Perinatal/Neonatal Case Presentation

Late-Onset Group B Streptococcal Infection in Identical Twins: Insight to Disease Pathogenesis

Kelly S. Doran, PhD
Vivian M. Benoit, BA
Robert E. Gertz, MS
Bernard Beall, PhD
Victor Nizet, MD

Late-onset group B streptococcal (GBS) infection affecting identical twins is described. Although exhibiting similar signs and symptoms at presentation, twin A suffered fulminant fatal meningitis while twin B recovered completely. The GBS isolates proved to be genetically identical and possessed equivalent abilities to invade and injure cells of the human blood–brain barrier in vitro. Clinical variables associated with the adverse outcome in twin A were longer duration of fever prior to antibiotics and the development of neutropenia. The case histories and experimental data are reviewed to underscore key features of GBS disease pathogenesis.
Journal of Perinatology (2002) **22,** 326–330 DOI: 10.1038/sj/jp/7210675

INTRODUCTION

Group B *Streptococcus* (GBS) is the leading cause of neonatal pneumonia, sepsis, and meningitis. Whereas programs for universal prenatal screening and intrapartum antibiotic prophylaxis appear to significantly reduce the incidence of early-onset GBS infections, late-onset disease incidence remains unchanged.[1] Late-onset GBS infection is manifested initially by bacteremia and is complicated in 40% to 60% of cases by bacterial penetration of the blood–brain barrier to produce meningitis.[2] Mortality in late-onset GBS infections is lower than for early-onset disease; however, 20% to 40% of infants with meningitis are left with permanent neurological sequelae including cerebral palsy, cognitive deficits, deafness, blindness, or seizures.[3–5]

Twin gestation is known to be a risk factor for development of invasive GBS disease.[6,7] Here we describe late-onset GBS infection affecting a pair of identical twins. The unique aspect of this case compared to all previous reports is the dramatic difference in outcomes. Despite similar initial signs and clinical findings, twin A suffered fulminant fatal meningitis, whereas twin B manifest only bacteremia with a rapid response to antibiotic therapy. We sought to examine genetic and phenotypic features of each twin's bacterial isolate, together with laboratory and clinical parameters, to uncover information that may be relevant to GBS disease pathogenesis.

Division of Pediatric Infectious Diseases (K.S.D., V.M.B., V.N.), University of California, San Diego, La Jolla, CA, USA; and Respiratory Diseases Branch (R.E.G., B.B.), National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA.

Address correspondence and reprint requests to Victor Nizet, MD, Division of Infectious Diseases, MC 0672, University of California, San Diego, La Jolla, CA 92093, USA.

CASE REPORTS

Birth History

Identical female twins were delivered at 34 weeks' gestation by repeat Cesarean section due to intrauterine growth retardation and absent end-diastolic flow on umbilical artery Doppler examination of twin B. Pregnancy and prenatal laboratory screening were otherwise unremarkable. GBS cultures were not performed and intrapartum antibiotics were not administered. Monochorionic, diamniotic twin placentas were observed on histopathologic examination. Birth weights were 1800 g for twin A and 1480 g for twin B. Delivery room and neonatal courses were unremarkable. The twins were discharged at 2 weeks of age and did well on formula feeds until their presentation at 9 weeks.

Twin A

This infant was brought to an outside Emergency Department with an 18-hour history of fever to 102°F and an 8-hour history of poor feeding and decreased activity level. Physical examination was notable for grunting respirations and decreased muscle tone. Blood, urine, and spinal fluid samples were collected, intravenous ceftriaxone administered, and the patient was transported by helicopter to our tertiary care center. Vital signs were: weight 3.7 kg, heart rate 212 beats/min, blood pressure 130/72 mm Hg, and respirations 60 breaths/min. Complete blood count (CBC) revealed WBC 1300/mm^3 with 16% neutrophils and 20% band forms, hematocrit 28.2%, and platelets 371,000/mm^3. Cerebrospinal fluid (CSF) collection showed RBC 3/mm^3, WBC 7/mm^3 (all neutrophils), glucose 0 g/dl, protein 643 g/dl, and abundant chaining Gram-positive cocci. GBS was isolated from both the initial blood and CSF cultures. The patient was managed with aggressive supportive care and high-dose ceftriaxone (100 mg/kg per day). A repeat CSF analysis on hospital day 2 showed RBC 3930/mm^3, WBC

Journal of Perinatology 2002; 22:326–330

120/mm^3, glucose 12 g/dl, and protein 2,006 g/dl; culture of this sample was sterile.

Within hours of ICU admission, the patient had deteriorating mental status necessitating airway protection and mechanical ventilation. Septic shock symptomatology ensued and the patient required substantial pressor and fluid support to maintain cerebral perfusion pressure, multiple blood products to correct disseminated intravascular coagulation, and an insulin drip to control hyperglycemia. The patient developed intractable seizures with persistent electroencephalographic abnormalities despite maximal anticonvulsant therapy, and severe diabetes insipidus with consequent hypernatremia. An intracranial pressure monitor was placed and normal parameters were maintained with hyperventilation and dextran continuous infusion. However, the patient remained comatose without spontaneous respirations. Magnetic resonance imaging on hospital day 10 (Figure 1) revealed severe diffuse encephalomalacia with multiple necrotic areas, formation of abscesses or cysts, subdural effusions, and meningeal enhancement. Given the patient's grave prognosis, a family conference was held and a decision was made to withdraw support. The patient died of respiratory failure on hospital day 12.

Twin B

Approximately 48 hours after admission of her sister to the hospital, this infant developed increased fussiness, decreased activity level, and poor feeding over a 6-hour period, at which point a fever of 102°F was noted. She was brought to the same outside Emergency Department within 2 hours of the measured fever. On examination, the patient was found to be crying but consolable, with a mottled appearance to the skin of the extremities. Vital signs showed weight 3.5 kg, heart rate 225 beats/min, blood pressure 114/48 mm Hg, and respirations 56 breaths/min. A blood culture was performed, ampicillin and cefotaxime administered, and the patient was transported by helicopter to our tertiary care center. CBC revealed WBC 6000/mm^3 with 44% neutrophils and 23% band forms, hematocrit 27.0%, and platelets 307,000/mm^3. The infant was admitted to the pediatric ward and CSF collection performed, showing RBC 0/mm^3, WBC 1/mm^3, and normal glucose and protein levels. Blood culture grew GBS, while CSF culture was sterile. The patient was given a fluid bolus and treated with ceftriaxone at 50 mg/kg per day. Her hospital course was uncomplicated; she did not have subsequent fevers or distress and exhibited good appetite and activity level. An antecubital peripherally inserted central catheter (PICC) was placed and she was discharged home on hospital day 5 to complete a 14-day course of intravenous antibiotics. She has remained healthy at routine pediatric visits through 1 year of age.

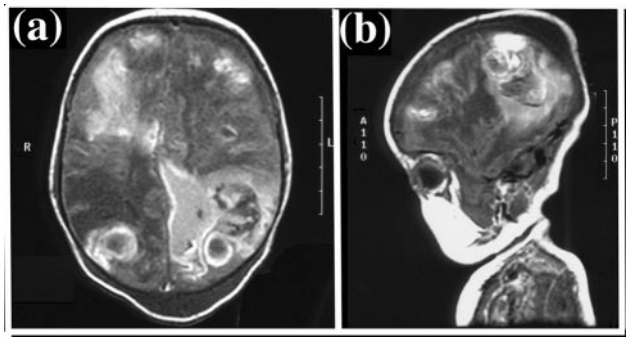

Figure 1. Neuroimaging of fatal GBS meningitis in twin A on hospital day 10. Transverse **(a)** and sagittal **(b)** views of magnetic resonance imaging showing diffuse necrotizing encephalomalacia and formation of parenchymal abscesses or cysts.

RESULTS AND DISCUSSION

GBS isolated from the CSF of twin A and from the blood of twin B were subjected to genetic and phenotypic analysis. Both isolates were determined to belong to capsular polysaccharide serotype III by microprecipitin reaction of bacterial acid extracts with type-specific antisera. Total chromosomal DNA was purified, digested with restriction enzyme *Sma*I, and studied by pulsed-field gel electrophoresis (PFGE) using published methods.[8] The PFGE comparison revealed an identical banding pattern for the isolates from each twin (Figure 2a). The surface polysaccharide capsule of GBS possesses antiphagocytic properties and is a known virulence factor for invasive infection.[9] To measure the amount of capsular polysaccharide expression by each isolate, we used a semiquantitative immunoblot assay with polyclonal rabbit antitype III antisera as previously described.[9] No differences in capsule expression were appreciated in paired analysis of serially diluted bacterial cell preparations (Figure 2b).

Development of GBS meningitis is felt to reflect, in part, the ability of the bacterium to invade and transcytose human brain microvascular endothelial cells (BMECs), the single cell layer comprising the blood–brain barrier.[10] Intracellular invasion of BMEC by GBS was measured by an in vitro antibiotic protection assay. The twin isolates were equivalent in BMEC invasion, with approximately 1.0% of the input inoculum penetrating intracellularly within 2 hours (Figure 2c). This result is comparable to published findings for various GBS strains,[10] although somewhat less than the invasive ability of two other serotype III GBS strains (COH1 and K79) tested in the experiment. *Bacillus subtilis* was used as a negative control.

Another proposed mechanism by which GBS could gain access to the CNS would be through direct lytic injury to BMEC resulting in disruption of blood–brain barrier integrity. GBS produce a β-hemolysin that aids in identification of the organism in the clinical laboratory. GBS expression of this β-hemolysin has been directly correlated with injury to a wide variety of human tissue culture cell lines.[11] We tested the GBS isolates from each twin for hemolytic and BMEC cytolytic activity using microtiter plate assays for hemoglobin and lactate dehydrogenase release, respectively.[12] The two clinical isolates exhibited equivalent hemolytic and cytolytic titers comparable to those of other serotype III GBS strains (Figure 2d). In

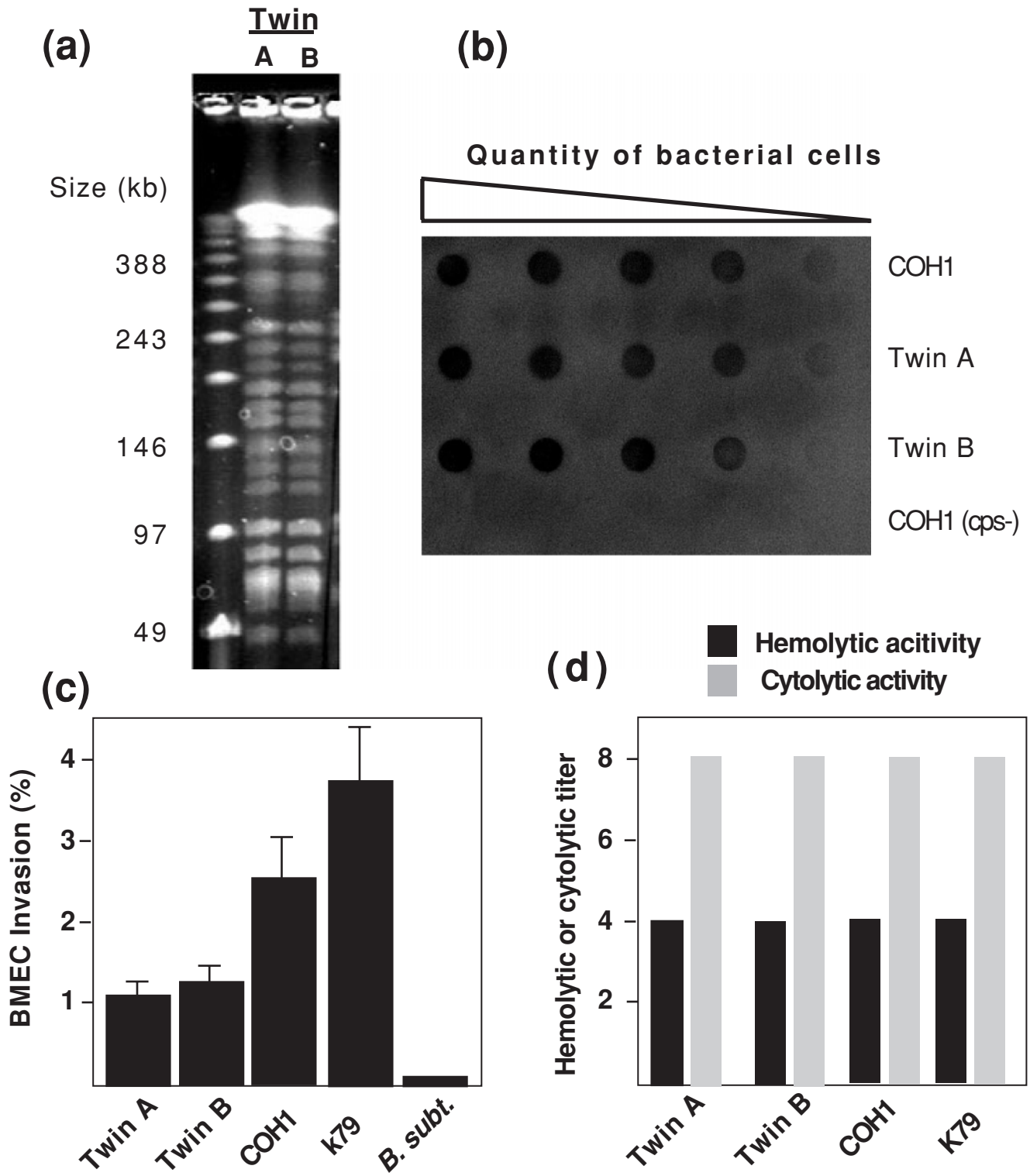

Figure 2. Comparative genotypic and phenotypic analysis of GBS strains from twins A and B. **(a)** PFGE of *Sma* I-digested total chromosomal DNA. **(b)** Immunoblot analysis of type III capsule expression on serial 2-fold dilutions of bacterial cell preparations ($\sim 1.5 \times 10^7$ cells in first column to $\sim 1 \times 10^6$ cells in last column). Type III GBS strain COH1 and its isogenic capsule deficient mutant HY106 serve as controls. **(c)** Intracellular invasion of human BMEC as determined by a gentamicin protection assay. Type III GBS strains COH1 and K79 and a noninvasive *B. subtilis* isolate serve as controls. **(d)** Hemolytic activity against sheep erythrocytes and cytolytic activity against human BMEC using microtiter plate assays for hemoglobin and lactate dehydrogenase release, respectively. The GBS isolates from twins A and B were found to be identical in all experiments.

summary, we found the GBS isolates from twins A and B to be serologically, genetically, and phenotypically identical.

In the more common early-onset form of disease, ascending GBS infection leads to fetal aspiration of contaminated amniotic fluid, with resultant pneumonia and a rapid evolution of clinical signs soon after birth. In contrast, the host and pathogen factors contributing to late-onset GBS infection are less well established. The infant presumably acquires the organism vertically upon vaginal delivery, or postnatally from maternal or caretaker contact, infected breastmilk,[12] or nosocomial sources.[13–15] In the present case, Cesarean delivery without signs of maternal infection and formula feeding suggests a postnatal common source contact exposure. Potential differences in the timing and inoculum size of GBS exposure between the two twins cannot be excluded.

Twin gestation is known to be a predisposing factor for both early- and late-onset GBS infections.[6,7] The relative risk of invasive GBS infection in the twin of an affected infant has been calculated as high as 25-fold.[2] Notably, premature twins are even more susceptible to concurrent infection than their term counterparts.[6] The increased susceptibility of twins to GBS infection is likely to be multifactorial. In early-onset disease, joint chorioamniotic invasion by GBS may produce simultaneous perinatal exposures. In late-onset disease, maternal, hospital, or community contact is expected to be shared by twins, as are potential predisposing factors to invasive GBS disease such as (1) low concentrations of maternal IgG specific for the capsular polysaccharide available for placental transport, (2) poor placental transport when birth occurs prior to 34 weeks' gestation, and (3) antecedent viral infections acquired postnatally.[2]

If the isolates acquired by each twin are identical, then enhanced bacterial virulence of particular GBS strains may contribute to a high simultaneous rate of bloodstream invasion. Serotype III strains account for the majority of late-onset GBS infection and the majority of meningitis cases regardless of age of onset.[2] In the limited numbers of twins with late-onset GBS disease in which serotyping was performed, all isolates have been identified as serotype III.[7,16] In a recent report in which genotyping was performed in a set of fraternal twins with recurrent uncomplicated bacteremia and cellulitis, the serotype III isolates were identical clones.[17] In the present study, PFGE and immunoprecipitin analysis once again revealed the GBS isolates from each twin to be genetically identical strains belonging to serotype III.

Twin A developed fulminant fatal meningitis, whereas twin B had no CNS involvement. Why some infants develop meningitis as a complication of GBS infection and others do not is poorly understood. This question becomes even more intriguing when genetic host factors are ruled out in the case of identical twins. One hypothesis for the pathogenesis of meningitis is that certain GBS strains, particularly those belonging to serotype III, possess specific virulence factors that facilitate penetration of disruption of the neonatal blood–brain barrier. By extension, expression of such virulence factors could be induced in a subpopulation of bacteria during the course of infection. In vitro, GBS are capable of adhering to, invading, and transcytosing polar monolayers of human BMEC, the cell layer comprising the blood–brain barrier, and β-hemolysin production by the organism can result in lysis and death of the infected BMEC.[10] We compared the CSF isolate from twin A and the blood isolate of twin B to determine whether differences in BMEC invasion or injury could be detected; however, none was identified. These studies suggest that twin B was infected with a GBS isolate with equivalent potential to invade and injure blood–brain barrier cells as her sister; yet, this did not occur.

Clinical signs of infection including fever, decreased activity level, poor feeding, tachycardia, and tachypnea, were noted for each twin at presentation; both initial CBCs demonstrated a pronounced shift toward immature neutrophil forms. However, twin A was markedly neutropenic ($470/mm^3$), whereas twin B maintained normal counts ($4020/mm^3$). Early exhaustion of mature marrow neutrophils is a characteristic feature of neonatal GBS septicemia in clinical studies[18] and experimental animal models.[19] Development of neutropenia in twin A appears to have heralded, or reflected, a more severe infection. Indeed, neutropenia is associated with poor outcome and a higher rate of meningitis in neonatal GBS disease.[20,21] The effects of low neutrophil counts are exacerbated by poor chemotaxis and abnormalities in complement function present in neonatal serum.[22]

Based on the above information, this case study could indicate that the complication of meningitis is less the result of specific host susceptibility or strain-specific bacterial virulence factors, and more the inevitable consequence of a sustained, high-level GBS bacteremia. When GBS replication in the bloodstream outpaces and/or exhausts the neutrophil phagocytic response, then it is possible that focal infection of the brain microvessels ensues. Of note, the risk of developing of GBS meningitis in the infant rat model is directly correlated to the duration and magnitude of bacteremia.[23]

An important clinical management question is raised by the current case presentation. For example, the 2000 American Academy of Pediatrics Red Book suggests that "because of the reported risk of coinfection, the twin, triplets, or any multiples of an index case with early- or late-onset (GBS) disease should be observed carefully and evaluated and treated empirically for suspected systemic infection *if any manifestations of illness occur*".[24] In contrast, certain experts have recommended empirical evaluation and antibiotic therapy for even asymptomatic twins of index cases with invasive GBS disease.[2,7] Although in our case emergence of symptoms in twin B allowed intervention in time to prevent meningitis and effect antibiotic cure, we recognize the merits of the more conservative approach of empirical evaluation and therapy. The extreme tachycardia of twin B at presentation indicated onset of the systemic response syndrome. Moreover, recent information on occult GBS bacteremia in the outpatient setting found that only the minority of infants <90 days of age exhibited high fever (28%) or leukocytosis (28%).[25] Clinical vigilance must be maintained even following empiric therapy, as late-onset GBS disease was reported to develop in a second twin despite intravenous antibiotics for 72 hours and an initially negative blood culture.[16]

In summary, the twin infants in this report were genetically and phenotypically indistinguishable; so were the bacteria that invaded their bloodstreams. One outstanding variable is evident: twin A was evaluated and treated for bacterial sepsis within 18 hours of onset of fever, twin B within 8 hours. Twin A suffered irreversible neurologic injury due to GBS meningitis; twin B benefited from heightened parental and physician concern following her sister's diagnosis. Above all, the microbiologic information gathered in this unique case serves as a reminder of the critical importance of prompt evaluation and empiric treatment of newborn infants at risk for bacterial sepsis.

Acknowledgements

We thank Alma Ruth Franklin (CDC) for performing the GBS serotyping and Craig E. Rubens for providing antitype III GBS antisera and the capsule-negative mutant HY106. This work was supported by NIH grant HD37224 (VN) and a United Cerebral Palsy Research Foundation Grant (VN). KSD is funded by a Burroughs Wellcome Fund Career Award.

References

1. Schrag SJ, Zywicki S, Farley MM, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Engl J Med 2000;342:15–20.
2. Edwards MS, Baker CJ. Group B streptococcal infections. In: Remington J, Klein JO, editors. Infectious Diseases of the Fetus and Newborn Infant, 5th ed. Philadelphia: WB Saunders; 2001. p. 1091–156.
3. Chin KC, Fitzhardinge PM. Sequelae of early-onset group B hemolytic streptococcal neonatal meningitis. J Pediatr 1985;106:819–22.
4. Edwards MS, Rench MA, Haffar AA, Murphy MA, Desmond MM, Baker CJ. Long-term sequelae of group B streptococcal meningitis in infants. J Pediatr 1985;106:717–22.
5. Harvey D, Holt DE, Bedford H. Bacterial meningitis in the newborn: a prospective study of mortality and morbidity. Semin Perinatol 1999;23:218–25.
6. Pass MA, Khare S, Dillon HC. Twin pregnancies: incidence of group B streptococcal colonization and disease. J Pediatr 1980;97:635–7.
7. Edwards MS, Jackson CV, Baker CJ. Increased risk of group B streptococcal disease in twins. JAMA, J Am Med Assoc 1981;245:2044–6.
8. Gherardi G, Whitney CG, Facklam RR, Beall B. Major related sets of antibiotic-resistant pneumococci in the United States as determined by pulsed-field gel electrophoresis and pbp1a–pbp2b–pbp2×–dhf restriction profiles. J Infect Dis 2000;181:216–29.
9. Rubens CE, Wessels MR, Heggin LM, Kasper DL. Transposon mutagenesis of type III group B *Streptococcus*: correlation of capsule expression with virulence. Proc Natl Acad Sci USA 1987;84:7208–12.
10. Nizet V, Kim KS, Stins M, et al. Invasion of brain microvascular endothelial cells by group B streptococci. Infect Immun 1997;65:5074–81.
11. Pritzlaff CA, Chang JC, Kuo SP, Tamura GS, Rubens CE, Nizet V. Genetic basis for the β-haemolytic/cytolytic activity of group B *Streptococcus*. Mol Microbiol 2001;39:236–48.
12. Nizet V, Gibson RL, Chi EY, Framson PE, Hulse M, Rubens CE. Group B streptococcal beta-hemolysin expression is associated with injury of lung epithelial cells. Infect Immun 1996;64:3818–26.
13. Olver WJ, Bond DW, Boswell TC, Watkin SL. Neonatal group B streptococcal disease associated with infected breast milk. Arch Dis Child Fetal Neonat Ed 2000;83:F48–9.
14. Paredes A, Wong P, Mason EO, Taber LH, Barrett FF. Nosocomial transmission of group B streptococci in a newborn nursery. Pediatrics 1977;59:679–82.
15. Easmon CS, Hastings MJ, Clare AJ, et al. Nosocomial transmission of group B streptococci. Br Med J (Clin Res Ed) 1981;283:459–61.
16. Rubin EE, McDonald JC. Group B streptococcal disease in twins: failure of empiric therapy to prevent late onset disease in the second twin. Pediatr Infect Dis J 1991;10:621–3.
17. Moylett EH, Fernandez M, Rench MA, Hickman ME, Baker CJ. A 5-year review of recurrent group B streptococcal disease: lessons from twin infants. Clin Infect Dis 2000;30:282–7.
18. Christensen RD, Rothstein G. Exhaustion of mature marrow neutrophils in neonates with sepsis. J Pediatr 1980;96:316–8.
19. Christensen RD, Shigeoka AO, Hill HR, Rothstein G. Circulating and storage neutrophil changes in experimental type II group B streptococcal sepsis. Pediatr Res 1980;14:806–8.
20. Bonadio WA, Jeruc W, Anderson Y, Smith D. Systemic infection due to group B beta-hemolytic streptococcus in children. A review of 75 outpatient-evaluated cases during 13 years. Clin Pediatr (Philadelphia) 1992;31:230–3.
21. Lannering B, Larsson LE, Rojas J, Stahlman MT. Early onset group B streptococcal disease. Seven year experience and clinical scoring system. Acta Paediatr Scand 1983;72:597–602.
22. Anderson DC, Hughes BJ, Edwards MS, Buffone GJ, Baker CJ. Impaired chemotaxigenesis by type III group B streptococci in neonatal sera: relationship to diminished concentration of specific anticapsular antibody and abnormalities of serum complement. Pediatr Res 1983;17:496–502.
23. Ferrieri P, Burke B, Nelson J. Production of bacteremia and meningitis in infant rats with group B streptococcal serotypes. Infect Immun 1980;27:1023–32.
24. Committee on Infectious Diseases. Group B streptococcal infections. In: Pickering LK, editor. 2000 Red Book: Report of the Committee on Infectious Diseases. Elk Grove Village, IL: American Academy of Pediatrics; 2000. p. 537–43.
25. Pena BM, Harper MB, Fleisher GR. Occult bacteremia with group B streptococci in an outpatient setting. Pediatrics 1998;102:67–72.