Clinical aspects of invasive infections with *Streptococcus dysgalactiae* ssp. *equisimilis* in Japan: differences with respect to *Streptococcus pyogenes* and *Streptococcus agalactiae* infections

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Abstract

Streptococcus dysgalactiae ssp. *equisimilis* (SDSE) is increasingly being identified as a pathogen responsible for invasive and non-invasive infections. We compared the clinical features of invasive SDSE infections with those of invasive infections caused by *Streptococcus pyogenes* (group A streptococcus (GAS)) and *Streptococcus agalactiae* (group B streptococcus (GBS)). Active surveillance for invasive SDSE, GAS and GBS was maintained over 1 year at 142 medical institutions throughout Japan. Clinical information was collected together with isolates, which were characterized microbiologically. Two hundred and thirty-one invasive SDSE infections were identified, 97 other patients had infections with GAS, and 151 had infections with GBS. The median age of the SDSE patients was 75 years; 51% were male and 79% had underlying diseases. Forty-two SDSE patients (19%) presented to the emergency department. Among the 150 patients (65%) for whom follow-up was completed, 19 (13%) died and eight (5%) had post-infective sequelae (poor outcome). Insufficient white blood cell responses (<5000 cells/μL) and thrombocytopenia on admission each suggested significantly higher risk of poor outcome (ORs 3.6 and 4.5, respectively). Of 229 isolates, 55 (24%) showed an stG6792 *emm* type, which was significantly associated with poor outcome (OR 2.4). Clinical manifestations of invasive SDSE infections were distinct from those of invasive GBS infections. Primary-care doctors should consider invasive SDSE infections when treating elderly patients.

Keywords: Invasive infections, non-invasive infections, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae* ssp. *equisimilis*

Original Submission: 7 June 2009; **Revised Submission:** 23 August 2009; **Accepted:** 24 August 2009

Editor F. Allerberger

Article published online: 2 September 2009

Clin Microbiol Infect 2010; **16**: 1097–1103

10.1111/j.1469-0691.2009.03047.x

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Introduction

Invasive infections caused by β-haemolytic streptococci apart from Lancefield groups A and B, as well as by *Streptococcus pyogenes* (group A streptococcus (GAS)) and *Streptococcus agalactiae* (group B streptococcus (GBS)), are reported increasingly worldwide [1,2]. The other streptococci include groups C, G, F and L; group G is notable because these streptococci can cause bacteraemia [3,4]. According to previous investigations [5], this group includes *Streptococcus dysgalactiae* ssp. *equisimilis* (SDSE), the *Streptococcus anginosus* group, and *Streptococcus canis*.

Recently, SDSE isolates possessing group G antigen have been recovered increasingly from severe invasive streptococcal infections [6]. Brandt *et al.* [7] characterized blood culture isolates of SDSE possessing Lancefield group A antigen. Infection with this pathogen (11 strains) was also sometimes found to lead to streptococcal toxic shock syndrome [8]. We have just completed whole genome analyses of two original isolates (GGS_124 (GenBank accession number

AP010935) and RE378) of SDSE, demonstrating a rate of genome overlap between this subspecies and GAS of 61–63%, whereas the overlap between the subspecies and GBS genomes was 15% (T. Akiyama, K. Ubukata & T. Kirikae, unpublished data).

However, active nationwide surveillance with a collection of large numbers of strains remains to be instituted, as for many years SDSE was considered to be non-pathogenic. We therefore collected isolates of this microorganism as well as of GAS and GBS, with accompanying detailed clinical information, from 142 medical institutions. Our aim was to compare clinical aspects of invasive diseases caused by SDSE with those caused by GAS or GBS in Japan.

Materials and Methods

Surveillance

Active laboratory-based surveillance for invasive SDSE, GAS or GBS infections was organized by the Laboratory of Molecular Epidemiology for Infectious Agents at the Graduate School of Infection Control Sciences, Kitasoto, Japan. SDSE included some isolates of the Lancefield C or A groups. We excluded *S. anginosus* group isolates that showed group C, G or F antigen in the process of isolate identification.

Surveillance was conducted for 1 year (1 August 2006 to 31 July 2007) in 142 medical institutions participating in the Invasive Streptococcal Disease Working Group established at the 19th Annual Meeting of the Japanese Society for Clinical Microbiology. Invasive streptococcal diseases were defined as conditions with isolation of organisms from a normally sterile site (i.e. blood, cerebrospinal fluid, joint fluid, ascites, or pleural effusion) [1,9,10]. Isolates were first identified as streptococci at local hospital laboratories, using standard commercially available latex agglutination assays. Detailed standardized categories of information regarding clinical features (e.g. hospital departments of initial presentation and underlying conditions) and laboratory findings (e.g. white blood cell (WBC) count, platelet count and C-reactive protein (CRP) serum concentration) were obtained from medical charts for all subjects enrolled. Clinical syndromes were assigned on the basis of physicians' diagnoses recorded in the medical charts. Poor outcomes were defined as either death from invasive infections within 3 weeks of onset or invasive disease-associated sequelae following the streptococcal infection (e.g. worsened paralysis or bedridden status). All isolates were sent to the Laboratory of Molecular Epidemiology for Infectious Agents to determine additional characteristics, including Lancefield group, species, M protein gene (*emm*) or capsular type, and antibiotic susceptibility.

Laboratory methods

Isolates were characterized with standard biochemical and enzymatic tests, and were identified as previously described [11]. The *emm* types of SDSE or GAS isolates [12] and the capsular types of GBS [13] isolates were determined as previously reported. All *emm* typing was based on the CDC database (ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/tsemm/). In addition, we quantified the susceptibility of streptococci to 14 antibiotics, including oral and parenteral antibiotics, by agar plate dilution methods using blood agar, as previously described [10]. Depending on the MICs, the presence of streptococcal genes associated with resistance to antimicrobials (e.g. *mef*(A), *erm*(A), or *erm*(B)) was determined. To assess the similarity of isolates, profiling using pulsed-field gel electrophoresis (PFGE) following DNA treatment with the restriction enzyme *Sma*I was also performed [10].

Statistical analysis

Microsoft Excel was used for data analysis. Patient or pathogen characteristics, clinical features and outcomes were compared between paired groups of isolates (SDSE and GAS, SDSE and GBS, or GAS and GBS), using the chi-squared test. To identify clinical laboratory findings associated with fatal outcome, ORs with 95% CIs and p-values according to the chi-squared test were calculated.

Results

We identified 231 patients with invasive infection caused by SDSE in the records from 142 medical institutions during the 1-year study period, during which time 97 GAS and 151 GBS cases were also collected. All isolates of SDSE, GAS or GBS were referred by the hospital laboratories for further microbiological characterization.

As shown in Fig. 1, the age distribution differed significantly between patients with invasive SDSE and those with GBS infection. All patients (n = 231) with invasive SDSE infection were adults, whereas GBS infected some patients 4 months old or younger in addition to adults, especially the elderly. We therefore chose to compare clinical aspects of invasive SDSE diseases with those caused by GAS (n = 82) or GBS (n = 123) in the adult population (≥15 years old).

Isolates (n = 12) of the *S. anginosus* group were excluded from the current surveillance. Lancefield groups G, C and A,