

Clonal Expansion of Multidrug-Resistant *Streptococcus dysgalactiae* Subspecies *equisimilis* Causing Bacteremia, Japan, 2005–2021

Appendix 2

Appendix 2 Table 1. Clinical characteristics of the patients with multiple episodes of *Streptococcus dysgalactiae* subsp. *equisimilis* bacteremia, Japan, 2005–2021*

Case no.	Age, y/sex	Underlying disease	Indwelling devices	Episode		Focus	ST	Antimicrobial drug duration, d
				no.	Date			
1	51/F	Active cervical cancer, post surgery, without metastasis	N	1	Mar 2012	Cellulitis, buttocks	ST671	14
				2	Jan 2013	Cellulitis, leg and foot	ST127	12
2	91/M	Diabetes mellitus with mild diabetic nephropathy, hypertension	Urethral catheter	1	May 2018	CAUTI	ST17	15
				2	Feb 2020	CAUTI	ST17	17
				3	Mar 2021	CAUTI	ST525	14
3	85/M	Congestive heart failure, hypertension	Cardiac pacemaker	1	Jul 2019	Vertebral osteomyelitis, psoas abscess, cervical lymph node adenopathy	ST525	58

Case no.	Age, y/sex	Underlying disease	Indwelling devices	Episode no.	Date	Focus	ST	Antimicrobial drug duration, d
				2	Apr 2020	Primary bacteremia without focus	ST525	26
4	74/M	Hemodialysis-dependent end-stage renal disease, Prostate cancer (post surgery) without metastasis, hypertension	N	1	Jun 2020	Primary bacteremia without focus	ST127	118
				2	Dec 2020	Vertebral osteomyelitis	ST17	not finished due to chronic suppression
5	83/F	Cervical cancer (post radiation therapy) without metastasis	Total hip replacement	1	Oct 2016	Cellulitis, left extremity	ST34	57
				2	Jan 2018	Cellulitis, left extremity	ST34	18
6	44/M	Diabetes mellitus with diabetic nephropathy	N	1	Apr 2017	Cellulitis, left extremity	ST17	16
				2	Dec 2017	Cellulitis, right extremity	ST628	16
7	81/M	Chronic kidney disease, aplastic anemia; immunosuppression via cyclophosphamide	N	1	Sep 2018	Cellulitis, right extremity	ST17	17
				2	Dec 2018	Cellulitis, right extremity	ST17	14
8	78/M	Cerebral infarction	N	1	Feb 2019	Cellulitis, left extremity	ST25	15
				2	Jun 2019	Cellulitis, left extremity	ST25	15

Case no.	Age, y/sex	Underlying disease	Indwelling devices	Episode no.	Date	Focus	ST	Antimicrobial drug duration, d
				3	Aug 2019	Cellulitis, left extremity	ST25	14
				4	Dec 2020	Cellulitis, left extremity	ST25	15
				5	Jun 2021	Cellulitis, left extremity	ST25	8
9	79/F	Anal canal cancer without metastasis, not treated within 5 years	N	1	Jan 2020	Cellulitis, right extremity	ST17	17
				2	Mar 2020	Cellulitis, right extremity	ST17	43

*CAUTI, catheter-associated urinary tract infection; ST, sequence type.

Appendix 2 Table 2. Patient demographics and clinical manifestations of 10 patients with nosocomial *Streptococcus dysgalactiae* subsp. *equisimilis* bacteremia, Japan, 2005–2021

Demographic and clinical data	No. (%)
Median age, y (IQR)	70.5 (67.5– 78.8)
Age group, y	
<50	0
50–59	1 (10.0)
60–69	3 (30.0)
70–79	4 (40.0)
80–89	1 (10.0)
≥90	1 (10.0)
Sex	
M	8 (80.0)
F	2 (20.0)
Medical history	
Malignant diseases	7 (70.0)
Hematological malignancy	1 (10.0)
Solid organ tumor	6 (60.0)
Cardiovascular diseases	2 (20.0)
Cerebral artery diseases	3 (30.0)
Chronic kidney diseases	1 (10.0)
Diabetes mellitus	3 (30.0)
Dementia	1 (10.0)
Chronic lung diseases	0
Collagen diseases	0
HIV	0
Chemotherapy	5 (50.0)
Immune suppressive therapy	1 (10.0)
Neutropenia	3 (30.0)
Clinical source of bacteremia, including duplicates*	
Cellulitis	2 (20.0)
Primary bacteremia without focus	4 (40.0)
Necrotizing fasciitis	0
Osteomyelitis and discitis	1 (10.0)
Psoas abscess	1 (10.0)

Demographic and clinical data	No. (%)
Septic arthritis	0
Infectious endocarditis	0
Urinary tract infection	0
Pneumonia	0
Catheter related blood stream infection	3 (30.0)
Multiple pathogen infection	4 (40.0)
<i>Escherichia coli</i>	1 (10.0)
<i>Staphylococcus aureus</i>	1 (10.0)
Viridans streptococci	1 (10.0)
<i>Staphylococcus epidermidis</i>	1 (10.0)
Clinical characteristics†	
Temperature $\geq 38^{\circ}\text{C}$	8 (80.0)
Mean arterial pressure <80 mm Hg, n = 9	5 (55.6)
Pulse rate >90 beats/min, n = 9	5 (62.5)
Disturbance of consciousness	6 (60.0)
Severe disease	
Streptococcus toxic shock syndrome	0
Vasopressor support required	0
Ventilator support required	0
Admission to intensive care unit required	0
Death	
In-hospital death	2 (20.0)
30-d mortality	1 (10.0)

*Vertebral osteomyelitis and psoas abscess, 1 case.

†Data on blood pressure was missed in 1 case-patient, and data on pulse rate was missed in 2 case-patients.

Appendix 2 Table 3. Correlation with *emm* type and clonal complex among *Streptococcus dysgalactiae* subsp. *equisimilis* isolates causing bacteremia, Japan, 2005–2021*

<i>emm</i> type	Clonal complex							Sequence type								Total no. (%)	
	17	25	29	34	146	15	128	129	471	70	8	285	674	675	677		525
<i>stG6792</i>	40	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41 (28.1)
<i>stG245</i>	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29 (19.9)
<i>stG840</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	16 (11.0)
<i>stG485</i>	0	0	10	0	0	0	4	0	0	0	0	0	0	0	0	0	14 (9.6)
<i>stG6</i>	0	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	5 (3.4)
<i>stG166B</i>	0	5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	6 (4.1)
<i>stG653</i>	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3 (2.1)
<i>stG2078</i>	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3 (2.1)
<i>stG10</i>	0	0	0	0	0	4	0	0	1	0	0	0	0	0	0	0	5 (3.4)
<i>stG480</i>	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	3 (2.1)
<i>stG4974</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2 (1.4)
<i>stC1400</i>	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	4 (2.7)
<i>stC36</i>	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2 (1.4)
<i>stG652</i>	1	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	5 (3.4)
<i>stG5420</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (0.7)
<i>stC74a</i>	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3 (2.1)
<i>stC6979</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1 (0.7)
<i>stG2574</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2 (1.4)
<i>stC15</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1 (0.7)
Total no.	50	41	13	5	3	6	4	1	1	1	1	1	1	1	1	16	146
(%)	(34.2)	(28.1)	(8.9)	(3.4)	(2.1)	(4.1)	(2.7)	(0.7)	(0.7)	(0.7)	(0.7)	(0.7)	(0.7)	(0.7)	(0.7)	(11.0)	

Appendix 2 Table 4. Prevalence of virulence factors according to the clonal complex or sequence type among *Streptococcus dysgalactiae* subsp. *equisimilis* isolates causing bacteremia, Japan, 2005–2021

Category, virulence factor	Assoc. gene	Clonal complex or sequence type, no. (%)						Severe disease			
		CC17	CC25	CC29	ST525	Others	Total	STSS	NF	Vasopressor need	In-hospital mortality
Adherence		N = 50	N = 41	N = 13	N = 16	N = 24	N = 146			N = 142	N = 143
Fibronectin binding protein	<i>fbp54</i>	50 (100)	41 (100)	13 (100)	16 (100)	26 (100)	146 (100)	NA	NA	NA	NA
	<i>GBS_65</i>	50 (100)	24 (58.5)	13 (100)	16 (100)	11 (42.3)	114 (78.1)	5/114 (p = 0.648)	10/114 (p = 0.118)	8/112 (p = 0.452)	8/112 (p = 1.000)
	<i>GBS_70</i>	50 (100)	24 (58.5)	13 (100)	16 (100)	11 (42.3)	114 (78.1)	5/114 (p = 0.648)	10/114 (p = 0.118)	8/112 (p = 0.452)	8/112 (p = 1.000)
Pilus island 1	<i>RS035</i>	48 (96.0)	24 (58.5)	13 (100)	16 (100)	11 (42.3)	112 (76.7)	5/112 (p = 0.665)	10/112 (p = 0.117)	8/110 (p = 0.483)	8/110 (p = 1.000)
	<i>85</i>	50 (100)	41 (100)	13 (100)	16 (100)	26 (100)	146 (100)	NA	NA	NA	NA
Laminin binding protein	<i>lmb</i>	50 (100)	41 (100)	13 (100)	16 (100)	26 (100)	146 (100)	NA	NA	NA	NA
C5a peptidase	<i>scpA/s</i>	50 (100)	41 (100)	13 (100)	16 (100)	26 (100)	146 (100)	NA	NA	NA	NA
	<i>cpB</i>	50 (100)	41 (100)	13 (100)	16 (100)	26 (100)	146 (100)	NA	NA	NA	NA
Exotoxin											
Streptococcal pyrogenic exotoxin	<i>speG</i>	0 (0)	0 (0)	13 (100)	16 (100)	15 (57.7)	44 (30.1)	5/44 (p = 0.026)	5/44 (p = 0.169)	4/43 (p = 869)	5/42 (p = 0.158)
Streptolysin O	<i>slo</i>	50 (100)	41 (100)	13 (100)	16 (100)	26 (100)	146 (100)	NA	NA	NA	NA
Exoenzyme											
Streptokinase	<i>ska</i>	50 (100)	41 (100)	13 (100)	16 (100)	26 (100)	146 (100)	NA	NA	NA	NA
	<i>sda</i>	1 (2.0)	5 (12.2)	0 (0)	0 (0)	1 (3.1)	7 (4.8)	0/7 (p = 1.000)	0/7 (p = 1.000)	0/7 (p = 1.000)	0/7 (p = 1.000)
Immune modulation											

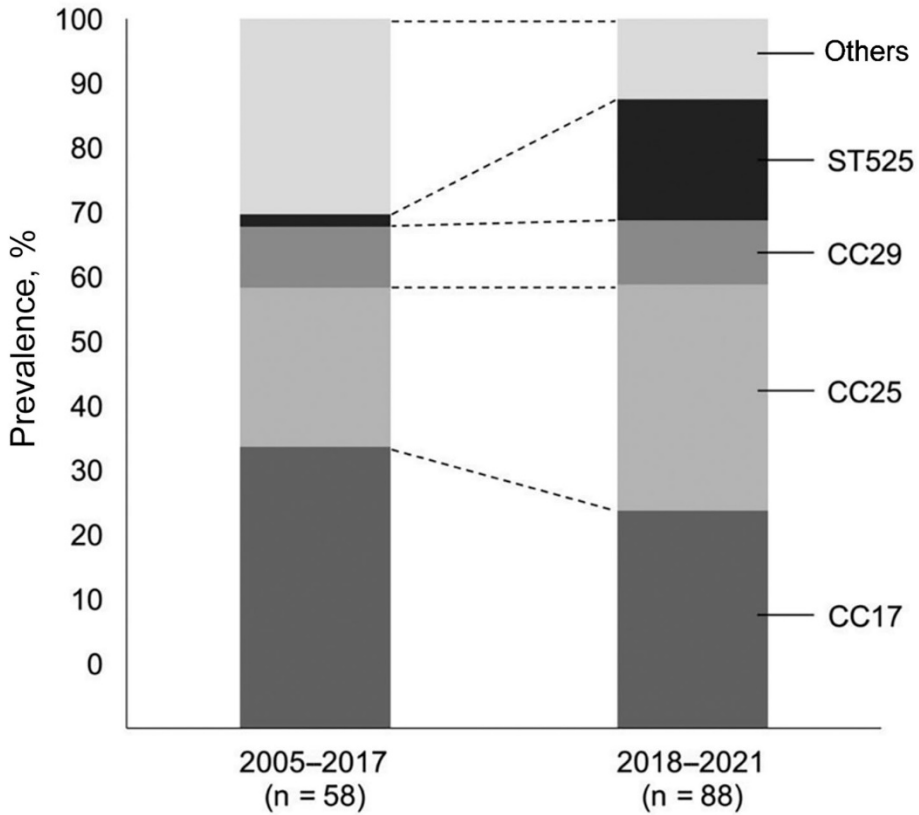
Category, virulence factor	Assoc. gene	Clonal complex or sequence type, no. (%)						Severe disease			
		CC17	CC25	CC29	ST525	Others	Total	STSS	NF	Vasopressor need	In-hospital mortality
Hyaluronic acid capsule	<i>hasC</i>	50 (100)	41 (100)	13 (100)	16 (100)	32 (100)	146 (100)	NA	NA	NA	NA

*CC, clonal complex; NF, necrotizing fasciitis; SSTS, streptococcal toxic shock syndrome; ST, sequence type.

Appendix 2 Table 5. Comparison of Tn3872-like integrative conjugative elements among other bacterial species and *Streptococcus dysgalactiae* subsp. *equisimilis* isolates causing bacteremia, Japan, 2005–2021*

Species	No. of alignments, total = 33	% Query cover
<i>Streptococcus agalactiae</i>	6	97
<i>Streptococcus pneumoniae</i>	13	96–97
<i>Streptococcus gordonii</i>	1	97
<i>Streptococcus pyogenes</i>	1	97
<i>Streptococcus salivarius</i>	1	97
<i>Streptococcus gwangjuense</i>	1	99
<i>Streptococcus oralis</i>	2	97
<i>Streptococcus mitis</i>	1	97
<i>Enterococcus faecalis</i>	1	97
<i>Enterococcus faecium</i>	2	97
<i>Gemella morbillorum</i>	1	97
<i>Lactobacillus iners</i>	2	97
<i>Fusobacterium nucleatum</i> subsp. <i>Animalis</i>	1	97

*We conducted a BLAST (<https://blast.ncbi.nlm.nih.gov>) search using Tn3872-like integrative conjugative elements (ICE) as a query. Differences in comparison with Tn3872-like ICE in ST525 are shown among species that lack the hypothetical protein between ORF20–ORF21.



Appendix 2 Figure. Temporal changes in the prevalence of CC17, CC25, CC29, ST525, and other clonal complexes and sequence types among *Streptococcus dysgalactiae* subsp. *equisimilis* isolates causing bacteremia, Japan, 2005–2021. CC, clonal complex; ST, sequence type.