

Citrobacter sedlakii Meningitis and Brain Abscess in a Premature Infant

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Received 17 April 1997/Returned for modification 10 June 1997/Accepted 10 July 1997

***Citrobacter sedlakii* was isolated from blood and cerebrospinal fluid cultures of a 5-day-old premature infant with sepsis, meningitis, and brain abscess. This newly described organism was difficult to identify due to discrepancies between the Vitek and API 20E identification systems. To our knowledge, this is the first report of the isolation of *C. sedlakii* from cerebrospinal fluid.**

Citrobacter species are gram-negative, oxidase-negative bacilli that are members of the family *Enterobacteriaceae*. The nomenclature of the various species within the genus *Citrobacter* has long been controversial, and it is known that many *Citrobacter* isolates from human clinical specimens do not have the biochemical characteristics of the previously described species, *Citrobacter freundii*, *C. koseri*, and *C. amalonaticus* (2, 6, 17). *Citrobacter* organisms can cause a variety of infections in adults and are most commonly isolated from urine, sputum, and soft-tissue specimens (12, 16). In children, *Citrobacter* species, especially *C. koseri* (formerly *C. diversus*) and *C. freundii*, are best known for their propensity to cause sepsis, ventriculitis, and neonatal meningitis with brain abscesses (5, 8, 11, 14, 15, 18). Recently (2), the genus *Citrobacter* was determined to contain 11 genetically distinct genomospecies; genomospecies 8 has been named *C. sedlakii* in honor of Jiri Sedlak, the Czechoslovakian microbiologist who first developed a serotyping scheme for *C. freundii* (23). To date, isolates submitted to the Centers for Disease Control and Prevention that were identified as *C. sedlakii* were isolated from human blood, wounds, and stool specimens (2). We report the first published case of neonatal meningitis and brain abscess caused by *C. sedlakii* and discuss the difficulties that were encountered in identifying this recently named species.

Case history. The patient was a 1,740-g, 30-week premature male born by vaginal delivery to a healthy 15-year-old primigravida without prenatal care. Apgar scores were 8 at 1 min and 9 at 5 min, and the physical examination was normal. Within 30 min after birth, the infant developed respiratory distress requiring intubation and mechanical ventilation. Intra-tracheal pulmonary surfactant was given, a blood culture was collected (no growth after 5 days), and empiric ampicillin and gentamicin were begun. The infant was extubated on the third day of life, but required reintubation and an evaluation for possible infection on day 5 because of respiratory distress, metabolic acidosis, thrombocytopenia, and disseminated intravascular coagulation. Piperacillin was added to ampicillin and gentamicin empirically. The blood culture from day 5 grew *Enterobacter cloacae* and *C. sedlakii*. The cerebrospinal fluid (CSF) contained 113 leukocytes/ml, 8,750 erythrocytes/ml, 105

mg of protein per dl, and 63 mg of glucose per dl. The CSF culture grew *C. sedlakii* in pure culture. Gentamicin was discontinued, and ampicillin, piperacillin, and cefotaxime were administered. A repeat blood culture on day 7 grew *C. sedlakii* alone; however, subsequent blood cultures were negative. A repeat lumbar puncture on day 9 revealed sterile CSF with 130 leukocytes/ml, 1,600 erythrocytes/ml, 232 mg of protein per dl, and 34 mg of glucose per dl. Urine and endotracheal tube cultures were negative. When susceptibility test results became available on the *C. sedlakii* blood and CSF isolates, it was found that all isolates had the same antimicrobial susceptibility pattern and the antibiotics were changed to intravenous (i.v.) cefotaxime and gentamicin. By day 14 the infant was clinically stable; gentamicin was discontinued, and therapy was changed to i.v. cefotaxime and oral cotrimoxazole.

During the infant's hospital course, several computed tomography scans and magnetic resonance imaging scans of the brain revealed abscesses in the right deep frontal, left frontal, and left occipitoparietal areas of the brain. An electroencephalogram showed bilateral temporal positive and negative sharp waves; however, the infant did not show clinical seizure activity. An auditory brainstem hearing-evoked response (ABER) test was normal bilaterally.

At 5 weeks of age, therapy was changed to intramuscular ceftriaxone and oral cotrimoxazole, and at 8 weeks of age the infant was discharged to complete 2 more weeks of oral cotrimoxazole therapy at home. At 3 months of age the infant was seen in a follow-up examination by the pediatric neurologist, who noted mild tone changes and possible hemianopia. There was no history of seizures, and a repeat ABER test was normal. At 4 months of age, tone and development were normal. An examination for possible hemianopia by the pediatric ophthalmologist was inconclusive, and follow-up ophthalmologic examinations were scheduled. At 5 1/2 months of age, a computed tomography scan of the brain showed resolution of all three abscess cavities, with a small area of residual calcification in the left parietal region.

Laboratory investigation. Two of three blood cultures and one of two CSF specimens collected for culture grew gram-negative bacilli on both sheep blood agar (SBA), and MacConkey agar (REMEL Laboratories, Lenexa, Kans.). On MacConkey agar the colonies were dry, pink, and flat. On SBA the colonies were gray-white, round, convex, and entire. The organism was oxidase negative and spot indole positive and fermented glucose. The Kligler's iron agar result was acid/acid

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with gas but no H₂S. The isolate was initially inoculated onto the Vitek GNI card and tested with the Vitek bacterial identification and susceptibility test system (bioMérieux-Vitek, Hazelwood, Mo.). The organism was identified by the Vitek system as *Enterobacter amnigenus* biogroup 2 (94%)/*C. freundii* (1%) (software version WSVTK-R04.01). Since *E. amnigenus* is an infrequently isolated species, the organism was inoculated into the API 20E system (bioMérieux-Vitek). The API 20E system identified the organism as a *Citrobacter* species (biocode 3344753; "very good" identification), with the choices being *C. diversus* and *C. amalonaticus*. Because of these discrepant identification tests, conventional biochemical tests were used. The organism was indole positive, methyl red positive, Voges-Proskauer negative, Simmons citrate positive, urease positive, and malonate positive. Arginine dihydrolase and ornithine decarboxylase tests were positive, but a lysine decarboxylase test was negative. The isolate fermented arabinose, cellobiose, maltose, mannitol, melibiose, sorbitol, rhamnose, trehalose, and xylose; adonitol, inositol, raffinose, salicin, and sucrose were not fermented. Discrepant reactions between conventional biochemical tests and the kit systems included a false-negative arginine dihydrolase test on the Vitek GNI card and false-negative urease tests on both the Vitek GNI card and the API 20E system. Resolved biochemical test reactions for these isolates were entered into the BioBASE (version 3.10) database (BioBASE, Inc., Boston, Mass.). This database identified the isolates as *C. sedlakii* (good identification with a 97.8% likelihood). The identity of the isolate was confirmed as *C. sedlakii* by the Microbial Diseases Laboratory, State of California Department of Health Services (Berkeley, Calif.).

Broth microdilution antimicrobial susceptibility testing (Pasco, Detroit, Mich.) of the isolate indicated that the organism was susceptible to gentamicin (MIC, 0.5 µg/ml), amikacin (MIC, ≤1 µg/ml), cefotaxime (MIC, ≤8 µg/ml), ceftizoxime (MIC, ≤4 µg/ml), ceftazidime (MIC, ≤2 µg/ml), ceftriaxone (MIC, ≤4 µg/ml), cefoperazone (MIC, 16 µg/ml), piperacillin (MIC, ≤8 µg/ml), cotrimoxazole (MIC, ≤1/16 µg/ml), ciprofloxacin (MIC, ≤0.25 µg/ml), and ofloxacin (MIC, ≤0.5 µg/ml). The organism was resistant to ampicillin (MIC, >16 µg/ml), cefazolin (MIC, >16 µg/ml), and cefuroxime (MIC, >16 µg/ml).

Discussion. The clinical aspects of our case of neonatal *Citrobacter* infection were not surprising. *C. koseri* (formerly *C. diversus*) has been the species most closely associated with meningitis and brain abscess in neonates, and the physical and radiographic findings of our case were not unusual for this clinical entity. The mode of acquisition of the organism in our case was not clear. Vertical transmission of *Citrobacter* from mother to infant has been reported (4, 7, 24), but in our case no cultures were obtained from the mother. Horizontal nosocomial transmission by nursery staff during *Citrobacter* outbreaks has been demonstrated (9, 10, 15, 21), but our patient represented a sporadic, isolated case which did not occur in an outbreak setting.

The optimal treatment regimen for *C. sedlakii* meningitis and brain abscess is not known. The initial choice of antibiotics in the present case was adequate, and there were no adverse clinical effects due to the delay in identification of the organism. The susceptibility pattern of the organism was typical of the more familiar species in the genus *Citrobacter*. After an initial course of exclusively i.v. antibiotics, oral cotrimoxazole was used (in addition to expanded-spectrum cephalosporins) because of its good absorption and penetration into the central nervous system (19). We are unaware of the previous use of cotrimoxazole for patients with *Citrobacter* brain abscesses, but it has long been used for central nervous system infections due

to *Salmonella* species. We used it with satisfactory results, and were able to avoid surgical i.v. line placement during the last few weeks of therapy. The total duration of in-hospital antimicrobial therapy was 6 weeks after sterilization of the CSF, which is consistent with recommendations in the literature (15). The use of oral cotrimoxazole allowed an additional 2 weeks of monotherapy to be given easily after hospital discharge while awaiting radiographic follow-up.

The tribe *Citrobacteraceae* consists of a single genus, *Citrobacter*. In 1993, Brenner and colleagues used DNA hybridization techniques to demonstrate that members of the genus *Citrobacter* belong to 11 genomospecies (2). *C. koseri* (formerly *C. diversus*), *C. amalonaticus*, and *C. farmeri* belong to genomospecies 2, 3, and 4, respectively, while organisms belonging to the "*C. freundii* complex" constitute the remaining eight genomospecies. The members of the *C. freundii* complex include *C. freundii* (genomospecies 1), *C. youngae* (genomospecies 5), *C. braakii* (genomospecies 6), *C. werkmanii* (genomospecies 7), *C. sedlakii* (genomospecies 8), *C. rodentium* (genomospecies 9), "*C. gilleni*" (proposed; genomospecies 10), and "*C. murlinae*" (proposed; genomospecies 11) (2, 22). With the exception of those of *C. koseri*, human isolates of *Citrobacter* species have been obtained primarily from stool specimens. In reviewing strains referred to the Centers for Disease Control and Prevention, Farmer and coworkers (6) cited *C. freundii* as a possible cause of diarrhea (although most fecal isolates do not appear to be associated with disease) and as a cause of occasional isolated cases of extraintestinal infections. In this 1985 report, a possible association between *C. koseri* and outbreaks of meningitis and brain abscesses in neonates was noted and the recovery of *C. amalonaticus* from blood cultures was reported (5). Janda and colleagues (13) at the Microbial Diseases Laboratory reported that *C. freundii* was the most common species isolated from all body sites except feces; in fecal specimens, *C. freundii* ranked fourth in prevalence behind *C. youngae*, *C. braakii*, and *C. werkmanii*. Members of the *C. freundii* complex have also been reported as possible causes of gastrointestinal illness associated with imported Brie cheese (3), and a *C. freundii* strain that carried a somatic antigen similar to that of *Escherichia coli* O157:H7 has been reported (1).

Although most frequently isolated from urine and respiratory tract specimens, *C. koseri* has been reported with increasing frequency as a cause of sporadic and epidemic meningitis in neonates and young infants (11, 12, 15, 17). Brain abscesses have been found in up to 75% of infants with *C. koseri* meningitis, a prevalence far greater than those for other bacterial agents of neonatal or infant meningitides (12, 15). One-third of infants with *C. koseri* meningitis die, and at least 75% of survivors have severe neurological impairment (11). To our knowledge, *C. sedlakii*, the organism isolated from the infant in the present report, has not previously been isolated from CSF and has only been isolated once before in our laboratory from a catheterized urine specimen.

Identification of *Citrobacter* species in clinical laboratories is hampered by the fact that the newly described species are not included in the databases of most commercially available identification systems. The lack of agreement between the Vitek and API 20E systems and the inability to easily identify this new organism are not surprising in retrospect. In 1995, O'Hara and colleagues (20) examined six isolates of *C. sedlakii* and found that five different commercial identification systems failed to accurately identify the isolates. In that study, as with our isolate, the Vitek system identified *C. sedlakii* strains as *E. amnigenus* and the API 20E system identified them as *C. amalonaticus* (20). The results for the arginine dihydrolase reaction

on the GNI cards were found to be falsely negative when they were compared to the results for the API 20E system and conventional methods. However, when the result for this reaction was changed to positive, the database for the Vitek GNI card still identified these isolates as *E. amnigenus* biogroup 2. Interestingly, when these biotype numbers were tested with the database of the recently released GNI+ card (bioMérieux-Vitek, Inc.), the isolates were labeled "unidentified organisms" (arginine dihydrolase-positive bionumber) or were identified as *C. freundii* complex (arginine dihydrolase-negative bionumber), which includes *C. sedlakii*. Both the GNI and GNI+ cards currently lack the biochemical substrates (i.e., melibiose and dulcitol) required for separation of these species. Similarly, the API 20E system lacks the biochemical tests (i.e., for malonate, adonitol, and dulcitol) that are necessary for separation of species within the genus *Citrobacter*. In addition, since this organism is spot indole positive and forms dry pink colonies resembling those of *E. coli* on MacConkey agar, the recovery of *C. sedlakii* from specimens for which spot indole tests are often performed (e.g., urine cultures) will also result in their misidentification.

Until all clinical laboratories are able to routinely and definitively assign *Citrobacter* isolates to the 11 recognized species, the medical community will not know the true proportion of neonatal meningitides and brain abscesses due to *C. sedlakii* or the other non-*C. koseri* citrobacters. This case report serves notice that complete biochemical assessment of clinically significant *Citrobacter* isolates should be undertaken if we are to accurately assess the pathogenic potential and epidemiology of this organism. To date, this is the first reported case of neonatal meningitis and brain abscess due to *C. sedlakii*.

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