Three Cases of Neonatal Meningitis Caused by *Enterobacter sakazakii* in Powdered Milk

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Three cases of neonatal infection caused by *Enterobacter sakazakii* are reported from the Department of Neonatal Intensive Care, the National University Hospital, Reykjavik, Iceland. These infections occurred during a 9-month period in 1986 and 1987. Two of the neonates, who were normal at birth, survived but were left with brain damage. The third, who had Down’s syndrome and severe cardiac malformations, died. The same organism was also grown from groin and anal swabs from a healthy neonate. *E. sakazakii* was not isolated from any environmental sources in the neonatal wards or in the milk kitchen, but it was grown from several lots of the powdered-milk formula used in the hospital. The four *E. sakazakii* strains isolated from the neonates were indistinguishable from 22 strains grown from the formula. Their biotypes, plasmid DNA profiles, and antibiograms were identical.

Approximately 50 to 75% of neonatal meningitis and septicemia cases are caused by *Escherichia coli* and *Streptococcus agalactiae* (7). The remaining of these cases are caused by a wide spectrum of bacteria, including many species of the family *Enterobacteriaceae*, among them strains belonging to the genus *Enterobacter*.

Urmenyi and Franklin (9) reported the first two known cases of neonatal meningitis and septicemia caused by *Enterobacter sakazakii*, described at that time as a yellow-pigmented *Enterobacter cloacae* strain. Jóker et al. (3) reported an additional case of meningitis caused by a yellow-pigmented *E. cloacae* strain in 1965. In 1980, the name *E. sakazakii* was proposed for this new species by Farmer et al. (2). Since then, a number of neonatal infections caused by this organism have been reported (10).

The natural habitat of *E. sakazakii* is unknown, but milk powders have been suggested as a possible mode of transmission in neonatal infections (7).

Three cases of neonatal meningitis caused by this organism were diagnosed in the National University Hospital, Reykjavik, Iceland, in 1986 and 1987. The last two cases, diagnosed within 1 month, prompted an epidemiological investigation which was focused on powdered milk when a literature search revealed it as a possible source of infection. The three cases are presented, and the investigation of the possible mode of transmission is described.

CASE REPORTS

**Case 1.** Case 1 was in a male infant born 18 March 1986 after 36 weeks of gestation (birth weight, 3,144 g). His mother was started on ampicillin the day before delivery because of suspected urinary tract infection. Her urine, however, was not cultured.

The child appeared to be healthy and fed well on breast milk and supplementary powdered-milk formula. On day 5, his condition deteriorated, and septicemia was suspected. His spinal fluid contained numerous polymorphonuclear cells, and *E. sakazakii* grew from cultures of spinal fluid and blood. Treatment with intravenous ampicillin and gentamicin was started immediately, and cefuroxime was added within 12 h. Two weeks after therapy was started, *E. sakazakii* still grew from the cultures of ventricular fluid. Gentamicin was subsequently administered intravenicularly, and chloramphenicol was added intravenously. Antibiotic therapy continued for 2 months. Computed tomography scans showed cystic cavities in the brain. The infant was discharged from the hospital at 3 months of age. His mental and physical development was considered to be markedly impaired. At 2 years of age he is severely mentally retarded and quadriplegic.

**Case 2.** Case 2 was in a full-term, male infant delivered spontaneously on 14 December 1986 after an uneventful pregnancy (birth weight, 2,508 g). The child had Down’s syndrome and an imperforate anus. Anoplasty was performed during the first 24 h after birth. Oral feedings with powdered-milk formula were started within hours after surgery. He did well until day 5, when he started feeding poorly and his condition deteriorated.

Electrocardiograms and ultrasonograms confirmed congenital heart defects and heart failure. Spinal fluid contained numerous polymorphonuclear cells, and gram-negative rods were seen. *E. sakazakii* was cultured from the fluid, but *E. coli* was isolated from blood. The boy was treated with ampicillin and cefotaxime. His condition deteriorated rapidly, and he died 5 days after birth. Autopsy revealed acute meningitis and fresh hemorrhages into both lateral ventricles as well as congenital heart defects.

**Case 3.** Case 3 was in a male twin born after 38 weeks of gestation on 6 January 1987 (birth weight, 3,308 g). He appeared to be healthy and fed well on breast milk and supplemental powdered-milk formula until day 5, when he started feeding poorly. On day 6 he was somnolent, he developed fever, and his condition became rapidly worse. His spinal fluid was cloudy and contained numerous leukocytes, predominantly polymorphonuclear cells. *E. sakazakii* grew from cultures of the fluid, but blood culture was negative. Antibiotic therapy with ampicillin and cefotaxime was started immediately. The clinical condition of the boy improved following initiation of therapy. Repeat cultures from the spinal fluid were negative. Antibiotics were discontinuation.

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continued after 3 weeks. The boy was discharged at 1 month of age with no obvious clinical signs of a neurological deficit. Computerized tomography scans of the brain did, however, show a cystic cavity in the left frontal lobe. The child has since developed a seizure disorder and is moderately delayed in all developmental areas.

MATERIALS AND METHODS

The powdered-milk formula used in the neonatal wards was prepared in a separate milk kitchen according to the recommendations of the manufacturers and was cooled right after preparation by placing the container in cold water. It was stored in a refrigerator after being dispensed into 250-ml bottles. Formula was prepared twice a day and delivered to the wards at 8 a.m. and 2 p.m., always within 2 h of preparation. It was not sterilized.

Numerous specimens were collected for culture from the environment in the maternity wards and in the milk kitchen. Attempts were made to isolate E. sakazakii from all utensils used to prepare the formula. Water samples were taken from bottle heaters used in the wards to warm the formula and were cultured both directly and by filtering 100-ml samples through filters (pore size, 0.22 μm) and culturing the filters.

Numerous cultures were made from formula freshly prepared in the milk kitchen by direct plating with 1-μl counting loops on blood agar plates, and on one occasion formula stored in a refrigerator in one of the wards was cultured.

Milk powder from seven unopened packages of five different lot numbers was cultured in the following way. Powder from a 500- or 1,000-g package was dissolved in the amount of water recommended by the manufacturers. This was done aseptically in sterile water, and the solution was divided into 100-ml samples in sterile containers. Of these containers, 154 were incubated in a water bath at 36°C for 4 h. Each of them was then subcultured with a 1-μl counting loop on one blood agar and one MacConkey agar plate.

The bacteria isolated were identified with the API 20E system. Biotyping, plasmid DNA profiles, and antibiograms were done at the Centers for Disease Control (N. C. Clark et al., unpublished results).

RESULTS

Careful scrutiny of the preparation process in the milk kitchen did not reveal any faults likely to cause contamination of the formula or multiplication of organisms during preparation. E. sakazakii was not isolated from the environment in the wards or in the milk kitchen or from utensils used for preparation of the formula.

By direct plating, the organism was isolated from one bottle of formula which had been stored for an unknown period of time in a refrigerator in one of the wards but not from any specimen of freshly prepared formula.

After incubation at 36°C for 4 h, 23 strains of E. sakazakii were isolated from some of the 100-ml samples made from five packages of milk powder, but E. sakazakii did not grow from any sample from two of the packages examined. All lot numbers examined contained the organism in at least one sample, and it was isolated from 70% of the samples from one 500-g package.

During this investigation E. sakazakii was grown from a urine specimen collected in a bag from a 3-day-old infant, who was under observation because he was delivered by cesarean section after 35 weeks of gestation. Two days later, urine was obtained by suprapubic aspiration, and the groin and anus were swabbed. E. sakazakii was grown from the swabs but not from the urine. This infant never showed any signs of infection.

The strains of E. sakazakii isolated from the four neonates and all but one of the 23 strains isolated from the formula turned out to be of the same biotype and had identical antibiograms and plasmid profiles (Clark et al., unpublished results).

DISCUSSION

Since E. sakazakii was first described as a separate species, it has been reported to have caused an outbreak of colonization of neonates (1) and 14 cases of neonatal meningitis or sepsis in addition to the 3 reported cases caused by E. cloacae of the yellow-pigmented variety (4, 5, 7, 8, 10; D. M. Adamson and J. R. Rogers, Clin. Microbiol. Newsl. 3:19-20, 1981). Our three cases bring the total of reported cases of neonatal infections caused by this organism to 20.

The natural habitat of the organism is not known, but it has been grown from different places in the hospital environment. E. sakazakii has been isolated from many brands of powdered milk (6), and Muytjens et al. (7) suggested powdered-milk formula as a likely mode of transmission, although they were unable to prove their hypothesis. Their cases were investigated retrospectively, and the strains cultured from powdered milk at the time of investigation were not identical to those isolated from the sick neonates.

The strains isolated from the infants in our hospital were, on the other hand, identical in all respects to the strains found in the formula used in the hospital. Because our investigation was performed in retrospect after the last case was diagnosed, we were unable to collect specimens for culture from the mothers. However, intrauterine infections seem unlikely, as none of the infants developed signs of infection until several days after birth. The infants who contracted E. sakazakii meningitis had all received powdered-milk formula before falling ill; however, so had many other infants, including the twin brother of patient 3, who never fell ill, and the healthy neonate from whom E. sakazakii was isolated from anal and groin swabs, indicating colonization without invasion.

The organism seemed to be present in the milk powder in low numbers, since it could only be isolated from freshly prepared formula by subculture after 4 h of incubation. Two of the infants who developed E. sakazakii meningitis were normal at birth, and an explanation is needed as to why they were infected by these low numbers. The most likely explanation appears to be that the rules pertaining to the handling of the formula in the wards were not always adhered to. There is some anecdotal evidence that the formula bottles were occasionally kept at 35 to 37°C for extended periods of time in bottle heaters, thus allowing for multiplication of the organism.

In the hope of preventing recurrence of these neonatal infections, bottle heaters were removed from the wards and policies for handling powdered-milk formula were revised and reinforced.

On one occasion, formula was plated directly and E. sakazakii was isolated from a bottle that had been kept in a refrigerator in a ward for an unknown period of time. This instance remains unexplained.

No attempt was made to quantitate the growth of bacteria other than E. sakazakii in the formula, but a number of other strains of the Enterobacteriaceae grew from it after 4 h of incubation, among them E. cloacae and E. agglomerans.
Now that it seems established that milk powder can be the mode of transmission for *E. sakazakii* meningitis or sepsis in neonates, it seems reasonable to pose the following questions. Is *E. sakazakii* fundamentally different from other members of the Enterobacteriaceae that are also found in milk powders, or have we only recognized its mode of transmission because it is an unusual pathogen? If so, is it possible that other species of the Enterobacteriaceae that cause neonatal infections can also be transmitted in powdered-milk formulas?

LITERATURE CITED


