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Case Report

First molecular evidence of intrauterine and surgical-site infections caused by *Streptococcus dysgalactiae* subsp. *equisimilis*

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Abstract

S. dysgalactiae subsp. *equisimilis* (SDSE) is infrequently associated with maternal infections during delivery in pregnant women. A rare case is presented of a woman with intrauterine infection and surgical-site infection due to SDSE after cesarean section, which had colonized her genital tract and, via the ascending pathway, reached her intact fetal membrane. All isolates were identified as *Streptococcus* Lancefield group G, and their *emm* genes that coded M protein belonged to stG6.1. The isolates tested negative for a series of streptococcal superantigen virulence genes but positive for nonsuperantigenic virulence genes. In particular, molecular typing using pulsed-field gel electrophoresis analysis disclosed that the three isolates from the different infection sites had identical profiles. Furthermore, multilocus sequence typing indicated that the three isolates belonged to a new sequence typing. Our results indicated that SDSE is potentially pathogenic for pregnant women and newborns if colonized.

Key words: *S. dysgalactiae* subsp. *equisimilis*; intrauterine infection; surgical-site infection; molecular evidence.

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Introduction

S. dysgalactiae subsp. *equisimilis* (SDSE) is a β-hemolytic streptococci that is increasingly prevalent in human infections, having the similar clinical spectrum of diseases caused by *S. pyogenes* (Group A Streptococcal infections, GAS) [1,2]. It presents as a pathogen in skin and soft-tissue infections, endocarditis, bacteremia, ocular infection, pneumonia, osteomyelitis, and meningitis [2-10]. However, the microorganism is rarely associated with maternal colonization and infections during the delivery.

Here we report the first evidence of SDSE that colonized the genital tract of a pregnant woman as a potential pathogen for maternal intrauterine infection (IAI) through intact fetal membrane, and a surgical-site infection (SSI) following cesarean section via the ascending pathway.

Case Report

A 29-year-old pregnant woman was admitted to the hospital in the 39th gestational week of her third pregnancy. The pregnancy process was normal except for gestational diabetes mellitus (GDM) that had been identified via screening test at the 17th gestational week. Her insulin-like growth factor was positive. Her liver and renal function tests were within normal limits. On admission, no abdominal pain was reported. Fetal distress was detected. Her temperature was 36.2°C, and her pulse rate was 82/min with blood pressure of 110/70mmHg. Ultrasonograhpy did not find abnormalities. She had a cesarean delivery 24 hours after admission. Her placenta was intact, and a full-term male baby was delivered with a birth weight of 3.375g. At birth, the infant was in good state of health with a heartbeat of 140/min. The Apgar scores were 10 at 1 minute and 10 at 5 minutes after delivery, respectively.

The patient's amniotic fluid was noted to be turbid; therefore, IAI was suspected and a sample of fetal membrane immersed in amniotic fluid was sent for histopathological examination and microbiological culture. Meanwhile, a vaginal swab was also collected for culture immediately after C-section.

Two days after delivery, the mother was febrile with a temperature of 37.7°C, and a pulse of 80/min. She

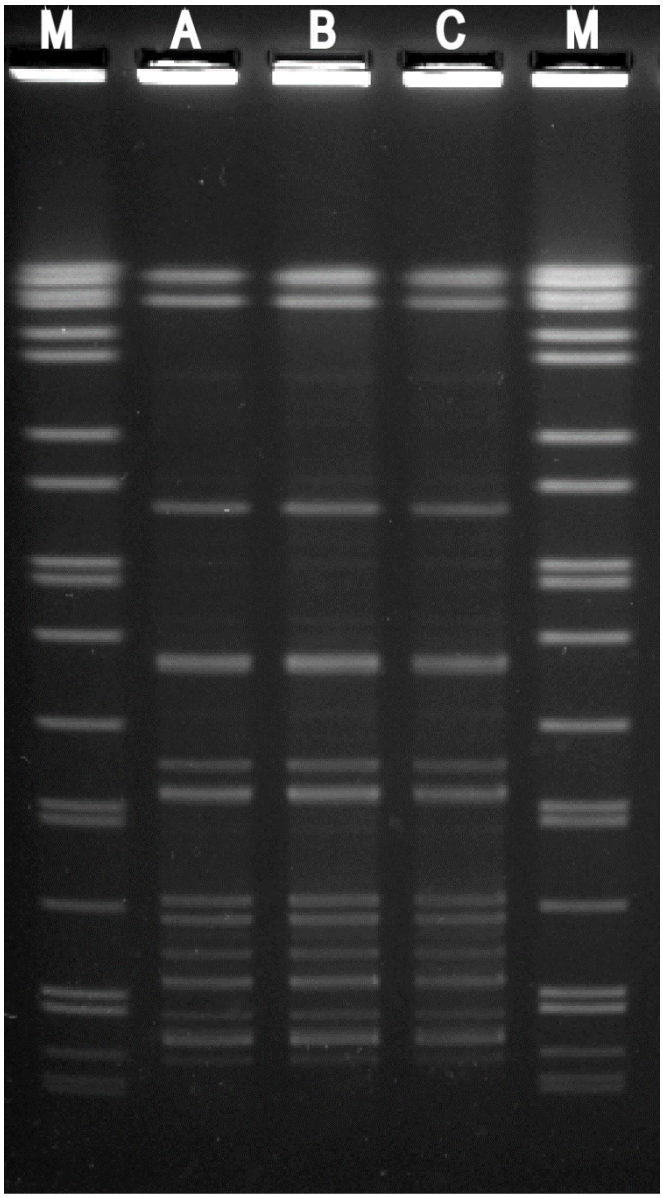
reported abdominal distension and discomfort. Maternal laboratory findings included a white blood cell count of 22.9×10^9/L with 91.1% neutrophils, hemoglobin of 108.0g/L, and a haematocrit of 35.4%. Her C-reactive protein level was 29.6mg/L. Histopathological examination revealed no obvious lesions in the chorionic and amniotic membranes or in the umbilical cord. Three days after the birth, she had fever and chills. On examination, her temperature had increased to 39.0°C and other laboratory findings showed a WBC of 11.0×10^9/L with 78.6% neutrophils and C-reactive protein 94.77mg/L. A post-discharge survey reported yellow purulent exudates at the left edge of the abdominal incision, but no incision dehiscence was detected. Ten-milliliter pus was discharged under slight pressure, which was collected for microbiological cultures. Blood cultures in two sets, each in aerobic and anaerobic bottles (bioMérieux, Bact/Alert, Marcy L'Etoile, France) were also collected. Intravenous treatment with ceftriaxone (1.5g every 8 hours) was initiated. Afterward, the surgical site underwent incision and drainage.

A monoculture of uniformly sized beta-hemolytic colonies was yielded on the culture of the swab from the vaginal tract (isolate A), the fetal membrane (isolate B), and the purulent drainage from the surgical site abscess (incision site, isolate C), both catalase and coagulase negative. The isolates, which were identified as SDSE (biotype no. 051454361301071) by Vitek Compact 2 (bioMérieux, Marcy l'Etoile, France), belonged to the Lancefield group G, as detected by a latex agglutination test (Streptococcal grouping kit, Oxoid, Basingstoke, UK). Antibiotic susceptibility testing (AST) was performed using the BD Phoenix SMIC/ID panel and Etest (bioMe´rieux, France) susceptibility testing. The interpretive criteria used for AST were the MIC Interpretive Standards for *Streptococcus* spp. β-Hemolytic Group as recommended by the Clinical and Laboratory Standards Institute. The MIC results were as follows: penicillin, ≤0.03125 (susceptible); ampicillin, ≤0.25 mg/L (susceptible); erythromycin, ≥8 mg/L (resistant); clindamycin, 2 mg/L (resistant); linezolid, 1 mg/L (susceptible); vancomycin, ≤0.5 mg/L (susceptible) and tetracycline, ≥16mg/L (resistant). The patient's blood culture gave a negative result after 5 days.

The patient became afebrile seven days after delivery and recovered from SSI. The baby was under strict supervision, and penicillin G (200 000 units twice daily, for three days) was given for neonatal infection prevention. During a seven-day period, he exhibited no signs or evidence of local or systematic infections, and was discharged with the mother. No recurrence of symptoms and signs were reported by the mother and son during the three-month outpatient follow-up.

The three SDSE isolates (A~C) from different sources were examined by DNA macrorestriction analysis using *Sma*I coupled with pulsed-field gel electrophoresis (PFGE) as previously described [5]. The PFGE profiles showed that strains isolated from the three sources were identical (Figure 1). This result was further confirmed by sequencing the *emm* amplicon and performing a BLAST search on the Centers for Disease

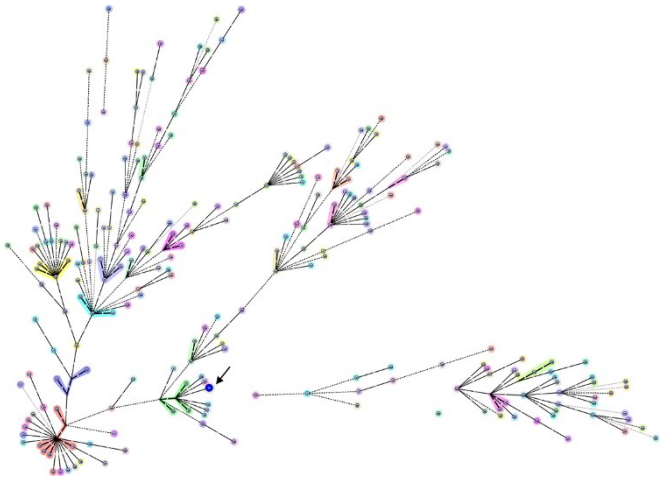
Figure 1. PFGE of *Sma*I-digested genomic DNA of the SDSE strains isolated from the vagina (Lane A); purulent drainage from the surgical site abscess (Lane B); and fetal membrane (Lane C). *Salmonella* serotype H9812 was used as a DNA size marker (M).



Control (CDC) streptococcal *emm* sequence database [11]. The isolates were identified as group G *streptococcus emm* type stG6.1.

Characterization of isolate virulence was conducted by multiplex PCR according to the primers and method previously described [5]. The three strains proved negative for a series of streptococcal superantigen virulence genes (*speA, speC, speG, speH, speI, speJ, speK, speL, speM, ssa, smez*); however, they were positive for nonsuperantigenic virulence genes (*scpA, ska, slo, and sagA*), as previously documented [5]. Furthermore, the strains were investigated by multilocus sequence typing (MLST) to determine their genotypic characteristics. A new allele of *atoB* was detected and submitted to the MLST database (http://sdse.mlst.net/), and afterward, a new sequence type (ST271) was assigned to the three strains (Figure 2). In the MLST database, the allele profile of ST271 was similar to that of ST25 except for *atoB*.

Figure 2. Minimum spanning tree analysis of the three SDSE isolates in the MLST database according to sequence type (ST). Each circle in the tree represents one ST; numbers in each circle represent their ST; ST271 of three isolates in this study indicated as arrow. The digits on the lines between two circles represent the different allele number of types.



Discussion

IAI or SSI have been regarded as important risk factors for maternal morbidity and mortality worldwide [12,13]. The organisms frequently found in IAI and SSI after cesarean section are those normally present in the lower genital tract, indicating that the lower genital tract is the most likely reservoir for causative microorganisms for maternal infections via the ascending pathway under the appropriate circumstances [13-16].

SDSE, increasingly recognized as an important human pathogen, is a group C or G pyogenic β-hemolytic *streptococcus* sharing similar virulence factors and disease spectrum with GAS [17]. It might act as a potential organism of infections in populations suffering from underlying diseases in adults. The mother in the current case had the typical risk factors associated with subsequent development of a SSI, such as GDM, chorioamnionitis [12], and slight anemia [18]. The patient experienced cesarean delivery and this exposure event could be a potential clue to the etiology of her SSI. Additionally, the molecular characteristics and AST results of the isolates from different specimens revealed 100% consistency, and the three isolates belonged to a new sequence typing. Furthermore, in our case, the fetal membrane was found intact before C-section, which might be explained by the ability of the microorganism to cross the intact membranes [13-15]. Despite the fact that SDSE has been rarely documented either to colonize the vaginal tract or as a cause of maternal infections [19], this case suggests that SDSE colonized in the lower genital tract may be a potential, if rare, cause of maternal IAI via the ascending route during pregnancy. During the cesarean delivery, the IAI allows bacteria to invade surgical sites, causing postoperative infection.

Isolates from our study remained sensitive to penicillin, cefotaxime, vancomycin and linezolid, but showed resistance to tetracycline, macrolides, and clindamycin. Ampicillin, vancomycin, and ceftriaxone are generally considered the preferred agent for SDSE [3,6]. The mother responded well to the therapy with ceftriaxone. The baby in our report was delivered at full term with normal weight and in a healthy state, and penicillin G was given for prevention of neonatal infections. This precaution might partially explain why there was no serious systemic infection.

The role of superantigens or associated toxic genes in the pathogenesis of SDSE infections remains unclear. GAS has virulence factors in common with SDSE, and among them is the M protein, which is encoded by *emm* genes [5]. However, several genes present in GAS that encode virulence factors, such as the superantigens, were absent in our SDSE isolate. Furthermore, our strain possessed several nonsuperantigenic virulence factors, including *scpA, ska, slo,* and *sagA*. These genes have been hypothesized to contribute to the pathogenesis of SDSE infection [5].

It is well accepted that Group B *streptococcus* (GBS) is the leading pathogen that might cross amniotic membranes resulting in IAI and exposure of the baby to infections [13]; however, other pathogens, including *Streptococcus gallolyticus* subsp. *pasteurianus* and

Streptococcus porcinus, documented to be asymptomatically colonized in genital tracts, might provoke IAIs, stillbirth, and maternal bacteremia via the ascending pathways [16,20]. Taken together, our study suggests that besides GBS, other microorganisms colonizing the genital tract, especially *Streptococcus* species, should be emphasized in clinical practice, as they might be the underlying pathogens in maternal or neonatal infections.

Conclusions

This case is unique because the SDSE strain is rarely detected colonizing in the low genital tract, or as a potential pathogen of either IAI or SSI via the ascending pathway during C-section. It has never been seriously considered that IAI and SSI caused by SDSE are derived from vaginal sources. Our observations provide the molecular evidence of vaginal colonization of SDSE as a portal of entry in cases of maternal infections.

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Authors' contributions

LB designed and wrote the paper. DB and FY performed PFGE and PCR amplification of virulence genes. SY, LX, ZF, LD, ZS and CY collected clinical data. WD analyzed and wrote the paper.

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