161).

Spreading

Streptokinase is a bacterial protein secreted by both SP and SDSE that is highly specific for plasminogen. By activation of plasminogen, plasmin is generated. Plasmin can degrade blood clots and connective tissue. Plasmin degradation of fibrinogen also causes enhanced blood vessel permeability and the accumulation of inflammatory cells. This tissue destruction and stimulation of the inflammatory response are considered to be important for the ability of the bacteria to spread (71, 121).

Antibiotic susceptibility and treatment

SP and SDSE are almost always susceptible to penicillin and other β -lactam agents (162, 163). The penicillin susceptibility of SP has not changed in spite of decades of use of penicillin (164). Penicillin is considered the drug of choice in the treatment of infections caused by SP and SDSE.

Antibiotic resistant organisms are those that possess a resistance mechanism demonstrated either phenotypically or genotypically and can be graded for instance as low-level or high-level resistance. Tolerance occurs when a substance that is usually bactericidal for the bacteria tested shows a diminished or absent bactericidal effect without loss of inhibitory action (165). Penicillin tolerance has been described both for SP and SDSE, but whether it has any clinical relevance is unclear (166, 167). The “Eagle effect” that implies a downregulation of the production of penicillin-binding proteins leading to impaired bacterial killing by penicillin has also been shown for both SP and SDSE (168, 169). Furthermore, failure or delayed response to treatment of pharyngitis in spite of penicillin sensitivity has been described (170, 171) and in the case of SP it has been hypothesized that it can escape penicillin by entering epithelial cells (172). Finally, no resistance against penicillin has been shown in SP, but penicillin-resistant isolates of SDSE were found in three epidemiologically linked patients (173). The isolates had mutations in multiple penicillin-binding proteins (PBPs).

Macrolides like erythromycin, azithromycin and clarithromycin have primarily been used for treating infections caused by SP and SDSE in patients allergic to penicillin.

Clindamycin is a lincosamide antibiotic also used in patients allergic to penicillin, but is also a recommended addition to penicillin in patients with necrotizing soft-tissue infections (NSTI) and toxic shock syndrome (TSS) (174, 175). Both macrolides and clindamycin inhibit bacterial proteins synthesis by binding to the 50S subunit of the bacterial ribosome (176). One of the incentives for the addition of clindamycin in more severe infections caused by SP and SDSE is the inhibition of bacterial protein synthesis, limiting the bacterial production of virulence factors like the M-protein and superantigens (177-179). Macrolide resistance in SP and SDSE is caused by two mechanisms, target drug reflux encoded by *mef* genes and target site modifications encoded by *erm* genes, the latter conferring resistance also against clindamycin (176). Geographical differences and temporal fluctuations in resistance to macrolides and clindamycin have been reported for both SP and SDSE (72, 180). The rates of resistance to macrolides and clindamycin reported have been higher in SDSE than in SP (64, 72, 181, 182).

Resistance rates to tetracyclines are variable in both SP and SDSE and tetracyclines are therefore not suitable for empirical treatment (181, 183). Susceptibility to fluoroquinolones is also variable (184).

Penicillin and aminoglycosides have a synergistic bactericidal effect against SP and SDSE in vitro unless they display high levels of resistance (185, 186). This effect is the rationale behind combination of aminoglycoside and penicillin in treatment of endocarditis. β -lactam agents are recommended for endocarditis caused by BHS in Swedish guidelines (187). International guidelines propose penicillin for endocarditis caused by SP and recommend the addition of aminoglycoside to the penicillin therapy for SDSE (188) based on studies that showed improved outcomes in patients treated with combination therapy (189), but a more contemporary study could not demonstrate any difference in outcomes (190). However, synergy between penicillin and gentamicin against some SDSE isolates has recently been demonstrated (99).

Intravenous immunoglobulins (IVIG) have been shown to be beneficial in severe invasive infections with SP, especially in cases with STSS and NSTI in some studies (191-193). For SDSE, case reports have documented positive effects (194). In the latest Cochrane review, the authors concluded that although IVIG reduced mortality among adults with sepsis, this benefit could not be validated from the examined studies, and current Infectious Diseases Society of America guidelines do not give a definitive recommendation on IVIG therapy (195, 196).

Streptococcus equi subspecies *zooepidemicus*

Epidemiology

Streptococcus equi subspecies *zooepidemicus* (SESZ) expresses the group antigen C when typed according to the Lancefield classification. SESZ primarily causes disease in animals like equids, cattle and dogs, but is also a zoonotic bacterium that can cause disease in humans (197).



Figure 3: "Dalahäst", traditional swedish painted wood-horse. Picture by Kristina Trell.

SESZ is a subspecies of *Streptococcus equi* (SE) (5). Other subspecies are *Streptococcus equi* subspecies *equi* (SESE) and *Streptococcus equi* subspecies *ruminantium* (SESR). SESE is believed to have evolved from an ancestral strain of SESZ and is thought to be a pathogen restricted to horses and other equids (Figure 3) (198), whereas SESZ and SESR occasionally cause infections in humans (199).

Since most clinical microbiology laboratories previously relied on grouping based on the Lancefield antigen rather than species determination, the true incidence of SESZ infections in humans is not known. Many of the cases reported have been related to the consumption of unpasteurized milk products or to contact with horses (8, 200-202).

Microbiological characteristics

SESZ are Gram-positive, facultative anaerobic cocci of the genus *Streptococcus*, belonging to the phylum Firmicutes. They grow in chains and the phenotype of SESZ on blood agar plates can vary from almost translucent mucoid to a matte grey or white appearance of around 1 mm in diameter. SESZ displays β -haemolysis on blood agar and is VP (Voges-Proskauer) and PYR (pyrrolidonyl arylamidase) negative (203). Another phenotypical characteristic of SESZ is that it ferments lactose and sorbitol in contrast to SDSE and SESE (203).

SESZ is a subspecies of SE (5) and the other subspecies are SESE and SESR. SESZ shares over 98% DNA sequence identity with SESE (198, 204). The separation between the two subspecies SESZ and SESE is therefore difficult, but SESR is genetically distinct from the two other subspecies (5, 6, 199).

Species identification and typing

SESZ expresses the group antigen C when typed according to the Lancefield classification (5). SDSE of human and animal origin also expresses the Lancefield group C antigen (43-45) and in clinical microbiology laboratories relying on the grouping of Lancefield antigen, SESZ has gone unnoticed among β -haemolytic GCS causing human infections. Other streptococcal species expressing the Lancefield group C antigen usually differ in haemolytic pattern and phenotypical characteristics like colony size and appearance (5).

Species identification of β -haemolytic streptococci was previously not routinely performed, but today MALDI-TOF MS is used for invasive isolates in clinical microbiology laboratories. This method can successfully discriminate SE to a species level (Figure 4) and some studies indicate that MALDI-TOF MS also can be used for accurate identification to the subspecies level (205, 206).

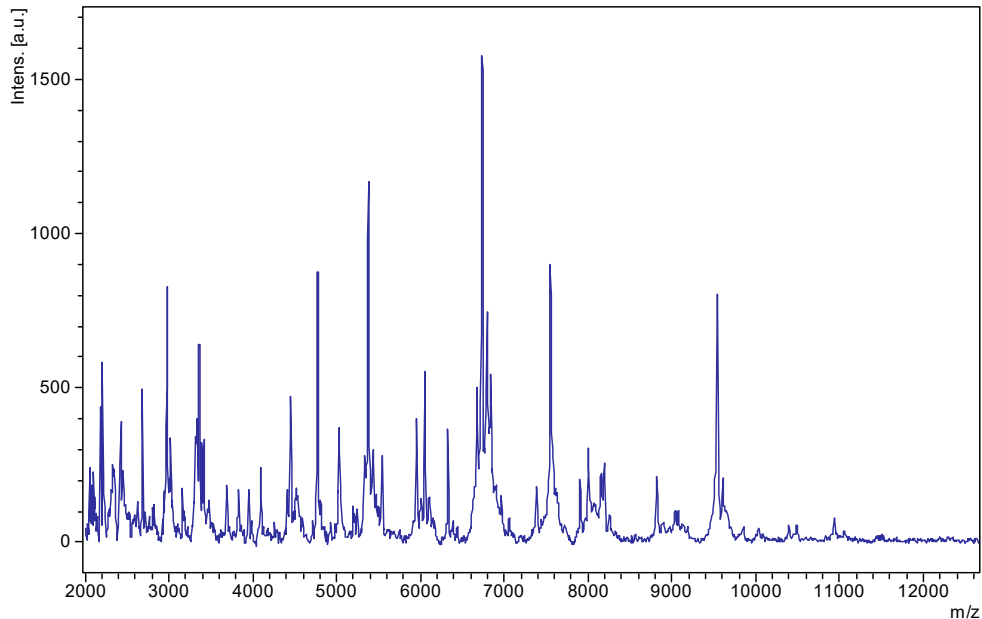


Figure 4: MALDI-TOF MS mass spectrum of SE. Courtesy of Bo Nilsson.

Combining sequencing of the 16S rRNA gene with species specific genes, like the *rpoB* can determine subspecies in many cases (207), but is time-consuming and can be difficult to interpret (208). MLST might also be utilized for this purpose (204). Finally, WGS has been used (209), however secure subspecies determination of SE remains precarious (6, 208).

To investigate epidemiological connections in infections caused by SESZ, typing of the M-like protein SzP can be used to differentiate isolates (39, 210).

Clinical manifestations

Infections with SE in humans have mostly been reported with SESZ and can present as bacteraemia with unknown focus, meningitis, arthritis, endocarditis or aortitis (200, 209, 211-216). Many of the cases reported have been related to the consumption of unpasteurized milk products or to contact with horses (8, 200-202). In some case series, the fatality rate has been high (8, 200, 216).

Virulence mechanisms

SESZ displays a M-like surface protein called SzP (210). Like other M-like proteins, SzP binds to fibrinogen and an anti-phagocytic activity is plausible (217). SzP has also been shown to protect against phagocytic killing by interaction with the complement system (218).

Streptolysin S (SLS) is the cytotoxin responsible for the beta-hemolytic phenotype and has been identified in the genome of SESZ, but not Streptolysin O (SLO) (219).

SESZ has been shown to display the superantigens SzeF, SzeN and SzeP (220). Screening of a diverse collection of SESZ isolates by qPCR revealed that 49% of these isolates were positive for these superantigen genes.

Antibiotic susceptibility

No EUCAST clinical breakpoints have been established for SESZ. In published case reports of SESZ in humans, the isolates when tested are typically susceptible to penicillin (200, 209, 211-216). In a large retrospective study of antibiotic susceptibility of BHS recovered from horses including 2893 isolates of SESZ, 98,9% were sensitive to penicillin (221).

Erysipelas

Erysipelas is a prevalent skin infection affecting the upper dermis (222-224). Clinical signs include an erythema with a sharp demarcation (Figure 5) and symptoms comprise fever, nausea and pain (225). Erysipelas and superficial cellulitis are often used to denote the same condition, whereas cellulitis is used to describe a skin infection that involves the whole dermis and subcutaneous tissues and these conditions can be difficult to clinically distinguish (226-228). Erysipelas most commonly affects the leg, followed by the arm and the face (94, 225, 229).



Figure 5: Erysipelas of the lower limb. Courtesy of Magnus Rasmussen

The major causative pathogens of erysipelas are by most researchers regarded to be BHS of group A, G and C (224, 226, 230, 231).

BHS are typically isolated from a minority of patients (94). Blood cultures are only positive in about 3-9% of the patients (94, 232, 233). Cultures are feasible when skin lesions are present, but if pathogenic bacteria are detected, it is difficult to assess their clinical significance (226, 228). Culturing of needle aspirates and skin biopsies from inflamed skin have also been evaluated, but rarely identify pathogenic bacteria (224, 234). Methods based on PCR have shown similar or even lower yield of positive findings (235, 236). Serology and direct immunofluorescence can also be used to investigate the causative pathogen (95, 237) but are less well suited for routine practice. Erysipelas therefore remains a clinical diagnosis. Two studies have shown that patients with erysipelas frequently are colonized with BHS in the perianal area (238, 239).

Recurrence is a known complication of erysipelas occurring in 21-29% of the patients, with lymphedema being the most common risk factor and prophylactic antibiotic treatment is sometimes considered (195, 223, 226, 240, 241).

Treatment of erysipelas typically relies on empirical antibiotics and elevation of the affected area. Hospitalization is recommended when a deeper or necrotizing infection is suspected, in immunocompromised patients or if outpatient treatment is not possible. Furthermore, source control with surgical management is recommended in cases of suspected necrotizing soft tissue infection (NSTI) (224). Current recommendations for treatment of erysipelas refer to different modes of spread of the infection, the pathogen involved and various clinical conditions, which make them difficult to follow and the applicability of the guidelines has therefore been questioned (242).

Postpartum *Streptococcus pyogenes* endometritis

Puerperal sepsis refers to a systemic infection in the postpartum period that became prevalent in the 1600s, when women started giving birth in hospitals (243, 244). Epidemics of puerperal sepsis occurred and the mortality rates could be very high. Semmelweis (Figure 6) reduced rates of puerperal sepsis from 11.4 in 1846 to 3.1 % in 1847 by introducing routines for hand hygiene in the maternity ward (245, 246). *Streptococcus pyogenes* (SP) is a well-known cause of postpartum infections (104, 245). The rates of puerperal sepsis declined, but from the 1980s onwards the rates have increased in concordance with an overall increase of invasive infections with SP (246, 247).



Figure 6: Stamp from Deutsches Bundespost

Postpartum women have a 20-fold increased risk for invasive SP infections compared with non-pregnant women (248, 249). Postpartum endometritis is the most common focus of infection and clinical signs includes fever and abdominal pain (104, 250, 251). The clinical diagnosis can be difficult and nonspecific symptoms are frequently present (247). Cultures are usually taken from the cervix and blood (104, 250). *Emm*28 is the most prevalent *emm*-type found in cases of postpartum endometritis and presumptive virulence factors like the R28 protein have been described (252, 253).

Treatment of SP postpartum endometritis usually consists of penicillin plus clindamycin as recommended for invasive SP infections and source control by surgical intervention is considered crucial (224, 254). However, in a recent retrospective study only a minority required surgical intervention and the rest made a full recovery with conservative medical treatment (255).

Nosocomial outbreaks of postpartum infections still occur and therefore surveillance of these infections is of great importance (256). *Emm*-typing can be used for this purpose and to further investigate clonal relatedness whole-genome sequencing (WGS) can be utilized (47, 257, 258). Outbreaks can be caused by inadequate hygiene measures or by a carrier among health care workers in the maternity ward (259, 260). Measures including environmental samplings and pharyngeal swabs from healthcare workers are parts of an outbreak investigation necessary for outbreak control (261, 262).

Present investigations

Aims

The overall aim of this thesis is to investigate infections caused by BHS from a microbiological as well as a clinical perspective.

The initial aims focused on in papers I and II were:

- To investigate if GGS and GCS from different sites of isolation differed in *emm*-types and if *emm*-types could be correlated to certain infections
- To investigate if certain *emm*-types predisposed for recurrent bacteraemia with GCS and GGS when compared to controls with only one episode of bacteraemia

The aims of the subsequent two studies followed from questions arising during the first two investigations:

- When the GCS were species determined we found cases of suspected zoonotic infections with SE, spurring the aims of the third paper: To investigate the clinical course of patients with SE bacteraemia and to elucidate epidemiological connections through sequencing of the gene encoding the M-like protein SzP
- When examining cases of recurrent bacteraemia with GCS and GGS we found that erysipelas was a risk factor for recurrence. A previous case report had shown a persisting perianal carriage of GGS in four cases of erysipelas. In the fourth study, we therefore wanted to examine the colonization of BHS in erysipelas with the aim to investigate the possibility of using perianal cultures for diagnostic purposes in patients with erysipelas

Finally, during my work in Infection Control we managed and brought an outbreak with postpartum endometritis with SP *emm75* to an end. *Emm75* is a rare cause of postpartum endometritis. The aim of the final study became:

- To investigate the use of WGS in the investigation of an outbreak of postpartum SP *emm75* infections in a maternity ward

Summary and Conclusions

For detailed descriptions of methods, materials and results in the present studies, the reader is referred to the separate papers.

Paper I: Species and *emm*-type distribution of group C and G streptococci from different sites of isolation

Summary

In the early 2010s, several studies indicated an increase in the incidence of beta-haemolytic streptococci (BHS) of groups C and G (GCS and GGS). Invasive forms of these infections were found in elderly patients with underlying comorbidities and the reported fatality rate was high. The disease spectrum reported was similar to that of *Streptococcus pyogenes*, including erysipelas, necrotizing soft tissue infection (NSTI) and streptococcal toxic shock syndrome (STSS). The vast majority of human isolates of GCS and GGS were verified as *Streptococcus dysgalactiae* (SD). At this time MALDI-TOF MS was introduced into clinical practice as a method for rapid species identification of bacteria including streptococci. Furthermore, *emm*-typing had been used in previous studies to investigate the distribution of SD *emm*-types in invasive and non-invasive infections and attempts had been made to link certain *emm*-types to prognosis.

The purpose of this study was to describe the *emm*-types of GCS and GGS from different sites of isolation and if possible correlate certain types to clinical presentation and severity of disease. Isolates from throat swabs and wounds were collected during two one-month periods in 2008 and 2011 and isolates from blood from the 1st of January 2008 to the 31st of December 2011. Species determination was made by MALDI-TOF MS. The species of the *Streptococcus equi* and *Streptococcus canis* isolates was confirmed by the sequence of the 16S rRNA gene. All isolates were subjected to *emm*-typing. The medical records were studied for data on mortality.

262 isolates, 183 GGS and 79 GCS were identified and 252 were speciated to SD by MALDI-TOF MS. *Emm*-typing revealed that the distribution of *emm*-types was different between GCS and GGS in our study (Figure 7).

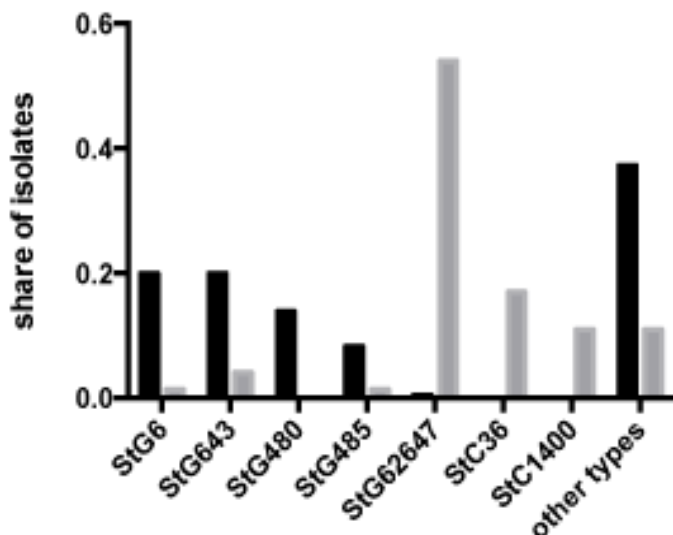


Figure 7: The type-distribution of GGS (black bars) and GCS (gray bars) expressed as proportion of total isolates (n = 183 for GGS and n = 73 for GCS).

The predominant *emm*-type among the GCS isolates was StG62647, constituting 54%, while StC36 and StC1400 also were prevalent. Among the GGS isolates the distribution of *emm*-types was more scattered and the four most prevalent were StG6 (20%), StG643 (20%), StG485 (14%) and StG480 (8%).

The GCS isolates from blood were more often StG62647, whereas the GCS isolates of *emm*-types StC36 and StC1400 were more common among throat isolates. For the GGS isolates, StG6 was more frequent in blood than in throat isolates and StG652 was prevalent in wound isolates as compared to in blood isolates. There was a statistically significant difference in *emm*-type distribution between throat and blood isolates for the GCS of our study, but not in the deviation in *emm*-type distribution between throat and blood isolates of GGS.

Study of patient medical records revealed that the patients with GCS or GGS bacteraemia were significantly older and more likely to be male than patients with the same bacteria isolated from throat or wound. No fatality within 28 days was

recorded among the cases with GCS bacteraemia, whereas 12% of the patients with GGS bacteraemia succumbed to the disease.

Conclusions

When species determination of the isolates of GCS and GGS was made by MALDI-TOF MS, the vast majority were found to be SD. Together with the phenotypical characteristics, the SD found in our study are probably SDSE as previously outlined. However, identification to the subspecies level was not possible with the methods used.

The GCS and GGS in our study predominantly expressed different *emm*-types, but some overlaps were seen. The differences in *emm*-type distribution of GCS and GGS at different isolation sites were small, suggesting that *emm*-types cannot be associated with certain disease presentations. Furthermore, there was no association between certain *emm*-types and mortality.

In conclusion, we were unable to link certain *emm*-types to disease severity in SD infections in our study. However, in analogy with the changing epidemiology of SP infections, *emm*-type surveillance of SD infection could prove important for the rapid detection of changes in type distribution leading to an increase in incidence and mortality.

Paper II: Recurrent bacteremia with *Streptococcus dysgalactiae*: a case-control study

Summary

GCS and GGS had in previous studies been shown to have a propensity to recur and recurrence rates of between 3 and 9 % were reported. Some studies had linked the tendency to recur to certain *emm*-types and examined if underlying clinical condition can predispose for the reappearance. The purpose of this retrospective case-control study was to evaluate the rate of recurrence in GCS and GGS infections and to evaluate if a relation to certain *emm*-types and clinical factors was present.

Cases of recurrent bacteraemia with GCS and GGS in the period 2003-2013 were identified and MALDI-TOF MS was used for species determination. The isolates were *emm*-typed. The cases of recurrent bacteraemia were then compared to controls with a single episode of bacteraemia with a case-control ratio of 1:4. Data on clinical presentation were taken from the medical records.

Among a total of 593 episodes, 23 episodes of recurrent bacteraemia with GCS and GGS were found in 2003-2013; GGS in 19 patients and GCS in 4 patients. When MALDI-TOF MS species identification was used, SD was demonstrated in 22 cases and *Streptococcus canis* in one case. Ninety-two control isolates (76 of GGS and 16 of GCS) were confirmed to be SD. The distribution of *emm*-types in the SD isolates causing recurrence mostly paralleled the isolates causing single episodes with the recurrence being caused by the same *emm*-type in 19 of 22 cases (Figure 8).

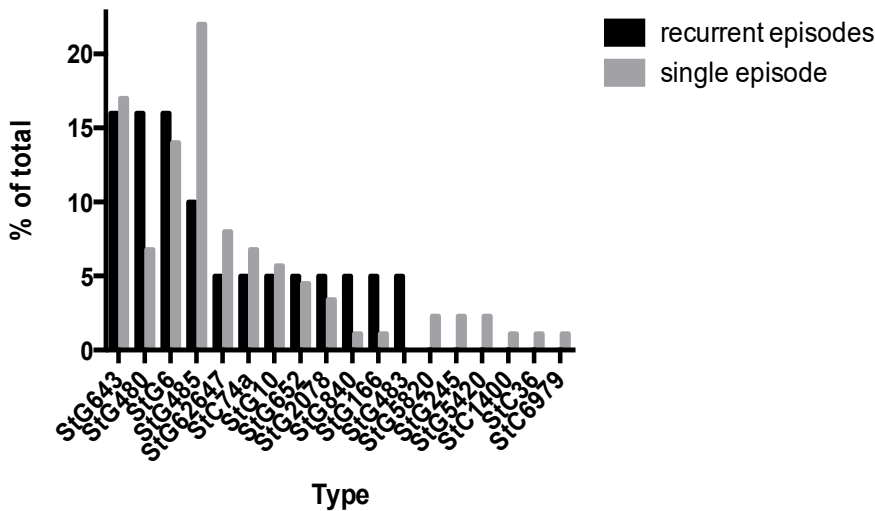


Figure 8: Distribution of types among isolates where the same *emm*-type caused the recurrent episode (n = 19) represented by black bars and among isolates from single episodes (n = 92) represented by grey bars.

In table 1, the demographic and clinical characteristics of patients with recurrent and single-episodes of SD bacteraemia are outlined. The patients of both groups had similar demographics and Charlson comorbidity score. Erysipelas was the most frequent clinical manifestation, seen in 50% of the recurrent cases and 38% of those with a single episode. No significant difference was found between the clinical characteristics of patients with recurrent and single-episodes of SD bacteraemia.

Table 1. Comparison of recurrent and single episodes of bacteraemia with SD

	Recurrent episode (n=22)	Single episode (n=92)	<i>p</i> for difference
Age (years, median)	74	74	<i>p</i> =0.6
Gender (% male)	64	63	<i>p</i> =1
Charlson comorbidity index (mean, range)	2 (0-6)	1 (0-7)	<i>p</i> =0.2
Underlying disease			
Diabetes (%)	32	24	<i>p</i> =0.5
Chronic leg ulcer (%)	27	11	<i>p</i> =0.09
Previous radiation or lymph oedema (%)	14	9	<i>p</i> =0.4
Previous erysipelas	23	8	<i>p</i> =0.05
Clinical Manifestation(%)			
Skin and soft tissue	67	56	<i>p</i> =0.3
Bacteraemia without focus	23	24	<i>p</i> =1
Abscess	0	4	<i>p</i> =1
Bone and joint	0	5	<i>p</i> =0.6
Post operative, device-related	0	8	<i>p</i> =0.3
Endocarditis	0	1	<i>p</i> =1
Severe sepsis at presentation (%)	23	32	<i>p</i> =0.6
Empirical treatment (%)			
Penicillin or cloxacillin	37	30	<i>p</i> =0.6
Cephalosporin or carbapenem	55	52	<i>p</i> =1
Other	5	11	<i>p</i> =0.7
No antibiotic	5	7	<i>p</i> =1
Treatment length (days, median)			
Intravenous	6.5	7	<i>p</i> =0.5
Total	14	14	<i>p</i> =1
Length of stay in the hospital (days, median)	7.5	11	<i>p</i> =0.2
Fatality proportion (%)	0	13	<i>p</i> =0.1

Conclusions

In our study, 22 patients with recurrent episodes of SD bacteraemia were seen and in 19 cases (86%), the same *emm*-type was encountered in both episodes. The high proportion of identical *emm*-types in recurrent episodes indicates a host-specific colonization.

Furthermore, in our study no specific *emm*-types were associated with recurrences. We did not identify clinical or microbiological factors that were significantly associated with an increased risk for recurrence, apart from the association with erysipelas in the first episode. We therefore argue that information to patients about the risk of recurrence and in some cases antibiotic prophylaxis can be warranted.

Paper III: Clinical and microbiological features of bacteremia with *Streptococcus equi*

Summary

During the previous studies, when the GCS were species-determined with MALDI-TOF MS, we found cases of infections with *Streptococcus equi* (SE). SE is a zoonotic pathogen causing human infections and has been related to consumption of unpasteurized milk products and to contact with horses. The purpose of this retrospective study was to investigate the clinical course of patients with bacteremia with SE and to elucidate epidemiological connections through sequencing of the gene encoding the M-like protein SzP.

Blood isolates of GCS from 1st of January 2003 to 31st of December 2015 were analyzed with MALDI-TOF MS and isolates identified as SE were then subjected to sequencing of the 16S rRNA and *rpoB* genes for confirmation of the species determination. Subspecies determination was not possible with the methods used as previously outlined. Subtyping based on sequencing of the M-like protein SzP was performed and patient records were reviewed.

167 blood isolates of GCS were subjected to MALDI-TOF MS and 149 SD and 18 SE (see table 2) were identified. Sequencing of the 16SrRNA and *rpoB* gene of the SE isolates confirmed species determination in all 18 cases. The most common clinical syndrome was bacteraemia of unknown origin in five cases. Septic arthritis was diagnosed in three patients. Furthermore, two cases of pneumonia, two cases of suspected catheter-related infection, one case of meningitis and mycotic aortic aneurysm respectively were seen. There were no fatalities. Review of the medical records revealed contact with domestic animals in ten cases. The gene encoding SzP was sequenced and the isolates were found to belong to different sequence types.

Table 2. Clinical features and findings of patients with SE bacteraemia.

Case	Age	Sex	Underlying disease	Presenting symptoms	Clinical diagnosis	Complications	Echo-cardio-graphy	Out-come	Group of SzPSe
1	41	F	AML; Allo-BMT; GVHD	Fever	Suspected Infection of Porta-cath	Removal of Porta-cath	Normal findings	Cured	HV2
2	41	F	Asthma	Fever; abdominal pain	Ovarial abscess	Thrombosis of vena ovarica	Normal findings	Cured	HV5
3	64	F	RA; HBP	Fever 2 weeks	Sepsis	Relapse of fever; Aortitis	Normal findings	Cured, but life-long AB.	HV4
4	95	M	ITDM; HBP; PM	Fever; abdominal pain; headache	Sepsis	-	-	Cured	HV1
5	75	F	CKD; HD	Fever; diarrhea	Strepto-coccal infection	Thrombosis in conjunction to CDC	-	Cured	HV4
6	86	M	Mitral PHV; AF; CHF	Chest pain	Pneumonia	-	-	Cured	HV4
7	59	K	HBP	Fever and chills	No diagnosis	-	Normal findings	Cured	HV5
8	67	M	IHD; CABG	Fever and confusion	Strepto-coccal septicemia	-	-	Cured	HV3
9	13 days	F	Low birth weight	Sleepiness, poor feeding	Septicemia in infant	-	-	Cured	HV5
10	63	F	Healthy	Abdominal pain; throat ache; fever	Abdominal pain	-	-	Cured	HV4
11	78	M	HBP; AF; TKA; CLL	Swelling and pain in prosthetic knee; fever	Prosthetic knee infection	Severe sepsis; infection of knee prosthesis requiring surgical revision	-	Cured	HV4
12	76	M	MDS; EAA; IBD	Cough; shortness of breath; fever	Pneumonia	-	-	Cured	HV4V
13	71	K	WM, AD	Confusion; fever; pain in the right wrist and hip	Septic arthritis	-	Normal findings	Cured	HV5

AML: acute myeloid leukemia; BMT: bone marrow transplant; GVHD :graft versus host disease; RA: reumatoid arthritis; HBP: high blood pressure; ITDM: insulin treated diabetes mellitus; PM: Pacemaker; CKD; chronic kidney disease; HD: hemodialysis; CDC: central dialysis catheter; PHV: prosthesis heart valve; AF: atrial fibrillation; CHF: congestive heart failure, IHD: ischemic heart disease; CABG, coronary artery bypass graft; TKA: total knee arthroplasty; CLL: Chronic lymphocytic leukemia; MDS: myelodysplastic syndrome; EAA: extrinsic allergic alveolitis; IBD: inflammatory bowel disease; WM: Waldenströms macroglobulinemia; AD: Adipositas dolorosa

Conclusions

Eighteen cases of SE were found during a thirteen-year period, which confirms that SE is a rare cause of infection in humans. No temporal or geographical clustering was found in our material. Furthermore, there were reports of domestic animals in ten of the patient records. This suggests sporadic transmission possibly from domestic animals. Our study indicates that sporadic cases of SE bacteraemia have a favourable prognosis. Determination of species with MALDI-TOF MS was possible and was shown to have a high specificity. We argue that species determination of GCS can offer important clues to the understanding of the mode of acquisition of SE.

Paper IV: Colonization of β -hemolytic streptococci in patients with erysipelas-a prospective study

Summary

Recent studies have concluded that SDSE is a prevalent cause of erysipelas. Erysipelas is a common infection causing significant morbidity and there are no established procedures for bacteriological sampling. Recurrent bacteraemia and erysipelas are particular features of SDSE and perianal carriage has been implicated as a mechanism for recurrence in erysipelas. In this prospective study we investigated the perianal colonization with beta-haemolytic streptococci (BHS) in patients with erysipelas.

Patients with erysipelas and a control group of patients with fever without signs of skin infection were prospectively included. Cultures for BHS were taken from the tonsils, the perianal area, and wounds from both groups. BHS when found were grouped according to Lancefield antigen and determination of species was made with MALDI-TOF MS. The BHS were then *emm*-typed. From patients with erysipelas and a positive culture for BHS, renewed cultures were taken after four weeks. Data on clinical presentation were taken from flow-sheets and the medical records. 25 patients with erysipelas and 25 with fever were included. In the group with erysipelas, 11 patients (44%) were colonized with BHS, ten patients were colonized in the perianal area and one patient in the throat. In contrast, only one patient in the control group was colonized. All the patients with erysipelas that were colonized with BHS had an erythema of the lower limb. The BHS were most commonly found to be SD. Nine of the 11 patients with erysipelas and BHS were

cultured again after four weeks and three of these were still found to be colonized with BHS (see table 3).

Table 3. The occurrence of BHS among the patients with erysipelas.

	First Visit				Return Visit			
	Tonsil	Perianal	Wound	Blood	Visit	Tonsil	Perianal	Wound
Patient 2	X ¹	GGs ² SD stG6.1	³	GGs SD stG6.1	Yes	x	x	
Patient 3	GAS SP emm77.0	GAS SP emm77.0	GAS SP emm77.0		Yes	x	x	
Patient 4	x	GGs SD stC74a.0	GAS SP emm4.0		Yes	x	GGs SD stC74a.0	
Patient 6	GAS SP emm4.0	x			Yes	x	x	x
Patient 8	x	GGs SD stG643.0	GGs SD stG643.0		Yes	x	GGs SD stG643.0	x
Patient 10	x	GGs SD stC74.0			Yes	x	x	
Patient 12	GCS SD stG62647.0	GCS SD stG62647.0	x	GCS SD stG62647.0	No			
Patient 14	x	GGs SD stG5420.0	GGs SD stG5420.0	x	No			
Patient 17	x	GGs ⁴		x	Yes	x	x	
Patient 18	x	GGs ⁴		x	No			
Patient 22	x	GGs ⁴			Yes	x	GGs SD stG166b.0	

¹ No growth of BHS. ² Abbreviations used are GGS, group G Streptococcus; GAS, group A Streptococcus; GCS, group C Streptococcus; SD, *Streptococcus dysgalactiae*; SP, *Streptococcus pyogenes*. ³ No culture taken. ⁴ The sample was not saved for species determination and emm-typing

Conclusions

In the group with erysipelas, 11 (44%) of the patients had positive cultures of BHS compared to one patient in the control group. In ten of these patients, BHS were found in cultures from the perianal area and nine were GGS/SD. Thus, we concluded that SD colonizes the perianal area in a substantial proportion of patients with erysipelas.

We therefore suggest that perianal cultures could be used as a diagnostic tool in cases of erysipelas. Since BHS are thought to be the major causative pathogens of erysipelas, a finding of BHS would support the diagnosis of erysipelas. In addition, the isolation of BHS would provide the clinician with guidance to antibiotic therapy.

Even if penicillin is the first choice for treatment of erysipelas, knowledge of the antibiotic susceptibility of the causative agent can prevent unnecessary broad spectrum antibiotics. Furthermore, in patients with allergy to penicillin, alternative antibiotics such as clindamycin or erythromycin might be used and resistance to these antibiotics is relatively common.

All patients carrying SD in the perianal area had erysipelas located on the lower limb. These findings are in agreement with recent evidence suggesting an association between SD and erysipelas of the lower limb. Moreover, in this study we demonstrate that some patients carry SD in the perianal area also after the conclusion of antibiotic treatment. Together with the propensity of SD to cause recurrent erysipelas, these observations would fit with a model where perianal colonization with SD provides a risk for subsequent erysipelas of the lower limb. This will have to be further examined, but could have implications for secondary prophylaxis in cases of recurrent erysipelas.

Paper V: Management of an outbreak of postpartum *Streptococcus pyogenes emm-75* infections

Summary

Surveillance and management of suspected outbreaks are assignments of physicians working in the Department of Infection Control and Prevention. In early autumn 2018, an outbreak of postpartum infections came to our attention. The purpose of this study was to describe the measures taken to control an outbreak of postpartum infections caused by SP and elucidate the role of whole-genome sequencing (WGS).

Patients presenting postpartum with signs and symptoms of infection are routinely cultured for BHS with cervical swabs and these are thereafter grouped according to Lancefield antigen, species-determined and *emm*-typed as part of an epidemiological surveillance. SP is a well-known cause of postpartum infections and the predominant type is SP of *emm28*.

During a 3-month period in the autumn of 2018, a total of six cases of postpartum infection with SP *emm75* were identified in the same maternity ward. Because of an ongoing surveillance of postpartum infections, an outbreak group consisting of the infection control team and the head of the maternity ward was formed early on in the outbreak. The infection control nurse examined all routines in the maternity ward to find possible sources of infection and to evaluate the hygiene measures.

Environmental swabs were taken from multiple areas in the delivery rooms, where contamination was suspected and these were screened negative for SP. Education in basic hygiene was given to all personnel. Health care workers in the maternity ward were screened with pharyngeal swabs for SP and all were negative. Furthermore, the times for the deliveries of the patients in the outbreak when compared with the work schedules revealed one health care worker as a possible common source of infection in five cases. After repeated pharyngeal swabs from this worker, a SP *emm75* was isolated. To investigate the relatedness of the SP *emm75* isolates, whole-genome sequencing (WGS) was undertaken. The five isolates from the patients epidemiologically linked to the health care worker were identical, whereas the sixth patient had an SP isolate of *emm75* of another sequence type (Table 4). Contemporary SP *emm75* isolates from blood cultures in the same geographical region were also different from the health care worker's isolate. The genomes were also examined for the presence of RD2 encoding the possible virulence factors like the R28 protein, but these were not found. Eradication antibiotic therapy was given to the implicated health care worker and this brought the outbreak to an end.

Table 4. Characteristics of the patients with SP postpartum infections.

Outbreak with SP <i>emm</i> -type 75	Number of days after delivery	Clinical diagnosis	Cultures positive for SP	Length of stay in hospital (days)	ST	Contact with carrier	SNPs compared to carrier	RD 2
Patient 1	6	Endometritis	Urine and cervix	2	49	Yes	0	Not found
Patient 2	2	Endometritis	Urine and cervix	4	49	Yes	0	Not found
Patient 3	4	Endometritis	Cervix	3	49	Yes	0	Not found
Patient 4	6	Endometritis	Cervix	4	49	Yes	0	Not found
Patient 5	1	Endometritis	Urine, cervix and blood	4	150	No	575	-
Patient 6	14	Endometritis and possible recurrence 1 month later	Urine and cervix	6	49	Yes	2	Not found

ST: sequence type, SNP: Single nucleotide polymorphism, RD 2: Region of difference 2

Conclusions

In this study, we describe an outbreak of postpartum endometritis caused by *emm75*. In our hospitals invasive isolates of SP are continuously *emm*-typed and *emm75* was not commonly encountered during this period. Furthermore, with WGS we could

demonstrate that the contemporary SP *emm75* isolates differed from the isolates in the outbreak. We therefore concluded that the outbreak did not reflect that SP of *emm75* was circulating in the community.

With WGS, this clonal outbreak of SP postpartum infections could be explained and a carrier could be identified. WGS has become a very important tool in the investigations of nosocomial outbreaks, but is time-consuming and expensive. Considering the cost and efforts involved in the management of a nosocomial outbreak, WGS provided the necessary information about the epidemiological connections. Cultures identified pharyngeal carriage of SP in a health care worker and WGS could ascertain the clonal relatedness. Throat carriage of SP in health care workers has been suggested as a source of nosocomial outbreaks and eradication of carriage is recommended. Eradication antibiotic therapy was given to the implied health care worker and thereafter no further cases of postpartum infections with SP *emm75* have been reported from the maternity ward.

To investigate the cause of this outbreak by examining the presence of putative virulence factors related to postpartum endometritis, analysis of the genome sequence of the isolates for the RD2 region was performed. These were not found and therefore we concluded that other virulence factors than those encoded by RD2 could be of importance in the pathogenesis of postpartum SP infections. It is noteworthy, however, that none of the patients in this outbreak had a severe course of the infection. It is therefore possible that this clone of SP *emm75* was less prone to cause severe invasive infection and that the outbreak rather resulted from an increased capability for pharyngeal carriage. Further studies of the genome of the outbreak clone would be necessary to evaluate this.

To conclude, source identification proved vital for control of this outbreak and WGS is a valuable tool for determining the epidemiology of nosocomial outbreaks.

Concluding remarks and future perspectives

In the department of Infectious Diseases in the early 2010s, infections caused by BHS of group C and G were considered to be rare events. Our studies of them emanated from an interest to learn more about these pathogens that were nowhere as well-studied as GAS. Also the awareness of the clinical importance of these BHS among colleagues was low. With the introduction of MALDI-TOF MS, rapid and accurate species identification of BHS became possible and most of these BHS were found to be *Streptococcus dysgalatiae* (SD). Together with *emm*-typing and other typing methods further studies into the microbiological and clinical features of these BHS became possible. Among BHS of group C, we found infections with *Streptococcus equi*, which previously had gone undiagnosed because of the use of the Lancefield classification of BHS. The role of SD in erysipelas was described by our research group among others and provided the questions behind our prospective study on erysipelas. Finally, the possibility of using WGS in management of outbreaks and the insight that outbreaks of postpartum endometritis caused by SP still occur was the reason for the last study.

An increase in the incidence of SD bacteraemia has been recognized and invasive disease is most commonly found in elderly patients with underlying comorbidities. With the use of MALDI-TOF MS in routine diagnostics, species identification is now performed on all BHS found in invasive isolates. This provides the necessary information for accessing the role of SD in invasive BHS disease and to compare the clinical and microbiological features of SD invasive disease with SP. A comparative study would provide further insights into the local epidemiology of these infections.

The increasing incidence of SD, that in some studies parallels that of SP, calls for measures and the causes remain unclear. Prolonged survival of adults with underlying chronic conditions has been suggested, but this would require further studies to elucidate. Other interesting aspects of SD disease that demand further investigations are the role of virulence factors. Furthermore, the interplay between virulence factors and host factors awaits further examination for an increased understanding of SD pathogenesis and why invasive disease occurs.

The clinical significance of SD infections has become more evident over the past decades, but the continuous reporting of SD as group C and G streptococci probably contributes to the lack of awareness of these infections. Recognizing these infections and the clinical presentation as an entity coupled to the species SD, might contribute an increased knowledge of these infections as in the case with SP. Taxonomic confusion and a still evolving knowledge of genetic relationships have probably hampered this shift in designation of these infections. Furthermore, the inability of MALDI-TOF MS to secure an identification to the subspecies level adds to this dilemma. Even if the vast majority of SD identified by MALDI-TOF MS probably is SDSE, this augments the remaining uncertainty concerning SD and SDSE. If improvement in MALDI-TOF MS techniques could offer definite identification to the subspecies level is another area that requires further studies.

For epidemiological surveillance, a proper speciation of BHS is of the outmost importance. Moreover, *emm*-typing is not routinely performed in invasive SD infections as is done in invasive SP infections. For SP infections, there is a national surveillance of circulating *emm*-types. Even if we could not find a correlation between *emm*-type and invasiveness of SD infections in our studies, other research groups have pointed towards possible connections. We therefore argue, that *emm*-typing of invasive SD infections is warranted, providing a surveillance method of emerging *emm*-types. With SP, clonal outbreaks have been recognized that have been coupled to the emergence of new *emm*-types and epidemics to the acquisition of virulence factors. To evaluate these changes in epidemiology of SD infections, *emm*-typing needs to be part of routine diagnostic of invasive isolates of SD.

For invasive SD infections, betalactam antibiotics are still safe treatment options. Penicillin should be considered the drug-of-choice for non-allergic patients, since BHS are almost always sensitive to penicillin. SD infections have therefore not presented the clinicians with a treatment dilemma thus far. The reports of isolates of SD with penicillin resistance and generally a higher degree of reduced susceptibility against antibiotics used in cases of penicillin allergy illustrate a need for proper sampling in invasive SD infections. The role of SD in erysipelas and the emergence of penicillin resistance in SD have really pinpointed the need for better diagnostic methods. The finding of perianal colonization of SD in cases of erysipelas could provide an easy assessable source of diagnostics, which can be used with already available routine sampling methods. We have found indications of the role of SD in recurrent erysipelas in concordance with several other studies and this field deserves further attention and studies.

The severity of invasive infections with SE has most likely been exaggerated in the past, since knowledge on clinical presentation relied almost entirely on case-reports. In general, it is more likely that spectacular cases are reported rather than less serious cases. However, the outcome of SE bacteraemia in our study was more

favourable than previously reported. Before MALDI-TOF MS, these infections went undiagnosed, but we found these infections to be rare events. However, to be able to detect an outbreak related to a common food source, species determination of GCS is of the outmost importance. During our study, we found that subspecies determination of SE was even more difficult than in the case of SD. Genetically, the subspecies of SE are quite similar providing a challenge for the various diagnostic methods to accurately differentiate between them. In this respect, the present method of MALDI-TOF MS would have to be further developed to be able to perform this.

The role of WGS in Infection Control is becoming more and more evident. The ability to establish epidemiological connections makes it an invaluable tool in outbreak investigations. To be able to elucidate the reason for an outbreak in terms of suspected increased virulence, the potential seems almost limitless. But still being time-consuming and dependent on advanced interpretation, the method requires further development.

Populärvetenskaplig sammanfattning

Beta-hemolytiska streptokocker är Gram-positiva bakterier som har fått sitt namn av den kompletta hemolys som ses vid växt på blodagarplattor. Sedan 1920-talet har de beta-hemolytiska streptokockerna delats in i grupper, idag finns ett tiotal grupper benämnda med bokstäver.

Mest studerad är grupp A streptokocker (GAS) som orsakar både vanliga infektioner som halsfluss och rosfeber samt mer allvarligare infektioner såsom barnsängsfeber och svåra hud och mjukdelsinfektioner, så kallade mördarbakterieinfektioner. Förr i tiden sågs i Sverige reumatisk feber efter obehandlade infektioner med hög risk för utvecklande av hjärtsjukdom.

Med upptäckten av penicillin under 1900-talet kunde infektioner behandlas effektivt, men på 1980-talet sågs en ökning av svåra infektioner orsakade av GAS. Under de senaste årtiondena har även en ökning av infektioner orsakade av grupp C och grupp G streptokocker noterats. Grupp C och grupp G streptokocker orsakar liknande infektioner som GAS hos människa.

Med modern diagnostik har man kunnat artbestämma betahemolytiska streptokocker. GAS tillhör nästan alltid arten *Streptococcus pyogenes* (SP) och grupp C och grupp G streptokocker tillhör oftast arten *Streptococcus dysgalactiae* (SD). En liten del av grupp C streptokocker som isoleras vid humana infektioner tillhör istället arten *Streptococcus equi* (SE), som normalt infekterar olika djurarter. Det har funnits otillräcklig kunskap om hur infektioner med SD och SE yttrar sig och om olika undertyper av dessa arter är kopplade till speciella kliniska tillstånd.

Under första delen av 2010-talet introducerades en ny analysmetod inom bakteriediagnostiken som var baserad på masspektrometri s.k. (MALDI-TOF MS). Med denna metod kunde artbestämning av beta-hemolytiska streptokocker ske både snabbt och tillförlitligt. I denna avhandling har artbestämning gjorts med MALDI-TOF MS samt även vidare klassificering av undertyper. Isolat av bakterier från olika lokaler (sår, hals och blod) har analyserats och jämförts och betydelsen av art och undertyp för olika infektionstillstånd har kunnat undersökas. Vidare har speciella kliniska tillstånd som återkommande bakteriemi, rosfeber och barnsängsfeber orsakade av beta-hemolytiska streptokocker undersökts.

I det första delarbetet beskriver vi art- och undertypsfördelningen av beta-hemolytiska streptokocker grupp C och G isolerade från blod, sår och hals.

Hypotesen var att speciella undertyper var kopplade till en ökad risk för allvarlig infektion. En sådan koppling mellan speciella undertyper och prognos kunde inte verifieras i vårt material. Däremot såg en koppling av undertyper till om det var grupp C eller grupp G streptokocker.

I det andra delarbetet undersökte vi tänkbara riskfaktorer för att få återkommande bakteriemi med SD. Det fanns då studien påbörjades ett par rapporter som beskrev risken för återkommande infektioner med SD. Med en fall-kontrollstudie undersöktes kopplingen mellan undertyper samt kliniska riskfaktorer och risken för recidiv. 22 patienter med recidiverande infektioner och 92 kontroller med endast ett infektionstillfälle identifierades och studerades. Studien visade att förekomst av rosfeber i första episoden var en riskfaktor för recidiv. I övrigt noterades inga statistiskt säkerställda skillnader mellan grupperna. Vidare sågs i vårt material att den upprepade episoden orsakades av samma undertyp som den initiala.

I det tredje delarbetet studerade vi fall av bakteriemi med SE. Tidigare beskrivningar av tillståndet har antytt att det var mycket allvarligt och ofta kopplat till opasteuriserade livsmedel och medförde en hög dödlighet. En kohort på 18 patienter undersöktes och ingen av dessa hade avlidit till följd av infektionen. Undertyper bestämdes och dessa resultat tillsammans med epidemiologiska variabler talade för att patienterna i denna kohort fått smittan av djur och att det inte fanns någon koppling till ett livsmedelsutbrott. Studien beskriver tydligt hur smittspridning av SE till människa kan identifieras snabbt och säkert med den nya masspektrometrin. Med tidigare metodik kunde inte denna smitta upptäckas i den kliniska rutindiagnostiken.

I det fjärde delarbetet undersökte vi om provtagning för bärarskap av beta-hemolytiska streptokocker kring ändtarmsmynningen skulle kunna användas för diagnostik vid rosfeber. Vid rosfeber finns ingen etablerad teknik för bakterieprovtagning och behandling ges därför oftast empiriskt. I läroböcker anges att rosfeber orsakas av SP, men SD har i nyare studier visat sig vara en minst lika vanlig orsak. Vi genomförde en prospektiv studie där 25 patienter med rosfeber och 25 patienter med feber av annan orsak provtogs vid insjuknandet. 10 av 25 patienter med rosfeber och endast en av 25 patienter med feber av annan orsak hade beta-hemolytiska streptokocker kring ändtarmsmynningen. Dessa resultat antyder att enkla bakterieodlingar kan ge viktig diagnostisk hjälp vid ett vanligt infektionstillstånd där adekvat diagnostik hittills saknats.

I det sista delarbetet beskriver vi hanteringen av ett utbrott av barnsängsfeber med SP. Utbrottshantering med provtagning och olika interventioner samt epidemiologisk kartläggning med helgenomsekvensering redovisas. Av särskilt intresse var att den undertyp av SP som tidigare inte beskrivits som en vanlig orsak till barnsängsfeber var den som koloniserade en sjukvårdsarbetare och orsakade barnsängsfeber hos fem kvinnor. Resultaten är beskrivna i manuskriptsform.

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Remember, only dead fish go with the flow....

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