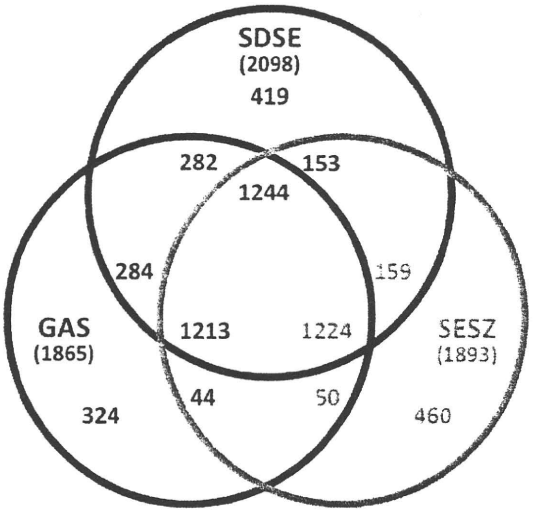

Figure 3 Venn diagram of gene content comparison among *S. dysgalactiae* subsp. *equisimilis* GGS_124 (SDSE), *S. pyogenes* MGAS315 (GAS) and SESZ MGCS10565. The inferred proteomes of SDSE, GAS, and SESZ were compared in a pairwise manner with their translated genomes by *in silico* Molecular Cloning and are presented as a Venn diagram. The numbers of products for each section are color coded to match the respective genomes. Genes showing more than 40% identity were considered homologues.

phage. This prophage was inserted at the predicted bacteriophage T12*att* site, which has been shown to be a gene that encodes a serine tRNA and is located between the CDS of SDEG_1100 and SDEG_1161 [20]. Six GAS strains, MGAS10394, MGAS315, MGAS5005, MGAS6180, MGAS8232, and SSI-1, have prophage elements: Φ10394.3, which carries *speK* and the streptococcal phospholipase A2 gene (*sla*); Φ315.2, which carries *ssa*; Φ5005.1, which carries *speA*; Φ6180.1, which carries the *speC* and Dnase (*spd*) genes; Φ8232.3, which carries *speL* and *speM*; and SPsP5, which carries *speC*, respectively [18,21-25]. In addition, ΦGGS_124.1 was found to contain a prophage-associated virulence factor gene for deoxyribonuclease (*sdc*).

(ii) Prophage GGS_124.2

We found that the ΦGGS_124.2 prophage is 35,814 bp in length, with a G+C content of 38.20% and 61 CDS. Ninety-five percent of the CDS in ΦGGS_124.1 have homology with genes in GAS prophages, making it likely that ΦGGS_124.2 is chimeric phage. The chromosomal phage attachment site (*attB*) and the ΦGGS_124.2 phage-encoded attachment site (*attP*) were not found, but the products of *attP/attB* recombination, *attL* and *attR*, with the same sequences as those of GAS prophages SPsP2 and Φ315.5 were identified. The genome context around the integration site for ΦGGS_124.2 was found to be conserved at the phage integration sites of 4 GAS strains, MGAS10394, MGAS315, SSI-1, and Manfredo, which contain the prophage elements Φ10394.6, carrying *sdn*; Φ315.5, carrying *speA*; SPsP2, carrying *speA*; and phiMan.1, carrying the DNase gene *mf3*, respectively [18,19,21,22]. No known prophage-associated virulence factor genes were found in ΦGGS_124.2.

(iii) Prophage GGS_124.3

We also found a prophage remnant, ΦGGS_124.3, which was about 12.6 kb length and homologous to the previously sequenced GAS prophage remnants Φ6180.4 and Φ10270.5 with a nucleotide identity of 73%. ΦGGS_124.3 and the two GAS phage remnants were found to be located between genes encoding a putative transcriptional regulator protein and the 30S ribosomal protein. In strain GGS_124, two truncated transposase proteins, SDEG_2117 and SDEG_2118, were found to be inserted upstream of GGS_124.3. No virulence factor genes are present in GGS_124.3.

Prokaryotes possess a system that mediates resistance to infection by foreign DNA, such as viruses [26,27]. When bacteria are exposed to phages, short fragments derived from phage DNA are integrated into clusters of regularly interspaced short palindromic repeat (CRISPR) regions of the bacterial genome as spacers [27]. CRISPR RNA transcripts and CRISR-associated proteins (Cas), act in complexes to interfere with virus proliferation [26]. This system has also been observed in GAS [20], SESZ [22,28], *S. mutans* [29], and *S. thermophilus* [30]. GGS_124 harbors a CRISPR/Cas system consisting of an array of genes, *can1*, *cas1*, *cas2*, and *csn2*, and CRISPR (Figure 5). The GGS_124 CRISPR has 19 direct repeats of 36 bp each and 18 spacer sequences 30 or 32 bp in length; 6 of these sequences are homologous to GAS prophage sequences, with more than 80% coverage (Additional file 5). When we analyzed the number of CRISPR spacers in an additional 7 SDSE isolates (Table 2), we found that the mean number of CRISPR spacers was higher in SDSE (18.0 ± 3.3 spacers) than in GAS strains (4.0 ± 1.0 spacers; range, 0 to 9) (Table 2). These results suggest that prophage infection of SDSE is somewhat restricted, resulting in a smaller number of virulence factors located in the prophage regions of SDSE. Alternatively, SDSE may be in contact with phages more frequently, with integrated phages having a fitness cost for SDSE.

Virulence factors encoded by the GGS_124 genome

An analysis of 58 SDSE strains isolated from human infections using targeted microarrays containing 216 GAS virulence genes composed of 70mer oligonucleotides showed that about 50% of the GAS virulence genes represented in the microarray were present in SDSE [31]. Three molecular markers, *speB*, the intergenic region upstream of the *scpG* gene and *virPCR*, have

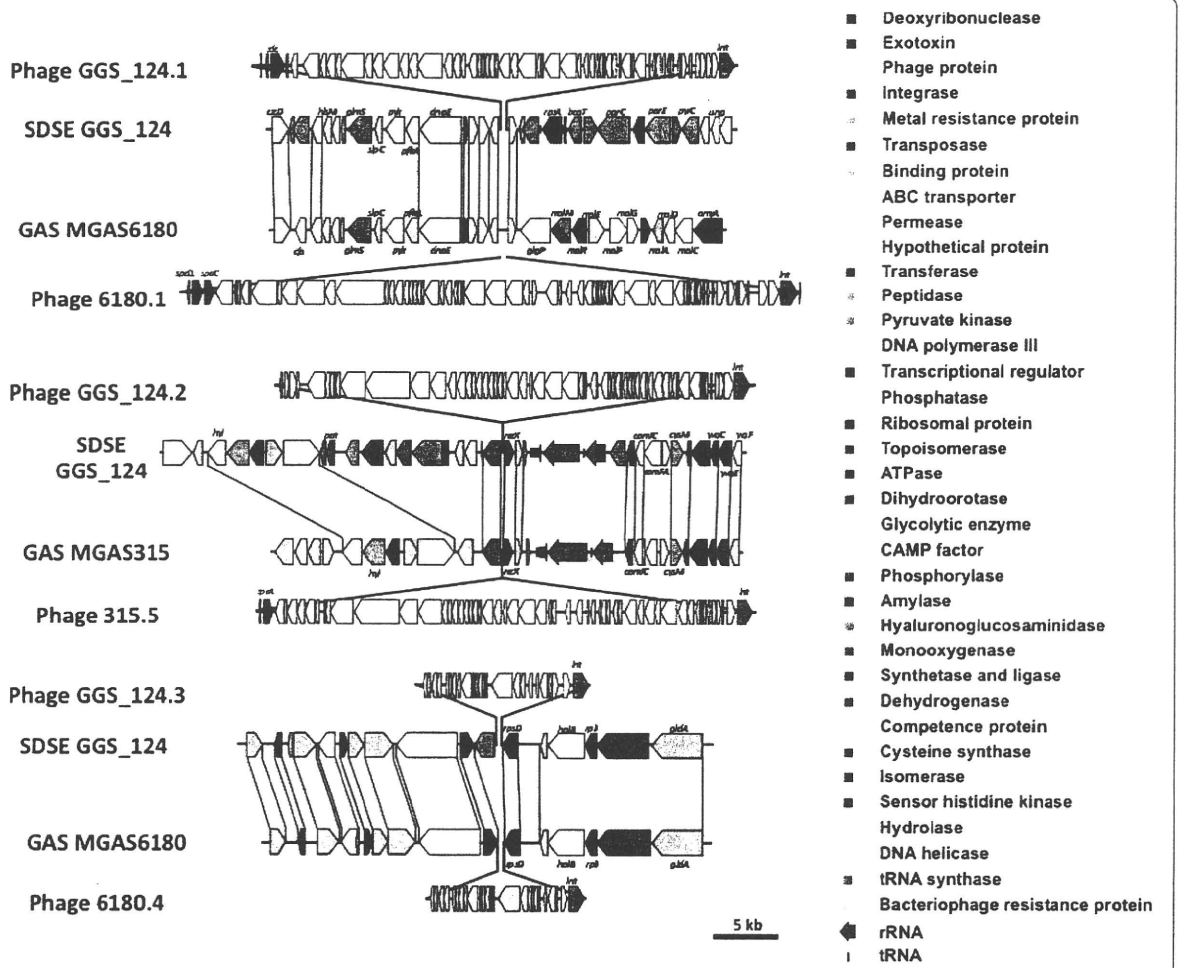

Figure 4 Prophage elements and the surrounding gene arrangements of GGS_124 and GAS. The organization of the genes located in the putative prophage regions found in GGS_124 and their insertion points in the genome were compared with those of GAS. Colored boxes between genes indicate level of similarity at the amino acid level (red, ≥90%; orange, 89%-80%; green, 79%-70%).

been shown helpful in discriminating between GAS and SDSE [32]. Based on homology analyses with known bacterial virulence factors, such as pore-forming toxins, a superantigen, proteases, FCT-like regions, adhesins, hyaluronidase, and a nuclease, we identified 71 putative virulence factor genes in the GGS_124 genome; their details are shown in Additional file 6. Comparison of the virulence factors in GGS_124 with those of other streptococcal species indicated that the putative virulence factors most similar to those of GGS_124 were found in GAS. In contrast, superantigen, SPE-B and the *has* operon of GAS are not conserved in GGS_124.

(i). Pore-forming toxins

GGS_124 has several putative hemolysins, including HlyX (SDEG_0427), HlyIII (SDEG_1015), and HlyA1 (SDEG_1483), which have also been detected in GAS, SESZ, SESE, *S. uberis*, and GBS. GGS_124 also has genes encoding streptolysin S (*sagA*) (SDEG_0705) and its biosynthesis proteins (*sagBCDEFGHI*) (SDEG_0706 to 0713), which are also present in GAS [33], SESZ, and SESE [22,28,34]. In addition, GGS_124 carries a gene for streptolysin O (SLO) (SDEG_2027), which is essential for GAS virulence and is required for the organism to escape from the endosome into the cytosol following invasion of host cells [35].

(ii). Superantigen

GGS_124 possesses only one superantigen gene, exotoxin G variant 4 (*spegg4*), which is homologous to GAS streptococcal exotoxin G (SpeG), with 79% amino acid identity (Additional file 6). In a previous analysis of the superantigenic activities of the *spegg4* product in human peripheral blood mononuclear cells [36], we found that its mitogenic activity was about 1% that of SpeG from GAS. Other genome-encoded superantigen

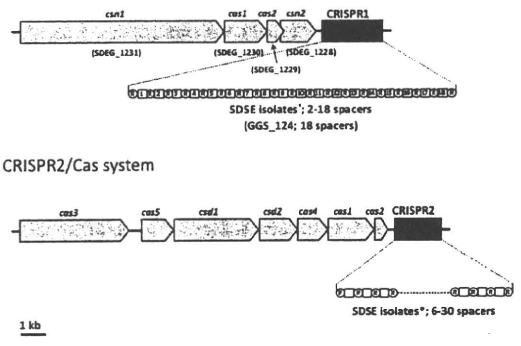

Figure 5 CRISPR/Cas system structures found in SDSE isolates. The CRISPR structures of SDSE isolates were analyzed by direct sequencing of PCR-amplified CRISPR regions. The repeat-spacer regions (CRISPR) are enlarged. Open circles marked "R" are direct repeats, whereas the square boxes indicate respective spacers with numbers. GGS_124 contained only CRISPR1, whereas some of the other strains also contained CRISPR2 (Table 2).

genes for mitogenic exotoxin Z (*smeZ*), which are present in GAS [37], were not found in the GGS_124 genome.

(iii) Proteases

We found that a putative proteinase (SDEG_1906) and streptococcal C5a peptidase (*scpB*) (SDEG_0933) [38] were conserved among GGS_124 and 5 closely related species. GGS_124 also has a gene with homology (42% amino acid identity) to exfoliative toxin A of *Staphylococcus aureus* strain Mu50 (SAV1173), which causes staphylococcal scalded skin syndrome [39]. GGS_124 also carries a gene for streptokinase (SDEG_0233), similar to streptokinase A of GAS, with 88% amino acid identity (Additional file 6). This protein complexes with plasminogen to form an activator, which has serine protease activity and cleaves free plasminogen. leading to activation of the zymogen [40]. Strikingly, GGS_124 lacks streptococcal cysteine protease (SpeB), an erythrogenic toxin produced by GAS with cysteine protease activity [41]. The GGS_124 genome lacks approximately 7 kb of

Table 2 Presence of Cas genes and the number of spacers in CRISPR1/Cas and CRISPR2/Cas

		CRISPR1/Cas			CRISPR2/Cas		
Species	**Strain**	**Cas genes**	**No. of spacers**	**Acc. No.**	**Cas genes**	**No. of spacers**	**Acc. No**
Streptococcus dysgalactiae subsp. *equisimilis*	GGS_124	+	18	AP010935.1	-	0	-
	168	+	2	AB553332	+	13	AB553333
	GGS_117	+	8	AB553338	+	12	AB553339
	170	+	9	AB553336	+	10	AB553337
	164	+	17	AB553343	+	6	AB553331
	GGS_118	+	8	AB553342	+	13	AB553341
	169	+	7	AB553334	+	30	AB553335
	163	+	3	AB553340	N. D.	N. D.	
Streptococcus pyogenes	MGAS8232	-	0	AE009949.1	-	0	0AE009949.1
	MGAS10394	-	0	CP000003.1	-	0	CP000003.1
	MGAS10750	+	0	CP000262.1	+	5	CP000262.1
	Manfredo	-	0	AM295007.1	-	0	AM295007.1
	MGAS10270	+	2	CP000260.1	+	3	CP000260.1
	MGAS315	+	0	AE014074.1	-	0	AE014074.1
	MGAS5005	+	3	CP000017.1	+	4	CP000017.1
	MGAS9429	+	0	CP000259.1	+	7	CP000259.1
	MGAS2096	+	0	CP000261.1	+	6	CP000261.1
	SF370	+	6	AE004092.1	+	3	AE004092.1
	SSI-1	+	0	BA000034.2	-	0	BA000034.2
	MGAS6180	+	4	CP000056.1	+	1	CP000056.1
	NZ131	+	4	CP000829.1	+	5	CP000829.1
Streptococcus equi subsp. *zooepidemicus*	MGCS10565	+	17	CP001129.1	+	9	CP001129.1
	H70	-	0	FM204884.1	+	18	FM204884.1
Streptococcus equi subsp. *equi*	4047	-	0	FM204883.1	-	0	FM204883.1

N.D.: No amplicon was obtained in PCR analyses.

the GAS strain MGAS315 sequence, including genes encoding SpeB (SpyM3_1742), the transcriptional regulator RopB (SpyM3_1744), and mitogenic factor 25K precursor (SpyM3_1745). Since several transposase and related genes (SDEG_0212, 0206, 0205, 0201, 0194) are located in the corresponding region, it is highly likely that the region that included *speB* was present in the common ancestor of GAS and SDSE but was not retained by SDSE after speciation.

We found that *speB* was not present in GGS_124, in agreement with the results of a microarray study, which showed that all of the 58 examined strains of group C and G SDSE isolated from patients lacked the *speB* gene [31,32]. We therefore examined whether SDSE strains have protease activity similar to that of SpeB (Additional file 7). We did not detect any SpeB-like protease activity in strains GGS_124 or GGS_118, which had been isolated from two patients with STSS. In contrast, a GAS strain produced a proteinase that was sensitive to E-64, which inhibits cysteine proteases, including SpeB.

(iv) FCT-like regions

Recently, GAS and GBS were shown to express pili, which are synthesized by proteins encoded by genes in FCT regions [1,42,43]. GGS_124 harbors 2 FCT-like regions, which are probable operons expressing different pilus-like structures (Figure 6). One of these contains genes encoding the transcriptional regulator RofA (SDEG_0156), two putative fimbrial structural subunit proteins (SDEG_0157 and SDEG_0158), two sortases (SDEG_0159 and SDEG_0160), and a putative fibronectin binding protein (SDEG_0161). It is similar to the FCT-6 region, which is conserved among M2 GAS, GBS, and SESZ [22,42,44]. The second region contains genes encoding a putative transcriptional regulator (SDEG_1782), a defective collagen binding protein (SDEG_1781), a signal peptidase I (SDEG_1780), a backbone protein (SDEG_1779), and an ancillary protein (SDEG_1778). It is similar to the FCT-3 region, which was found in M3, M5, M18, and M49 GAS [43].

(v) Adhesins

GGS_124 possesses genes that encode putative adhesion proteins, including proteins similar to putative fibronectin binding proteins (SDEG_0161, 1263, and 1984), pullulanase (SDEG_0237), phosphopyruvate hydratase (SDEG_0704), laminin binding protein (SDEG_0935), internalin protein (SDEG_1372), and collagen binding protein (SDEG_1781), all of which bind to the extracellular matrix (Additional file 6). SGGS_124 also possesses genes encoding immunoglobulin G binding protein (SDEG_1358) [45] and multifunctional streptococcal plasmin receptor (Plr)/streptococcal surface dehydrogenase (SDH)/glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which binds to complement component C5a (SDEG_1936) [46] (Additional file 6), although the product of SDEG_1936 lacks a signal peptide.

(vi) Hyaluronan capsule synthesis

Hyaluronic acid (HA), synthesized *via* the *hasABC* operon, is considered a pleiotropic virulence factor involved in

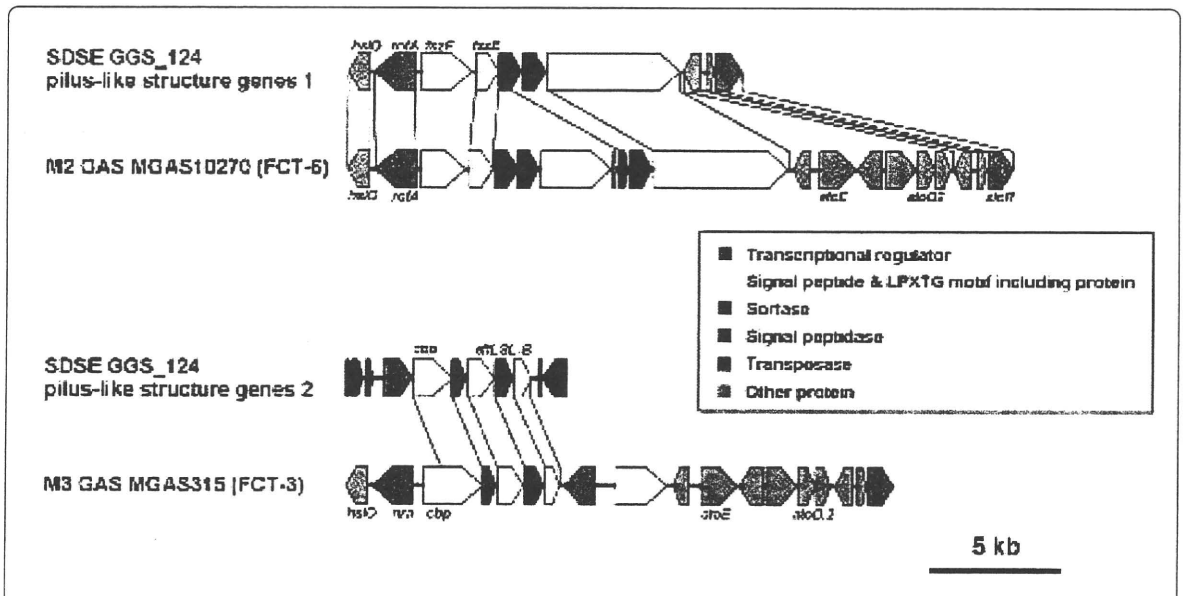

Figure 6 Organization of genes encoding pilus-like structure proteins in *S. dysgalactiae* subsp. *equisimilis* GGS_124. The organizations of genes encoding pilus-like structure proteins in GGS_124 were compared with those in GAS and SESZ. Colored boxes between genes indicate similarity at the amino acid level (red, ≥90%; orange, 90%-80%; yellow-green, 79%-70%; green, 69%-60%; purple, 59%-40%; gray, <40%).

many aspects of GAS infection [47]. GGS_124, however, does not contain an *hasABC* operon, in contrast to the genomes of GAS, SESZ, SESE, and *S. uberis*. Rather, GGS_124 possesses only one gene, encoding glycosyl transferase (SDEG_0628), which shows a low level of similarity to *hasA* of GAS (20% amino acid similarity). Although one SDSE strain has been shown to possess a hyaluronan synthase (AF023876.1), very similar to the product of *hasA* [48], GAS gene microarray analysis of 58 SDSE strains isolated from human infections showed that all harbored only hasC [31]. We found that GGS_124 also harbors only *hasC* (SDEG_1980) (Additional file 6), making it unlikely that SDSE produces HA *via* the *hasABC* operon.

(vii) Hyaluronidase

GGS_124 possesses a gene in a non-prophage region of the genome that encodes a putative hyaluronidase (SDEG_0654), with 66% identity to *hylB* in SESZ (Additional file 6). Hyaluronidase in GAS is thought to contribute to the spread of bacteria in tissues and may allow GAS to utilize host hyaluronic acid or its own capsule as an energy source [49]. The hyaluronidase in GGS_124 may have a function similar to that in GAS.

(viii) Nucleases

GGS_124 possesses 5 genes that encode putative nucleases with a secretion signal peptide: genome-encoded streptodornase (SDEG_0541), extracellular nuclease (SDEG_0714), DNA-entry nuclease (SDEG_0732), cell surface 5'-nucleotidase (SDEG_0825), and prophage-associated deoxyribonuclease (SDEG_1103), all of which are predicted to code for a secretion signal peptide. Two of them, SDEG_0714 and SDEG_0825, code for potential cell wall anchor motifs, LPKAG and LPMAG, respectively (Additional file 6). The putative streptodornase SDEG_0541 and DNA-entry nuclease SDEG_0732 are homologous to phage-encoded extracellular streptodornase D Sda1 of GAS (PHA01790) [50] and DNA-entry nuclease EndA of *S. pneumoniae* TIGR4 (SP_1964) [51], respectively (Additional file 6). Sda1 and EndA have been found to degrade neutrophil extracellular traps (NETs) [50,51], which are composed of granule proteins and chromatin released by neutrophils and can catch and kill surrounding bacteria [52]. The putative extracellular DNase SDEG_0714 is similar to M1 GAS cell-wall-located DNase SpnA (Spy0747), which has been reported important for virulence, *e.g.*, dispersion in host tissue [53] (Additional file 6).

(ix) Other virulence factors

GGS_124 possesses genes encoding the multifunctional M protein (*stg480.0*) (SDEG_0230). The M protein of GAS shows antiphagocytic and adhesin activities, whereas the adhesion function of the GGS M protein may be due to a collagen binding motif [54,55]. Since the product of *Stg480.0* lacks this motif, the M protein of GGS_124 may not act as an adhesin.

Streptococcal inhibitor of complement (SIC) and distantly related to SIC (DRS) are some extent of homology. DRS binds complement factors but does not inhibit complement mediated cell lysis [56,57], whereas SIC inhibits complement mediated cell lysis [58]. GGS_124 harbors a putative DRS gene (SDEG_0932), which consists of a signal sequence, two repeat regions, and a proline-rich region typical of DRS, and is homologous to the Drs12.04 protein of GAS strain *emm12* with 48% amino acid identity [59] (Additional file 6).

The GGS_124 genome harbors a gene encoding a collagen-like protein (SDEG_1113), similar to the collagen-like repeat phage protein of SESE 4047 (SEQ_0837), with 41% amino acid identity. Streptococcal collagen-like proteins (Scl) are cell-surface molecules of GAS with domains containing tracks of repeated Gly-Xaa-Yaa sequences that form a mammalian collagen-like triple-helical structure. These proteins mediate the internalization of GAS into human cells upon binding of Scl to the human collagen receptor integrin [60]. The GGS_124 gene encoding collagen-like protein does not contain a signal peptide or LPXTG motif, suggesting that the gene product may not be expressed on the cell surface. In contrast, GGS_124 does not harbor genes encoding proteins similar to the other collagen-like proteins (*sclA* and *sclB*) in GAS.

NAD glycohydrolase (SDEG_2029), which is located in the NADase-streptolysin O operon of the GAS genome [61], was found to be conserved in the same operon in GGS_124 (Additional file 6). This enzyme is expressed after streptolysin O is injected into host cells and accelerates cell death [61,62].

(x) Distribution of virulence factors among Streptococci

We also assessed the presence or absence of representative virulence factors among sequenced streptococcal species, including GAS (MGAS315), SESE (4047), SESZ (MGCS10565), GBS (A909), and *S. uberis* (0140J) (Additional file 8). Among 30 virulence factors, most of those located in the core genome, but not those located in streptococcal phages, are conserved in GGS_124, except for *speB*. In contrast, other *streptococci* lack genes encoding streptolysin O, NAD glycohydrolase and DRS (or SIC), suggesting the importance of these proteins in the pathogenicity of SDSE and GAS in humans, causing STSS.

Putative virulence factors unique to SDSE

We identified 20 gene products in GGS_124 containing signal peptides and LPXTG cell wall surface anchor motifs that showed low similarity to known proteins. Using PCR, we analyzed the distributions of these putative virulence factors in 8 SDSE isolates (Table 3 arranged according to their decreasing lethality in mice). A putative T-antigen-like protein structural subunit

Table 3 Putative virulence factors found in GGS_124 and their prevalence in the SDSE isolates based on the results of PCR analyses

	S. dysgalactiae subsp. *equisimilis* GGS_124				Best hit strain			PCR analysis							
Locus tag	Product name	Length (aa)	LPXTG motif	% Identity	Strain	Product name	Reference sequence	GGS_124	168	GGS_117	170	164	GGS_118	169	163
	fimbrial subunit				*Streptococcus equi* subsp. *zooepidemicus*	fimbrial subunit									
SDEG_0157	Protein	645	IPNTG	40.48	MGCS10565	protein	YP_002124169.1	Yes	x	x	x	x	x	x	x
	T-antigen-like fimbrial				*Streptococcus equi*	T-antigen-like fimbrial									
	structural subunit				subsp. *zooepidemicus*	structural subunit									
SDEG_0158	protein	315	IPKTG	49.84	MGCS10565	protein	YP_002124168.1	Yes	Yes	Yes	x	x	x	x	x
SDEG_0180	hypothetical protein	184		-	No hit	-	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	hypothetical					hypothetical									
	membrane associated				*Streptococcus pyogenes*	membrane-associated									
SDEG_0267	protein	242		44.35	MGAS2096	Protein	YP_601272.1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	cell surface serine				*Streptococcus*										
SDEG_0574	endopeptidase	216		49.36	*Agalactiae*	CspA	AAN85092.1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	cell wall surface				*Streptococcus equi* subsp. *zooepidemicus*	cell wall surface									
SDEG_0805	anchor family protein	486	LPKAG	43.73	MGCS10565	anchor family protein	YP_002123384.1	Yes	Yes	x	x	Yes	x	x	Yes
					Streptococcus equi subsp. *zooepidemicus*	histidine triad protein									
SDEG_0918	histidine triad protein	153		51.35	MGCS10565	HtpA	YP_002123384.1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	complement inhibitor				*Streptococcus pyogenes*	complement inhibitor									
SDEG_0932	protein	226		47.92	MGAS2096	protein	YP_601343.1	Yes	x	x	x	x	Yes	x	x
	nisin resistance				*Streptococcus*	nisin resistance									
SDEG_0979	protein, putative	322		42.17	*agalactiae* 2603V/R	Protein	NP_687984.1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
					Streptococcus										
SDEG_1327	YaeC family protein	280		49.47	*agalactiae* 2603V/R	YaeC family protein	NP_687791.1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	probable surface					probable surface									
	antigen negative				*Streptococcus suis*	antigen negative									
SDEG_1429	regulator	185		52.22	98HAH33	regulator Par	YP_001200806.1	Yes	x	Yes	Yes	Yes	Yes	x	Yes
					Streptococcus pyogenes										

Table 3 Putative virulence factors found in GGS_124 and their prevalence in the SDSE isolates based on the results of PCR analyses *(Continued)*

SDEG_1480	hypothetical protein	299	LPVTG	33.33	MGAS6180	hypothetical protein	YP_280631.1	Yes	x	Yes	Yes	Yes	Yes	Yes	Yes
SDEG_1511	hypothetical protein	546		27.59	*Streptococcus suis*	hypothetical protein	ABQ42885.1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
					Streptococcus uberis										
SDEG_1573	adhesion protein	661	LPKTG	38.8	UT888	adhesion protein	ABB52003.1	Yes	Yes	x	x	x	x	x	Yes
SDEG_1601	hypothetical protein	249		-	No hit	-	-	Yes	x	Yes	x	x	Yes	x	x
					Streptococcus pyogenes										
SDEG_1773	hypothetical protein	210	FPSTG	37.96	M1 GAS	hypothetical protein	NP_268519.1	Yes	Yes	Yes	x	x	Yes	Yes	Yes
					Streptococcus sanguinis										
SDEG_1969	hypothetical protein	234		32.77	SK36	hypothetical protein	YP_001035903.1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	protein F2-like				*Streptococcus equi*										
	fibronectin binding				subsp. *zooepidemicus*	fibronectin binding									
SDEG_1984	Protein	528	LPATG	40.46	ATCC 35246	protein	ABC87919.1	Yes	x	x	Yes	Yes	x	Yes	Yes
SDEG_2022	hypothetical protein	106		-	No hit	-	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
SDEG_2141	hypothetical protein	175		-	No hit	-	-	Yes	x	x	x	Yes	x	x	Yes

Genes encoding proteins, which contain predicted signal peptide based on SignalP http://www.cbs.dtu.dk/services/SignalP and the LPXTG motif based on HMMER http://bamics3.cmbi.kun.nl/jos/sortase_substrates/help.html and were not highly homologous to putative virulence factors of GGS_124. Distribution of the putative virulence factors of GGS_124 in the 16 SDSE strains was analyzed by PCR, and the results are presented as SDSE strains in order of decreasing lethality in mice. Yes: PCR analysis postive; x: PCR analysis negative.

(SDEG_0158), encoded in the FCT-6-like region in GGS_124, was detected in the 3 most virulent strains, GGS_124, GGS_168, and GGS_117. In both GGS_168 and GGS_117, the FCT-6-like region was not detected by PCR (data not shown). Interestingly, the SDEG_1601 gene encoding a functionally unknown hypothetical protein was amplified in isolates that cause STSS. However, no virulence factors associated with mouse lethality or Lancefield groups were found.

Relatedness of *emm* type and pathogenicity in SDSE

Particular M (or *emm*) types of GAS have been associated with certain streptococcal diseases [63]. In investigating the pathogenic traits of M4, M12, M1, and M3 GAS clinical isolates in a murine model [64], we found that murine lethality was closely associated with M type. The M1 and M3 types of GAS, which are isolated at high frequency from patients with STSS, showed higher virulence in mice than did M12 and M4. Using this mouse model, we assessed the virulence of the *emm* types of 8 group G SDSE isolates to analyze whether most frequent *emm*-type of isolates in epidemiological studies (see below) are more virulent in mice (Table 1). We found that the lethality in mice of these isolates was not associated with their isolation from patients with STSS or with the frequently isolated *emm*-type in humans such as stG10.0 and stG643.0 (see below). To determine whether the *emm* type of SDSE was associated with pathogenicity in humans, we reviewed the epidemiological data regarding the isolation frequencies of GCS and GGS in relation to their *emm* types [3,6,7,65,66] (Additional file 9). We found that *stG10.0* was the most frequent *emm* type in Portugal and Japan, *stG643.0* was the most frequent in western Norway, and *stG6.0* was the most frequent in the USA. When we calculated the degree of correspondence among the isolation rates of *emm* types in each area using the Kendall tau rank correlation coefficient, we found no significant correlations among all regions (data not shown), suggesting that there is no linkage between *emm* type of SDSE and infectivity in humans or mice.

Discussion

Comparative analysis of *Streptococcus* 16S rRNA sequences had indicated that SDSE was more closely related to GBS than to GAS [67,68]. In contrast, we found that SDSE, which belongs to Lancefield groups C and G [3,65], is more closely related to GAS than to other sequenced streptococci based on genome wide and gene level comparisons

SDSE is known to cause diseases very similar to those caused by GAS, such as pharyngitis, cellulitis, infective arthritis, vertebral osteomyelitis, and STSS [10-16]. This similarity may be due, at least in part, to their conservation of a large number of genes for virulence factors. Figure 7 shows a summary of putative virulence factors and proposed virulence functions in SDSE. SDSE shares most of the virulence factor genes of GAS, including streptolysin O, streptokinase, FCT-like regions, NADase, and DRS. However, GGS_124 and probably almost all other SDSEs lack SpeB, superantigens except for SpeG, and hyaluronan synthesis *via hasABC* [31]. As *spegg4*, which has about 1% of the mitogenic activity of GAS-derived SpeG, is the only gene encoding a protein homologous to superantigens, and most SDSE isolates do not harbor superantigen-like genes other than *spegg* [31,69], it is highly unlikely that superantigens play a significant role in the pathogenesis of SDSE infection in humans.

Particular M (or *emm*) types of GAS have been associated with certain streptococcal diseases [63]. We therefore analyzed the virulence in a mouse model of several SDSE strains bearing different *emm*-types, but we were unable to find a significant correlation between *emm*-type of SDSE and virulence. Further studies are needed to provide further insight into the linkage between *emm* type of SDSE and infectivity in humans.

Streptococcal phages are considered critical in horizontal gene transfer, especially in the transport of virulence factors [28,70], in *Streptococci*. Three prophage elements in GGS_124 were found to be homologous to GAS prophages in both nucleotide and amino acid sequences. The positions of prophage insertion were also conserved between GGS_124 and previously sequenced GAS prophages, suggesting that SDSE and GAS share the same phage species, and that horizontal gene transfer between SDSE and GAS has occurred. However, GGS_124 does not contain prophages that encode genes for superantigens, Sla, or MF. These phage encoded genes were also missing from all strains previously analyzed by a GAS microarray [31]. Sdn is an exception, since it was detected in 4 of these 58 SDSE strains [31]. These results showed that SDSE may have some resistance to infection by GAS phages carrying genes encoding virulence factors.

Prokaryotes possess the CRISPR/Cas system, which mediates resistance to infection by foreign DNA, such as viruses [26,27]. GGS_124 has a CRISPR/Cas system, designated CRISPR1/Cas, whereas the other SDSE isolates analyzed in this study had another CRISPR/Cas system, designated CRISPR2/Cas. We found that SDSE strains usually have a higher total number of spacers than GAS, suggesting that prophage infection of SDSE was restricted to some extent, resulting in a smaller number of virulence factors located in the prophage regions of SDSE. Similar restrictions were observed in SESZ when compared with SESE. For example, the SESE 4047 genome, which contains no CRISPR, contains genes encoding virulence factors in prophage regions. In contrast, SESZ MGCS10565

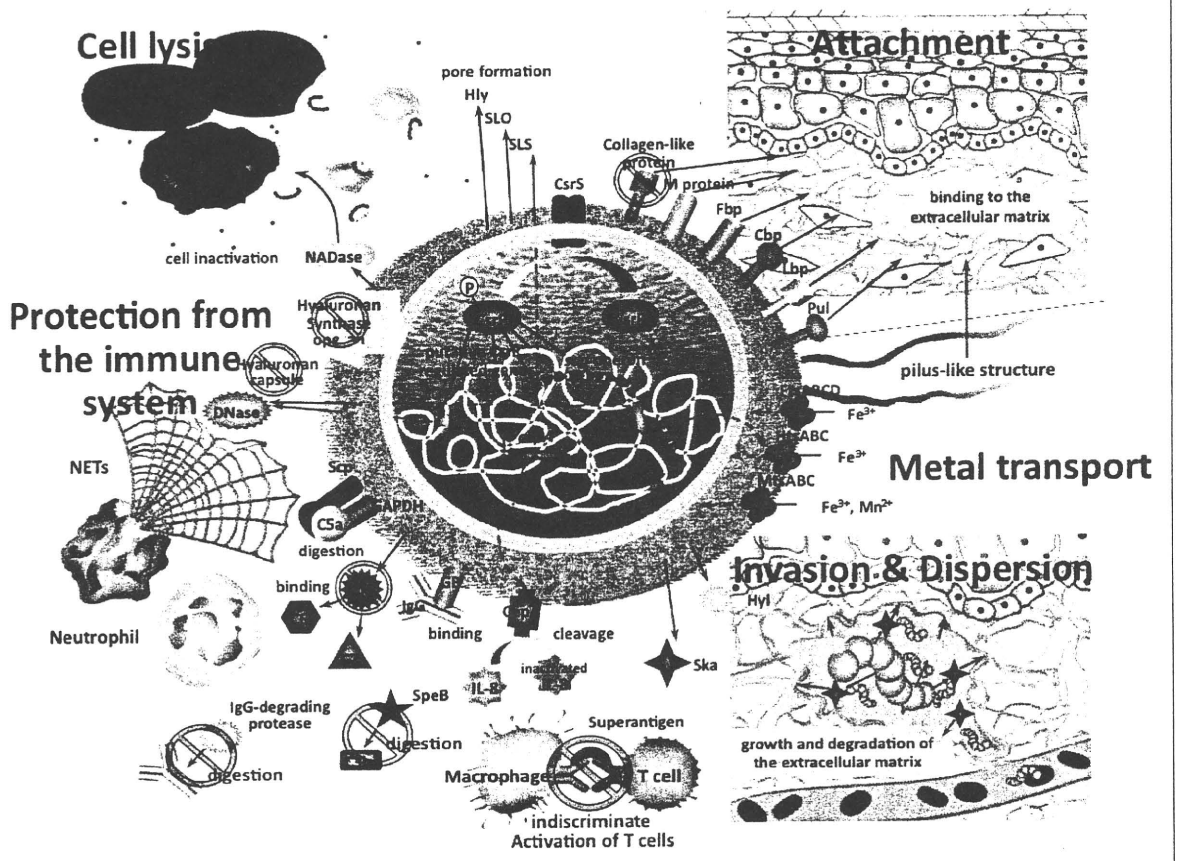

Figure 7 Putative virulence factors and posited virulence function of SDSE. Cell-surface proteins, extracellular secreted proteins, metal transporters, and the two-component regulator CsrR/CsrS, which affect the expression of approximately 10% of all genes in a GAS strain [78,79], are shown. The putative virulence factors on the cell surface of SDSE are adhesins, including M protein, fibronectin binding protein (Fbp), collagen binding protein (Cbp), laminin binding protein (Lbp), and pullulanase (Pul) [80]. Factors that protect bacteria from the host immune system are shown, including cell envelope proteinase A (CepA), which cleaves within the interleukin-8 (IL-8) C-terminal α-helix [38]; immunoglobulin G binding protein (GB) [49]; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and C5a peptidase (Scp) [46]. Putative pathogenic roles of the virulence factors of SDSE, including hyaluronidase (Hyl), streptokinase (Ska), extracellular nuclease and streptodornase (DNase), which digest neutrophil extracellular traps (NETs) released from dead neutrophils [50], and the pore-forming proteins, such as hemolysin (Hly), streptolysin S (SLS), streptolysin O (SLO), and NAD glycohydrolase (NADase), are indicated. FtsABCD and HtsABC are ferrichrome transporters and MtsABC is a metal transporter. Black arrows show protein secretion, red arrows show expression of genes regulated by CsrR, blue arrows show protein attachment to the extracellular matrix, brown arrows show metal transport from the extracellular environment into the cell, and purple arrows show degradation of extracellular matrix by secreted Hyl or Ska. The factors marked with a Stop sign, which are major virulence factors of GAS, do not function in SDSE.

and H70, which contain 26 and 18 spacers, respectively, do not carry any prophages. Thus, the CRISPR system in streptococci sharing prophages may play a substantial role in the spread of virulence factors among species. Alternatively, these virulence factors may not benefit to SDSE during carriage or disease, such that the integration of these specific phages is not selected for.

Conclusions

We have shown here that SDSE likely acts as a pathogen, based on its genome sequence and close relationship with GAS. As the frequency of isolation of SDSE from patients has increased, it should not be overlooked as a source of infection.

Methods

Bacterial strains

Three *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) isolates, GGS_124, GGS_117, and GGS_118, were obtained from 3 patients with STSS, and 5 SDSE isolates were isolated from 5 patients with non-STSS (Table 1). All SDSE isolates were classified as Lancefield

group G. *Streptococcus pyogenes* (GAS). NIH9 [71] was used as the SpeB-producing strain.

emm typing

The *emm* types were classified according to a protocol for *emm* typing of the Centers for Disease Control and Prevention (CDC) http://www.cdc.gov/ncidod/biotech/strep/protocol_emm-type.htm.

SDSE infection in mice

All animal experiments were performed according to the guidelines of the Ethics Review Committees of Animal Experiments of Tokyo Women's Medical University and the National Center for Global Health and Medicine. Virulence in mice was determined as described [64]. Briefly, LD_{50} values were determined by intraperitoneal (*i.p.*) injection of each SDSE strain into 5 6-7 week old female ddY mice.

Genome sequencing

We obtained draft contig data of GGS_124 from a commercial service (454 Life Sciences, Branford, CT), and the gaps between the contigs were tiled by PCR after closure to validate assembly using specific primers or by primer walking and an ABI 3100 genetic analyzer (Applied Biosystems Inc., Foster City, CA). The percentage of QV40+ bases, an index of the quality of data from pyro-sequencing, was 99.87%. The genome sequence of GGS_124 has been deposited in the DDBJ database (accession no. AP010935).

Genome annotation and bioinformatics

Transfer RNAs (tRNAs), transfer-messenger RNA (tmRNA), and rRNA sequences were predicted using ARAGORN [72]. Coding sequences (CDS) were predicted using *in silico* Molecular Cloning (In Silico Biology Inc., Yokohama City, Kanagawa, Japan) for selection of optimal start sites. Predicted genes and intergenic regions were compared using the NCBI sequence database http://blast.ncbi.nlm.nih.gov/Blast.cgi, and predicted CDS and start sites were adjusted accordingly. A genome-wide homology search was performed using the discontiguous megaBLAST algorithm http://blast.ncbi.nlm.nih.gov/Blast.cgi with a word size of 11 and rewards and penalties (2, -3) that optimize for alignments of about 85% identity. The phylogenetic tree of all sequenced *Streptococcus* species was constructed based on CVTree http://tlife.fudan.edu.cn/cvtree/[73]. Secretion signal peptides were predicted using SignalP http://www.cbs.dtu.dk/services/SignalP[74]. Sortases and cell wall sorting signals were predicted with available hidden Markov models using HMMER http://bamics3.cmbi.kun.nl/jos/sortase_substrates/help.html[75]. Clustered, regularly interspaced, short palindromic repeat (CRISPR) spacers were detected using CRISPR Finder http://crispr.u-psud.fr/[76]. The phylogenetic tree of all sequenced *Streptococcus* was constructed based on the CVTree http://tlife.fudan.edu.cn/cvtree/[73]

Detection and sequencing of CRISPRs and CRISPR-associated protein (Cas) genes in the 8 SDSE strains

CRISPRs and Cas genes in the SDSE strains were detected by conventional PCR using ExTaq (Takara Bio Inc., Otsu, Shiga, Japan) and the primers listed in Additional file 10. Template DNA was isolated as described [64]. The PCR cycling conditions were 94°C for 2 min followed by 30 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 4 min, and a final extension at 72°C for 6 min. The amplified CRISPR fragments were sequenced using an ABI 3100 genetic analyzer (Applied Biosystems Inc.). The CRISPR spacers were identified using CRISPR Finder http://crispr.u-psud.fr/[76]. The sequences of CRISPR and the spacers have been deposited in the DDBJ database (accession numbers are listed in Table 2).

Determination of SpeB-like protease activity

SpeB-like protease activity was assayed as described [77], with slight modifications. Briefly, DTT was added to bacterial culture supernatants to a final concentration of 10 mM and incubated at 37°C for 30 min. An equal amount of 2 g/L azocasein was added, and incubation was continued for an Additional 30 min at 37°C. Trichloroacetic acid was added to a final concentration of 5%, and incubation was continued for 15 min at 4°C. After centrifugation, an equal amount of 5 M NaOH was added to the supernatant, and its absorbance at 450 nm was measured.

Distribution of putative virulence factors unique to GGS_124 among the other SDSE

The distribution of putative virulence factors of GGS_124 among SDSE strains was analyzed by conventional PCR using ExTaq (Takara Bio Inc.) and the primers listed in Additional file 10. Template DNA extraction and PCR were performed as described [64].

Additional material

Additional file 1: Overview and comparison of the genome sequences of GGS_124 and genus Streptococcus available in databases as of January 2010.

Additional file 2: Unrooted phylogenetic tree of genus *Streptococcus*, including *S. dysgalactiae* subsp *equisimilis* GGS_124. The phylogenetic tree of all sequenced *Streptococcus* was constructed based on CVTree http://tlife.fudan.edu.cn/cvtree/[73], which constructs whole genome based phylogenetic trees without sequence alignment by using a Composition Vector (CV) approach. The genetic distances between the major nodes are shown.

Additional file 3: Genome rearrangement map of SDSE strain GGS_124 relative to GAS strains MGAS315 and strain SSI-1, and *S. uberis* 0140J. The genes were aligned from the predicted replication

origin of each genome. The colored bars separating each genome represent similarity matches identified by *in silico* molecular cloning. BLASTP comparisons of CDS with GAS MGAS315 and SSI-1 and *S. uberis* 0140J are shown as amino acid identities of ≥90% (red), 89%-80% (orange), 79%-70% (yellow), 69%-60% (green), 59%-50% (light blue), and 49%-40% (dark blue). Prophages are highlighted as green boxes.

Additional file 4: GGS_124 genes showing higher similarity to genes from bacteria other than GAS, or no similarity to genes in the databases

Additional file 5: Features of CRISPR found in the GGS_124 genome and phages derived from GAS containing sequences homologous to GGS_124 spacers

Additional file 6: Putative virulence factors found in GGS_124 and their homologous genes in other *streptococcal* species

Additional file 7: Determination of SpeB-like protease activity in the SDSE isolates. SDSE (GGS_124 and GGS_118) and GAS (NIH9) were cultured in BHI in the presence or absence of E-64, and the culture supernatants were analyzed for protease activity using azocasein as a substrate. The background activity of BHI is also shown.

Additional file 8: Distribution of the virulence factors found in SDSE among other *streptococci*

Additional file 9: Comparison of *emm*-type of GCS and GGS isolates from humans reported from 4 countries. Shown are *emm*-specific differences of SDSE in invasive and noninvasive infections from 1998 to 2004 in Portugal [65], the *emm* types of 128 strains of SDSE collected from 11 medical institutions in Japan from September 2003 to October 2005 [7], the *emm* types of 64 GCS and GGS isolates associated with noninvasive disease in western Norway from February 2005 to March 2006 [66], and the *emm* types of 212 invasive SDSE isolates collected in Atlanta, Georgia, from July 2002 to June 2004 and in the San Francisco Bay Area of California from January 2003 to December 2004 in the USA [3]. Each stack was ordered from higher (top) to lower isolation frequency (bottom).

Additional file 10: List of oligonucleotide primers used in this study.

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Authors' contributions
YS and TMA performed the molecular genetic studies, participated in sequence alignment and drafted the manuscript. KO performed the molecular genetic studies and participated in sequence alignment. SYM and KU analyzed the genome sequences. JY, YS and TMA performed animal experiments. TK drafted the manuscript. UK, JY and TK conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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感染症法とガイドライン

Ⅰ／感染症法制定とその改正の背景と経緯

1　感染症の予防及び感染症の患者に対する医療に関する法律の制定について

従前の感染症対策の中心的法規であった伝染病予防法は，明治30年に制定されたものであったが，制定後，感染症の状況は大きく変化した。昭和45年以降，世界で少なくとも30以上の新興感染症（エボラ出血熱，エイズ，C型肝炎等）が出現し，また，近い将来克服されると考えられていた感染症が再興感染症（結核，マラリア等）として人類に再び脅威を与えている。また，医学・医療の進歩，公衆衛生水準の向上，国民の健康に関する意識の向上，人権の尊重や行政の公正透明化への要請，国際交流の活発化や航空機による迅速大量輸送時代の到来等，感染症を取り巻く状況も著しく変化した。このような状況のもと，これまでの伝染病予防法を中心として実施してきた感染症対策を全面的に改めるとともに，併せて個別対策法としての性病予防法とエイズ予防法を廃止統合し，統合的に感染症対策を推進するために，感染症の予防及び感染症の患者に対する医療に関する法律（以下感染症法，平成10年法律第114号）が制定された。

2　感染症法の改正について

平成10年に感染症法が制定された後，新たに発生し世界的にも大きな問題となったSARS（重症急性呼吸器症候群）や，ウエストナイル熱をはじめとする動物由来感染症対策の強化のため，平成15年に感染症法の改正（平成15年法律第145号）が行われた。

また，平成18年に行われた感染症法の改正（平成18年法律第106号）では，病原体等の管理体制を確立し，SARSの終息宣言を踏まえての二類感染症への変更や，結核予防法を廃止して結核を二類感染症に追加する等の改正が行われた。

さらに，新型インフルエンザ対策の整備が必要であったことから，平成20年に感染症法の改正（平成20年法律第30号）が行われた。

Ⅱ／感染症法のポイント

1　対策の基本的考え方

今日，多くの感染症の予防・治療が可能になってきており，従来の集団の感染症予防に重点を置いた考え方から，個々の国民の予防および良質かつ適切な医療の積み重ねによる社会全体の感染症の予防の推進に基本的考え方を転換していくこととしている。

2 事前対応型行政の構築

これまでのように感染症が発生してから防疫措置を講じるといった事後対応型行政から，普段から感染症の発生・拡大を防止するため，

①感染症発生動向調査体制の充実

②国が策定する基本指針や都道府県の策定する予防計画

③エイズや性感染症等を対象に国が施策の総合的な方向性を示す特定感染症予防指針

の３つの柱を軸とした事前対応型行政へと転換した。

3 感染症類型と医療体制の再構築

感染症法においては，法律の対象とする感染症を，その感染力や罹患した場合の症状の重篤性等に基づいて，一類感染症から五類感染症に分類するとともに，新型インフルエンザ等感染症，指定感染症，新感染症の制度を設けている（表 1-1，1-2）。

また医療体制について，従前の伝染病予防法においては，市町村における伝染病院等の設置義務が規定されていたが，感染症法においては，各感染症に応じて良質かつ適切な医療を提供

◎表 1-1　感染症の類型とその性格について

新感染症	・人から人に伝染すると認められる疾病であって，既知の感染症と症状等が明らかに異なり，その伝染力及び罹患した場合の重篤度から判断した危険性が極めて高い感染症
一類感染症	・感染力，罹患した場合の重篤性等に基づく総合的な観点からみた危険性が極めて高い感染症
二類感染症	・感染力，罹患した場合の重篤性等に基づく総合的な観点からみた危険性が高い感染症
新型インフルエンザ等感染症	[新型インフルエンザ] 新たに人から人に伝染する能力を有することとなったウイルスを病原体とするインフルエンザであって，全国的かつ急速なまん延により国民の生命及び健康に重大な影響を与えるおそれがあると認められるもの [再興型インフルエンザ] かつて世界規模で流行したインフルエンザであってその後流行することなく長期間が経過しているものが再興したものであって，全国的かつ急速なまん延により国民の生命及び健康に重大な影響を与えるおそれがあると認められるもの （告示で指定）
三類感染症	・感染力，罹患した場合の重篤性等に基づく総合的な観点からみた危険性が高くないが，特定の職業への就業によって感染症の集団発生を起こし得る感染症
四類感染症	・人から人への感染はほとんどないが，動物，飲食物等の物件を介して感染するため，動物や物件の消毒，廃棄などの措置が必要となる感染症
五類感染症	・国が発生動向調査を行い，その結果等に基づいて必要な情報を一般国民や医療関係者に提供・公開していくことによって，発生・拡大を防止すべき感染症
指定感染症	・既知の感染症の中で上記一～三類に分類されない感染症において一～三類に準じた対応の必要が生じた感染症 （政令で指定，１年限定）

表 1-2　感染症法の対象疾病について

新感染症	（該当なし）
一類感染症	（法）エボラ出血熱，クリミア・コンゴ出血熱，痘そう，南米出血熱，ペスト，マールブルグ病，ラッサ熱
二類感染症	（法）急性灰白髄炎，ジフテリア，重症急性呼吸器症候群（SARS コロナウイルスに限る），結核，鳥インフルエンザ（病原体がインフルエンザウイルス A 属インフルエンザ A ウイルスであってその血清亜型が H5N1 であるものに限る。以下「鳥インフルエンザ（H5N1）」という）
三類感染症	（法）腸管出血性大腸菌感染症，コレラ，細菌性赤痢，腸チフス，パラチフス
四類感染症	（法）E 型肝炎，A 型肝炎，黄熱，Q 熱，狂犬病，炭疽，鳥インフルエンザ（鳥インフルエンザ（H5N1）を除く），ボツリヌス症，マラリア，野兎病 （政令）ウエストナイル熱，エキノコックス症，オウム病，オムスク出血熱，回帰熱，キャサヌル森林病，コクシジオイデス症，サル痘，腎症候性出血熱，西部ウマ脳炎，ダニ媒介脳炎，チクングニア熱，つつが虫病，デング熱，東部ウマ脳炎，ニパウイルス感染症，日本紅斑熱，日本脳炎，ハンタウイルス肺症候群，B ウイルス病，鼻疽，ブルセラ症，ベネズエラウマ脳炎，ヘンドラウイルス感染症，発しんチフス，ライム病，リッサウイルス感染症，リフトバレー熱，類鼻疽，レジオネラ症，レプトスピラ症，ロッキー山紅斑熱
五類感染症	（法）インフルエンザ（鳥インフルエンザ及び新型インフルエンザ等感染症を除く），ウイルス性肝炎（E 型肝炎及び A 型肝炎を除く），クリプトスポリジウム症，後天性免疫不全症候群，性器クラミジア感染症，梅毒，麻しん，メチシリン耐性黄色ブドウ球菌感染症 （省令）アメーバ赤痢，RS ウイルス感染症，咽頭結膜熱，A 群溶血性レンサ球菌咽頭炎，感染性胃腸炎，急性出血性結膜炎，急性脳炎（ウエストナイル脳炎，西部ウマ脳炎，ダニ媒介脳炎，東部ウマ脳炎，日本脳炎，ベネズエラウマ脳炎及びリフトバレー熱を除く），クラミジア肺炎（オウム病を除く），クロイツフェルト・ヤコブ病，劇症型溶血性レンサ球菌感染症，細菌性髄膜炎，ジアルジア症，水痘，髄膜炎菌性髄膜炎，性器ヘルペスウイルス感染症，尖圭コンジローマ，先天性風しん症候群，手足口病，伝染性紅斑，突発性発しん，破傷風，バンコマイシン耐性黄色ブドウ球菌感染症，バンコマイシン耐性腸球菌感染症，百日咳，風しん，ペニシリン耐性肺炎球菌感染症，ヘルパンギーナ，マイコプラズマ肺炎，無菌性髄膜炎，薬剤耐性緑膿菌感染症，薬剤耐性アシネトバクター感染症，流行性角結膜炎，流行性耳下腺炎，淋菌感染症
新型インフルエンザ等感染症	新型インフルエンザ，再興型インフルエンザ
指定感染症	（該当なし）

していく観点から，厚生労働大臣が指定する特定感染症指定医療機関，都道府県知事が指定する第一種感染症指定医療機関および第二種感染症指定医療機関を法定化している。

4　患者等の人権に配慮した入院手続の整備

感染症患者が感染症法に基づいて入院する場合においては，手続保障のための数多くの規定が設けられている。

まず，説明と同意に基づいた入院を期待する入院勧告制度の導入があげられるが，この勧告に応じない患者に対してのみ入院措置が講じられることになる。

また，都道府県知事（保健所長）が72時間に限って入院勧告等を行うとする応急入院制度を導入しており，その後72時間を超えた入院の必要性やさらに10日（結核の場合は30日）毎に入院継続の必要性を判断する際には，感染症の診査に関する協議会（原則として保健所毎に設置）の意見を聴いたうえで行わなければならないことを法定化している。

さらに，30日を超える長期入院患者からの行政不服審査請求に対しては，厚生労働大臣が厚生科学審議会の意見を聴いたうえで5日以内に裁決を行わなければならないといった行政不服審査法の特例を設けている。

5 まん延防止措置の再整理

従前の伝染病予防法においては，まん延防止のための措置として，消毒や物件の廃棄のほかに，集会・祭礼の禁止等の規定が数多く設けられていたが，感染症法においては，個々の規定の必要性について十分に吟味し，今日においてもなお，まん延防止のために必要とされた措置を規定する一方，不必要な規定は削除した。なお，それぞれの措置の発動に際しては，その発動を必要最小限とすることが規定されている。

6 動物由来感染症対策の充実

サルがエボラ出血熱やマールブルグ病の病原体を媒介する危険があるとして，これまでも問題とされてきたが，伝染病予防法では，感染の危険があるサルに対して，輸入禁止や輸入検疫を実施する規定は法律上設けられていなかった。感染症法においては，サル等の動物に対する輸入禁止，輸入検疫の規定を設けた。また，動物の輸入に係る届出制度の創設，獣医師等の公衆衛生上の責務，感染症を媒介するおそれのある動物についての疫学調査の実施等についても規定されている。

さらに，狂犬病予防法により，イヌ，ネコ等の狂犬病を媒介する危険性のある動物を輸入検疫の対象とすることとしている。

7 国際協力の推進

感染症の問題は，もはや1つの国のみで解決できるものではなく，世界各国が協力しながら対策を進めていかなければならない地球的規模の問題である。したがって，感染症法においては，国の責務として感染症の情報収集や研究の推進について，国際的な連携の規定を明記している。

また，検疫法の改正により，一類感染症および新型インフルエンザ等感染症等を検疫感染症として規定するなど，世界の情勢を踏まえた検疫体制の強化を図っているところである。

Ⅲ／ガイドラインの趣旨

感染症法における消毒については，都道府県知事が，感染症の病原体に汚染された場所等に

ついて，その感染症の患者やその場所を管理する者等に対し消毒を命じ，この命令で発生の予防やまん延の防止が困難である場合には市町村に消毒するよう指示し，又は都道府県の職員に消毒させることができる（感染症法第27条）。

この場合の消毒の方法については，厚生労働省令で定められることとされているが，この厚生労働省令については次のように規定されている（感染症法施行規則第14条）。

①対象となる場所の状況，感染症の病原体の性質その他の事情を勘案し，十分な消毒が行えるような方法により行うこと

②消毒を行う者の安全並びに対象となる場所の周囲の地域の住民の健康及び環境への影響に留意すること

これらの措置の実際の実施に当たっては，実務の参考となるものがあれば関係者の実務の便宜になるので，このガイドラインを作成することとしたものである。

消毒・滅菌法―基礎と実際