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Bacillus cereus Bacteremia in a Preterm Neonate

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Bacillus cereus **is an uncommon but potentially serious bacterial pathogen causing infections of the bloodstream, lungs, and central nervous system of preterm neonates. A case of bacteremia caused by *B. cereus* in a 19-day-old preterm neonate who was successfully treated with vancomycin, tobramycin, meropenem, and clindamycin is described. Implications for the diagnostic laboratory and clinicians when *Bacillus* species are detected in normally sterile sites are discussed, and the small numbers of infant infections proven to be due to this organism that have been described previously are reviewed.**

CASE REPORT

A 585-g male infant, who was a second twin, was born by spontaneous vaginal delivery after a 24-week gestation in June, 2001. Prior to delivery his mother received ampicillin and azithromycin for prolonged premature rupture of membranes, with chorioamnionitis and betamethasone to aid in fetal lung surfactant production. Except for premature labor, her pregnancy had been otherwise normal. The infant had respiratory distress immediately after birth and was treated with exogenous surfactant and mechanical ventilation. He received empirical ampicillin and gentamicin for suspected sepsis. Blood cultures obtained on admission to the neonatal intensive care unit gave negative test results, and antibiotics were discontinued after 3 days. On postnatal day 19, the infant became hypotensive, requiring isotonic saline volume expansion and exogenous catecholamine support. The leukocyte count was 12×10^9/liter and the platelet count was 125×10^9/liter. Antibiotic treatment with vancomycin and tobramycin was initiated. In tests using a BacTAlert system (BioMérieux, Durham, N.C.), two pediatric aerobic blood culture bottles obtained at this time were positive for gram-positive bacilli after 2 days of incubation at 35°C. Cerebrospinal fluid (CSF) was sterile. The organism produced large, spreading yellowish colonies that were beta-hemolytic on Trypticase soy agar with 5% sheep blood and were catalase positive. Using a BBL Crystal Gram-Positive ID kit (Becton Dickinson, Cockeysville, Md.), the bacterium was identified as *Bacillus cereus*. Identification was confirmed by the Alabama State Public Health Reference Bacteriology Laboratory on the basis of motility, production of lecithinase on egg yolk agar, and production of acid from glucose, maltose, and salicin. Clindamycin and meropenem were added after culture results were available, because a preliminary agar disk diffusion susceptibility test indicated possibly reduced susceptibility to vancomycin and ampicillin. The neonate received 10 days of total antibiotic treatment. Additional blood cultures performed after completion of antimicrobial chemotherapy were negative. He was discharged home on day 162 of hospitalization.

B. cereus is a motile, aerobic or facultatively anaerobic, spore-forming, gram-positive or gram-variable bacterium of the family *Bacillaceae* that is found worldwide in dust, air, and water. As a human pathogen, the organism is perhaps best known for its role as a mediator of self-limited foodborne illness. However, in recent years there has been an increasing appreciation for its potential as an opportunistic pathogen in immunocompromised, critically ill, or otherwise debilitated patients, those with foreign bodies, and intravenous drug abusers (6). Among members of this genus, only *B. anthracis* is potentially more significant as a cause of human disease (21).

Advances in neonatal intensive care over the past several years have improved survival of very-low-birthweight neonates, but due to their immature immune systems (15) as well as their being subjected to prolonged mechanical ventilation and frequent use of long term, intravascular catheters, members of this vulnerable population are particularly susceptible to disseminated disease caused by environmental organisms such as *B. cereus*. The number of documented cases of clinically significant invasive infections in neonates or older infants due to *B. cereus* is small. A computerized search of the English language literature in the National Library of Medicine Database provided a total of 22 cases in infants of clinically significant systemic infection proven to be due to *B. cereus*. These cases are summarized in Table 1. Of the 22 reported infections, 16 occurred in neonates, most of whom were born prematurely. Among 15 individual published reports describing *B. cereus* infections in infants since 1970, only 5 have come from the United States, with the most recent in 1993 (9). Thus, neonatologists as well as clinical microbiologists in this country may not always appreciate the significance of isolations of *B. cereus* from clinical specimens obtained from newborns due to its apparent rarity as a nosocomial pathogen; the significance is further clouded by the frequent appearance of the species in cultures as a contaminant (11). In our case, when the preliminary laboratory report for positive blood cultures indicated the presence of gram-positive bacilli, clinicians first assumed it

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TABLE 1. Published reports of clinically significant *Bacillus cereus* infections in infants[a]

No. of cases	Sex/birthweight (g)/gestational age (weeks)	Age at first positive culture	Predisposing factors	Primary infection site(s)	Treatment	Outcome	Mode of transmission	Reference
1	Male/1,580/26	30 days	Prematurity, MV[b]	Blood, CSF	Amikacin, vancomycin	Died 9 days after onset	ND[c]	3
3	Male/895/28	11 days	Prematurity, MV	Blood, CSF, trachea, skin lesion	Amoxicillin, cefotaxime	Died 1 day after onset	Ventilator equipment and hands of staff	19
	Female/1,000/26	12 days	Prematurity, MV	Blood, synovial fluid	Meropenem, vancomycin	Recovered		
	Male/2,780/37	20 days	Asphyxia, MV	Blood, CSF, IV catheter tip	Meropenem, vancomycin	Recovered		
1	Male/735/24	14 days	Prematurity, MV, CNS hemorrhage	Blood, bone, marrow	Ciprofloxacin, clindamycin, gentamicin, imipenem, vancomycin	Died 49 days after onset	ND	16
4	ND/880/27	1 day	Prematurity, MV	Endotracheal aspirate	Vancomycin	Recovered	Possible contamination of ventilator circuits	7
	ND/880/28	1 day	Prematurity, MV	Endotracheal aspirate	ND	Died		
	ND/640/28	2 days	Prematurity, MV	Endotracheal aspirate	ND	Died		
	ND/530/26	3 days	Prematurity, MV	Endotracheal aspirate	ND	Died		
2	Male/920/27	1 day	Prematurity, MV, intrauterine growth retardation	Blood, CSF, lung (postmortem)	Ampicillin, gentamicin	Died at 2 days of age	Possible contamination of resuscitation devices, drugs, or hands of caregivers	9
	Female/690/25	1 day	Prematurity, MV	Blood, lung (postmortem)	Ampicillin, gentamicin	Died at 2 days of age		
1	Female/2,715/36	5 days	Prematurity, MV, Arnold-Chiari malformation	CSF, neural tissue	Ampicillin, cefotaxime, ceftazidime, vancomycin	Recovered	ND	20
1	Female/830/26	7 days	Prematurity, MV, thalamic hemorrhage	Blood, CSF	Amikacin, vancomycin	Died 1 day after onset	ND	13
1	Female/1,500/32	7 weeks	Prematurity, MV, IV catheterization	CSF, IV catheter tip	Ampicillin, chloramphenicol	Recovered	IV catheter	4
1	Female/ND/32	8 days	Prematurity, MV, central line for hyperalimentation	CSF	Ampicillin, erythromycin, gentamicin	Died 3 days after onset	ND	8
1	Female/ND/ND	8 days	ND	Eye	ND	Recovered	ND	1
2	ND/ND/ND	Newborn	ND	Blood, CSF	ND	ND	ND	18
	ND/ND/ND	10 months	ND	Blood	ND	ND		
1	Female/1,320/32	2 days	Prematurity, asphyxia, intestinal perforation	Blood, brain	Ampicillin, gentamicin	Died at 4 days of age	Umbilical catheter, necrotic tissue	17
1	ND/ND/ND	8 mo	Hydrocephalus, VP shunt	Blood, CSF, tip of VP shunt	Ampicillin, gentamicin, trimethoprim-sulfamethoxazole, VP shunt removal	Recovered	VP shunt tip	14
1	ND/ND/ND	18 wk	Dandy Walker cyst, VP shunt	CSF	Ampicillin, gentamicin, VP shunt removal	Recovered	ND	10

[a] Reports included in the table are limited to those in which *Bacillus* species known to be *B. cereus* were isolated from infants with clinically significant systemic infections.
[b] MV, mechanical ventilation.
[c] ND, not described.

would be *Listeria monocytogenes* and expressed initial skepticism when the organism was identified as *B. cereus*, thereby indicating their unfamiliarity with this organism as a potential cause of neonatal bacteremia.

Despite the small number of reported cases of *B. cereus* systemic infections in infants, many of which were fatal, it seems likely that the actual occurrence of this organism and perhaps other *Bacillus* species is more common as a true pathogen than the published literature would suggest. This belief is based on the fact that clinical laboratories may not attempt to determine the complete species identification of *Bacillus* organisms even when the organisms are isolated from sterile sites such as blood or CSF (4, 21), arbitrarily designating them as contaminants without adequate consultation with clinicians, and on the confusing taxonomy of the genus *Bacillus* that in the past may have been responsible for the inappropriate attribution of infections due to *B. cereus* to other species such as *B. subtilis* (14, 18). According to one report (14), management of an infant with *B. cereus* meningitis was complicated by the assumption that multiple previous isolations of the same organism from blood cultures were merely contaminants.

Clinical manifestations of diseases due to *B. cereus* have been ascribed to the production of various exotoxins, including the enterotoxin responsible for foodborne disease, lecithinase, phospholipases, proteases, and hemolysins that produce extensive damage and liquefactive necrosis of infected tissues (18). Histopathological examination of tissues collected at autopsies of fatal cases of neonatal *B. cereus* infections have demonstrated that tissue invasion with multiplication of organisms in various organs can also occur (8, 9,13). The case reports summarized in Table 1 have shown that infections caused by this organism usually involve the bloodstream, CSF, and/or respiratory tract (1, 3,4, 7–10, 13, 14, 16–20).

Assessment of the origin of infections due to organisms such as *B. cereus* that are widely disseminated in the environment is often difficult and may not yield an obvious source. Nonetheless, a few epidemiologic investigations of outbreaks that have occurred in Europe provide some insights on likely vehicles for infections with *B. cereus* in neonatal units.

A report from The Netherlands (19) described 35 neonates in a hospital unit that were colonized in the respiratory tract with a single *B. cereus* clone. Three of them showed signs of respiratory infection, and one died. The epidemic strain of *B. cereus* was detected on the hands of nursing staff and in mechanical ventilation equipment. Three additional reports from Great Britain (2, 7, 22) also described outbreaks of *B. cereus* in hospital neonatal units. The first report (2) showed that 28 of 57 (49%) neonates in a unit were colonized asymptomatically in the umbilicus over a surveillance period of 2 weeks, 90% of them with the same *B. cereus* serotype. The same strain was also identified in the unit's diaper supply. Another report (22) described dissemination of *B. cereus* over a 4-month period in 48 of 259 (19%) umbilical swabs of asymptomatic neonates in a single hospital unit, most isolates of which belonged to a single serotype. The primary source of the organism could not be established. The third report (7) described an outbreak in which *B. cereus* was isolated over a 2-week period from endotracheal secretions of six intubated neonates, four of whom developed clinical illness and one of whom died. The *B. cereus* isolates from four of the neonates were proven to be of the same serotype. Contaminated ventilator equipment was suspected to be the source of infection. Intravenous catheters and ventriculoperitoneal shunts have also been documented as modes of transmission in case reports of neonatal *B. cereus* meningitis (4, 14).

Policies in the University of Alabama Hospital Clinical Microbiology Laboratory mandate that fluid from positive blood culture bottles is to be subcultured for a qualitative direct susceptibility test using the agar disk diffusion technique. This practice enables preliminary susceptibility data to be communicated to physicians if requested, pending final organism identification and standardized susceptibility testing according to guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (12) when indicated. This practice also assists technologists in determining whether bacterial isolates growing from more than one blood culture bottle are likely to be of the same species. While standardized testing procedure and interpretation guidelines are available from the NCCLS for agar disk diffusion testing and broth-based MIC testing for gram-positive cocci such as staphylococci and streptococci, no such interpretive guidelines are available for gram-positive bacilli such as *B. cereus* (12). Therefore, the laboratory does not routinely perform and report susceptibility tests on such organisms without consultation with the physician caring for the patient. When requested under these circumstances, quantitative susceptibility testing has been performed using the agar-gradient diffusion (Etest) technique in accordance with the manufacturer's instructions and interpreted using the NCCLS MIC breakpoint criteria for staphylococci (12). A disclaimer indicating the nonstandardized methodology for the test performed under these conditions is reported in the medical record. The *B. cereus* isolate from the neonate described in this report had an Etest vancomycin MIC of 24 μg/ml (rounded to 32 μg/ml) and was interpreted as resistant according to NCCLS criteria for staphylococci (12). The test was repeated two additional times in accordance with the manufacturer's instructions, with results of 16 and 24 μg/ml.

Due to concern about the possible emergence of vancomycin resistance, since the *vanA* gene responsible for vancomycin resistance in enterococci has been documented previously in *B. circulans* (5), a broth-based reference method was eventually used at Mayo Medical Laboratories, Rochester, Minn., to determine the vancomycin MIC. The MIC was 4 μg/ml, which would be considered indicative of susceptibility (12).

In view of the increasing awareness of the potential role of *Bacillus* species as human pathogens, especially in neonates and other immunosuppressed hosts, and in direct response to the *B. anthracis* bioterrorism events of 2001, our laboratory now pays greater attention to all *Bacillus* isolates from clinical specimens. We routinely perform a motility test and examine blood agar plates for typical colonial morphology of *B. anthracis* (11). All *Bacillus* isolates that grow in two blood culture bottles from the same set or in at least two bottles from 2 sets collected at the same time and any isolate grown on primary culture plates from CSF are subjected to biochemical tests for species identification. Whenever *Bacillus* species of possible clinical significance are encountered, prompt notification of the species identity and interaction with clinicians are extremely important in view of the high mortality that has been

reported for neonatal infections due to these organisms and the variability of antimicrobial susceptibilities (21).

Most strains of *B. cereus* are resistant to penicillins and cephalosporins. They have been reported to be susceptible to the aminoglycosides, clindamycin, vancomycin, carbapenems, chloramphenicol, and erythromycin (6). This is important to consider, since the other gram-positive bacillus of major significance in neonates, *L. monocytogenes*, is usually treated with ampicillin. Our experience and that of others (21) with potentially misleading in vitro susceptibility tests using agar-based methods suggests that quantitative broth-based MIC tests performed using NCCLS methods are advisable to guide specific therapy. Combination therapy with vancomycin and another drug such as an aminoglycoside or clindamycin given empirically while awaiting susceptibility data has been recommended for systemic infections (6). Removal of contaminated foreign bodies such as intravascular catheters or intraventricular shunts is also of great importance to avoid persistent or recurrent infection.

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