

25 **Abstract**

26 Beta-hemolytic streptococci of groups C and G, designated as *Streptococcus*
27 *dysgalactiae* (SD), can cause severe and recurring invasive infections. In this case-
28 control study, we aimed to identify clinical and molecular risk factors for recurrence
29 of SD bacteremia. Twenty-two cases of recurrent SD bacteremia were identified and
30 median time between episodes was six months. The most frequent clinical
31 manifestation was skin- and soft-tissue infection. Cases and 92 controls, with single
32 episode SD bacteremia, showed similar demographics, had similar Charlson
33 comorbidity scores, and had similar clinical presentations. Thirty days fatality was 13
34 % among controls whereas none of 22 cases died. In 19 (86%) cases, the same *emm*
35 type was encountered in both episodes. SD isolates from recurrent episodes and from
36 single episodes had a similar *emm* type distribution. Thus, we did not identify clinical
37 risk factors for recurrences. The high proportion of identical *emm* types in recurrent
38 episodes indicates a host-specific colonization.

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41 **Keywords:** bacteremia, beta-hemolytic streptococci, recurrent bacteremia,
42 *Streptococcus dysgalactiae*, *emm*-type.

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45 **1. Introduction**

46 Human pathogenic beta-hemolytic streptococci of groups C and G (GCS and GGS)
47 have become increasingly recognized as important causes of severe infection [1-3].
48 The vast majority of GGS and GCS causing human infections belong to
49 *Streptococcus dysgalactiae* (SD) and cause a spectrum of disease similar to that of
50 *Streptococcus pyogenes* [3-6]. SD is further divided into *subsp. equisimilis* which is
51 beta-hemolytic and can have different Lancefield groups and *subsp. dysgalactiae*
52 which is not beta-hemolytic and display Lancefield group C [7]. Otherwise, the two
53 subspecies are not easily separated by standard methods such as biochemistry or
54 sequencing of the 16S rRNA gene [7,8]. Among GCS causing human infections,
55 some isolates are *Streptococcus equi* [5,9], and some isolates among GGS are
56 *Streptococcus canis* [10]. Typing of SD is commonly performed through sequencing
57 of the *emm* gene encoding the M protein. The most common focus of invasive SD
58 infection is skin- and soft tissue, followed by bacteremia of unknown origin, but also
59 septic arthritis, and infective endocarditis occurs [3]. Invasive infections are
60 frequently seen in elderly patient and those with underlying medical conditions [5,11].
61 The mortality rates in SD bacteremia have been reported to be between 8 and 15 %
62 [5,11,12]. SD bacteremia has a tendency to recur and rates of recurrence between 3
63 and 9 % have been reported [6,13-16]. In such reports, up to four recurrences were
64 described with a median interval of 8 months (range 1-64 months) between the
65 episodes [3,6,15,17]. There has been some speculation whether or not recurrence is
66 associated with a specific *emm* SD type or- clinically- with specific underlying
67 conditions. In previous case series, recurrence was caused by the same *emm* type SD
68 in 7 of 8, 3 of 4, and in 2 of 5 patients and isolates from a given patient displaying the
69 same *emm* type were highly genetically related [3,6,15]. The SD *emm* type causing

70 recurrence has varied between reports but StG485.0 [6,17] and StG6 [3] have been
71 isolated from many cases. These studies were underpowered to detect if particular SD
72 types are more prone to cause recurrent bacteremia. Studies looking at the clinical
73 associations reported the presence of genital cancer (particularly cervical cancer), a
74 history of cellulitis [6], or “chronic lymphatic abnormalities” [15] more frequent in
75 cases with recurrent SD bacteremia. The present case-control study was performed to
76 identify bacterial and host factors associated with increased risk for recurrence of SD
77 bacteremia.

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79 **2. Materials and methods**

80 Cases of recurrent bacteremia with GCS and GGS between 2003 and 2013 were
81 identified in the databank of the department of clinical microbiology in Malmö/Lund.
82 The laboratory is the only one serving a population of around 1.2 million inhabitants.
83 For each case, the two preceding and the two following patients with bacteremia with
84 SD of the same streptococcal group were selected as controls (i.e. case-control ratio
85 1:4). The identification of the bacteria by the diagnostic laboratory had depended on a
86 typical appearance upon Gram-staining and on blood agar as well as on latex
87 agglutination (Streptex, Remel, Lenexa, KS, USA). We cultured the bacteria on blood
88 agar in 5% CO₂ at 37°C over night and directly transferred them to grids and
89 subjected them to analysis with Ultraflex extreme matrix-assisted laser desorption
90 ionization - time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics,
91 Bremen, Germany), using the Biotyper version 3.0 software. A score of above 2.0
92 was considered to confirm the identification to the species level. The *emm* gene
93 encoding the SD M protein was sequenced and type was determined as described
94 (<http://www.cdc.gov/streplab>).

95 Data on clinical presentation was gathered from the medical records of the respective
96 patient according to a predefined questionnaire modified from [18]. Sepsis was
97 assessed as described [19]. The study was approved by the Regional Ethics
98 Committee in Lund (no 2013/31). Statistical analyses were performed using the Prism
99 6 software. If not otherwise stated, Fischer's exact test was used to compare
100 categorical variables and Mann-Whitney-U test used for continuous variables. A p-
101 value < 0.05 was considered significant.

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103 **3. Results**

104 **3.1 Patients with recurrence**

105 Twenty-three patients with recurrent episodes of bacteremia with GCS or GGS were
106 identified among a total of 593 episodes. Their features are described in table 1. In 19
107 patients GGS, and in 4 patients GCS was the microorganism causing recurring
108 bacteremia. When MALDI-TOF MS species identification was used, SD was
109 demonstrated in 22 cases, and *Streptococcus canis* (GGS) in one case. In 20 patients
110 two episodes of bacteremia were recorded, and in three patients three episodes were
111 identified. The median time between the first and the second episodes was six months
112 (range 1-53 months), whereas the time interval between the second and third episode
113 was four, six, and nineteen months respectively. In 19 patients, the recurrence was
114 caused by the same *emm* type. In three cases, different *emm* types were noted. The *S.*
115 *canis* isolate was non-typeable.

116 Skin- and soft-tissue infection was the most frequent clinical manifestation (17 of 22
117 patients). Erysipelas was the single most common entity comprising 50 %. In 77 % of
118 the cases, the clinical presentation was identical in the first and the second episode.

119 Treatment for the first episode was given with a beta-lactam antibiotic for a minimum

120 of 10 days. More SIRS criteria (median 3) were noted on the presentation of a
121 recurrent episode when compared with the initial episode (median 2), though this
122 difference was statistically not significant ($p=0.2$ with Wilcoxon's matched-pairs
123 signed rank test). There were no differences in CRP levels or the number of patients
124 fulfilling criteria for severe sepsis on initial presentation and presentation at
125 recurrence. No fatalities were recorded among the 23 patients with recurrence.

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127 **3.2 Comparison of recurrent and single episodes of SD bacteremia**

128 **3.2.1 Bacteriological aspects**

129 92 control isolates (76 of GGS and 16 of GCS) were confirmed to be SD by MALDI-
130 TOF MS. The type-distribution of the isolates where the same type caused recurrence
131 ($n=19$) were similar to types of isolates ($n=92$) causing a single episode (Figure 1).
132 Types StG643, StG480, and StG6 were common in both groups whereas StG485 was
133 more common in the group with a single episode.

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135 **3.2.2 Clinical characteristics**

136 Table 2 compares the demographic and clinical characteristics of patients with
137 recurrent and single episode SD bacteremia. A previous history of erysipelas was
138 more common in the group with recurrence but this difference was not statistically
139 significant. Table 2 also compares the clinical presentations of patients with recurrent
140 and single episode SD bacteremia. Erysipelas was the single most common entity
141 comprising 50 % of patients who later recurred and 38 % of those with a single
142 episode. Infection foci such as abscesses, bone and joint infections and infections
143 related to medical procedures and foreign materials were more common in the group
144 with a single episode. The differences were, however, not statistically significant.

145 Five patients (23 %) from the group with recurrent SD bacteremia had an initial
146 presentation with severe sepsis; in comparison 30 patients (32 %) presented with
147 severe sepsis in the group with a single SD bacteremia episode ($p=0.6$). Empirical
148 treatment was initiated with a broad-spectrum beta lactam (e.g. a cephalosporin or
149 carbapenems) in 52 % of cases and with a penicillin or cloxacillin in 34 % of cases
150 with no significant differences between the groups. The total time for intravenous
151 antibiotics was similar between the groups (7 and 6.5 days respectively) as was the
152 total time of antibiotic treatment (Table 2). The length of hospital stay was non-
153 significantly longer in the group with a single episode.
154 There were 12 fatalities (13 %) within 30 days of the positive blood culture in the
155 control group whereas none of the 23 patients experiencing recurrence died within 30
156 days of the recurrence ($p=0.1$).

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158 **4. Discussion**

159 We identified 23 patients with recurrent GCS and GGS bacteremia in 11 years
160 underlining the observation that SD bacteremia has a tendency to recur. Our study,
161 despite of being retrospective, is larger than previous case reports/series, has defined
162 controls, and is population based. We did not identify factors that were significantly
163 associated with an increased risk for recurrence, apart from the association with
164 erysipelas in the first episode. Due to the nature of retrospective case-control studies,
165 the conclusions are limited by the estimates (e.g. clinical assessment) noted in the
166 patient chart and the relatively small sample size of cases. Nevertheless, the fact that
167 almost 90% of the recurrences are caused by a pathogen revealing the same *emm* type
168 is indicative for a specific host-pathogen colonization association. Moreover, none of

169 our patients had a history of intravenous drug use, suggesting an endogenous or host-
170 specific source of recurrent bacteremia.

171 *SD subspecies equisimilis* is beta-hemolytic and can display different Lancefield
172 groups as opposed to *subsp. dysgalactiae* which is not beta-hemolytic and carries a
173 Lancefield group C antigen [7]. The SD isolates described here all displayed beta-
174 hemolysis and a majority are Lancefield group G and thus they likely all belong to the
175 *subspecies equisimilis* [7], but since MALDI-TOF MS cannot separate this subspecies
176 from *subspecies dysgalactiae* we chose not to indicate subspecies [8]. The types
177 StG6, StG480, and StG485 prevalent in this study have also been reported to be so by
178 others [2,12,14,20]. Type StG643 comprising 17 % of our isolates was very recently
179 shown to be common in Norway [21]. Also, looking solely at *emm*-types, there was
180 no significant association with increased risk for recurrence. We were not able to
181 analyze host factors (e.g. HLA types) to review a host-pathogen association as shown
182 previously with GAS [22]. The risk factors reported previously; genital cancer [6] and
183 “chronic lymphatic abnormalities” [15], were infrequent among our cases whereas a
184 history of cellulitis, reported by Liao *et al.* to be associated with recurrence, was also
185 observed frequently among our patients with recurrence.

186 The risk for recurrence in GCS/GGS bacteremia has been reported to be between 3
187 and 10 % and the majority of recurrences occur within the first year [6,13-16]. We
188 could confirm this observation in our study. Since bacteremia with SD is associated
189 with significant mortality and morbidity, the patients and their relatives should be
190 informed of the risk for recurrence and informed to seek medical care immediately
191 upon suspicion of recurrence. Intensified efforts to eliminate potential risk factors
192 such as wounds are also warranted. The use of penicillin prophylaxis is a
193 controversial issue and the dosing and duration of such treatment is unexplored. In

194 selected cases prophylaxis could be considered and we would favor the use of
195 prophylaxis to patients after recurrence if there is a defined risk factor that cannot be
196 eliminated. To improve the understanding of SD recurrent bacteremia, future studies
197 should address if the patients with recurrence are constantly colonized or if they are
198 reinfected as well as if their antibody response to SD differ from that of patients with
199 single episodes.

200

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296 **Legend to figure**

297 Figure 1. Distribution of types among isolates where the same *emm* type caused the
298 recurrent episode (n=19) represented with black bars and among isolates from
299 single episodes (n=92) represented by grey bars. Other isolates include
300 StG5820, StG245, and StG5420 (n=2), as well as single isolates of StC1400,
301 StC36, and StC6979.

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303 **Table 1 Characteristics of patients with recurrent episodes of bacteremia with**
 304 **GCS/CCS**

Age & gender	Underlying condition	Clinical syndrome 1 st /2 nd episode	Group	Type	Months to recur
90 M	CHF, RF	Erysipelas	GGS	StG480/485	305
87 F	De	UF/bursitis	GGS	nt	307
83 M	PE, PN	Erysipelas	GCS	StG652	308
71 M	DM, KT, CW	WI	GGS	StG480	3
69 F	DM, RF, CW, COPD	WI	GGS	StG10	309
62 M	DM, Ob, CW	Erysipelas	GGS	StG6792/652	310 311
56 F	LO, PE	Erysipelas	GGS	StG166	312
87 M	De, PC	UF/Erysipelas	GGS	StG485	313
84 F	S, CHF	Erysipelas	GGS	StG480	313
81 M	DM, CW, PD	Erysipelas/WI	GCS	StG62647	314
79 M	PE, cyt for LC	Erysipelas	GGS	StG6	315
69 F	AML, PAC	UF/PAC-infection	GGS	StG2078	316
68 F	SLE, CHF, COPD	Necrotizing cellulitis/UF	GCS	StG643	317
79 M	PH, PE	Erysipelas	GGS	StG480	318 319
62 F	DCM, Sa	Erysipelas	GGS	StG6	320
80 F	AVP	UF/Erysipelas	GCS	StG643	320
81 M	PC, CW	WI/WI/osteitis	GGS	StC74a	2, 21
75 M	PH	Cellulitis	GGS	StG485	322
70 F	RT against AC	Myositis/UF	GGS	StG6	323
71 M	Ob, DM, UC	Erysipelas	GGS	StG6792/6/652	16, 24 325
60 M	DM, HL, Ps	Cellulitis/Erysipelas	GGS	StG643	9 326
57 M	Cyt for VC, PAC	UF/PAC-infection	GGS	StG480	327
65 M	PE, Ob, PN	Erysipelas	GGS	StG483	327 328

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Abbreviations used are: CHF, congestive heart failure; RF, renal failure; nt, non-typable; De, dementia; UF, unknown focus; PE, previous erysipelas; PN, polyneuropathy; DM, diabetes mellitus; KT, kidney transplanted; CW, chronic wound; WI, wound infection; COPD, chronic obstructive pulmonary disease; Ob, obesitas; LO, lymphedema; PC, prostate cancer; S, stroke; PD, Parkinsons disease; cyt, cytostatic drugs; LC, lung cancer; AML, acute myeloic leukemia, PAC, port-á-cath; SLE, systemic lupus erythematosus; ICU, intensive care unit; DCM, dilated cardiomyopathy; Sa, sarcoidosis; AVP, aortic valve prosthesis; PH, prostate hyperplasia; RT, radiation therapy; AC, anal cancer; UC, urinary catheter; HL, Hodgekin lymphoma; Ps, psoriasis, VC, ventricular carcinoma.

355 **Table 2. Comparison of recurrent and single episodes of bacteremia with SD**

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

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357 Statistical testing was performed with Mann-Whitney U test for continuous variables
 358 and with Fischer's exact test for categorical variables.

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