Human Infections Due to *Streptococcus dysgalactiae* Subspecies *equisimilis*

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Human streptococci that belong to *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) have long been known under the name of β-hemolytic groups C and G streptococci. Extensive taxonomic studies during the past years have distinguished most of the veterinary pathogens belonging to Lancefield groups C and G from those of human origin. After being considered nonpathogenic for many years, SDSE is now recognized as an important bacterial pathogen. The clinical spectrum of diseases caused by this species closely resembles *Streptococcus pyogenes* infections, including the occurrence of poststreptococcal sequelae. In accordance with these observations, many of the virulence factors present in *S. pyogenes* can also be found in SDSE strains. High nucleotide-sequence identities in virulence genes and the association of these genes with mobile genetic elements support the hypothesis of extensive horizontal gene-transfer events among streptococcal species of the pyogenic group. Recent epidemiological studies have shown increasing numbers of invasive SDSE infections, often among immunocompromised patients, and suggest that this species will probably gain even more clinical importance in the near future. For a better understanding of the changing epidemiology and pathogenicity of SDSE, an increased awareness of these microorganisms as human pathogens and proper identification are mandatory.

*Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) belongs to the group of pyogenic streptococci, which are often referred to as β-hemolytic streptococci. According to recent taxonomic studies, large colony–forming human groups C and G streptococci are currently classified as SDSE [1, 2]. The pathogenicity of these microorganisms has been increasingly recognized, with a wide spectrum of disease similar to that caused by *Streptococcus pyogenes* [3].

**TAXONOMIC STATUS**

Historically, the species *S. dysgalactiae* consists of at least 5 distinct subgroups (table 1). In 1996, Vandamme et al [1] first suggested the division of *S. dysgalactiae* into 2 subspecies: SDSE subspecies nova for human groups C and G strains and *S. dysgalactiae* subspecies *dysgalactiae* (SDSD) subspecies nova for all strains of animal origin. The genetic relationship between human and animal isolates of *S. dysgalactiae*, however, remains controversial. The most widely used classification to date was proposed by Vieira et al [2] in 1998 and is based on DNA-DNA hybridization tests and multilocus enzyme electrophoresis. It defines all β-hemolytic groups C and L and human group G streptococci as SDSE and only the α-hemolytic or nonhemolytic group C streptococci as SDSD.

**EPIDEMIOLOGY AND CLINICAL SYNDROMES**

Pyogenic streptococci of Lancefield groups C and G emerged as important human pathogens in the late 1970s and early 1980s [3, 4]. Only a few laboratories identify groups C and G streptococci to the species level; therefore, exact numbers of human SDSE infections are not available. In a recent population-based study, the burden of invasive SDSE infections approximated that of invasive *S. pyogenes* infections [5]. SDSE is not unusual as a colonizer of the human upper respiratory, gastrointestinal, and female genital tracts, and it is often present in skin lesions. Sites of colonization and focal infections serve as principal reservoirs for transmission. Infections due to SDSE are transmitted person to person; an animal reservoir for these strains has not been reported. Zoonotic group C or G streptococcal infections are comparatively rare and are mostly caused by other...
streptococcal species after animal contact or are associated with the consumption of unpasteurized dairy food products. Community-acquired outbreaks [6, 7] and clusters of hospital-acquired SDSE infections have been reported [8].

SDSE causes a variety of superficial, deep, toxin-mediated, or immunologically mediated diseases in humans. The spectrum ranges from harmless superficial skin infections to life-threatening streptococcal toxic shock–like syndromes. Pharyngitis is a classic presentation in adult patients [9], and SDSE has clearly been responsible for epidemic outbreaks of pharyngitis in children [6]. The significance of this pathogen as a cause of endemic or sporadic pharyngitis in children, however, is less firmly established. An association of pharyngitis with streptococcal species after animal contact or are associated with the consumption of unpasteurized dairy food products. Community-acquired outbreaks [6, 7] and clusters of hospital-acquired SDSE infections have been reported [8].

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SDSE primarily presents as skin and soft-tissue infections, including pyoderma, cellulitis, wound infections, abscesses, erysipelas, and necrotizing fasciitis [5]. Severe infections often affect injection drug users, elderly patients with underlying immunosuppressive comorbidities or conditions predisposing them to skin breakdown, and burn patients in whom the infections may lead to graft failure [15].

For invasive SDSE infections, sites of colonization or primary focal infections are most likely ports of entry [16]. Invasive infections comprise arthritis, osteomyelitis, pleuropulmonary infections, peritonitis, intra-abdominal and epidural abscesses, meningitis, endocarditis, puerperal septicemia, neonatal infections, necrotizing fasciitis, myositis, and streptococcal toxic-like syndrome. An increase in cases of bacteremia and of severe infection due to group G β-hemolytic streptococci in humans has been recognized lately [17]. Data on systemic diseases in England and Wales have demonstrated that, in contrast to group C streptococcal diseases, group G streptococcal invasive diseases have increased significantly in number since 1985 [3, 18], even though the presence of virulence determinants does not appear to be different between group C and group G SDSE [19]. Moreover, a high rate of relapse and recurrent group G streptococcal bacteremia has been noted [20]. The literature suggests a distinct association between group G streptococcal bacteremia or other invasive infection and underlying conditions, such as diabetes mellitus, alcoholism, or cardiovascular or neoplastic diseases [21]. Even though SDSE strains are closely related to Streptococcus agalactiae, invasive neonatal infections are rare [22].

**Table 1. History of the Taxonomic Status of *Streptococcus dysgalactiae* Subspecies equisimilis**

<table>
<thead>
<tr>
<th>Historical subgroups of S. dysgalactiae</th>
<th>Initial description of S. dysgalactiae subsp. equisimilis [1]</th>
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<tbody>
<tr>
<td>α-Hemolytic or nonhemolytic bovine group C streptococci (S. dysgalactiae)</td>
<td>S. dysgalactiae subsp. equisimilis subsp. nov.: human large colony–forming groups C and G streptococci</td>
</tr>
<tr>
<td>β-Hemolytic porcine group C streptococci (Streptococcus equisimilis)</td>
<td>S. dysgalactiae subsp. dysgalactiae subsp. nov.: all animal large colony–forming group C streptococci and group L streptococci</td>
</tr>
<tr>
<td>β-Hemolytic animal (pig, dog, and cattle) and, rarely, human group L streptococci</td>
<td>Current taxonomic status of S. dysgalactiae subsp. equisimilis [2]</td>
</tr>
<tr>
<td>β-Hemolytic human group C streptococci (S. equisimilis)</td>
<td>S. dysgalactiae subsp. equisimilis: all β-hemolytic large colony–forming groups C and L streptococci and human group G streptococci</td>
</tr>
<tr>
<td>β-Hemolytic human group G streptococci</td>
<td>S. dysgalactiae subsp. dysgalactiae: α-hemolytic or nonhemolytic large colony–forming group C streptococci</td>
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Table 2. Putative Virulence Determinants among Pyogenic Streptococci

<table>
<thead>
<tr>
<th>Proteins with highly similar sequences</th>
<th>Genes or homologues described in</th>
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<tbody>
<tr>
<td></td>
<td>SDSE</td>
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<tr>
<td>Fibronectin binding proteins</td>
<td>X</td>
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<tr>
<td>Plasminogen binding proteins</td>
<td>X</td>
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<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>X</td>
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<tr>
<td>Streptococcal surface enolase</td>
<td>X</td>
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<tr>
<td>Protein S (vitronectin) binding protein</td>
<td>X</td>
</tr>
<tr>
<td>Laminin binding protein</td>
<td>X</td>
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<tr>
<td>Streptolysin O</td>
<td>X</td>
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<tr>
<td>Streptolysin S</td>
<td>X</td>
</tr>
<tr>
<td>Superantigens (SpeA, Spec, SpeG, SpeM, Ssa, and Smez)</td>
<td>X</td>
</tr>
<tr>
<td>Dysgalactin</td>
<td>X</td>
</tr>
<tr>
<td>M protein</td>
<td>X</td>
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<tr>
<td>Capsule</td>
<td>X</td>
</tr>
<tr>
<td>C5a peptidase</td>
<td>X</td>
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<tr>
<td>Protein G</td>
<td>X</td>
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<tr>
<td>Streptokinase</td>
<td>X</td>
</tr>
</tbody>
</table>

**NOTE.** GAS, group A streptococcus; GBS, group B streptococcus; SDSD, *Streptococcus dysgalactiae* subspecies dysgalactiae; SDSE, *S. dysgalactiae* subspecies equisimilis.

* Genes with low similarity.

isolates. Fortunately, most of these species are of primarily veterinary origin. Moreover, cross-reaction between *Streptococcus pneumoniae* and group C streptococcal latex reagent has been reported [23]. Nonhuman large colony–forming β-hemolytic group G streptococci usually belong to *Streptococcus canis*, an animal pathogen that has been found only rarely in humans [24], or to *Streptococcus intestinalis* [25]. Group L β-hemolytic streptococci are rare in human specimens and also belong to SDSE [2]. Furthermore, SDSE strains carrying the Lancefield A antigen have been identified [26, 27].

**Species identification by phenotype and biochemical properties.** A negative Voges–Proskauer reaction is helpful for the distinction of SDSE strains from streptococci of the *S. agalactiae* group. Biochemical differentiation of human group C from group G SDSE strains is hardly ever necessary and can be achieved by testing for the production of L-proplyl-L-arginine aminopeptidase [28]. On the basis of 16S ribosomal RNA (rRNA) gene-sequencing data, some biochemical panels correctly identify SDSE. The API system and Vitek system (bioMérieux Vitek) confidently identified 99% and 94% of isolates, respectively. Although the ATB Expression system (bioMérieux Vitek) contains the largest number of biochemical reactions, only 74% of isolates were identified correctly [29]. To distinguish group A SDSE from *S. pyogenes*, the absence of pyrrolidonyl arylamidase production in SDSE is characteristic [26].

**Molecular approaches.** Sequence analysis of the 16S rRNA gene allows a reliable species identification of *S. dysgalactiae*, and phylogenetic analysis shows that it is most closely related to *S. agalactiae* within the pyogenic group of streptococci [30]. For subspecies information on *S. dysgalactiae*, the analysis of the 23S rRNA gene appeared to be better suited to distinguish SDSD from SDSE [31]. In addition, amplification of a species-specific part of the 16S–23S rRNA intergenic spacer region allows a reliable species identification of *S. dysgalactiae* of Lancefield groups C, G, and L; however, its reliability for subspecies identification within *S. dysgalactiae* needs to be further evaluated [32]. Alternatively, identification and phylogenetic analysis of *S. dysgalactiae* can be achieved by sequencing of the superoxide dismutase gene [33, 34].

**VIRULENCE DETERMINANTS AND MICROBIAL PATHOGENESIS**

SDSE causes a similar spectrum of diseases in humans as does *S. pyogenes*, and molecular studies have described virulence determinants that are nearly identical to the known alleles in *S. pyogenes* or in other pyogenic streptococci [35] (table 2). Putative virulence determinants of SDSE include adhesins, toxins, and factors important for dissemination in human tissues and for interference with the host immune responses. The molecular events involved in the emergence of human invasive SDSE strains, however, are poorly understood. Besides a slow evolution through the accumulation of point mutations, the acquisition of genetic material through horizontal gene transfer may have increased bacterial fitness or conferred the ability to colonize a new ecological niche.

**Adhesins.** Adhesion to basement membrane components can mediate the colonization of damaged epithelium and can
facilitate the invasion of bacteria into the bloodstream. Pathogenic bacteria can interact with extracellular matrix components that are found ubiquitously in different tissues within the host. Several fibronectin-binding proteins (FnB, FnB, FnB, and FnB) have been described in S. dysgalactiae [36], and their presence has been shown to correspond to the capability of streptococci to adhere to human skin fibroblasts [37]. Plasmin(ogen) binding, which has been found in human groups C and G streptococci [38], is incriminated in tissue invasion processes. In addition to the sequences of 2 related M-like proteins, a gapC gene encoding a glyceraldehyde-3-phosphate dehydrogenase that also functions as a plasmin(ogen)-binding protein has been identified in the SDSE isolate H46A [39]. Moreover, a novel, strong plasmin(ogen)-binding protein SEN (streptococcal surface enolase) has been characterized [40]. Besides the interaction with fibronectin and plasminogen, SDSE binds specifically to the human S protein vitronectin [41], mediating the adherence to human epithelial and endothelial cells [42]. Another major component of basement membranes is the glycoprotein laminin. The gene lmb, which encodes a laminin-binding protein with structural features common to the streptococcal lipoprotein receptor family, was first identified in S. agalactiae [43]. A virtually identical copy of lmb is present in SDSE [34].

**Toxins.** The phenotype of β-hemolytic streptococci is caused by hemolysins. The gene slo, encoding the pore-forming streptolysin O of SDSE, is almost identical to that of S. pyogenes [44]. Thus, elevated antibody responses to streptolysin O are not specific to S. pyogenes or SDSE infections and do not have a protective role in the host. Streptolysin S is another cytotoxin that is present in groups A, C, and G streptococci. In SDSE, a functional homologue of the sag operon (from sagA to sagI) of S. pyogenes is presumably involved in streptolysin S production [45].

Streptococcal pyrogenic exotoxins (Spe) activate T cell receptor molecules in direct association with major histocompatibility complex class II on antigen-presenting cells and lead to massive T cell proliferation and release of inflammatory cytokines [46]. In S. pyogenes, the streptococcal pyrogenic superantigen SpeA has been associated with the pathogenesis of the streptococcal toxic shock syndrome [47], whereas the role of the newer superantigens has not been fully elucidated. Several streptococcal superantigen genes are located on mobile DNA elements, such as bacteriophages integrated in the bacterial genome [48]. Superantigen genes speA, speC [49], speM, ssa, and smezZ [50] have been found in SDSE strains and are nearly identical to S. pyogenes genes. In S. pyogenes, the superantigens SpeA and SpeC are considered to be responsible for scarlet fever. Only a single report of scarlet fever associated with group C pharyngitis has been published, but there are no data available on the superantigenic activity of the responsible isolate [51]. In SDSE, the gene speG<sup>769</sup>, with an 87% similarity to speG of S. pyogenes, and a high similarity between the genomic segment upstream of speG<sup>769</sup> and the corresponding region in S. pyogenes, was first identified by Sachse et al [52]. Subsequently, new alleles of speG<sup>769</sup>, with a higher similarity of 98% to known speG sequences, were detected in SDSE [53]. However, isolates harboring speG/speG<sup>769</sup> lacked expression of the respective genes and consistently revealed only low mitogenic activity [53]. Recently, a novel bacterial superantigen, the S. dysgalactiae−derived mitogen, has been characterized in 3 animal S. dysgalactiae strains but in none of 28 S. pyogenes strains [54].

A bacteriocin (dysgalactacin) has been identified in a single SDSE strain. It is directed primarily against S. pyogenes and presumably provides an ecological advantage in the common human microenvironment [55].

**Evasion of the host immune system.** The M protein, encoded by the emm gene, is a major virulence factor of S. pyogenes. Not only does it confer resistance to phagocytosis, but it also mediates adherence to and internalization into human epithelial cells, interferes with the coagulation system, and inhibits the complement cascade [56]. For groups C, G, and L streptococci, analysis of the corresponding genes revealed a polymorphism comparable to that in S. pyogenes, with currently >100 distinct stC, stG, and stL sequence types [57]. The relationship between the potential for invasiveness and the sequence type of SDSE isolates, which has been investigated by different groups, so far has not revealed any particularly invasive stC or stG sequence type [20, 54, 58]. In S. pyogenes, emm/emm-like genes are located in the mga regulon between the mga and scpA genes, whereas the genetic loci surrounding these genes in SDSE isolates are more heterogeneous and display mosaic structures [59]. In S. pyogenes, protective immunity is associated with antibodies against the hypervariable N-terminal portion of the M protein; a corresponding mechanism in SDSE infections is not fully elucidated.

Resistance to phagocytosis in S. pyogenes is primarily mediated by the M protein, which is also consistently found in human SDSE strains but is absent from any of the animal-associated group G streptococcal strains [60, 61]. Homologues of the S. pyogenes capsular synthesis genes are present in SDSE [62]; the role of the capsule for the pathogenicity of SDSE, however, has not been thoroughly studied.

The human chemotaxin C5a that recruits phagocytes to the site of infection can be destroyed by a streptococcal cell surface endopeptidase. The gene encoding this C5a peptidase has been found in S. pyogenes (scpA), S. agalactiae (scpB), and human group G β-hemolytic streptococci (scpG) [63], whereas group G streptococci isolated from dogs and cows lack C5a peptidase activity. scpG/scpC reveals significant similarities with both scpA and scpB [34, 63]. In contrast to S. pyogenes, in SDSE and S. agalactiae, the C5a peptidase gene is associated with mobile
genetic elements [34] but is not a member of the mga regulon [64].

Protein G of SDSE represents a unique type III immunoglobulin G (IgG) Fc receptor and reveals distinct binding sites for serum albumin and α1-macroglobulin [59]. The fact that protein G binds the heavy chains not only of IgG Fc but also of Fab fragments that are conserved among diverse species may explain its wide IgG species reactivity [65].

**Spread**. Streptokinase contributes to the dissolution of blood clots by activating plasminogen and is considered an important factor in the invasiveness of streptococci. Streptokinase has been isolated from human and animal groups C and G streptococci and also from *S. pyogenes*. It exhibits a strong specificity for the plasminogen of their respective hosts and, therefore, may be a determinant for the host range of streptococcal species [66]. The fact that *skc* and *skg* alleles of SDSE are closely related to the *ska* subcluster 2a of *S. pyogenes* that is strongly associated with throat isolates of *S. pyogenes* may possibly contribute to the throat tropism of SDSE. Streptokinase production of the SDSE strain H46A is so abundant that it has been used to commercially generate streptokinase activity.

**EPIDEMIOLOGICAL TYPING**

The most common serological typing scheme for SDSE is based on the antigenic specificity of the surface-expressed T and M proteins [58]. For molecular typing purposes, the N terminus of the M protein encoding *emm* genes is considered the reference standard. A large database has been developed by the Centers for Disease Control and Prevention and includes sequences of β-hemolytic groups A, C, G, and L streptococci [57]. For outbreak investigations, restriction-enzyme profiles have proved to be highly specific [67]. Molecular typing schemes on the basis of pulsed-field gel electrophoresis and random amplified polymorphic DNA have also been published for SDSE [68, 69]. For various streptococcal species, multilocus sequence typing emerged as a highly discriminatory epidemiological method, but a multilocus sequence typing scheme has not yet been developed for SDSE.

**ANTIMICROBIAL SUSCEPTIBILITIES AND TREATMENT STRATEGIES**

SDSE isolates remain almost uniformly susceptible to penicillin and other β-lactam agents, and penicillin is considered the drug of choice. Isolates with a slightly increased minimum inhibitory concentration of 0.25 μg/mL for penicillin were recently reported in both Europe and North America [70]. The clinical significance of this intermediate susceptibility remains uncertain, similar to the previously described phenomenon of penicillin tolerance among group C and G streptococci [71, 72]. To avoid delayed or poor responses of infections because of failure of penicillin, the addition of an aminoglycoside to the cell wall–active agent should be considered for serious infections. In more-aggressive SDSE infections, particularly those associated with streptococcal toxic shock syndrome, the patient may benefit from the addition of clindamycin for reasons such as bacterial viability, toxin production, or host response. Moreover, treatment with intravenous immunoglobulin preparations may reduce the inflammatory activity [73].

Macrolides play an important role in the treatment of streptococcal infections, particularly for patients with β-lactam hypersusceptibility. Resistance to macrolides in SDSE has been shown to be widespread in many countries, with rates up to 16% in Europe, 19% in the United States, and 24% in Hong Kong. Major geographical differences are associated with the 2 currently recognized mechanisms of macrolide resistance that have been previously detected in *S. pyogenes* or other gram-positive cocci [70, 74, 75]. The target site modification that is mediated by various *erm* genes has been found to be the predominant resistance type in group G streptococci from Europe [76, 77]. However, erythromycin-resistant group G streptococci from Asia and the United States showed a prevalence of *mef* genes [70, 78, 79]. The fact that a new *mef* sequence variant was recently identified in group G streptococci suggests that macrolide resistance continues to evolve [76].

Because of its excellent coverage, the recent generation of fluoroquinolones has been increasingly used in clinical practice, resulting in a noticeable increase in the resistance rate among viridans streptococci and *S. pneumoniae* during the past several years. Ongoing surveillance of the spread of resistance genes is required as the selective pressure for fluoroquinolone resistance continues among the adult population. In North America and Europe, the incidence of fluoroquinolone-resistant β-hemolytic streptococci (<1%) remains low [70]. In the SENTRY Antimicrobial Surveillance Program, all SDSE isolates remained susceptible to vancomycin, quinupristin-dalfopristin, and the newer agent linezolid [70]. Tetracycline no longer represents an option for the empirical treatment of SDSE isolates; resistance rates of ~60% and higher have been reported [70, 75].

To improve understanding of the changing epidemiology of and medical therapy for severe SDSE infections, all invasive β-hemolytic streptococci should be identified to the species level and accurately tested for antimicrobial susceptibility.

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**References**