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Streptococcus dysgalactiae subsp. *equisimilis* infection and its intersection with *Streptococcus pyogenes*

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SUMMARY *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) is an increasingly recognized cause of disease in humans. Disease manifestations range from non-invasive superficial skin and soft tissue infections to life-threatening streptococcal toxic shock syndrome and necrotizing fasciitis. Invasive disease is usually associated with co-morbidities, immunosuppression, and advancing age. The crude incidence of invasive disease approaches that of the closely related pathogen, *Streptococcus pyogenes*. Genomic epidemiology using whole-genome sequencing has revealed important insights into

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global SDSE population dynamics including emerging lineages and spread of anti-microbial resistance. It has also complemented observations of overlapping pathobiology between SDSE and *S. pyogenes*, including shared virulence factors and mobile gene content, potentially underlying shared pathogen phenotypes. This review provides an overview of the clinical and genomic epidemiology, disease manifestations, treatment, and virulence determinants of human infections with SDSE with a particular focus on its overlap with *S. pyogenes*. In doing so, we highlight the importance of understanding the overlap of SDSE and *S. pyogenes* to inform surveillance and disease control strategies.

KEYWORDS *Streptococcus*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae*, group C/G *Streptococcus*

INTRODUCTION

Streptococcus dysgalactiae subsp. *equisimilis* (SDSE) is an increasingly recognized cause of human infection. Almost all cases of large-colony, beta-hemolytic Lancefield group C/G streptococcal disease in humans are caused by SDSE (1, 2). SDSE is closely related to, and shares overlapping clinical manifestations with the more well-known pathogen, *Streptococcus pyogenes* (1, 3). The two pathogens occupy similar ecological niches on the human pharynx and skin, and genomic investigations have demonstrated evidence of extensive shared gene content, including many virulence factors and evidence of interspecies horizontal gene transfer (4–7). While *S. pyogenes* is considered exclusively a human pathogen, SDSE is also associated with animal infections. However, human-adapted SDSE strains are genetically distinct from those of animal origin and zoonotic infection is rare. Similarly, zoonotic infection by the other *Streptococcus dysgalactiae* subspecies, *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD), an important animal pathogen, is also rare (8).

SDSE disease can be divided into two broad categories: invasive disease, which is generally defined as a manifestation where SDSE is isolated from a normally sterile site such as the bloodstream or joint fluid, and non-invasive disease, such as pharyngitis or impetigo. Epidemiological studies from different regions have consistently demonstrated increasing incidence of invasive SDSE and group C/G streptococcal disease, predominantly in older adults, over the last two decades. Some regions report crude rates of invasive SDSE disease exceeding that of *S. pyogenes*, including multiple countries in Western Europe and East Asia (9–13). Despite increasing recognition of the burden of SDSE disease, routine genomic surveillance and an understanding of the molecular basis of virulence in SDSE lag behind those of *S. pyogenes*. With increasing efforts to control *S. pyogenes* disease including vaccine development (14, 15), a greater understanding of the genomic and clinical overlap between these two related pathogens is required.

In this review, we summarize the clinical and molecular epidemiology of SDSE, including disease pathogenesis, molecular virulence determinants, clinical presentation, and treatment with a particular focus on the clinical and genomic overlap between SDSE and *S. pyogenes*. As human- and animal-adapted strains of SDSE are distinct, we will focus on human-adapted SDSE strains and SDSE as it relates to human disease.

TAXONOMY AND MICROBIOLOGY

S. pyogenes and SDSE belong to the order Lactobacillales and family Streptococcaceae and are large-colony (>0.5 mm after 24 hours of incubation), beta-hemolytic organisms when cultured on blood agar. Both species grow well when incubated at 35°C–37°C in air, but growth can be enhanced with CO_2 enrichment (16). Identification of streptococcal species in clinical laboratories has traditionally been based on a combination of hemolysis pattern on blood agar, colony morphology, and the Lancefield carbohydrate antigen (16). The eponymous Lancefield antigens are cell wall carbohydrates expressed by beta-hemolytic streptococci, which can be used in bacterial identification by group-specific sera and have been central in streptococcal taxonomy (17).

Taxonomy

Classification of *S. dysgalactiae* has been subject to much confusion and change in the late 20th century. The use of hemolysis pattern on blood agar and Lancefield grouping for species and/or subspecies identification of *S. dysgalactiae* has been hampered by similar beta-hemolysis and carriage of Lancefield group C and G antigens in other streptococcal species such as *Streptococcus equi* and *Streptococcus canis* (3, 18).

S. dysgalactiae currently consists of two subspecies with their division proposed in 1996 and refined in 1998 based on phenotypic, multi-locus enzyme electrophoresis and DNA-DNA hybridization characterization: (i) SDSE, which are beta-hemolytic group C, G, or L isolates of human or animal origin, and (ii) SDSD with the Lancefield group C antigen, of animal origin and demonstrating alpha-hemolytic or non-hemolytic pattern on blood agar (19, 20). SDSE isolates carrying the Lancefield group L antigen are usually of animal origin and rarely cause human disease (8). However, exceptions to these

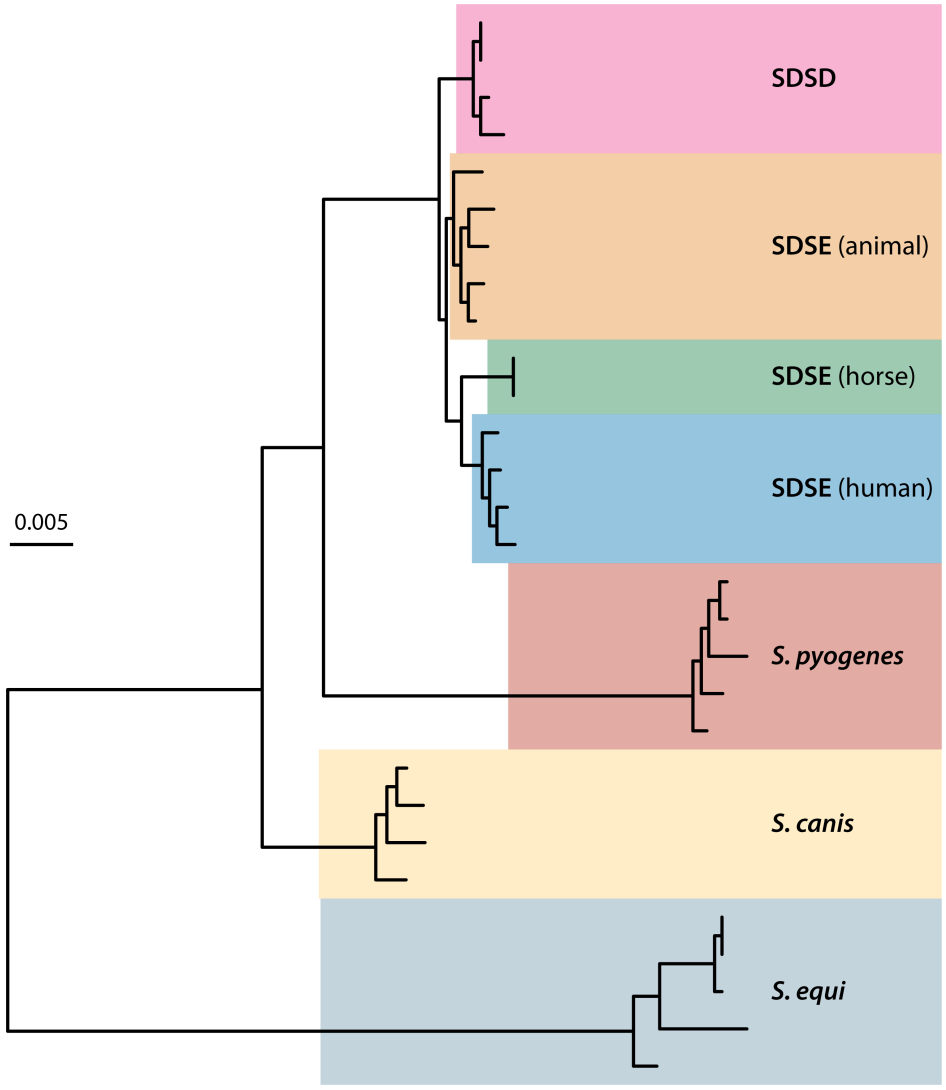

FIG 1 Midpoint-rooted phylogenetic tree of *Streptococcus pyogenes*, *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) from human and animal reservoirs, *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD), and other clinically relevant large-colony Lancefield group C/G streptococci. Strains of SDSE isolated from animal hosts are phylogenetically distinct from strains causing human disease. Phylogeny inferred from selected National Center for Biotechnology Information RefSeq genomes (Table S1) using GTDB (24) bac120 marker genes and FastTree (25) v.2.1.11 under the WAG model. Scale represents amino acid substitutions per site. SDSE (animal) clade includes strains isolated from dogs, pigs, cows, fish (tilapia), and rhinos.

definitions exist, and delineation of the *Streptococcus dysgalactiae* subsp. remains a topic of active discussion.

With the advent of whole-genome sequencing (WGS), there has been a growing appreciation of the close relationship between SDSE isolates from animal hosts and SDSD, and their distinction from human-adapted strains of SDSE (Fig. 1) (8, 21–23). At the whole-genome level, there appears to be phylogenetic separation of SDSE strains of human and animal origin, supported by carriage of distinct genetic repertoires of the two groups (22). It is likely that as more WGS becomes available, particularly of strains from animal hosts, there will be further refinements in the taxonomy of SDSE and associated subspecies.

Laboratory identification

While colony morphology, hemolysis pattern, and Lancefield grouping will correctly identify most cases to subspecies level according to criteria proposed by Vieira et al. in 1998, exceptions exist when compared to higher resolution speciation based on genomic classification (21). The Lancefield group A antigen has traditionally been uniquely associated with *S. pyogenes*. However, there are multiple reports of Lancefield group A carbohydrate-expressing SDSE, and therefore, it should not be used as a marker to delineate *S. pyogenes* from SDSE (26–30). Phenotypic analysis using the pyrrolidonyl aminopeptidase test in most cases can differentiate *S. pyogenes* from group A SDSE (Table 1).

The *Streptococcus anginosus* group may also carry the Lancefield group C/G antigen and demonstrate beta-hemolysis but can be differentiated from SDSE as they are small colony forming (<0.5 mm after 24 hours of incubation) and are Voges-Proskauer reaction positive (16, 18). Uncommonly, human disease can be caused by zoonotic beta-hemolytic streptococci carrying Lancefield group C/G antigens such as *S. equi* subsp. *zooepidemicus* (31) or *S. canis* (32, 33), which may be misidentified as SDSE if classified solely on colony morphology, hemolysis pattern, and Lancefield grouping (Table 1) (1, 18).

Automated commercial bacterial identification systems such as the VITEK system (GPI) can identify SDSE to subspecies level with varying accuracy, with one study reporting >93% correct identification when compared to 16S rRNA sequencing, which itself has limitations in identifying subspecies as discussed below (34). Other reports of the VITEK 2 Gram-positive identification card have found correct, high confidence identification of SDSE as low as 64% when compared to conventional phenotypic testing, although identification to species level was correct in >90% of cases (35).

Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry can only identify *S. dysgalactiae* to species level. However, evaluation of commercially available databases has demonstrated that confident identification of *S. dysgalactiae* may be as low as 51%–66% (36, 37). Frequently, a lack of identification to species level was due to difficulty distinguishing *S. dysgalactiae* from *S. canis* or *S. pyogenes*, with more than one species attaining a high confidence score (score >2) (36–38). Studies have suggested removal of select spectra in the commercial MALDI-TOF reference database may improve the proportion of correctly identified isolates (36, 38).

Sequencing of 16S rRNA can robustly distinguish different streptococcal species but can lack the resolution to delineate closer subspecies relationships between SDSE and SDSD. Human SDSE strains are distinct from SDSD on 16S rRNA sequencing. However, the 16S rRNA sequence from many SDSE isolates from animal hosts clusters with SDSD rather than human SDSE isolates (3, 23, 39). WGS has described a similar phenomenon where SDSE isolates from animal hosts appear host adapted and distinct from human SDSE isolates (8, 22). Despite its limitations, 16S rRNA sequencing remains useful for distinguishing *S. dysgalactiae* from other streptococcal species (3).

McMillan et al. proposed two gene targets as a molecular method for differentiating *S. pyogenes* and SDSE: (i) *speB* encoding a cystine protease which is present ubiquitously in *S. pyogenes* but absent in SDSE and (ii) a highly conserved open reading frame in the intergenic region upstream of the gene for C5a peptidase, *scpG*, in SDSE (40). Primers

TABLE 1 Phenotypic characteristics of clinically relevant Lancefield group A, C, and G streptococci[b]

Characteristic	*S. pyogenes*	*S. dysgalactiae* subsp. *equisimilis*	*S. dysgalactiae* subsp. *dysgalactiae*	*S. canis*	*S. equi* subsp. *zooepidemicus*	*S. anginosus* group
Lancefield group	A	A, C, G, L	C	G	C	A, C, G, F, or none
Hemolysis pattern	β	β	α or non-hemolytic	β	β	α, β, or non-hemolytic
Colony size[a]	Large	Large	Large	Large	Large	Small
Bacitracin susceptibility	+	–	–	–	–	–
Pyrrolidonyl aminopeptidase	+	–	–	–	-	–
CAMP reaction	–	–	–	+	–	–
Voges-Proskauer reaction	–	–	–	–	–	+
Hippurate hydrolysis	–	–	–	–	–	–
Trehalose	+	+	+	Variable	–	+
Sorbitol	–	–	Variable	–	+	–

[a]Large colony defined as >0.5 mm after 24 hours of incubation.
[b]Adapted from *Manual of Clinical Microbiology*, 13th Edition (16), and Facklam (18).

for these regions were evaluated in a diverse collection of *S. pyogenes* and SDSE isolates from endemic areas in Australia and India and were able to distinguish all 167 isolates tested (40). In a separate study, a multiplex PCR assay was designed to differentiate between human and equine-adapted strains of SDSE, targeting the 16S rRNA sequence combined with two sets of primers targeting the streptokinase precursor gene of SDSE (41). Validation across 17 SDSE strains from humans, 30 from horses, and 18 from dogs confirmed the targets could distinguish the strains tested (41). However, these methods have not been widely adopted.

Ultimately, accurate subspecies identification may not be possible in every case in clinical laboratories. Fortunately, this is unlikely to affect clinical management for an individual patient in most situations. Most cases of large-colony group C/G beta-hemolytic streptococci in humans will be caused by human-adapted strains of SDSE. Zoonotic infections with animal isolates of SDSE are rare. Depending on differing workflows at individual clinical laboratories, a combination of MALDI-TOF and biochemical testing should be able to differentiate *Streptococcus dysgalactiae* spp. from zoonotic streptococcal species and group A SDSE from *S. pyogenes*. Surveillance programs should, however, consider WGS for accurate subspecies identification.

SDSE PATHOGENESIS AND VIRULENCE DETERMINANTS

SDSE carries a repertoire of virulence factors to facilitate host colonization, invasion, immune escape, and dissemination in the case of invasive disease, generally defined as manifestations where SDSE is cultured from a normally sterile site (Fig. 2). These virulence factors span adhesion molecules targeting the host extracellular matrix, exotoxins, and immune evasion mechanisms. Many of these virulence factors have been discovered as homologs originally described in *S. pyogenes*, which may reflect a common origin or horizontal gene transfer (Table 2) (1, 42). For example, many exotoxins are carried on mobile genetic elements (MGEs) capable of horizontal transfer across species. Despite similarities, the repertoire of SDSE virulence factors has not been as comprehensively studied as *S. pyogenes*, and the sequence of events leading to invasion of normally sterile sites in the human host is poorly described. Additionally, shared virulence factors are not found in the bacterial population at the same frequency in each species. These differences have been hypothesized to underlie some of the clinical and epidemiological differences between the pathogens. The virulence repertoire of animal-adapted strains is not well studied, and the following section will focus on virulence factors described in human-adapted strains of SDSE.

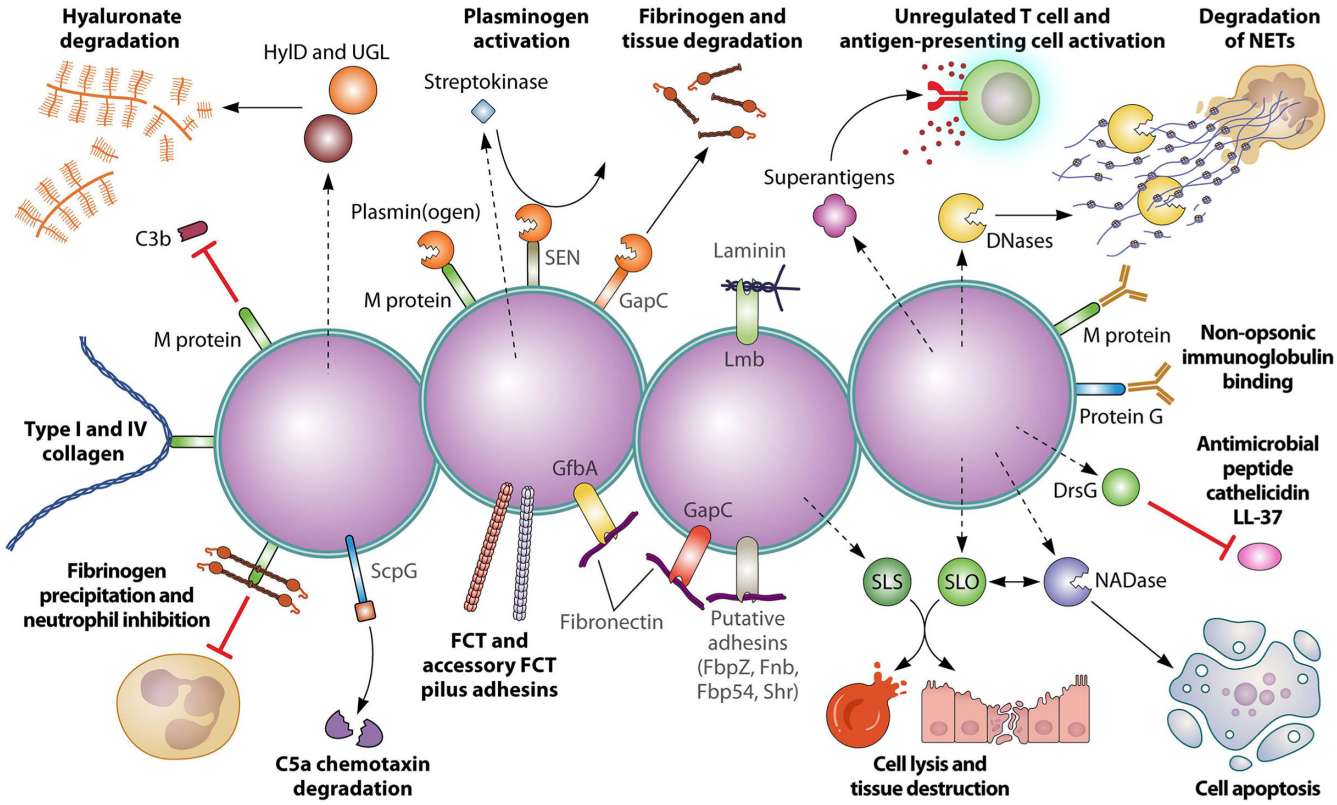

FIG 2 Virulence factors expressed by *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) to facilitate adhesion, immune escape, and invasion in the human host. The multi-functional M protein inhibits complement deposition, binds host IgG, acts as adhesin to fibrinogen and collagen, and binds plasmin(ogen), which can then be activated by bacterial streptokinase (Skc/Skg). Plasmin(ogen) is also bound by streptococcal surface enolase (SEN) and glyceraldehyde-3-phosphate dehydrogenase (GapC) and mediates fibrinogen and tissue degradation. C5a peptidase ScpG degrades the chemotaxin C5a. DNases such as Sdc degrade neutrophil extracellular traps (NETs). Protein G and M protein bind and inhibit IgG function. DrsG inhibits the innate anti-microbial peptide, cathelicidin LL-37. Pili encoded by two loci, FCT and a variably present accessory FCT locus, putatively mediate tissue adhesion to fibronectin and collagen. GfbA, GapC, and other putative adhesins mediate adhesion to fibronectin. Lmb putatively binds laminin and is near identical to a laminin-binding protein in *S. pyogenes*. Streptolysin O (SLO) and streptolysin S (SLS) cause cell lysis and tissue destruction. Nicotinamide glycohydrolase (NADase) causes host cell apoptosis and enhances SLO. Superantigens other than SpeG are uncommonly present but can cause uncontrolled inflammation through activation of T cells. HylD and unsaturated glucuronyl hydrolase (UGL) degrade hyaluronate in the host extracellular matrix.

M protein

SDSE expresses a surface M protein, encoded by the gene *emm*, which shares significant homology in its conserved C-terminal region to the M protein of *S. pyogenes* (43). Like the M protein of *S. pyogenes*, the M protein of SDSE is a cell surface multi-functional major virulence factor contributing to immune escape, adhesion, and host invasion (44, 45). While the M protein of SDSE is also predicted to form a coiled-coil structure, it is less well characterized than its counterpart in *S. pyogenes*. The function of the M protein in *S. pyogenes* is not homogenous, and genomic clustering of variants of the *emm* gene in *S. pyogenes* has been found to correlate with varied functions such as substrate binding (46). The same genomic or functional analysis has not been performed for variants of the M protein in SDSE, and the full function and the mechanisms underlying its virulence properties are incompletely understood.

Like the M protein of *S. pyogenes*, the N-terminal region of the SDSE M protein is hypervariable, and variants likely have different functional properties (45, 46). The hypervariable N-terminal region also forms the basis of a molecular typing scheme shared between SDSE and *S. pyogenes* strains, the *emm* type (47). Descriptions of the SDSE M protein have also been confounded by alternative names given to variants of

TABLE 2 Characterized virulence factors in *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) and their homologs in *Streptococcus pyogenes*[b]

SDSE virulence factor	SDSE gene name	*S. pyogenes* homolog	*S. pyogenes* gene name	Function
Multi-functional				
M protein	*emm* (*fog*)	M protein	*emm*	Adhesin, anti-phagocytic, plasminogen binding
Adhesins				
GfbA	*gfbA*	SfbI/PrtFI	*sfbI* and *prtF1*	Fibronectin binding
FCT pilus	FCT and accessory FCT	FCT pilus	FCT	Multi-functional adhesin
GapC	*gapC*	GAPDH/Plr	*plr*	Plasmin(ogen) binding and putative fibronectin binding
SEN	*sen*	SEN	*sen*	Plasmin(ogen) binding
Lmb[a]	*lmb*	Lmb	*lmb*	Laminin binding
Toxins and proteases				
Streptolysin S	*sagA*	Streptolysin S	*sagA*	Hemolysin and cytotoxin
Streptolysin O	*slo*	Streptolysin O	*slo*	Hemolysin and cytotoxin
Streptokinase	*skc, skg*	Streptokinase	*ska*	Plasminogen activation
Nicotinamide glycohydrolase (NADase)	*nga*	NADase	*nga*	Host cell energy depletion and augmentation of other virulence factors
SpeG	*speG, speG*^{dys}	SpeG	*speG*	Superantigen toxin
SpeA, SpeC, SpeI, SpeM, SSA, and SmeZ	*speA*, *speC*, *speI*, *speM*, *ssa*, and *smeZ*	SpeA, SpeC, SpeI, SpeM, SSA, and SmeZ	*speA*, *speC*, *speI*, *speM*, *ssa*, and *smeZ*	Superantigen toxin infrequently found in SDSE
Phospholipase A2[a]	*slaA*	Phospholipase A2	*slaA*	Cytotoxin
Hyaluronate lyase	*hylD*	Hyaluronate lyase	*hyl*	Nutrient acquisition from host extracellular matrix
Unsaturated glucuronyl hydrolase	*ugl*	Unsaturated glucuronyl hydrolase	*ugl*	Nutrient acquisition from host extracellular matrix
Immune escape				
Streptodornase	*sdc* (*sdg*) and *spd1*	Streptodornase	*sdn*, *spd1*	DNase
C5a peptidase	*scpG* (*scpC*)	C5a peptidase	*scpA*	Complement degradation and anti-phagocytic
Protein G	*spg*	–	–	Immunoglobulin G binding
DrsG	*drsG* (*sicG*)	SIC-DRS	*drs*	Resistance to host innate anti-microbial peptides

[a]Not experimentally verified but shares almost 100% identity with gene in *S. pyogenes*.
[b]Alternative gene names are given in parentheses.

the protein including references to it as fibrinogen-binding protein of G streptococci (FOG) or an M-like protein (48–50). Some lineages of *S. pyogenes* carry additional M-like proteins, Mrp and Enn, which share structural and functional similarity with the M protein (51). These additional proteins have not been described in SDSE.

A variant of the SDSE M protein, FOG (GenBank accession number AY600861.1), resembles *emm* sequence type (ST) *stG*11. Presence of FOG has been shown to be essential for survival *in vitro* in blood compared to an *emm*/FOG-negative strain and was found to form aggregates mediated by binding of fibrinogen at the N-terminal domain of the protein (50). Precipitation of fibrinogen by FOG contributed to the inhibition of neutrophil function possibly through mechanisms leading to neutrophil exhaustion (50). FOG has also been shown to bind to the Fc region of human IgG subclasses IgG1, IgG2, and IgG4 at its C-terminal domain, as well as IgG from multiple animal species (52). However, unlike IgG binding by another virulence factor protein G, FOG still permitted the binding of complement C1q in non-immune sera (52). The significance of this interaction is not clear and does not override the overall immune escape functions of the protein.

The SDSE M protein is capable of mediating resistance to phagocytosis through inhibition of the alternative complement pathway (53, 54). In *S. pyogenes*, alternative

complement inhibition occurs through binding of complement-inhibitory proteins such as factor H and binding of the Fc domain of immunoglobulins by the *S. pyogenes* M protein (55). Factor H binding and immunoglobulin binding have also been demonstrated by SDSE M proteins and localized to the conserved C-terminal domain (50).

The SDSE M protein has been shown to bind plasma proteins such as plasminogen, fibrinogen, and albumin (48, 56). M proteins carrying a plasminogen-binding domain at its N-terminal end, can activate bound plasminogen by streptokinase (48). This interaction may facilitate invasion and completement escape through the serine protease and fibrinolytic activity of activated plasmin and may even facilitate adherence to host cells (56, 57).

Ex vivo experiments have indicated the importance of the SDSE M protein in adhesion to human skin mediated by binding to collagen I (58). Some M proteins are also able to bind collagen IV and facilitate colonization through a motif in the N-terminal hypervariable region, "peptide associated with rheumatic fever" (PARF), so named because it has been implicated in autoimmunity and acute rheumatic fever (ARF) in *S. pyogenes* (45). Such binding to type IV collagen was found for more than 10 distinct SDSE *emm* types and representative of 11% of SDSE isolates from a study of predominantly non-invasive disease-causing isolates in Vellore, India (45). It is not known if the PARF motif contributes to autoimmunity in SDSE, and its role in *S. pyogenes* remains controversial.

Unlike *S. pyogenes*, where clinical syndromes are associated with particular *emm* types in some geographical settings, there has been no consistent correlation between SDSE *emm* type and disease manifestation or outcome (55). Possible associations have been reported in case series such as an association between *stG*2078, *stG*10, *stG*485, and *stG*6 with invasive disease, *stG*6792, *stG*166b, *stC*36, and *stC*839 with non-invasive isolates and mixed findings for *stG*480 (3, 59, 60). Conflicting reports of both higher mortality and a lack of association with invasive disease caused by *stG*6792 have been published in Japan, where it was the most isolated *emm* type in clinical samples (11, 12). Other studies comparing non-invasive and invasive cases have found no association between *emm* type and site of disease (61–63). Therefore, there has not been a consistent correlation between *emm* type and clinical manifestation or outcome. However, correlations are limited by small isolate numbers, differences in study design, and heterogenous strains in different countries. Additionally, in SDSE, the same *emm* type has been found in multiple genetic backgrounds as defined by multi-locus sequence type (MLST) and WGS and may therefore obscure disease associations when used in isolation (7, 64–67). The incorporation of WGS in disease surveillance will assist with delineating differences between lineages and clinical associations.

In *S. pyogenes*, the *emm* gene, which encodes the M protein, lies within the *mga* regulon with genes encoding additional M-like proteins such as *mrp* and *enn*, and *scpA* encoding a C5a peptidase (68). The arrangement of genes making up the *mga* regulon determine the M pattern in *S. pyogenes*, which has been associated with tissue tropism (69). There is a homologous region designated *mgrC* (multi-gene regulon-like genomic segment) in SDSE which appears to exist in two forms: (i) a small form consisting of *nrd*, a gene encoding a putative ribonucleotide reductase, *mgc*/*mgg*, a virulence factor regulator, *emm*, and *rel* a protein involved in the synthesis and degradation of guanosine 3′,5′-bipyrophosphate; and (ii) a large form consisting of *nrd*, *mgc*, *emm*, *cpdB*, a putative 2′,3′-cyclic-nucleotide 2′-phosphodiesterase, and *rel* (Fig. 3). A homolog of the C5a peptidase gene, *scpG*, is located remote to *mgrC* unlike *scpA*, which lies within the *mga* regulon in *S. pyogenes* (70, 71). SDSE does not encode additional M-like proteins in the *mgrC* regulon. No association between *mgrC* type and isolation from blood or non-invasive sites has been reported (71). In a data set with limited geographical diversity, organization of the *mgrC* regulon was correlated with *emm* types, but some *emm* types were present in both variants (71).

Adhesins

In addition to the M protein, SDSE expresses several other cell surface adhesion factors targeting components of the human extracellular matrix such as fibronectin, fibrinogen, collagen, and laminin. These factors are critical to host binding, colonization, and invasion. Many of these factors are homologous to adhesion proteins found in *S. pyogenes*.

SDSE possesses regions homologous to the FCT locus in *S. pyogenes*, which consists of genes encoding fibronectin-binding proteins, collagen-binding proteins, and T antigens (pilus structures) (72–74). Unlike *S. pyogenes*, SDSE isolates carry two FCT loci which have been referred to as the FCT and accessory FCT locus. The FCT locus is present in all isolates, whereas the accessory FCT locus is variably present in 80%–90% of isolates (7, 73). A similar dual FCT arrangement is also present in *Streptococcus agalactiae* (73). Both the FCT and accessory FCT loci encode putative pili structural proteins and sortases for pilus assembly and cell wall anchoring (Fig. 4) (73, 74). The FCT locus may also encode additional fibronectin-binding proteins. The putative pilus structures of the SDSE FCT loci have not been experimentally characterized.

The organization of genes in the primary FCT loci among 53 invasive isolates in Norway led Oppegaard et al. to propose three types of the primary FCT locus (originally named the FCT_1 locus but referred to as the "primary FCT" or "FCT" locus here to avoid confusion with *S. pyogenes* FCT patterns) and a single type of accessory FCT (originally named FCT_2) (Fig. 4) (73). However, a comprehensive analysis of FCT patterns in global SDSE and non-invasive isolates has not been performed, and there is likely an additional uncaptured variation.

The primary FCT locus variably encodes a fibronectin-binding protein, GfbA, near its 5′ end with homology to the *S. pyogenes* fibronectin-binding protein Sfbl/PrtFI. The gene encoding GfbA is variably present in approximately 10%–50% of isolates (73, 77, 78). GfbA has been shown to bind human skin fibroblasts through fibronectin in the presence of regions homologous to the fibronectin-binding repeat domains of PrtF (78). Like *sfbl*/*prtF1* in *S. pyogenes*, *gfbA* in SDSE lies immediately downstream from a positive-acting transcriptional regulator *rofA*, which regulates multiple pathways in *S. pyogenes* but has yet to be characterized in SDSE (74, 77). Most isolates also contain a putative fibronectin-binding gene, *fbpZ*, at the 3′ end of the locus (73).

In *S. pyogenes*, FCT organizational types share linkage with *emm* patterns and may have a role in the tissue tropism reported with *emm* patterns (75). It is unclear whether this also holds for SDSE. A study of invasive SDSE isolates manifesting as osteoarticular infections in Norway found genetic variation in the primary FCT region correlated with *in vitro* binding to collagen and fibronectin (73). However, these differences did not predict

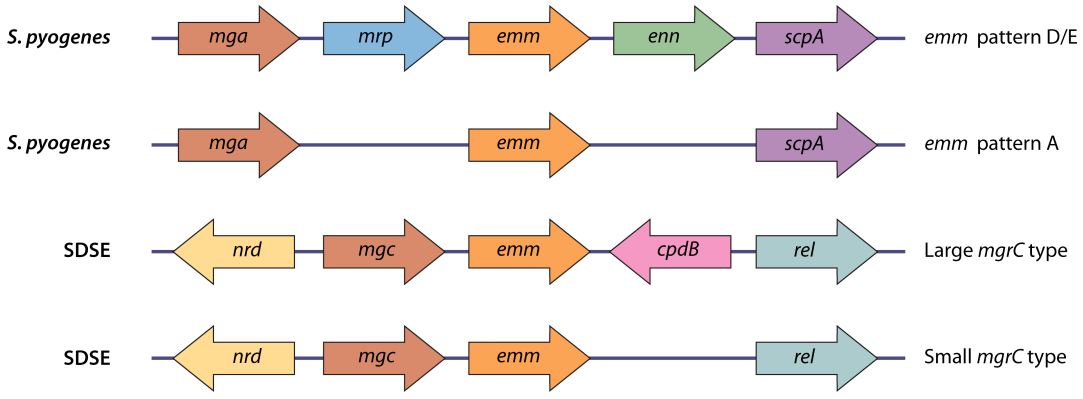

FIG 3 Schematic organization of the *mgrC* regulon of *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) in comparison to the two most common configurations of the *mga* regulon in *S. pyogenes*. Homologous genes share the same color. The direction of the arrow indicates the orientation of the gene. A homolog of *scpA*, *scpG*, is located distant to the *mgrC* regulon in SDSE. Schemes are based on Frost et al. (51) and Geyer et al. (70).

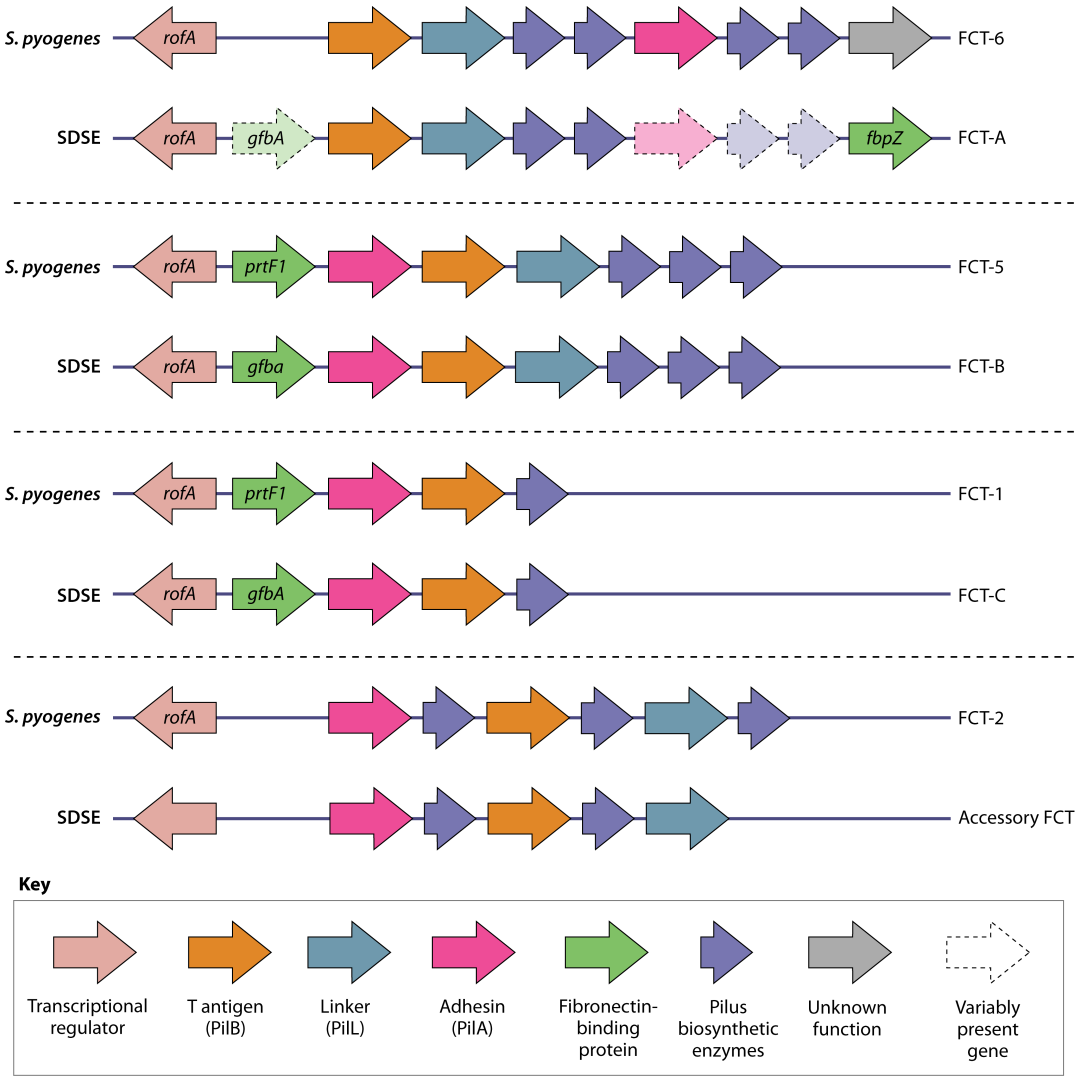

FIG 4 Genetic arrangement of the FCT and accessory FCT loci in *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) in comparison to homologous FCT types found in *S. pyogenes*. All SDSE strains carry the FCT locus, and 80%–90% carry the accessory FCT locus. Pilus biosynthetic enzymes include sortase genes. Schemes are based on Kratovac et al. (75), Nakata and Kreikemeyer (76), and Oppegaard et al. (73).

clinical manifestations when compared with invasive isolates from patients without osteoarticular manifestations.

Among other adhesins in SDSE, glyceraldehyde-3-phosphate dehydrogenase (GapC) and an alpha-enolase [streptococcal surface enolase (SEN)] bind plasminogen and plasmin (79, 80). The gene, *gapC*, shares 95% sequence identity with *plr*, a plasminogen and fibronectin-binding protein in *S. pyogenes* (79). However, binding of fibronectin by GapC has not been experimentally characterized. Alpha-enolase, named SEN, is a glycolytic enzyme found in most beta-hemolytic streptococci and is shared by SDSE and *S. pyogenes* (80). The binding of plasminogen and plasmin to SEN appears stronger than the interaction with *plr* and therefore likely also *gapC* and provides pathogenic streptococci with multiple pathways for plasminogen binding (80).

SDSE and *S. pyogenes* also bind to human vitronectin, a glycoprotein component of the extracellular matrix. However, the protein mediating binding has not been defined (81). Binding of vitronectin mediates epithelial and endothelial adhesion and may also play a role in immune invasion as vitronectin inhibits the formation of the complement membrane attack complex (81, 82).

Other adhesion molecules predicted by sequence homology in SDSE include putative fibronectin-binding proteins *fnb*, *fbp54*, and *shr* (65, 73, 83). A fibronectin-binding protein, FnB, has been characterized in an animal group C streptococcal isolate, which was reported as *S. equisimilis* (84). However, this sequence appears distinct from the putative *fnb* in human SDSE. The gene encoding a laminin-binding protein, *lmb*, has also been detected in SDSE and is present in almost all human isolates (4, 65, 73, 83, 85). The sequence of *lmb* is highly conserved and is shared between *S. pyogenes*, *S. agalactiae*, and SDSE (85).

Toxins

Bacterial toxins are thought to be essential in mediating many disease manifestations in SDSE and *S. pyogenes*, particularly invasive disease, or severe manifestations such as necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). After colonization, many of these factors are integral to tissue invasion and dissemination of infection.

Cytolytic/pore-forming toxins

Like in *S. pyogenes*, SDSE expresses two secreted cytolytic toxins, streptolysin S and streptolysin O. The clear zone of beta-hemolysis around colonies of SDSE on agar media is mediated by the oxygen stable streptolysin S (SLS). The gene encoding SLS, *sagA*, lies within the *sag* operon with other genes encoding proteins for post-translation modification and export (from *sagA* to *sagI*) and is homologous to the *sag* operon in *S. pyogenes* (86, 87). The *sagA* gene shares approximately 90% identity with that in *S. pyogenes* (87). In a murine necrotizing fasciitis model, knockout of the *sagA* gene in SDSE isolates from patients with necrotizing fasciitis attenuated disease and resulted in lower tissue bacterial loads with less histologic inflammation (87). Other postulated functions of SLS facilitating invasion include quorum sensing, paracellular invasion, host immune cell lysis, and iron acquisition (88). However, given that SLS in present in almost all strains, its presence likely does not explain possible variable invasive potential of different strains (88).

The second hemolysin, streptolysin O (SLO) is encoded by *slo*, a thiol-activated cytolysin, and shares >99% amino acid homology to that of *S. pyogenes* (89). SLO is inactivated by oxygen and is not responsible for the beta-hemolytic phenotype. Increased SLO expression in invasive compared to non-invasive SDSE isolates has been implicated in human keratinocyte cell lines and a three-dimensional skin model as responsible for keratinocyte cytotoxicity (90). In *S. pyogenes*, SLO prolongs bacterial survival in human macrophages by facilitating escape from endosomes and, in synergy with nicotinamide glycohydrolase (NADase), has been shown to prolong intracellular survival in human oropharyngeal keratinocytes (91, 92).

NADase, encoded by *nga*, is located within the NADase-streptolysin O operon in *S. pyogenes* and is also present in the same configuration in SDSE (74, 93). Experimental data in *S. pyogenes* demonstrate a close interaction between secreted SLO and NADase to enhance virulence through improved stability of the toxins and SLO-mediated delivery of NADase to cause direct cell toxicity via depletion of host NAD (94, 95). NADase activity has been implicated in the pathogenesis of STSS in *S. pyogenes*, and a SDSE mutant expressing elevated NADase activity has also been described in a case of STSS (96).

Prophage encoded secreted streptococcal phospholipase A2 (*slaA*), which has been associated with the emergence of M3 *S. pyogenes* outbreaks, has also been described rarely in SDSE with the same nucleotide sequence (97, 98). In *S. pyogenes*, phospholipase A2 has activity against multiple phospholipid head and acyl groups and has been implicated in pharyngeal colonization and invasive disease in animal models (98, 99). The significance of *slaA* in SDSE pathobiology is unclear but is unlikely to be significant, given its rarity in SDSE.

Superantigens

More than 15 superantigen exotoxin genes have been described in *S. pyogenes* and are thought to be important for disease manifestations such as STSS and scarlet fever. Not all superantigen genes have been identified consistently in SDSE and, when found, are infrequent except for *speG* (4, 61, 100, 101). Streptococcal pyrogenic exotoxins are secreted superantigens with the ability to stimulate large proportions of T cells by activation of MHC molecules on host CD4 cells independent of the antigen-binding cleft resulting in an uncontrolled immune response (55). Superantigens SpeA and SpeC have also been implicated in facilitating nasopharyngeal infection in *S. pyogenes* (102–104). Most superantigens in *S. pyogenes* are located on prophages integrated in the bacterial genome except a few chromosomally encoded factors such as *smeZ*, *speG*, and *speJ* (105). Despite their physiological effects, associations between disease severity or outcome and the superantigen repertoire for *S. pyogenes* have been mixed (55). Reports of infrequent SDSE isolates carrying *speA*, *speC*, *speH*, *speI*, and *smeZ* by PCR amplification or whole-genome sequencing have been reported, but consistent associations between virulence repertoire and disease manifestations have not been found (4, 27, 97, 106, 107). SDSE isolates have been described with two alleles of *speG*: (i) an allele which is 98% similar to *speG* found in *S. pyogenes* and (ii) an allele, occasionally named *speG*^{dys}, which shares approximately 85% nucleotide identity to *speG* in *S. pyogenes* (100, 101). *speG* or *speG*^{dys} are the most frequently detected superantigen genes in SDSE in up to 65% of invasive and non-invasive isolates (4, 7). However, presence of *speG/speG*^{dys} in SDSE may not produce a significant exotoxin or superantigen effect in humans. *In vitro*, no *speG* or *speG*^{dys} mRNA was detected in a collection of predominantly invasive isolates with findings supported by little or no mitogenic activity of the same SDSE isolates compared to *S. pyogenes* strains (101). Additionally, expression of recombinant *speG*^{dys} alleles in *Escherichia coli* demonstrated little stimulation of human peripheral blood mononuclear cells (108). These findings suggest that the superantigen repertoire of SDSE is unlikely to be solely responsible for its virulence and clinical presentations.

Secreted enzymatic toxins

Streptococcal interaction with host plasminogen and plasmin has been extensively studied as a pathogenic factor. Streptokinase is present in almost all SDSE isolates and activates plasminogen to degrade clots and tissue barriers after secretion to facilitate invasion and spread. The gene encoding streptokinase in SDSE, variably named *skc* and *skg* after isolation from group C and G SDSEs, respectively, is highly conserved (>95% nucleotide identity) in human-adapted strains and is homologous to *ska* in *S. pyogenes* (109–112). The streptokinase genes are present in a conserved region downstream of the *mgrC* or *mga* regulon in SDSE and *S. pyogenes*, respectively (70). An invasive isolate of SDSE isolated from a patient with necrotizing fasciitis was found to produce higher levels of streptokinase than comparator *S. pyogenes* isolates, and its activity was necessary to cause fibrinolysis (113). SDSE *skc*/*skg* show high sequence identity (>95% nucleotide identity) with one of two major clusters of streptokinase alleles in *S. pyogenes*, specifically a cluster associated with throat colonization, suggesting possible throat tropism in SDSE reflected by high pharyngeal colonization rates in population studies (110). SDSE streptokinase also displays strong host preference with differential activity demonstrated by human, porcine, and horse isoforms in species-matched plasma and may be a key factor in determining host-pathogen specificity (114, 115). Supporting host specificity of streptokinase, amino acid identity of streptokinase from SDSE isolated from animal hosts is as low as 25% compared to streptokinase secreted by human-adapted SDSE strains or strains from heterologous animal hosts (115).

SDSE secretes a hyaluronate lyase, HylD, and unsaturated glucuronyl hydrolase, which are upregulated in infection and have been shown *in vivo* to be more effective at hyaluronate degradation than homologs in *S. pyogenes* and *S. agalactiae* (116). Hyaluronate is a major component of the human extracellular matrix. Laboratory knockout of *hylD* in SDSE attenuated virulence in a murine severe sepsis and skin

wound model (116). Since SDSE lacks a hyaluronic acid capsule, which is present in many *S. pyogenes* strains, this suggests a potentially SDSE-specific virulence pathway for acquiring nutrients from the host.

A notable virulence factor found in most isolates of *S. pyogenes*, the cysteine proteinase SpeB, is not present in SDSE. SpeB participates in immune escape through cleavage of immunoglobulins and anti-microbial peptides and is implicated in invasive disease through degradation of host proteins (55). SpeB also appears critical in murine models of *S. pyogenes* disease and is implicated in models of cutaneous infection (117–119). No verified reports of *speB* in SDSE have been published, and again this indicate an important divergence in pathways of disease pathogenesis in SDSE compared to *S. pyogenes*.

Virulence regulation

Transcriptional regulators acting via two-component systems have been described in SDSE and influence *in vitro* and *in vivo* disease phenotypes. Characterized regulator operons in SDSE display significant homology with *S. pyogenes*. In a global data set of SDSE isolates, 13 two-component regulators originally described in *S. pyogenes* were found to have homologs in SDSE with 10 of 13 found in >99% of isolates (7).

The CovRS/CsrRS two-component regulatory system is one of the most well-described virulence regulators in SDSE and *S. pyogenes*. *covRS* and *fasCAX* are opposing two-component regulators in SDSE which control multiple virulence factors including *covRS*-mediated repression and *fasCAX*-mediated induction of SLS and streptokinase (96, 120). Knockout of *covS* in SDSE upregulates expression of SLS and hemolysis in a murine model (121). *covRS* has also been implicated as a negative regulator of *emm*, *scpG*, *nga*, *lmb*, and *spg* in SDSE (121, 122). Regulation by the *covRS* system in SDSE also extends to genes involved in carbohydrate metabolism (121). These findings mirror the function of *covRS* in *S. pyogenes*, which has been shown to regulate approximately 15% of genes including repression of *speA*, *sagA*, *ska*, *sda1*, and capsule genes while upregulating *speB* (123). The *covR* and *covS* genes of SDSE and *S. pyogenes* share over 95% amino acid identity. The genes in the *fasCAX* operon of SDSE similarly share significant homology with the *fasBCA* operon of *S. pyogenes* (120).

The strongest producer of streptokinase found to date is from SDSE strain H46A, which carries a stop mutation in *covR*, resulting in a derepressed phenotype (120). Natural *covRS* mutants have also been detected in *S. pyogenes* and are associated with upregulation of virulence genes and murine models (124, 125). An SDSE mutant with an Ala105Asp mutation in *covR* has been co-isolated with a wild-type strain with the same *emm* and MLST in a patient with STSS (96). The *covR* mutant exhibited six times more NADase activity than the wild-type isolate, and the hyperproduction was attenuated with the introduction of a wild-type *covR* gene (96). In *stG*6792 ST17 isolates which had caused STSS in Japan, heterogenous mutations in *covRS* were detected in 19% of isolates and associated with increased expression of *sagA* (122). Conversely, no mutations were detected in non-invasive isolates of the same *emm* and MLST suggesting *covRS* mutations may partially contribute to invasive phenotypes (122).

An additional regulatory small RNA named *srrG* immediately upstream of *sagA* has been described in *stG*6792 ST17 STSS isolates in Japan. This small RNA appears to be a negative regulator of the *sag* operon, and mutations in *srrG* were found in 14% of isolates in a collection from Japan, mostly in those without *covRS* mutations (122). In contrast, no *srrG* mutations were found in non-invasive strains (122). Isolates with mutant *srrG* demonstrated a similar increase in SLS expression and mortality in a murine model compared to isolates with mutations in *covS* (122).

The frequency of *covRS* mutations in clinical isolates of SDSE has only been analyzed in limited data sets, and its influence on global lineages is uncertain (83, 96, 121). However, as the aforementioned study described isolates of the same *emm* and MLST but found heterogenous mutations in *covRS* and *srrG*, it has been suggested that many of these mutations may develop *in vivo* after invasion rather than being lineage

specific (122, 125, 126). Selection of *covRS* mutations after invasion has previously been described in *S. pyogenes* (125). This is also supported by the simultaneous finding of both mutant and wild-type *covR* SDSE mutants in the bloodstream a patient with STSS (96).

The streptococcal invasion locus, *sil*, is another two-component regulatory system in SDSE which acts as a negative regulator of virulence genes in the presence of the peptide pheromone, SilCR (127). The *sil* operon consists of a putative two-component system encoded by *silA* and *silB*, ABC transporter genes *silD* and *silE*, a small peptide encoded by *silC* which has been linked with virulence, and *silCR*, which encodes a peptide resembling a bacterial pheromone peptide (128, 129). The *sil* locus demonstrates high identity between SDSE and *S. pyogenes* but is not present in all isolates. The presence of the *sil* locus was found in most invasive SDSE isolates from a retrospective collection in Jerusalem compared to only 25% of corresponding invasive *S. pyogenes* isolates (127). In a global collection of SDSE genomes, the *sil* locus was found in approximately 80% of isolates across both invasive and non-invasive strains (7). When present, the *sil* locus of SDSE and *S. pyogenes* may interact within and across species through SilCR-mediated quorum sensing (130). On microarray transcriptional analysis, SilCR can upregulate genes encoding bacteriocins with cross-species activity (130, 131). In keeping with its negative regulatory function, presence of secreted SilCR in a murine SDSE necrotizing fasciitis model significantly attenuated infection and mortality (127). In *S. pyogenes*, a clinical M14 strain isolated from a patient with necrotizing fasciitis harbored a missense mutation in the start codon of *silCR* (132). When exposed to exogenous SilCR, the isolate demonstrated attenuated virulence and chemokine proteolysis (132). Deletion of *silA* and *silB* in *S. pyogenes* has been associated with upregulation of *sagA*, possibly through unopposed action of SilC with an inability to sense SilCR (130). Interestingly, an emerging invasive disease-associated *stG*62647 ST20 SDSE lineage in Norway carries an insertion sequence (IS) element in *silB* which may disrupt signaling by SilCR (83, 130). However, experimental verification of the impact of the IS element in the *stG*62647 ST20 strain has yet to be described. Taken together, the differential presence of an intact *sil* operon between SDSE and *S. pyogenes* could also contribute to differences in virulence and clinical presentations between the two pathogens.

Mutations in the stand-alone regulator *ropB* in the *rgg* family of regulators have been described in *S. pyogenes* and implicated in the downregulation of *speB* (133). However, like *speB*, *ropB* is not present in SDSE. Other regulators implicated in *S. pyogenes* phenotypic variation, including *mga* and *rofA/nra*, have homologous genes in SDSE as *mgg*/*mgc* and the regulators in the FCT loci, respectively (7). In fact, 24 of 28 of *S. pyogenes* stand-alone regulators have been found to have homologs in a global database of SDSE genomes (7). However, their influence on SDSE gene expression and phenotype has not been experimentally characterized.

Immune escape

Extracellular DNases (streptodornase) secreted by *S. pyogenes* and SDSE are frequently encoded by genes present on prophage elements. Multiple DNases have been described in *S. pyogenes*, and some isolates carry multiple DNase genes. SDSE strains have been described to carry between zero and two DNase genes (7). The first DNase gene described in SDSE was *sdc*, which shares 48% homology with *sdaD* and 98% with *sdn* in *S. pyogenes* (74, 134, 135). Other putative DNases have since been described in SDSE based on sequence homology, including *sda1*, *spd1*, and *spnA* (7, 27, 74, 136). DNases, best exemplified by the *S. pyogenes* Sda1, facilitate immune escape through degradation of DNA-based neutrophil extracellular traps, suppression of Toll-like receptor 9 host immune responses and macrophage killing (126, 137, 138). However, the role of DNases in SDSE disease pathogenesis has not been independently experimentally determined.

C5a is a major chemotaxin of the human innate immune system. *scpG* encodes a surface, cell wall anchored C5a peptidase which is present in all human SDSE isolates and shares significant homology with *scpA* in *S. pyogenes* (85). Similar to ScpA in *S. pyogenes*,

ScpG cleaves C5a and interferes with complement-mediated neutrophil phagocytosis (139).

Protein G encoded by *spg* is present in all human isolates of SDSE and is a well-described virulence factor contributing to immune escape. In fact, protein G is used widely in laboratories for IgG purification. It is expressed on the surface of SDSE and binds the heavy-chain Fc and Fab domains of all classes of human IgG with high affinity (140). Through this interaction, protein G interferes with IgG-mediated activation of C1q and activation of the classical complement pathway (52, 140). Protein G is also able to bind serum albumin and the human proteinase inhibitors kininogen and alpha-2-macroglobulin to prevent cleavage of surface virulence determinants (141, 142). Different isolates of SDSE carry variants of *spg* with varying numbers of repeats (143). Not only does protein G bind human IgG, but it is also able to interact with IgG from a wide range of animal hosts including rabbits, mice, goats and cows likely reflective of common ancestry from a multi-host predecessor to modern lineages of SDSE (144). A separate protein in *S. pyogenes*, protein G-related alpha-2-macroglobulin-binding protein or GRAB, has sometimes been mentioned as a possible homolog of protein G but only shares an alpha-2-macroglobulin-binding domain and otherwise has little sequence or functional similarity (145).

Anti-microbial peptides such as cathelicidin LL-37 are a critical component of the human innate immune system. SDSE secretes a protein, DrsG, which shares sequence similarity with distantly related to SIC (DRS) in *S. pyogenes*, and like DRS, inhibits the anti-microbial peptide cathelicidin LL-37 (146). DrsG has also been described as SicG in the literature (147, 148). DrsG does not inhibit complement-mediated lysis of sheep erythrocytes (146). Several *drsG* alleles have been described, which mostly differ in the length of repeat units and have been found to be present in strains from at least 14 *emm* types without a clear association with clinical phenotype (146, 147). Unlike SIC and DRS which are restricted to a small number of *emm* types in *S. pyogenes* but are universally present within those *emm* types, *drsG* was variably present in SDSE strains with the same *emm* type (83, 146). However, at a WGS resolution which overcomes issues of *emm* type carriage across different lineages, *drsG* was found to be enriched across related strains (7).

Polysaccharide or polypeptide capsules are major immune evasion and virulence factors in many bacteria including multiple streptococcal species such as *S. agalactiae* and *S. pneumoniae*. They play important roles in immune evasion through inhibition of phagocytosis and complement binding and may contribute to virulence through mechanisms such as host-binding (149). Hyaluronic acid capsule is a key virulence factor mediating inhibition of opsonophagocytosis in *S. pyogenes* and plays a role in early pharyngeal colonization (150). *S. pyogenes* encodes hyaluronic capsule synthesis through the *hasABC* operon consisting of *hasA*, *hasB*, and *hasC* (151). *hasC* has been detected in SDSE but does not harbor the entire *hasABC* operon responsible for hyaluronic acid capsule synthesis (4, 74). Previous reports of a hyaluronan synthase gene in *S. equisimilis* describe a gene with >99% nucleotide identity to *S. equi* and has not been found in modern SDSE strains studied using WGS.

GENETIC OVERLAP AND EXCHANGE BETWEEN SDSE AND *S. PYOGENES*

SDSE has a median genome length of 2.1 Mbp and GC content of 39.5% (7, 152). This is slightly larger than a median length of 1.8 Mbp and GC content of 38.5% for *S. pyogenes* (153). An analysis of a global collection of 501 SDSE and 2,083 *S. pyogenes* genomes found that 75% of SDSE and 88% of *S. pyogenes* core genes (genes present in nearly all strains of a species) were shared across species in conserved genomic locations (7). The genetic similarity between the two organisms provides a background that may facilitate genetic exchange across species. The exchange of virulence or regulatory genes may propagate shared virulence pathways and disease phenotypes. Similarly, cross-species exchange of anti-microbial resistance genes may enable dissemination of resistance.

The genomic similarity between SDSE and *S. pyogenes* and their cohabitation within similar ecological niches in the human host, the throat, and skin provides the conditions and opportunity for horizontal gene transfer (HGT) (6). Indeed, multiple examples of HGT including homologous recombination and gene transfer between the species inferred from gene sequence similarity and shared MGEs harboring virulence factors and anti-microbial resistance genes have been described (5–7). Additionally, several *S. pyogenes* vaccine candidates share homologs in SDSE including 12 which were present in >99% of a collection of global SDSE and *S. pyogenes* genomes (7, 55, 153). Therefore, HGT may influence not only genome diversity and bacterial adaptation in the face of environmental and host pressures but also coverage and diversity of vaccine candidate antigens across both species (7).

Recombination

HGT has classically been described to occur by three mechanisms. Transformation involves uptake of exogenous DNA from the environment followed by homologous recombination; transduction occurs through phage-mediated transfer and integration of genetic material; and conjugation uses direct transfer by conjugation machinery often encoded by plasmids or integrative conjugative elements (ICEs) (154). Although highly suggestive evidence of recombination has been reported, neither *S. pyogenes* nor SDSE is naturally competent for transformation under standard laboratory conditions (155). However, both organisms carry genes predicted to encode the full competence system and analysis of a globally diverse collection of *S. pyogenes* genomes, and a separate analysis of SDSE suggests that recombination is likely responsible for much of the observed genomic variation (7, 153, 156). *S. pyogenes* has been demonstrated to exhibit competence under biofilm conditions but at much lower rates than other streptococci such as *S. pneumoniae* (157). It is therefore possible that despite difficulty achieving competence *in vitro*, recombination via transformation can occur *in vivo*, giving rise to the observed genomic patterns.

HGT involving homologous recombination of whole genes occurs between SDSE and *S. pyogenes*. Alleles of the *emm* gene usually associated with SDSE, such as *stG*1750, *stG*485, and *stG*643, have been found in *S. pyogenes*, and similarly, *emm* genes from *S. pyogenes* such as *emm*12 have been detected in SDSE (60, 158). SDSE and *S. pyogenes* share six of seven MLST genes with some alleles shared across species. Identical alleles of MLST genes *recP* and *xpt* have been described in both species and a phylogenetic tree of *recP* was unable to separate the species (66). Additionally, alleles of *gki* and *mutS* found in some SDSE isolates share greater homology with alleles described in *S. pyogenes* than those in other SDSE strains (64). These findings provide strong evidence of gene exchange between SDSE and *S. pyogenes*.

Analysis of shared core genes found that 34% (393 of 1,166) had evidence of cross-species recombination events (7). These genes were spread across the entire genome without any hotspots or enrichment of gene functional classes. Overall frequency and net directionality of transfer could not be inferred, but the findings suggest that interspecies homologous recombination can occur across much of the SDSE and *S. pyogenes* genome.

Predicted interspecies recombination has also been reported within genes implicated in anti-microbial resistance and virulence factors. Fluoroquinolone resistance in *S. pyogenes* and SDSE is mediated by mutations in *gyrA* and *parC*, which are components of DNA gyrase and topoisomerase IV, respectively. An analysis of quinolone resistance-determining regions in SDSE isolates found *parC* alleles which clustered with sequences from *S. pyogenes* rather than SDSE (159). Examination of alignments of *parC* suggested interspecies recombination events involving part of the gene gave rise to the observed sequences (159). The FCT region in *S. pyogenes* is a known hotspot of recombination (75). Although recombination analysis over the entire length of the two FCT regions in SDSE has not been studied, evidence of recombination has been detected between

homologous genes encoding fibronectin-binding proteins, *gfbA* in SDSE and *sfbI* in *S. pyogenes* (77).

Recombination of genomic segments covering multiple genes has also occurred and is likely responsible for the emergence of the Lancefield group A carbohydrate in SDSE. The group A carbohydrate consists of a polyrhamnose backbone with a glycerol phosphate-modified *N*-acetylglucosame side chain and is encoded by the conserved *gacA-gacL* operon (160, 161). Within this operon, *gacI*, *gacJ*, *gacK*, and *gacL* are essential for side-chain synthesis, and deletion of *gacI* attenuates virulence and increases susceptibility to innate immune clearance (160). While SDSE with group G or C carbohydrates carries a homologous *gac* operon, the composition and number of genes in the operons differ between each other and that of the group A carbohydrate (7, 27, 160, 162). This reflects the different side chains of the group C and G polysaccharides and a backbone of alternating a-1,3-linked rhamnose and galactose in the group G polysaccharide rather than polyrhamnose in groups A and C (162–165). Comparison of the *gac* operon between a group A ST184 SDSE isolate, group G strain GGS_124, and *S. pyogenes* strain MGAS5005 suggested a recombination event involving the entire *gac* operon (160). An analysis of group A ST128 SDSE isolates from USA suggested a distinct recombination event involving a recipient group C SDSE isolate and most of the *gac* operon between *gacE* and *ebsA*, two genes downstream of *gacL* (27). While additional isolates of group A SDSE from Japan are very closely related to reported genomes from the USA, a report of azithromycin resistant group A SDSE from the Gambia are from an unrelated ST (30). Globally sampled group A SDSE isolates appear to belong to at least four distinct lineages (7). These findings suggest that more than one recombination event may have occurred between *S. pyogenes* and SDSE to give rise to distinct group A SDSE lineages.

Evidence has also emerged of recombination involving the highly conserved penicillin-binding protein (PBP) genes of *S. pyogenes* and SDSE. Despite decades of widespread penicillin use, no cases of penicillin-resistant *S. pyogenes* have ever been reported, and there has only been a single report of four penicillin-resistant SDSE isolates suggesting an as yet undefined constraint in acquiring resistance in *S. pyogenes* and SDSE (166). *S. pyogenes* carries four high-molecular-weight PBP genes, *pbp1a*, *pbp1b*, *pbp2x*, and *pbp2a*, while SDSE encodes the same PBP genes with >90% amino acid identity to those in *S. pyogenes* and an additional gene, *pbp2b* (167). Unlike *Streptococcus pneumoniae* where mutations in PBP genes are frequently acquired by recombination with commensal viridans group streptococci, most variation in PBP genes in *S. pyogenes* occurs by non-synonymous mutations (167–169). In particular, amino acid substitutions near the active site motifs in PBP2x are associated with an increase in penicillin MIC but remain below clinical breakpoints (167, 169–175). An analysis of over 26,000 publicly available whole-genome sequences of *S. pyogenes* identified rare divergent *pbp1b* and *pbp2x* alleles which demonstrated segments of similarity to SDSE alleles suggestive of interspecies recombination (170). An *emm*81 *S. pyogenes* isolate from France with 55 amino acid mutations in *pbp2x* from likely recombination including A551V, M593T, and G600D in the transpeptidase enzymatic domain was associated with a threefold rise in penicillin MIC without a clear loss in fitness (176). Additionally, rare *S. pyogenes* isolates have been identified with a *pbp2b* gene identical to alleles found in SDSE (170). The phenotypic effects of acquisition of *pbp2b* in *S. pyogenes* are unclear. Although some isolates with mildly elevated penicillin MICs have been documented, PBP dynamics are complex and may involve contributions of multiple PBPs to achieve high-level resistance or compensation for fitness costs as demonstrated in *S. pneumoniae* (177, 178). *S. pyogenes* and SDSE may be limited in their ability to acquire multiple mutations simultaneously through recombination due to a lower degree of competence than *S. pneumoniae*. Nevertheless, penicillin resistance has been documented in SDSE, which may have greater tolerance for mutations due to the presence of an additional PBP compared to *S. pyogenes*. PBP changes acquired in SDSE may serve as a reservoir of gene

exchange with *S. pyogenes*. However, large-scale genomic analyses of PBP variation in SDSE have not been performed.

Vaccine candidate genes

Recent efforts to progress a *S. pyogenes* vaccine have intensified as a key pillar of *S. pyogenes* disease control (14, 15). There are currently no SDSE-specific vaccine candidates for humans. A J8 peptide vaccine encoding a conserved region of the M protein has been the only candidate to be tested against human SDSE strains in a mouse model (179). Studies on *S. dysgalactiae* vaccines have largely focused on animal disease and SDSD rather than SDSE. However, early-stage animal vaccine candidates such as alpha-enolase, which was highly efficacious in a cobia fish challenge model (180), and GapC epitopes which are immunogenic in mice (181), have high identity homologs in human SDSE and *S. pyogenes* strains and may serve as a model for human candidates.

In the setting of extensive genomic overlap between the two species, 12 leading *S. pyogenes* vaccine candidate genes were found to be present in almost all isolates of both species (7). The predicted amino acid sequence of the SDSE alleles were highly conserved and most demonstrated >90% similarity to the *S. pyogenes* vaccine sequence (7). Although theoretical coverage by 5 of 11 leading preclinical multi-component vaccines was predicted to be >99% in both SDSE and *S. pyogenes*, a correlate of protection or potential cross-protection between alleles is lacking for confirmation (7).

Shared vaccine antigen genes suggest possible cross-species effects with these candidate vaccines. Conversely, cross-species recombination could provide a reservoir of increased antigenic diversity particularly in the context of vaccine selection pressures. In this context, six vaccine candidate genes, which were present in >99% of isolates in both species, demonstrated evidence of interspecies recombination (7). It is anticipated that with further surveillance and WGS, evidence of cross-species recombination may also become evident in other vaccine candidates.

Mobile genetic elements

In addition to recombination between SDSE and *S. pyogenes*, MGEs such as prophages and ICE contribute to most of the gene content variation between strains for each species (7, 153). Reports from the 1970s demonstrated that a phage isolated from group G streptococci cultured from human pharynx was able to transduce group A streptococci (182, 183). Systematic mapping of sites where MGEs insert into the bacterial genome (insertion regions) across the two species found that 29% (16 of 55) of SDSE MGE insertion regions were also present in *S. pyogenes* (7, 184). Clustering of MGE sequences at these shared regions found that 25% of the clusters were shared across the species, suggesting possible broader sharing of MGE families than previously appreciated (7). These findings are mirrored by earlier observations of homologous MGE segments/fragments across species which likely extend beyond SDSE and *S. pyogenes* to include other organisms such as SDSD (22).

Several examples of near identical MGEs have been found in both species. The prophage ΦMGAS5005.3 carries a streptodornase gene, *sda1*, which is thought to be important in the emergence of one of the most successful modern *S. pyogenes* lineages M1T1 (138, 185). A near identical prophage has been found at a conserved MGE insertion region across multiple SDSE lineages supporting cross-species exchange and dissemination (7). Investigation of group A SDSE isolates in the USA identified an isolate carrying the exotoxin gene, *speC*, and the DNAase gene, *spd1*, on a prophage element highly similar to that found in *S. pyogenes* (7, 27). Several other prophage and ICE carrying anti-microbial resistance genes such as *tet*(M), *mef*(A)/*msr*(D), and *erm*(B) have been found at varying levels of identity across species and may be particularly prevalent in settings with extensive co-circulation of the two species (6, 186, 187).

While prophage content may be shared across SDSE and *S. pyogenes*, prophage-related exotoxin genes are rarely found in SDSE, suggesting there are constraints on phage-mediated HGT (5, 7). In *S. pyogenes*, up to half of the accessory gene pool is

contributed by prophages, and individual isolates may carry an average of two to four (7, 105, 153). SDSEs carry less prophage elements than *S. pyogenes* with an average of one per genome (7, 188). SDSE and *S. pyogenes* carry type II-A and I-C CRISPR arrays which are variably present and provide adaptive defense against foreign DNA such as phages (189). However, the mean number of spacers encoding target sequences for the CRISPR arrays are up to four times greater in SDSE than *S. pyogenes*, possibly contributing to the restricted prophage repertoire in SDSE (74). It is also unknown what role possible phage receptors such as the hyaluronic acid capsule, which is only present in *S. pyogenes*, or restriction-modification systems have in cross-species prophage differences.

ICE and ICE-like elements including integrative mobilizable elements may provide a greater contribution to accessory genome variation in SDSE and contribute to the dissemination of anti-microbial resistance genes (7, 186, 190–192). SDSEs on average carry more ICE than *S. pyogenes* genomes (7, 193). *In vitro* evidence of interspecies transfer is supported by an ICE, ICE*Sde*3396, from SDSE, which was shown to be mobilizable cross-species to *S. pyogenes* and *S. agalactiae* (194). Another ICE*Sde*3396-like element has been shown to mediate in *trans* mobilization of a small plasmid carrying the macrolide resistance gene *erm*(T) between SDSE and *S. pyogenes* (195). Almost identical plasmids have been described in *S. pyogenes* and *S. agalactiae* (195). However, small plasmids are uncommon in SDSE, and although they may carry anti-microbial resistance determinants, they do not contribute significantly to the accessory gene pool (196).

In summary, there is mounting evidence of ongoing gene transfer between SDSE and *S. pyogenes* involving homologous recombination, transduction, and conjugation. HGT between the species can facilitate the transfer of virulence and/or AMR genes as well as potential broadening of protein diversity, which could have implications for vaccine design and impact. As increased diversity of SDSE WGS is obtained, it is likely that even greater HGT between SDSE and *S. pyogenes* will be uncovered, particularly if surveillance is integrated to provide co-collected cohorts of both pathogens.

EPIDEMIOLOGY

Clinical incidence

Since the 1980s, there has been improved identification of SDSE with increasing recognition of it as a cause of human disease. In recent decades, there have been numerous epidemiological reports of increasing incidence of SDSE disease. However, historic comparisons are confounded by changing taxonomy and imprecise identification of *Streptococcus dysgalactiae* subsp. Furthermore, group C and G streptococci or SDSE were often separated, although there is no systematic difference in disease phenotype or virulence potential between the two groups for SDSE. However, as most human isolates of large-colony, beta-hemolytic, Lancefield group C/G streptococci are SDSEs, this review will include both in the following section (2, 10, 197).

Epidemiological studies reporting incidence since the 1990s have demonstrated a consistent increase in invasive group C/G *Streptococcus* or SDSE disease in many regions including Asia, Australia, Europe, and North America (2, 9, 10, 198–202). Recent studies describe an annual incidence of invasive disease in some regions up to 7.6 cases per 100,000 population per year, which is similar to and, in some cases, exceeds that of *S. pyogenes* (2, 9, 198). However, in contrast to *S. pyogenes*, which has a bimodal age distribution affecting the extremes of age, invasive SDSE predominantly affects older adults (2, 10–12, 197, 203). In Western Norway, SDSE was the fifth most common cause of bloodstream infection in 2021 (2). In Japan, SDSE has been responsible for more than twice the number of bloodstream infections as *S. pyogenes* (11, 12). The increase in invasive SDSE incidence in some jurisdictions may be associated with an aging population and/or increasing prevalence of co-morbidities associated with SDSE disease such as diabetes (12, 67, 198, 202). Despite multiple studies from high-income countries, reports of the epidemiology of invasive disease in low- and middle-income countries have been limited (204, 205). Data from India and Fiji suggest that the incidence of *S.*

pyogenes disease may be four to five times higher than SDSEs in these regions which traditionally are considered hyperendemic for *S. pyogenes* disease (204, 205).

Estimates of incidence of non-invasive disease is much less certain. Most epidemiological studies focus on invasive disease and non-invasive isolates are often collected as convenience samples or from a single anatomical site such as the pharynx. Reports of pharyngeal carriage and incidence of pharyngitis are mixed and may reflect differences between study settings. Pharyngeal carriage is discussed in further detail in the section "Clinical Presentation and Transmission." Retrospective laboratory-based surveillance over 15 years in Western Norway suggested a possible decline in incidence of group C/G streptococci in non-invasive samples up to 2013 (9). However, the cause of the decline was unclear, and the findings have not been replicated elsewhere.

Epidemiological markers

The most widely used molecular epidemiological marker for SDSE is the *emm* sequence type (*emm* type) determined by 180 nucleotides of the *emm* gene consisting of the hypervariable 150 nucleotides encoding the first 50 codons of the mature surface M protein and 30 nucleotides encoding 10 codons of the conserved signal sequence (49). The typing scheme is identical to that used for *S. pyogenes* and correlates with, and has now supplanted, traditional serological typing of the M protein (47). Isolates are grouped into an *emm* type (e.g., *stG*10) when nucleotide identity of the hypervariable sequence shares ≥92% identity to an existing *emm* type and a new *emm* subtype assigned if there is no exact match within that *emm* type (e.g., *stG*10.1) (206). As of May 2022, there were more than 100 *emm* types and 380 *emm* subtypes assigned to SDSE (206).

Based on a similar method used for *S. pyogenes*, T-typing performed by antibody agglutination directed at the T pilus antigen was also historically used for epidemiological typing of SDSE (207, 208). However, using commercially available type-specific sera designed for *S. pyogenes*, studies showed that SDSE isolates were often non-typeable, and T-typing is no longer a widely used method for epidemiological typing (10).

MLST is a traditional approach designed to estimate the population structure for many bacterial pathogens, including SDSE (209). The MLST scheme for SDSE uses seven housekeeping genes: *gki*, a glucose kinase; *gtr*, a glutamine transport protein; *murI*, glutamate racemase; *mutS*, a DNA mismatch repair protein *recP*, a transketolase, and *xpt*, a xanthine phosphoribosyl transferase, which are shared with *S. pyogenes* (64, 197). The seventh gene is *atoB* (acetoacetyl-coathioloase), also known as *yqiZ* but renamed to reduce confusion with *yqiL* in *S. pyogenes*, which occupies a different locus. Each combination of alleles is assigned a unique ST and maintained in a centralized database by PubMLST (209).

As a multi-gene approach, MLST provides greater resolution than *emm* type as a molecular epidemiological marker. Individual STs are generally restricted to a single-group carbohydrate, but the same is not true of *emm* type (64). Each *emm* type can be present across multiple STs but show better correlation with MLST single-locus variant clusters, also known as clonal complexes (64, 66). However, the same *emm* allele can also be found on very different genetic backgrounds, potentially as the result of recombination (7, 64, 66, 67). Conversely, MLST clusters or clonal complexes can also contain multiple *emm* types and be represented across multiple genetic backgrounds when compared to WGS suggesting they also may not be lineage specific (7, 64, 66, 67). Despite the limitations of *emm* and MLST alone, when applied to defined geographical regions and time periods, such as in jurisdictional surveillance studies, strains denoted by a ST and *emm* combination generally correlate with WGS lineages.

However, *emm*, MLST and even MLST clonal complexes demonstrate limitations within a global context. MLST designations do not factor the genetic distance between two alleles; one nucleotide difference in an MLST allele is designated as a new allele just as would an allele with a dozen mutations. Whole-genome clusters or lineages assigned by WGS provide the greatest resolution as assessment often includes both total gene content and sequence variation (7). Multiple STs can be found within a

single WGS cluster or lineage, and conversely, MLST clonal complexes may span multiple whole-genome clusters (7). Additionally, the MLST loci and the *emm* gene are affected by homologous recombination, which may obscure epidemiological inferences based on these traditional markers in the global context (7, 64). However, it should be noted that *emm* type and MLST retain significant utility in allowing historical comparisons and in settings where WGS may not be available.

Molecular epidemiology

There is diversity among *emm* types described by epidemiological studies globally of invasive and non-invasive disease with 18 distinct *emm* types represented when counting the five most frequent *emm* types from each region (Fig. 5). Within each region, the top five *emm* types consist of approximately 50% or less of circulating isolates. This is indicative of multiple circulating strains associated with both colonization and disease. However, within a region, strains carrying certain *emm* types may predominate, such as *stG*6792 in Japan since 2006 or *stG*62647 in Western Europe in the last decade (11, 12, 63, 67, 83, 107, 202, 210–212). Although trends may be evident in *emm* types in some regions, sampling of SDSE is heavily biased to a small number of countries in Eastern Asia, Europe, Australia, and Northern America, and to invasive disease. This limits the true understanding of global diversity, particularly in low- and middle-income regions. Reports of *emm* type diversity have only been reported from just over 20 countries with no reports from the continent of Africa except for a single study reporting 10 group A SDSE isolates from the Gambia (30). Additionally, as noted in the previous section, *emm* type and even MLST have limitations as epidemiological markers for SDSE and do not describe factors which may be associated with the emergence of novel lineages.

WGS has increasingly been used for pathogen surveillance. WGS is routinely used in *S. pyogenes* surveillance in several health jurisdictions in the USA and the UK. The

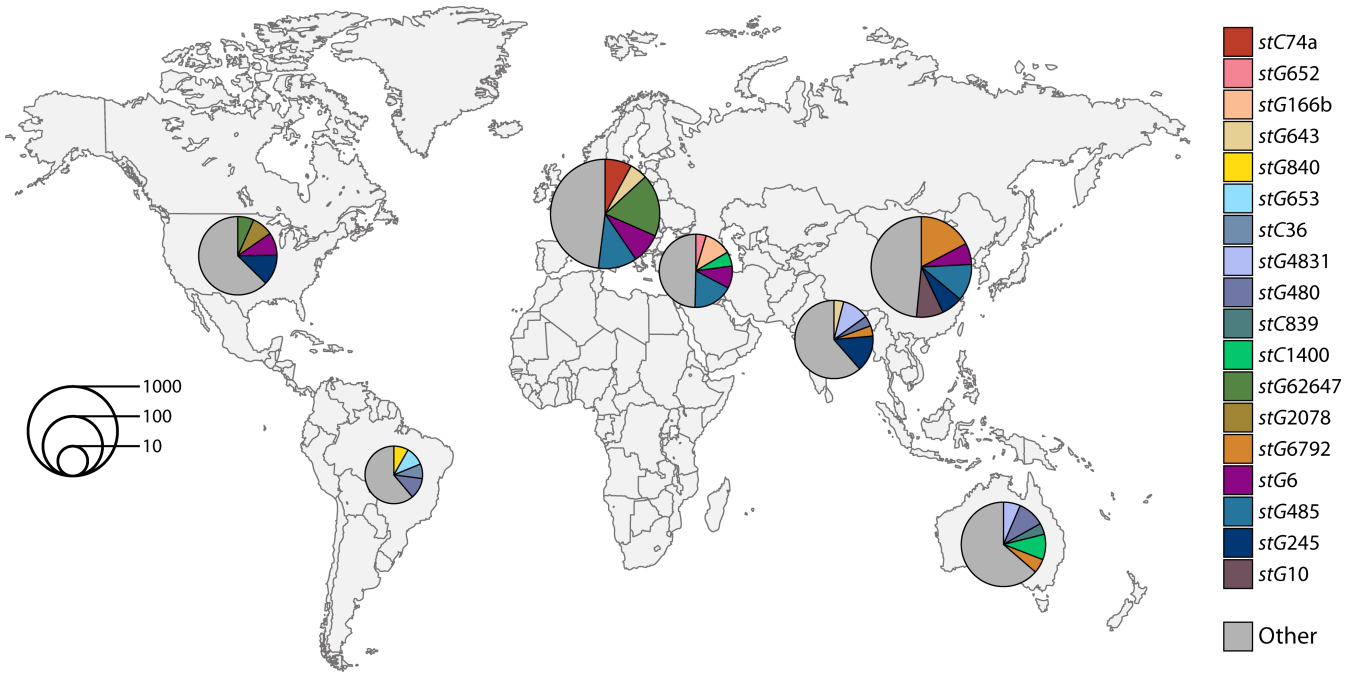

FIG 5 *Streptococcus dysgalactiae* subsp. *equisimilis emm* type diversity from published population or healthcare center-based epidemiological studies describing ≥20 SDSE or large-colony, beta-hemolytic group C/G *Streptococcus* isolates. Only the five most frequent *emm* types in each region are highlighted with all remaining *emm* types grouped as "other." Studies were grouped by region: Western Europe (2, 3, 10, 60, 62, 67, 97, 107, 159, 202, 213–218) (16 studies, *n* = 4,618), Western Asia (219, 220) (2 studies, *n* = 284), Southern Asia (221, 222) (2 studies, *n* = 420), Eastern Asia (11, 12, 61, 71, 192, 210–212, 223–226) (12 studies, *n* = 2,344), Oceania (4, 227, 228) (3 studies, *n* = 677), Northern America (65, 66, 197) (3 studies, *n* = 423), Southern America (229–231) (3 studies, *n* = 90). There were no eligible studies from Africa.

discovery of the hypervirulent $M1_{UK}$ lineage, which differed by only 27 single-nucleotide polymorphisms from the prevalent $M1_{global}$ lineage, was only discovered by WGS as the two lineages otherwise shared the same *emm* type and MLST (232, 233). Longitudinal WGS surveillance of SDSE has thus far been largely academic led yet has yielded new insights into SDSE genomic epidemiology.

Oppegaard et al. reported increasing incidence of a lineage carrying the *emm* type, *stG*62647, since 2013, which was potentially associated with more severe clinical manifestations in Norway (2, 83). Strains carrying this *emm* type have also emerged to be the most frequent cause of invasive SDSE disease in multiple other jurisdictions in Western Europe and North America (63, 65, 202, 213). Isolates from Norway were associated with a disrupted gene in the regulatory *sil* locus, which is a known negative regulator of multiple virulence genes (83). Isolates carrying *stG*62647 from France were shown to be closely related and also carried a disrupted *sil* locus, indicating that the disrupted regulator may be a lineage characteristic (234). In a mouse necrotizing myositis model, *stG*62647 isolates caused greater mortality and were associated with higher bacterial loads in comparison to a *stC*74a isolate without the *sil* locus (234). However, the strains compared were not closely related, and therefore phenotype-genotype associations could not be drawn. The drivers of the emergence of the lineage carrying *stG*62647 as a cause of invasive disease remain to be elucidated.

WGS has also been used to characterize the emergence of lineages associated with anti-microbial resistance. Analysis of SDSE bloodstream isolates from hospitals in the Kyoto-Shiga region of Japan from 2005 to 2021 detected an increase in macrolide resistance (192). While isolates carrying the *stG6792 emm* type were historically the most frequent cause of invasive disease in the region, WGS of isolates delineated the clonal emergence of a *stG*840 MLST525 lineage with macrolide resistance and tetracycline resistance genes (*erm*B and *tet*M, respectively) carried on a Tn916-like ICE (192). The authors speculated that the emergence of the new lineage in the region may be related to selective pressures of increased antibiotic usage in Japan.

Increasing use of WGS promises to provide greater resolution for the understanding of transmission and emergence of global SDSE lineages and may also shed light on pathogen biology, genotype-phenotype associations, and factors underlying bacterial population dynamics.

CLINICAL PRESENTATION AND TRANSMISSION

SDSE was historically considered a colonizing bacterium and an uncommon cause of opportunistic infection before increasing awareness in the 1980s of its capacity to cause human disease (1, 208). SDSE is commonly isolated from the throat and intermittently among other sites such as the the skin, the gut, and the female genital tract (1, 208, 235). SDSE has been reported to cause a spectrum of illness overlapping with *S. pyogenes*, including cellulitis, tonsilitis, necrotizing fasciitis, STSS, septic arthritis, osteomyelitis, meningitis, post-partum sepsis, pneumonia, and endocarditis (Fig. 6) (9, 11, 12, 73, 74, 87, 214, 235–240).

Transmission

Transmission of SDSE occurs from human to human. WGS reconstruction of transmission chains has demonstrated transmission within and between households from asymptomatic throat colonization (6). Transmission of strains associated with invasive disease within a household have been described although less well established than for invasive *S. pyogenes* (241). Data are currently insufficient to indicate if household contacts are at significantly increased risk of invasive infection and if anti-microbial prophylaxis in close contacts is warranted for invasive SDSE disease. In addition to community outbreaks of pharyngitis, nosocomial outbreaks have been described in hospital burn units, wound clinics, and maternity units with group C/G *Streptococcus* (208). However, modern nosocomial outbreaks appear uncommon, and there are no established healthcare screening guidelines.

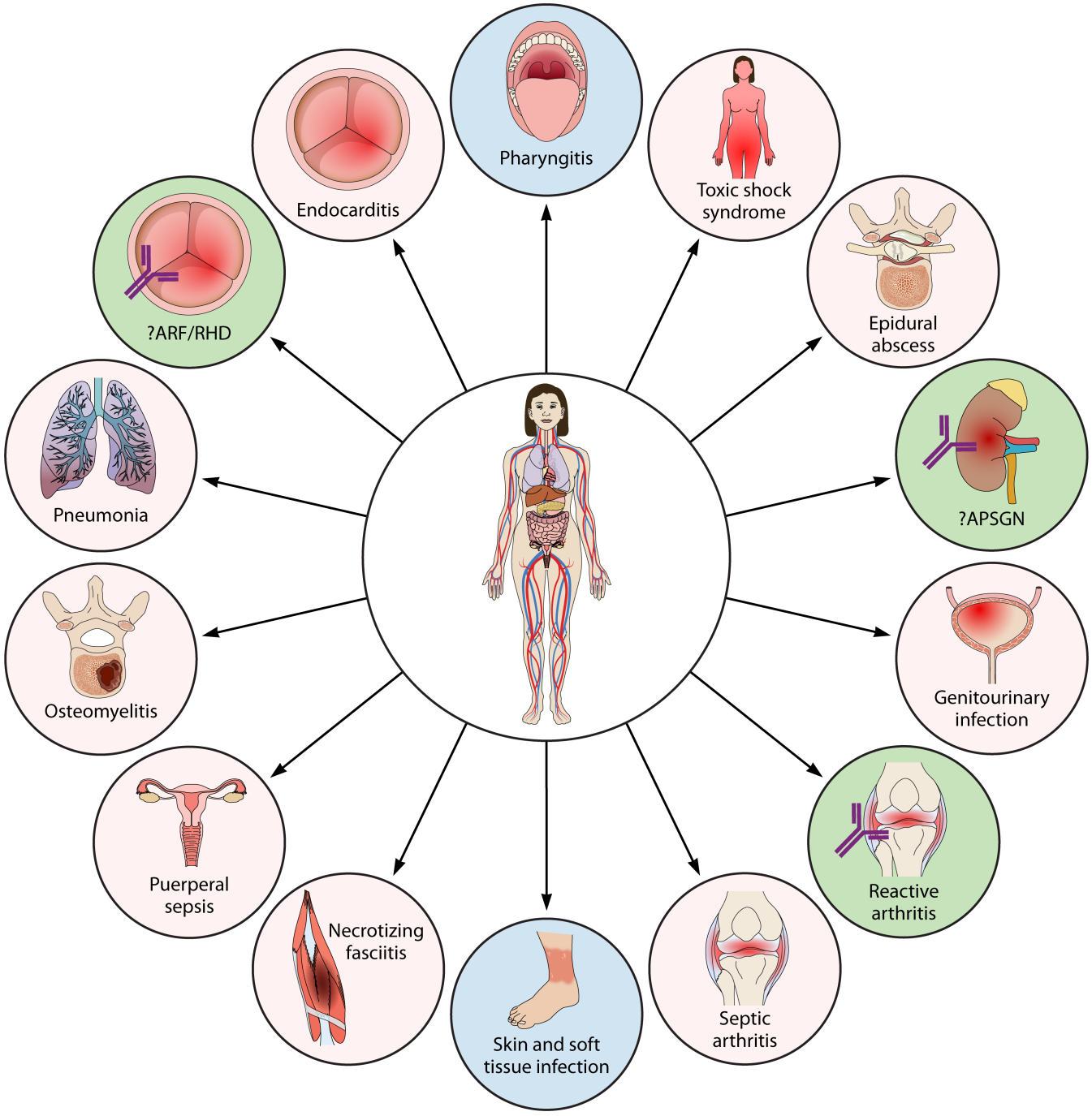

FIG 6 Disease manifestations caused by *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) in humans. Non-invasive manifestations are highlighted in blue, invasive manifestations in red, and post-infectious immune-mediated manifestations in green. Putative immune-mediated manifestations are denoted with question marks. Skin and soft tissue manifestations such as cellulitis can seed bacteremia and is a common source of invasive disease. Between 20% and 50% of invasive cases presenting with bacteremia may not have a clinically evident source of infection. SDSE infection has been linked with acute rheumatic fever, rheumatic heart disease, and acute post-streptococcal glomerulonephritis, but a firm causal relationship has not been established. APSGN, acute post-streptococcal glomerulonephritis; ARF, acute rheumatic fever; RHD, rheumatic heart disease.

Infection or colonization with group G SDSE in humans occurs more frequently than group C SDSE (11, 62, 227). In some reports, group G SDSE was isolated two to five times more frequently than group C SDSE, although there have been no proven differences in disease phenotype between the two groups (11, 62, 215).

Throat carriage and pharyngitis

Throat colonization rates vary from 2% to 20% and tend to be higher in areas which are considered hyperendemic for streptococcal disease, such as Northern Australia or Fiji, and may be more common in older children and adolescents (221, 227, 228, 242–246). Co-isolation of SDSE and *S. pyogenes* from the same sample appears to be uncommon, although rates may be underestimated by standard microbiological methods as *S. pyogenes* and SDSE colonies appear identical on blood agar (6, 227, 244). Indeed, although SDSE and *S. pyogenes* may carry quorum sensing receptors and bacteriocins with cross-species activity, analysis of real-world transmission did not demonstrate evidence of interspecies transmission interference (6). Future studies incorporating multi-isolate identification or unbiased deep sequencing of surveillance specimens may help address this gap in knowledge.

Improved identification methods and recognition of SDSE and group C/G *Streptococcus* in the 1980s described outbreaks of pharyngitis, in some cases foodborne (208, 247, 248). Pharyngitis with group C/G *Streptococcus* is clinically indistinguishable from *S. pyogenes*, and both can cause elevated titers of anti-streptolysin O (1, 249, 250). Larger epidemiological studies seeking to describe the role of SDSE in sporadic or endemic pharyngitis by comparing rates of isolation between symptomatic cases and controls have found variable associations (228, 238, 242, 243, 245, 251, 252). Comparisons of the contribution of *S. pyogenes* and SDSE in pharyngitis have also varied with an incidence reported as much as two times higher to seven times lower for SDSE or group C/G *Streptococcus* compared to *S. pyogenes* (223, 245, 246). However, many epidemiological studies were hampered by low isolation rates of group C/G *Streptococcus*, potential confounding with non-SDSE group C/G *Streptococcus* species, and differences in study population.

Pharyngitis in *S. pyogenes* can occasionally be followed by scarlet fever, thought to be mediated by streptococcal superantigen exotoxins (253). SDSE has not been linked with scarlet fever and infrequently carries superantigens implicated in scarlet fever pathogenesis. A case report in 1989 described a single patient with pharyngitis caused by a group C beta-hemolytic *Streptococcus* associated with a scarlatiniform rash (254). However, the species of the group C *Streptococcus* was not described, and there have been no definitive epidemiological associations described between SDSE and scarlet fever.

Skin carriage and infection

Beta-hemolytic streptococci including SDSE are the most common cause of erysipelas and cellulitis. Erysipelas is characterized by pain, warmth, and erythema with a sharp demarcation caused by inflammation in the upper dermis (255). Cellulitis is defined as infection additionally involving the subcutaneous tissue manifested with diffuse edges to the rash (255). However, in clinical practice, these two terms are often used interchangeably, and clinical management does not differ. These entities should be differentiated from necrotizing fasciitis, which is life threatening and characterized by rapid clinical progression, extreme pain, systemic toxicity, and, at later stages, blistering necrosis. While a microbiological diagnosis of erysipelas and cellulitis is often not successful despite careful sampling of wounds, serology, and even skin biopsy, most cases in which an organism is found are caused by *S. pyogenes* or group C/G *Streptococcus* (255–259). Serological studies have suggested that a significant rise in anti-streptolysin O titer without a concurrent rise in anti-DNAse B may reflect SDSE infection rather than *S. pyogenes* (259, 260). Identification of cellulitis etiology based on serological profile and culture suggest approximately 70% of cases of cellulitis in adults are caused by beta-hemolytic streptococci with 20% to >50% of these cases fulfilling a profile consistent with SDSE infection (259, 260). In fact, in a single-hospital observational study in Japan between 2012 and 2015, rates of SDSE as the cause of bacteremic cellulitis were greater than that of all other beta-hemolytic streptococci combined (261).

Systematic studies on carriage frequency of SDSE on human skin are limited. SDSE has not been detected at high abundance in skin metagenomic studies (262, 263). Based on culture, perianal colonization with SDSE has been described in patients with erysipelas and may be more frequent than colonization in the interdigital areas of the feet, throat, or nasopharynx in these patients (264–266).

Although a common cause of cellulitis, SDSE appears to be an infrequent cause of pyoderma or impetigo. A throat and skin sore carriage study in remote northern Australia demonstrated very few isolates of SDSE associated with pyoderma despite high rates of isolation from asymptomatic throat carriage (6, 227, 244). This contrasts with *S. pyogenes*, which is a common cause of impetigo. The genetic mechanism behind the disparity is unclear but may reflect key differences in adhesion factors, metabolic pathways, or the virulence repertoire of the two pathogens.

Zoonotic infection

SDSD is a major animal pathogen, but recently, animal-adapted strains of SDSE have been increasingly recognized. SDSE has been isolated from multiple animal hosts, including pigs, horses, sheep, and dogs, where it has been detected as a colonizer but also associated with clinical disease (8, 22, 23, 267, 268). Manifestations of clinical disease in animal hosts include genito-pelvic and disseminated disease in horses and invasive infection in piglets (8, 23, 269, 270).

Isolates of SDSE from animals are genetically distinct from human isolates with a host-specific virulence repertoire and do not appear to be a reservoir of human infection apart from possible rare zoonotic transmission (1, 23, 271). Almost all available whole-genome sequences of SDSE from human infections are human-adapted strains. The same SDSE strain based on *emm* type and MLST has been described in humans and companion animals such as dogs (268). Isolates with the same MLST have also been detected in carriage isolates from humans and equine hosts (23, 271). While higher-resolution WGS confirmation was not available, it appears many of these cases cluster with human-adapted strains and may represent human to animal transmission (23, 271). In a later study, an "intermediate group," again described based on clustering of MLST, contained isolates from both equine and human hosts (59). WGS of a small number of isolates from the intermediate group clustered with animal-adapted SDSE strains isolated from various hosts including dogs, pigs, and horses rather than forming a new multi-host adapted clade (59). Strains isolated from humans within this group may therefore reflect zoonotic transmission.

While zoonotic infection leading to clinical disease appears to be rare, systematic sampling of humans and companion animals or those with close contact to animals known to be colonized with SDSE has not been performed. It is therefore possible that transient colonization of humans with animal-adapted SDSE strains or animals with human-adapted strains may be more commonly what has been thus far described in the literature.

Invasive disease

Most reports of SDSE infection in humans have focused on invasive disease characterized by syndromes associated with isolation of the organism from a normally sterile site, often bloodstream. While rates vary slightly based on definition of invasive disease, studies report between 60% and 95% of invasive SDSE cases are blood culture positive and comparable to the rate described for *S. pyogenes* (9, 197, 198, 201, 202, 224). Invasive SDSE can manifest with a spectrum of clinical syndromes including bacteremic cellulitis, bacteremia without focus, necrotizing fasciitis, STSS, pneumonia, urogenital infection, post-partum or puerperal sepsis, infective endocarditis (IE), epidural abscess, and meningitis (Fig. 6) (11, 12, 65, 192, 197, 199, 272)

Invasive SDSE infection is associated with older age, particularly those older than 65 years, and >75% of patients have co-morbidities including vascular disease, obesity,

diabetes, or malignancy (10–12, 197, 198, 203, 235, 272). Invasive disease is uncommon in children (2, 10, 11). In contrast to *S. pyogenes*, invasive SDSE disease occurs more frequently in males, making up to 60% of cases (9, 10, 192, 202). All-cause crude mortality is estimated to be approximately 10%–20% and is comparable to *S. pyogenes* (11, 62, 65, 197, 201, 273). However, adjusted for age and/or co-morbidities, *S. pyogenes* has been associated with greater mortality (201, 273).

Studies comparing the relative frequencies of manifestations of invasive disease have varied based on study population and case definitions. However, bacteremic cellulitis and bacteremia without a focus have been identified as the most common manifestations of invasive SDSE, each responsible for 20%–50% of cases, similar to *S. pyogenes*, followed by osteoarticular disease (11, 12, 65, 192, 197, 199, 272). Although cellulitis is the most common source of invasive SDSE infection, bacteremia is detected in only <10% of cases of cellulitis and routine blood cultures are not generally recommended for cellulitis or erysipelas in the absence of clinical concern or severity (274, 275). Comparisons with *S. pyogenes* suggest *S. pyogenes* may be associated with greater severity of illness and higher incidence of necrotizing fasciitis and STSS (273, 276–278). Other manifestations of invasive disease occur less frequently and include pneumonia, urogenital infection, post-partum or puerperal sepsis, IE, epidural abscess and rarely meningitis (9, 11, 12, 73, 74, 87, 214, 235–240).

Incidence of IE is more frequent in the context of bloodstream infection with SDSE than *S. pyogenes* (214, 279). SDSE has recently been included as a "typical" cause of endocarditis in the 2023 updated Duke-ISCVID criteria for IE (280). The implication of this change is that SDSE requires only two positive blood cultures to meet a major IE criterion compared to the previous Modified Duke Criteria, which required three (281). The change was prompted by a population linkage study in Denmark which reported an endocarditis prevalence of 6.4% among bacteremic SDSE cases with an adjusted odds ratio (OR) of 6.16 compared to low-risk *Streptococcus pneumoniae* as a reference (279). However, SDSE IE prevalence in bacteremic patients in other studies have been lower including a meta-analysis which reported a pooled prevalence of 3.0% (13, 282). Differences in rates may reflect heterogeneity of risk in different populations and regions (13). The greatest risk of IE appears to be in those risk factors such as prosthetic heart valves, previous IE, native valve disease or an intracardiac device (283). In patients with SDSE IE, the clinical course has been described to be aggressive with rates of embolic phenomena as high as 50% and comparable mortality to more common causes of IE such as *Staphylococcus aureus* (214, 284).

SDSE may cause more recurrent disease than *S. pyogenes*. Recurrent invasive SDSE infection has been described in up to of 10% of patients in case series but varies based on cohort (9, 198, 219, 272). Lower rates, 2%–3% of cases, have been reported in larger series and population-based surveillance, suggesting there may be differences in study populations (9, 10, 285). While patients may have recurrent infection with unrelated strains, reports suggest many of these cases may be caused by the same strain based on molecular typing of the *emm* gene or other methods such as pulsed-field gel electrophoresis (215, 272, 286). Recurrence is often manifested with cellulitis and in the presence of risk factors such as lymphoedema, previous cellulitis, skin ulcers, and/or regional radiotherapy (215, 219, 272, 286, 287). Recurrence may therefore reflect a SDSE-specific capability for persistence in hosts with predisposing factors. However, the mechanism of persistence and the reservoir site in those with recurrent disease is unclear.

Post-infection immune-mediated disease

A large burden of disease caused by *S. pyogenes* is related to post-infectious immune-mediated complications such as ARF, rheumatic heart disease (RHD), and acute post-streptococcal glomerulonephritis (APSGN) (288). SDSE has been linked by various levels of evidence to these conditions and remains an area of active research.

The Northern Territory of Australia (NT) carries a disproportionately high burden of ARF/RHD but historically low rates of clinical pharyngitis, which has traditionally been

considered the trigger for ARF and RHD (244, 289). This has led to an interest in other potential triggers. Sera from children in the NT have been demonstrated to possess antibodies against group C/G streptococci, which cross-react with human cardiac myosin *in vitro* (289). In a rat model, injection with a strain of SDSE isolated in children from the NT who presented with ARF, triggered myocarditis and valvulitis, which were indistinguishable from that caused by *S. pyogenes* (290). However, large epidemiological studies have not been performed to establish a definitive clinical link between SDSE and ARF/RHD. It remains unclear what role SDSE may play in immune priming for ARF/RHD, particularly in high-incidence regions, and is an unmet need in efforts to combat ARF/RHD.

Distinct to ARF, SDSE, akin to *S. pyogenes*, also causes reactivate arthritis, which generally has a shorter period between infection and onset of arthritis than with ARF and does not satisfy the Jones criteria for ARF (291). Case series suggest that group C/G *Streptococci*, which generated an increase in anti-streptolysin O titer and therefore likely SDSE, could cause reactive arthritis that was indistinguishable from that caused by *S. pyogenes* (250, 292, 293). Reactive arthritis should also be distinguished from septic arthritis after seeding of infection into a joint. Treatment of reactive arthritis is generally symptomatic and may involve non-steroidal anti-inflammatory medications or even corticosteroids in the acute phase after control of sepsis (291).

SDSE has been associated with APSGN, but evidence is limited to small series or case reports. A study from Trinidad of group G *Streptococcus* isolated from skin lesions associated with APSGN between 1976 and 1980 did not describe species and reported only seven cases of which two had concurrent *S. pyogenes* isolated from the throat (294). A case report in 1987 described a case of bacteremic group G streptococcal disease associated with acute deterioration in renal function and hematuria (295). However, the timeline of the case more accurately reflects that of infection-related glomerulonephritis from an active infection rather than the classic clinical course of APSGN (296). Modern outbreaks of group C *Streptococcus*-related APSGN have been found to be *S. equi* subsp. *zooepidemicus* related rather than SDSE (297). In *S. pyogenes*, antibodies to virulence factors streptococcal inhibitor of complement (SIC) and distantly related to SIC (DRS) have been associated with APSGN (298, 299). Indirect evidence of a possible link between SDSE and APSGN is suggested by a serosurvey which demonstrated a correlation between antibodies to SDSE DrsG, a homolog to *S. pyogenes* DRS, and APSGN in India (300).

TREATMENT AND ANTI-MICROBIAL RESISTANCE

Primary therapy

SDSE has remained almost universally susceptible to penicillin despite over 80 years of clinical use (166, 224). Penicillin remains the preferred therapy for all presentations, from pharyngitis to invasive disease (Table 3). No SDSE-specific treatment guidelines or trials exist, and therefore, treatment regimens recommended for other beta-hemolytic streptococci and syndrome-specific guidelines are also recommended for SDSE (275, 301–304).

There has only been one published report of *in vitro* penicillin resistance in four isolates from three separate patients in Denmark (166). SDSE do not harbor beta-lactamases, and resistance was associated with multiple mutations in high-molecular-weight PBPs (166). Although *S. pyogenes* remains universally susceptible to penicillin, isolates with raised MICs have been detected as being associated with mutations in *pbp2x* (169–175). Systematic genomic surveillance of PBP genes has not been carried out for SDSE, but studies which reported penicillin MICs have not detected significant numbers of isolates with raised MICs (224, 305). However, as susceptibility testing for beta-hemolytic streptococci is not universally performed in clinical laboratories, identification of isolates with raised MICs may be limited (306).

Despite *in vitro* susceptibility to penicillin, clinical failure has been demonstrated in cases of pharyngitis, a phenomenon that has also been described in *S. pyogenes* (307).

TABLE 3 Initial anti-microbial treatment options and adjunctive therapies for *Streptococcus dysgalactiae* subsp. *equisimilis* disease[a]

Therapy	Efficacy
Primary therapies	
Penicillin	Preferred therapy for invasive and non-invasive disease; resistance extremely rare, only four resistant isolates reported in the literature. Treatment failure in pharyngitis has been reported and may be related to intracellular invasion/persistence.
Aminopenicillins, cephalosporins, and carbapenems	May be used as an alternative to penicillin, e.g., allergy, but have a broader spectrum of activity not required in monomicrobial infection
Macrolides	Alternative to penicillin, e.g., allergy, for non-invasive disease. Anti-microbial susceptibility should be confirmed if used due to increasing resistance globally.
Lincosamides, e.g., clindamycin	Alternative to penicillin, e.g., allergy, for non-invasive disease. Anti-microbial susceptibility should be confirmed if used due to increasing resistance globally.
Glycopeptides	Alternative to penicillin in severe disease, e.g., allergy; resistance rare
Linezolid	Alternative to penicillin, e.g., allergy, but limited clinical experience; resistance rare
Trimethoprim-sulfamethoxazole	Alternative to penicillin, e.g., allergy, for non-invasive disease. However, clinical data are limited. Resistance is considered uncommon, but surveillance data are limited and resistance may be emerging in some regions such as India.
Fluroquinolones, e.g., moxifloxacin or levofloxacin	Not preferred therapy. Consider alternatives such as macrolides or clindamycin if unable to give a beta-lactam. Anti-microbial susceptibility should be confirmed if used due to increasing resistance globally.
Adjunctive therapies	
Lincosamides, e.g., clindamycin	May be considered as adjunctive therapy for antitoxin effect in addition to a beta-lactam in cases of severe disease, e.g., STSS[b] or necrotizing fasciitis. However, clinical data are limited. Unknown if efficacious as an adjunctive therapy in the setting of resistance.
Linezolid	Alternative to clindamycin as adjunctive therapy, in addition to a beta-lactam, but data are very limited, and clindamycin is preferred as adjunctive therapy
Intravenous immunoglobulin	May be considered as adjunctive therapy for antitoxin effect in addition to a beta-lactam and/or clindamycin in cases of severe disease, e.g., STSS or necrotizing fasciitis. However, data on use in SDSE disease are very limited.

[a]No randomized clinical trials have been performed for SDSE disease, and treatment options listed here reflect the authors' experience and have been based on recommendations for other beta-hemolytic streptococci and syndrome-specific guidelines.
[b]STSS, streptococcal toxic shock syndrome.

It has been hypothesized that clinical failure of penicillin in *S. pyogenes* may be related to intracellular invasion and evasion of killing by extracellular penicillin (308). SDSE also appears able to invade epithelial cells and may therefore share a common basis for clinical penicillin treatment failure (216, 289).

Alternatives to penicillin include other beta-lactams such as cephalosporins or carbapenems, macrolides, clindamycin, linezolid, glycopeptides, trimethoprim-sulfamethoxazole, and fluoroquinolones. Susceptibility testing should be performed if using macrolides, clindamycin, or fluoroquinolones in the setting of increasing anti-microbial resistance in many jurisdictions. Rates of erythromycin and clindamycin resistance vary by region and approach 10%–40% for both anti-microbials in reports from Japan, Korea, Norway, Spain, and the UK, and resistance may be increasing in frequency (186, 192, 212,

217, 218, 309). Resistance determining genes such as *ermA*, *ermB*, and *mef*(A)/*msr*(D) are present on MGEs, which may facilitate dissemination of anti-microbial resistance (186, 192). Similarly, resistance to fluoroquinolones such as levofloxacin is increasing, with rates more than 10% reported related to mutations in *gyrA* and *parC* (159).

Historically, many beta-hemolytic streptococci were thought to be resistant to trimethoprim-sulfamethoxazole (310, 311). However, these results may have in fact been due to high thymidine content in culture media which bypasses the inhibitory effects of trimethoprim-sulfamethoxazole on folate metabolism (312). Low thymidine is now specified in modern Mueller-Hinton agar used in susceptibility testing (313). Recent studies suggest trimethoprim-sulfamethoxazole susceptibility may be higher for SDSE than other non-beta-lactam anti-microbials such as clindamycin in some regions (314, 315). However, clinical experience is limited; resistance genes such as *dfrF* encoding a trimethoprim-insensitive dihydrofolate reductase have been described in SDSE, and high rates of resistance may be emerging in some regions such as India (7, 316). Despite limited data for SDSE-specific indications, trimethoprim-sulfamethoxazole may be used for superficial SDSE infections such as cellulitis extrapolated from its efficacy in trials and retrospective studies of skin and soft tissue infection, including non-purulent cellulitis, where a significant proportion are expected to be caused by SDSE (317–319). However, in these studies, microbiological confirmation was often unable to be obtained or streptococcal species were not reported. Additionally, caution should be exercised when extrapolating from studies performed in populations such as children where SDSE infection is rare (311, 320).

Adjunctive therapies

Adjunctive clindamycin is frequently used in severe *S. pyogenes* infection such as STSS or necrotizing fasciitis in combination with surgical debridement and beta-lactam therapy. Streptococcal exotoxins and virulence factors are thought to be crucial in the pathogenesis of STSS and necrotizing fasciitis. Clindamycin inhibits protein synthesis and toxin production *in vitro* and in murine models and may be more effective than penicillin in large inoculum infections such as myositis (321–323). In a large propensity matched retrospective study, adjunctive clindamycin was associated with reduced in-hospital mortality for invasive disease caused by *S. pyogenes* (277, 322). The same study found no such benefit in cases of invasive group C/G disease and, in fact, a trend toward poorer outcomes with the use of clindamycin (277). However, species identification was not available for most isolates and a number of non-SDSE cases were included in the analysis. While isolates with clindamycin resistance were excluded when tested, clindamycin susceptibility was not available for most cases. Thus, in the context of a trend toward higher rates of resistance in group C/G *Streptococcus*, it is not clear if resistance to clindamycin contributed to observed poorer outcomes in this specific study. Additionally, fewer patients with invasive group C/G streptococcal disease received clindamycin than for *S. pyogenes*, resulting in a less precise estimate. However, it should be noted that the interaction between clindamycin resistance and adjunctive treatment remains unclear, with the possibility that clindamycin may still retain an antitoxin effect at high doses despite *in vitro* resistance in *S. pyogenes* (322). A separate nationwide retrospective cohort study in Japan also found no benefit with adjunctive clindamycin but included group B *Streptococcus* (*Streptococcus agalactiae*) with group C/G *Streptococcus* and did not exclude cases with clindamycin resistance (324). Possible differences in response to adjunctive clindamycin between the organisms may be related to differences in toxin profiles with SDSE harboring fewer exotoxin-related genes. Further studies are required to verify these findings and characterize *in vitro* and *in vivo* differences in response to clindamycin between the organisms. Currently, caution should be applied to extrapolating the use of adjunctive clindamycin to SDSE infections.

In the face of rising clindamycin resistance, the oxazolidinone, linezolid, has been suggested as a possible alternative adjunctive agent. Resistance to linezolid in beta-hemolytic streptococci is considered rare (325). Experimental evidence indicates that

linezolid is also able to reduce streptococcal exotoxin production, but clinical data are limited, with clindamycin remaining the preferred agent (321, 326).

Intravenous immunoglobulin (IVIg) is also frequently used as an adjunctive treatment in cases of *S. pyogenes* STSS or necrotizing fasciitis with mixed findings in observational studies (327, 328). IVIg is thought to act through neutralization of streptococcal exotoxins and may have additional activity, such as promoting pathogen opsonophagocytic killing (329, 330). Small, uncontrolled case series suggest a possible benefit in severe invasive group C/G infection manifesting as STSS (331). In a murine infection model, IVIg neutralization of the fibrinolytic virulence factor, streptokinase, attenuated lesion size (113). However, the literature on the clinical use of IVIg in SDSE STSS or necrotizing fasciitis is very limited (276).

While adjunctive therapies may hold promise to reduce the risk of severe outcomes with invasive SDSE disease, limited observational evidence hampers wider applicability of these treatments. Randomized clinical trial evidence is required, ideally nesting invasive SDSE disease with *S. pyogenes* in the trial design, with a survey of clinicians suggesting there is adequate equipoise for enrollment in randomized trials of both adjunctive clindamycin and IVIg (332). Given the relatively low incidence of severe manifestations of SDSE disease such as STSS or necrotizing fasciitis, an adequately powered trial would require international multi-center participation.

CONCLUSION

SDSE is an increasingly recognized cause of human disease with recent reports describing a crude burden of invasive disease comparable to the closely related and significant human pathogen, *S. pyogenes*. With increasing use of WGS, an improved understanding of SDSE diversity and lineage dynamics is being unraveled. While there are clear geographical differences, globally successful lineages have emerged raising further questions regarding drivers of SDSE population diversity. However, many regions of the world to date have reported scarce epidemiological and genomic data leaving gaps in our understanding of the global epidemiology of SDSE.

As our understanding of the pathobiology of SDSE improves, it is also becoming clear that there is significant genomic and biological overlap between SDSE and *S. pyogenes*. Concurrent genomic analyses of the two species demonstrate extensive shared gene content and evidence of cross-species gene transfer through recombination and movement of MGEs. Genes encoding virulence factors and anti-microbial resistance determinants have been found on cross-species MGEs. Additionally, many leading *S. pyogenes* candidate vaccine antigen candidates are present in SDSE, which could provide an additional health benefit and should be considered in future vaccine readouts. Considering that several *S. pyogenes* vaccine antigens exhibit signatures of cross-species recombination, selection pressure from vaccines could encourage adaptive evolution which should be monitored.

Although SDSE remains almost universally susceptible to penicillin, increasing rates of resistance to other anti-microbials such as macrolides and clindamycin have been described. A paucity of high-quality evidence exists for treatment strategies in serious disease such as the use of clindamycin or IVIg in STSS or necrotizing fasciitis. As such, many current treatment strategies are extrapolated from observational evidence and *S. pyogenes* treatment strategies, which should be interpreted with caution.

Taken together, there is a mounting need to further understanding of SDSE pathobiology, epidemiology, and its interaction with *S. pyogenes*. While SDSE causes a similar spectrum of disease to *S. pyogenes*, further work is required to understand the role of SDSE in immune priming for post-infectious immune-mediated diseases such as ARF/RHD. SDSE could be incorporated with *S. pyogenes* surveillance to gain a better understanding of the burden of disease and genomic overlap and to optimize disease control strategies. At the bedside, high-quality randomized clinical trials are also required to inform treatment strategies for serious disease and offers a further opportunity to integrate SDSE and *S. pyogenes* within the same study framework.

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ADDITIONAL FILES

The following material is available online.

Supplemental Material

Table S1 (CMR00175-23-s0001.xlsx). Genomes used to infer *Streptococcus* genus phylogenetic tree.

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