Streptococcus gallolyticus subsp. pasteurianus infection in twin infants

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ABSTRACT

*Streptococcus gallolyticus* subsp. *pasteurianus*, previously known as *Streptococcus bovis* biotype II.2, is an uncommon pathogen in neonates. Nevertheless, it can cause severe neonatal sepsis and meningitis often clinically indistinguishable from group B *streptococcus* and has been associated with considerable morbidity. We report the first known cases of *S. gallolyticus* subsp. *pasteurianus* infection in twin infants.

CASE REPORT

Twin B, a 3-week-old diamniotic-dichorionic twin male at Texas Children’s Hospital Pavilion for Women had a 1-day history of pale color, lethargy, poor feeding and increased irritability. He was born at 32 weeks gestation, weighing 2120 g to a 26 year-old primigravida mother via Cesarean section for severe pre-eclampsia. The mother had unknown group B *Streptococcal* status and did not receive antibiotic prophylaxis during delivery. Immediately following his birth, the patient developed respiratory distress requiring intubation and surfactant. He was transported to the neonatal intensive care unit (NICU) for further management and continued to improve in the NICU until day of life (DOL) 24 when he was found to have pale color, loose stool, respiratory distress and hypoxemia. Within the next few hours, he also experienced mild hypothermia. Full sepsis work-up was initiated and empiric parenteral vancomycin (15 mg/kg every 12 hours), gentamicin (4 mg/kg every 24 hours) and ceftazidime (40 mg/kg every 8 hours) were initiated. Approximately 6 hours after the onset of his symptoms, the child developed acute respiratory failure requiring intubation and refractory hypotension requiring dopamine infusion. Two hours later, he developed new-onset seizures that progressed to status epilepticus. The chest radiograph revealed coarse markings within the lungs with superimposed mild volume loss in the right upper lobe.
Initial laboratory studies are presented in Table 1. The CSF white blood cell (WBC) differential was not available. Degenerated inflammatory cells including monocytes and neutrophils were reported. The CSF Gram stain revealed Gram-positive cocci in clusters* with many WBCs. The peripheral, aerobic blood culture was positive for Gram-positive cocci in pairs and chains at 8 hours in the VersaTREK Microbial Detection System (TREK Diagnostic Systems, Cleveland, OH). The initial CSF culture for twin B revealed abundant gamma-hemolytic Streptococcus, not Enterococcus, identified as Streptococcus gallolyticus subsp. pasteurianus with 99% probability and excellent identification (confidence level), using the GP identification card on the Vitek® 2 platform, software version 4.01, bioMérieux, USA). The urine culture was negative. Parenteral ampicillin (75 mg/kg every 6 hours) was initiated and all other antibiotics were discontinued.

Over the next few days, twin B’s clinical course continued to deteriorate. He experienced continuous seizures accompanied by myoclonus with abnormal electroencephalographic studies. He was given multiple blood products for worsening anemia and thrombocytopenia and electrolyte supplementation for moderate hyponatremia and hyperkalemia. A head ultrasound obtained on the first day of illness showed normal anatomy; however, a magnetic resonance imaging of the brain with and without contrast taken four days after onset demonstrated moderate lateral, third and fourth ventricular hemorrhage with secondary mild dilatation of the lateral and third ventricles and without evidence of acute infarction, parenchymal abscess or empyema (Figure 1). Repeat head ultrasound 2 days later showed grade 3 intraventricular hemorrhage, which then remained stable and the infant gradually improved without further

* The initial gram stain from the original specimen was reported as “in clusters” likely due to appearance of very small clusters (1-3 cocci) along with pairs. However, when the gram stain was performed from colonies on solid media, Gram-positive cocci in pairs were found consistent with Streptococcus species.
complications for the rest of his admission. Repeat blood cultures after 24h and 48h of antibiotic treatment were negative. A repeat CSF culture after 6 days of antibiotic treatment showed Gram-positive cocci by Gram stain but the culture was negative. Twin B was treated with ampicillin for a total of 14 days from CSF clearance.

Twin A, the 3-week old sister of twin B, was born weighing 1474 g with similar symptoms of respiratory distress immediately after delivery. Resuscitation included positive pressure ventilation and continuous positive airway pressure, but no intubation or surfactant was required. On DOL 24, she experienced intermittent tachypnea and underwent a full sepsis work-up due to severe infection in twin B. Subsequent laboratory studies are presented in Table 1. Initial CSF studies showed colorless, hazy cerebrospinal fluid and the CSF Gram stain revealed no organisms and was culture negative. The blood culture was positive for Gram-positive cocci in pairs after 11 hours. Twin A remained hemodynamically stable despite positive blood cultures and was treated with IV ampicillin (75 mg/kg every 6 hours). She completed a 10-day course and was discharged upon completion of therapy.

Further analysis of the organisms growing in the blood culture from twin A and blood and CSF cultures from twin B were identified as Streptococcus gallolyticus using pyrosequencing of three hypervariable regions (V1, V3, and V6) of the 16S rRNA gene (1). Briefly, extracted bacterial DNA (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche Applied Science, IN) was used as template in PCR reactions with universal primers targeting the conserved regions directly flanking the variable regions. The resulting sequence was searched against the Ribosomal Database Project (RDP) maintained by Michigan State University and the SmartGene Integrated Database Networking System (IDNS) (SmartGene, Inc., Raleigh, NC) (2, 3). The 16S rRNA gene was amplified by PCR using primers 8F and 1541R (1) and the products
were sequenced at Seqwright Inc. (Houston, TX). The sequences were compared to the corresponding region of published 16S sequences of related species and subspecies, deposited in GenBank, using MEGA 5.1 (4). The neighbor-joining tree (Figure 2) shows an alignment of the twin isolates with *S. gallolyticus* subsp. *pasterurianus* and a 100% sequence match was observed by Nucleotide BLAST align analysis to ATCC43144 (http://blast.ncbi.nlm.nih.gov/) (5-8).

Analysis of the twins’ isolates by pulsed field gel electrophoresis using previously described methods and digestion with Smal revealed that the twins’ isolates were identical, suggesting the twins were infected by the same strain (data not shown) (9, 10). Antimicrobial susceptibility testing by E test using CLSI guidelines (ref) demonstrated susceptibility to ampicillin (MIC, 0.125µg/ml), penicillin (MIC-0.125µg/ml), cefotaxime (MIC-0.25µg/ml) and vancomycin (MIC-0.5-1 µg/ml) for all the isolates.
DISCUSSION

Streptococcus gallolyticus subsp. pasteurianus is a highly unusual pathogen in neonates. Only a handful of case reports exist. This is the first report of infection by this organism in neonatal twins. The pathogen has been associated with considerable morbidity and, in some cases, mortality over the last four decades. Previously recognized as Streptococcus bovis biotype II/2, the taxonomy has been revised. Originating as one of the six DNA groups of the S. bovis/S. equinus complex (11), it was later subdivided into biotype II based on its inability to ferment mannitol (11) and then into biotype II/2 based on phenotypic testing and sequencing of its sodA gene (12). In the last ten years, biochemical tests have further differentiated the biotypes I and II into distinct subspecies and new species nomenclature has replaced the old biotype classification (11). The three species formerly known as S. bovis biotype II.2, S. waius, and S. macedonicus were grouped into one DNA cluster but with limited DNA–DNA relatedness and sufficient divergence in their 16S rDNA sequences to be separable (5, 12, 13). Reclassified as S. gallolyticus subsp. pasteurianus, S. gallolyticus subsp. gallolyticus, and S. gallolyticus subsp. macedonicus, the three subspecies differ biochemically (12, 14). S. gallolyticus subsp. pasteurianus can be differentiated from other subspecies based on beta-galactosidase (BGAL) and beta glucuronidase (BGAR) tests, by which S. gallolyticus subsp. pasteurianus is positive while S. gallolyticus subsp. gallolyticus and S. gallolyticus subsp. macedonicus are negative.

While the Vitek platform results are commonly only reported at the species level by the clinical laboratory, a study of gram-positive bacteria using the Vitek 2 GP identification card showed 100% accuracy in the identification of three Streptococcus gallolyticus subsp. pasteurianus isolates; none of the other 214 isolates of the Streptococcaceae family, including 4 subsp. gallolyticus were identified as this species (15). The Vitek® biochemical panel includes the BGAL and BGAR biochemical tests. The present case report shows a 100% 16s rRNA
alignment of two neonatal isolates to the previously confirmed *Streptococcus gallolyticus* subsp. *pasteurianus*, ATCC43144, and positive BGAL and BGAR biochemical tests, confirming the identity of the isolated pathogens and further supporting the previous notion that distinct differences between subspecies can be reliably detected by Vitek and confirmed by 16S sequencing (5, 13, 15).

While *S. bovis* (biotype I), part of normal colonic microbiota, has long been associated with colonic neoplasia and bacterial endocarditis in adults, the clinical significance of *S. bovis* biotype II, particularly within the neonatal population has been less well established (16). Over the last two decades, however, a small, but growing pool of literature has been describing invasive infections in neonates and infants. Most of these cases have been sporadic and document bacterial sepsis with or without accompanying meningitis in neonates less than 24 hours to 2 months of age with severe disease rarely described in those older than 1 week of age (13, 17). Almost all of the patients presented with gastrointestinal symptoms and responded well to a two-week course of a penicillin-derivative with minimal complications (13). In recent years, similar infections in adults have been reported and some have even documented relative penicillin and gentamicin resistance making careful evaluation and awareness of this pathogen imperative (16, 18-20).

Similar to patterns of early and late-onset Group B *Streptococcus* (GBS) disease, invasive *S. gallolyticus* subsp. *pasteurianus* infection presumably occurs either via an ascending route (early-onset) or via vaginal delivery or postnatally from maternal or caretaker contact, infected breast milk or nosocomial sources (21). Fikar and Levy reported a neonate with *S. bovis* meningitis, whose mother had the same organism growing from rectal and vaginal cultures (22).
In 2006, the first outbreak of *S. gallolyticus* subsp. *pasteurianus* bloodstream infections was reported involving five preterm infants in a university hospital neonatal intensive care unit in France implicating hand carriage and poor sterile procedure (23). The exact pathophysiology of *S. bovis* group meningitis is unclear, though recent studies have suggested the gastrointestinal tract as a possible source. Noble demonstrated that *S. bovis* was isolated more frequently from the stool samples of neonates (5/21, 23.8%) compared with adults (2/39, 5%) while Gerber et al. reported a higher prevalence of abdominal symptoms (5/7, 71%) in infants with systemic *S. bovis* infection without isolation of the pathogen from the stool (24, 25). More recently, Takahashi et al. isolated identical isolates of *S. gallolyticus* subsp. *pasteurianus* from blood, CSF and stool suggesting translocation of previously commensal bacteria with initiation of bacteremia and subsequent systemic dissemination and infiltration into CSF as a possible mechanism (26). In our case, no further investigation into the source of the organism was pursued.

Twin gestation is a known risk factor for the development of invasive Group B *Streptococcus* (GBS) disease, but its role in other neonatal infections remains unclear. Within the former process, the relative risk of invasive GBS infection in the twin of an affected neonate has been reported to be as high as 25-fold (27). Prematurity and precipitous labor further increase susceptibility to infection (28). While studies of neonatal *S. gallolyticus* subsp. *pasteurianus* infections are limited, we hypothesize that the pathogenesis of the disease is similar. Furthermore, the extreme variability of the clinical presentation of these twins raises important questions regarding management of neonatal infection in twins. The neonates in our case not only differed in their presentation, with prominent respiratory rather than gastrointestinal symptoms, but also demonstrated two extremes of possible sequelae in the disease course. While one twin was mildly symptomatic, the other twin faced an unusually severe course for a late-
onset infection including overwhelming sepsis with serious neurologic damage, a complication that has rarely been reported. One previous report regarding *S. gallolyticus* subsp. *pasteurianus* meningitis included two infants with seizure-like activity, but none with neurologic sequelae (13).

In summary, these cases broaden the clinical diversity of neonatal infection in twins and also highlight the importance of thorough evaluation of both twins in the setting of suspected sepsis, regardless of the presence of symptoms (28).
REFERENCES


11. Poyart C, Quesne G, Trieu-Cuot P. 2002. Taxonomic dissection of the *Streptococcus bovis* group by analysis of manganese-dependent superoxide dismutase gene (*sodA*) sequences: reclassification of ‘*Streptococcus infantarius* subsp. *coli*’ as *Streptococcus lutetiensis* sp. nov and...
of *Streptococcus bovis* biotype II.2 as *Streptococcus pasteurianus* sp. nov. Int J Syst Evol Microbiol. 52: 1247-55.


Table 1. Blood and cerebrospinal fluid results.

<table>
<thead>
<tr>
<th>Laboratory results (reference range)</th>
<th>Twin B</th>
<th>Twin A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOL 24</td>
<td>DOL 30</td>
</tr>
<tr>
<td>White blood cell (6-19 x10³/μL)</td>
<td>1,310</td>
<td>---</td>
</tr>
<tr>
<td>Neutrophils (32-67%)</td>
<td>9.5%</td>
<td>---</td>
</tr>
<tr>
<td>Bands (0-8%)</td>
<td>7.6%</td>
<td>---</td>
</tr>
<tr>
<td>Lymphocytes (25-37%)</td>
<td>60%</td>
<td>---</td>
</tr>
<tr>
<td>Monocytes (0-9%)</td>
<td>1.9%</td>
<td>---</td>
</tr>
<tr>
<td>Hemoglobin (13-22 g/dL)</td>
<td>10.3</td>
<td>---</td>
</tr>
<tr>
<td>Platelet (150-450 x10³/μL )</td>
<td>312</td>
<td>---</td>
</tr>
<tr>
<td>C-reactive protein (&lt;1mg/dL)</td>
<td>3.9</td>
<td>---</td>
</tr>
<tr>
<td>CSF white blood cell² (0-25/μL)</td>
<td>61</td>
<td>3,693</td>
</tr>
<tr>
<td>CSF red blood cell (0-2/μL)</td>
<td>84</td>
<td>30,250</td>
</tr>
<tr>
<td>CSF glucose (24-63 mg/dL)</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>CSF protein (&lt;150mg/dL)</td>
<td>404</td>
<td>846</td>
</tr>
<tr>
<td>CSF gram stain</td>
<td>Many Gram + cocci in pairs</td>
<td>Rare Gram + cocci in pairs</td>
</tr>
<tr>
<td>CSF culture</td>
<td><em>Streptococcus galolyticus</em> subsp. <em>pasteurianus</em></td>
<td>Negative</td>
</tr>
<tr>
<td>Blood culture⁵</td>
<td><em>Streptococcus galolyticus</em> subsp. <em>pasteurianus</em></td>
<td>---</td>
</tr>
</tbody>
</table>

CSF: cerebrospinal fluid, DOL: day of life.

² CSF WBC differential was not available.
⁵ Repeat blood cultures on DOL 25 and 26 were negative for both patients.
Figure 1

Magnetic resonance imaging of the brain with contrast. A. Moderate lateral ventricular hemorrhage. Lateral ventricles are mildly enlarged. B. Hemorrhage in the cisterna magna inferior to the cerebellar vermis.
Figure 2

Neighbor-joining tree of the 16S rRNA partial gene sequences (1433 bp) of the clinical isolates from Twin A and B in comparison to corresponding sequences of strains representing related species/subspecies, available in GenBank. The neighbor-joining tree was constructed using MEGA5.1. The comparator strains were downloaded from GenBank: *Streptococcus galolyticus* subsp. *pasteurianus* ATCC43144, NCBI reference sequence NR_102811.1;*Streptococcus galolyticus* subsp. *galloyticus* ATCC43143, GenBank AF104114.1;*Streptococcus infantarius*, NCBI reference sequence NR_028761.1;*Streptococcus macedonicus*, GenBank EU163501.1.