We investigated an outbreak of *Serratia marcescens* in the neonatal intensive care unit (NICU) of the University Hospital of Zurich. *S. marcescens* infection was detected in 4 children transferred from the NICU to the University Children’s Hospital in Zurich. All isolates showed identical banding patterns by pulsed-field gel electrophoresis (PFGE). In a prevalence survey, 11 of 20 neonates were found to be colonized. *S. marcescens* was isolated from bottles of liquid theophylline. Despite replacement of these bottles, *S. marcescens* colonization was detected in additional patients. Prospective collection of stool and gastric aspirate specimens revealed that colonization occurred in some babies within 24 hours after delivery. These isolates showed a different genotype. Cultures of milk from used milk bottles yielded *S. marcescens*. These isolates showed a third genotype. The method of reprocessing bottles was changed to thermal disinfection. In follow-up prevalence studies, 0 of 29 neonates were found to be colonized by *S. marcescens*. In summary, 3 consecutive outbreaks caused by 3 genetically unrelated clones of *S. marcescens* could be documented. Contaminated milk could be identified as the source of at least the third outbreak.

*Serratia marcescens* is an important nosocomial pathogen, especially in neonatal intensive care units (NICUs), and it may cause serious infections, including meningitis, bacteremia, and pneumonia, with significant associated morbidity and mortality in newborns [1]. Normally, *S. marcescens* does not constitute part of the intestinal bacterial flora of neonates [2]. This bacterium may be transmitted to neonates through feeding [3] and use of soaps [4], contaminated antiseptics [5], breast pumps [6], and tocograph transducers [1], and it can be spread via contact with the patient. Risk factors for acquisition of nosocomial infections in NICUs are low birth weight, long duration of hospitalization, and receipt of critical care [7]. We report the investigation of outbreaks of *S. marcescens* infection in infants transferred from the NICU of a tertiary care university hospital.

**MATERIALS AND METHODS**

**Background.** From April 1998 through May 1999, 4 children developed invasive infections with *S. marcescens* while they were hospitalized at the University Children’s Hospital in Zurich, Switzerland. All had been transferred from the NICU of the University Hospital of Zurich (USZ). *S. marcescens* isolates showed identical banding patterns by pulsed-field gel electrophoresis (PFGE; see the subsection about microbiological studies and genotyping), which suggested the presence of an outbreak in the NICU. This prompted an epidemiologic investigation.

**Epidemiologic investigation.** Data from medical records were gathered retrospectively. Case patients were neonates hospitalized in the NICU during the period of January 1998 through May 1999 in whom
and sink surfaces. Analyzed data included gestational age, birth weight, mode of delivery, information on the use of breast milk or other sources of enteral nutrition, medications used, presence of underlying diseases, receipt of critical care (i.e., parenteral nutrition, oxygen therapy, mechanical ventilation, catheterization), length of stay in the NICU, and location within the NICU.

A prospective study of stool or rectal colonization with S. marcescens was performed in July 1999 to determine the prevalence of S. marcescens colonization and/or infection in the NICU. The recorded data were the same as the data in the retrospective study, with the addition of information about medications used by the mothers. The data for colonized and noncolonized neonates (control patients) were compared. Environmental samples were obtained from incubators, the blood-gas analyzer, medications, milks, soaps, sonography gel, taps, and sink surfaces.

A second prospective survey that focused on the onset of S. marcescens colonization was performed during an 8-week period from December 1999 through January 2000 to determine sources and routes of colonization. Stools and gastric aspirate samples were obtained from neonates admitted to the NICU or to the maternity ward for bacterial culturing at birth and on days 4, 7, and 21 of life. Additional prevalence studies of neonatal stool colonization were performed to assess the impact of infection-control interventions.

Microbiological studies and genotyping. For investigation of colonization, stool samples, hand swabs of health care workers, and specimens of other material were plated on agar selective for Serratia species. (DNase Test Agar with methyl green; Difco Laboratories, Becton Dickinson) that contained cefazolin and vancomycin, 30 mg/L each (Eli Lilly), and colistine, 3 mg/L (Sigma). Colonies with a decolorized zone due to DNase activity were identified as follows.

Bacterial isolates were identified as S. marcescens by use of a biochemical identification system [8] and oxidation of xylose (1% xylose in oxidative-fermentative basal medium; Difco Laboratories). In situations in which a clear identification could not be made, the isolates were also identified by use of the commercial Api20E system (bioMérieux).

Molecular analysis of each isolate was performed by PFGE. Bacteria were embedded in agarose, then lysed in situ by lysozyme and proteinase K, followed by digestion of chromosomal DNA with infrequently cleaving restriction endonucleases (SpeI). PFGE was performed by use of the CHEF-DR III system (Bio-Rad). The initial switch time was 5 s, the final switch time was 60 s, and the run time was 16 h at 6 V/cm. Gels were stained with ethidium bromide and viewed by ultraviolet transillumination, and the pattern of restriction-fragment bands was interpreted. Isolates were considered to be the same genotype by use of the criteria described by Tenover et al. [9]. In addition, the generated profiles were subjected to numerical analysis by use of Gel Compar II software (Applied Maths), which resulted in the construction of phylogenetic trees.

Statistical analysis. Categorical variables were analyzed by use of the Pearson $\chi^2$ statistic or, when cell sizes were small, Fisher’s exact test. Continuous variables were analyzed by use of Student’s $t$ test. A $P$ value of <.05 was considered statistically significant. All tests were 2-sided. Variables found to be statistically significant ($P<.05$) in simple regression analysis were entered into a logistic regression model. Computations were performed by use of Statview software, version 4.5 (Abacus Concepts).

RESULTS

Evolution of the outbreaks. Figure 1 shows a synopsis of the evolution of the outbreaks, timing of interventions, and surveillance activities. The index cases were 4 children who had been transferred from the NICU of the USZ to the University Children’s Hospital. S. marcescens had been isolated from blood cultures (3 cases) and cultures of ascites (2 cases) obtained at some point from April 1998 through May 1999. All isolates showed identical banding patterns by PFGE (strain A; figures 1 and 2). The ensuing retrospective analysis of microbiological records of children hospitalized in the NICU of the USZ during the period of January 1998 through May 1999 revealed 28 clinical specimens that yielded S. marcescens, which had been obtained from 19 patients. All of the patients had been born at USZ. Clinically relevant infections were found in 17 (89%) of these patients (retrospective analysis; figure 1), including 8 patients with conjunctivitis, 3 with septicemia (1 patient died), 1 had a brain abscess, and 1 had thoracic empyema, 2 with urinary tract infections, 2 with otitis externa, 1 with soft-tissue infection after herniotomy, and 1 with enterocolitis and omphalitis. Two patients were found to be colonized in their lower respiratory tract without evidence of infection; therefore, data from these patients are not included in figure 1. No isolate from this retrospective series was available for genotyping, except for 1 blood culture isolate recovered from a neonate with thoracic empyema. The banding pattern of this isolate was identical to the pattern of the isolates from the 4 index cases (strain A).

Prevalence study. To determine whether neonates showed gastrointestinal colonization with S. marcescens, we conducted a prospective prevalence study in July 1999 (prevalence study 1; figure 1). This survey revealed that 11 (55%) of 20 investigated neonates harbored S. marcescens (strain A) in their stool specimens. No significant differences with respect to mode of delivery, birth weight, gestation age, use of medication in chil-
Outbreaks of *S. marcescens* in an NICU

Figure 1. The number of infected or colonized neonates during an outbreak of *Serratia marcescens*, shown in time relation to the different strains and to the different interventions used, in a study of an outbreak of *S. marcescens* in the neonatal intensive care unit of the University Hospital of Zurich.

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Prospective study. These aforementioned 4 additional colonizations prompted an investigation of potential sources and mechanisms of colonization of infants with *S. marcescens* in the NICU. As part of a prospective study conducted in December 1999 and January 2000, stool and gastric aspirate samples were obtained on days 1, 4, 7, and 21 of life. Colonization occurred in 18 (35%) of 51 neonates (prospective study; figure 1); in 2 of these neonates, it occurred within 24 h after birth. In some patients, gastric colonization preceded stool colonization.

In addition to stay in the NICU, birth weight of <2000 g or <1500 g, gestational age of <33 weeks, receipt of parenteral nutrition, and placement of an umbilical artery or peripheral venous catheter were identified as significant risk factors (table 1). However, no parameter was identified as an independent risk factor for colonization in a logistic regression model. All isolates studied in this survey showed a new banding pattern; however, among the isolates, the banding pattern was again identical (strain B; figures 1 and 2).

The emergence of a second clone, as well as the dynamic of colonization, again suggested the presence of a common source of the epidemic and excluded a maternal source. Although the first environmental cultures in July 1999 were negative (i-
Figure 2. Banding patterns of *Serratia marcescens* isolated during 3 different periods, including analysis of the degree of genetic relatedness (expressed as percentages) of individual strains performed by use of GelCompar II software.

including random samples of each different milk formula), the milk kitchen was considered a likely source of the outbreak, because *S. marcescens* had been found in the milk kitchen sink during the very same sampling.

**Milk kitchen intervention.** Observation of health care workers preparing milk formulas suggested that contamination could occur during these procedures as a result of inadequate hand disinfection. Therefore, in June 2000, we cultured milk samples after preparation of formula. *S. marcescens* was found in 6 (31.6%) of 19 samples. All bacterial isolates showed a third banding pattern by PFGE (strain C). Strain C was also found to be colonizing neonates in March 2000 (prevalence study 3; figure 1) and in blood culture isolates in June and July 2000 recovered from 2 very-low-birthweight neonates with fatal sepsisemia (1 case due to *S. marcescens* and 1 case due to a polymicrobial infection with *S. marcescens*, *Klebsiella pneumoniae*, *S. aureus*, and viridans streptococci). The same strain was isolated in stool samples obtained from 8 neonates with diarrhea during the summer of 2000. After these observations, a clean sector (for preparation of milk), which was strictly separated from the dirty sector (for cleaning of bottles), was installed within the milk kitchen. In addition, reprocessing of all bottles by thermal disinfection was outsourced to the Central Sterilization Department. Strict infection-control practices were enforced and teaching sessions on alcoholic hand disinfection were held. To evaluate the effectiveness of this approach, a fourth prevalence study was performed 2 months later. None of the 19 investigated neonates was found to be colonized with *S. marcescens* in October 2000 (prevalence study 4; figure 1), nor were any of the 10 neonates investigated in January 2001 (prevalence study 5; figure 1). The PFGE with a dendrogram of the strains isolated at the 3 different time periods is shown in figure 2. Comparison of the antibiotic susceptibility patterns of isolates from the 3 time periods failed to distinguish among clones.

**Impact on University Children’s Hospital.** To determine the impact of our infection-control measures in the NICU of the USZ on the prevalence of *S. marcescens* in patients transferred to the intensive care unit (ICU) of the University Children’s Hospital, 2 prevalence studies were conducted. In July 1999, *S. marcescens* (strain A) was found in rectal swabs from

Table 1. Risk factors for colonization with *Serratia marcescens* in the gastrointestinal tract of children during a prospective survey of an outbreak in the neonatal intensive care unit of the University Hospital of Zurich, December 1999–January 2000.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) of patients</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colonized $(n = 18)$</td>
<td>Not colonized $(n = 33)$</td>
</tr>
<tr>
<td>Intensive care unit stay</td>
<td>17 (94.4)</td>
<td>13 (39.4)</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2000</td>
<td>14 (77.7)</td>
<td>8 (24.2)</td>
</tr>
<tr>
<td>&lt;1500</td>
<td>9 (50)</td>
<td>5 (15.2)</td>
</tr>
<tr>
<td>Gestational age &lt;33 weeks</td>
<td>14 (77.8)</td>
<td>6 (18.2)</td>
</tr>
<tr>
<td>Receipt of parenteral nutrition</td>
<td>7 (38.9)</td>
<td>4 (12.1)</td>
</tr>
<tr>
<td>Catheter placed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Umbilical artery catheter</td>
<td>11 (61.1)</td>
<td>5 (15.2)</td>
</tr>
<tr>
<td>Peripheral venous catheter</td>
<td>11 (61.1)</td>
<td>8 (24.2)</td>
</tr>
</tbody>
</table>

* Determined by use of the χ² test.
3 (60%) of 5 children admitted from the USZ but in 0 swabs from 26 children from other neonatology units \( (P = .002) \). In May 2000, 2 (25%) of 8 children referred from the USZ were found to be colonized with \( S. \) marcescens (strain C), compared with 0 of 17 children referred from other neonatology units \( (P = .09) \), by Fisher’s exact test.

**DISCUSSION**

Our study expands the knowledge of outbreaks of \( S. \) marcescens in NICUs by documenting that genetically unrelated clones may be sequentially introduced. Although we were only able to unequivocally identify contaminated milk as the source during the third outbreak (strain A), the fact that the first strain (strain A) was found in the sink of the milk kitchen during the first environmental investigation serves as circumstantial evidence for our hypothesis that milk contamination played an important role in the propagation of pathogens in the first 2 episodes as well. This hypothesis is supported by the observation that colonization of neonates and associated outbreaks stopped only after the reorganization of procedures in the milk kitchen (figure 1).

Milk is a known source of nosocomial infections in neonates. Abrahammsen et al. [10] reported an outbreak of *Flavobacterium meningosepticum* in an NICU caused by contaminated rubber stoppers of milk bottles. In another NICU, 5 patients developed primary bacteremia caused by *K. pneumoniae* after feeding with contaminated breast milk from a single donor; the breast-pump tubing and safety trap were grossly contaminated [11]. Human milk contaminated with *Salmonella kottbus* caused an outbreak that involved 7 of 22 infants in a third NICU. There were no other risk factors in this outbreak, except for the consumption of milk from a single donor, whose milk was subsequently found to be contaminated with *S. kottbus* [12]. Three cases of neonatal infection caused by *Enterobacter sakazakii* were reported from Iceland. The organism was not isolated from any environmental sources in the neonatal wards or in the milk kitchen, whereas cultures of several lots of the powdered-milk formula used in the hospital had positive results [13]. Finally, in a maternity hospital in Birmingham, England, the same types of *Enterobacter aerogenes* (formerly *Klebsiella aerogenes*) and *Pseudomonas aeruginosa* were found in milk feeds and in feces samples obtained from babies who had received the milk. The bacteria were also found in the tap of a mixing container, which was considered to be the major source of contamination of feeds [14].

Milk is also known to be a source of nosocomial infections caused by *S. marcescens*. Gransden et al. [15] reported an outbreak of *S. marcescens* infection associated with contaminated breast pumps in a special-care baby unit. The outbreak was brought under control when the method of disinfection of pumps was changed from soaking in hypochlorite solution to washing at 80°C. Berthelot et al. [1] linked an *S. marcescens* outbreak not only to contaminated transducers of 2 internal tocographs but also to a bottle of enteral feed additive and the breast milk of 2 mothers.

On the basis of the results of our investigation, the most likely cause of bacterial contamination of the milk was the lack of separation between a clean and a dirty sector within the milk kitchen. The cleaning of used bottles and the preparation of milk were performed in the same place. The following hypothesis regarding the cause for propagation of the outbreak may be entertained: (1) a bottle was contaminated by the hands of a carrier of *S. marcescens*, followed by secondary transmission of the bacteria to other bottles during preparation of fresh milk; and/or (2) the bacteria were introduced into the milk kitchen via the colonized hands of health care workers. Indeed, we detected *S. marcescens* on the hands of a person charged with cleaning bottles during our investigation. Contamination of the water supply of the hospital is unlikely, because we did not observe an increase in the rate of *S. marcescens* infection outside of the NICU.

Milk seems to be a good culture medium for *S. marcescens*. This species is often found in milk samples obtained from lactating dairy cows [16] and was associated with a high prevalence of mastitis in a dairy herd [17].

Our study demonstrates the need for comprehensive infection-control measures, including the separation of procedures used to process used bottles and prepare fresh milk, to stop an ongoing outbreak. Other investigators have already shown that *S. marcescens* may spread rapidly [18] and that the introduction of standard hygienic measures may be insufficient to halt an epidemic. In such situations, cohort nursing and even temporary closure of the affected department may become necessary [18–20].

Reported morbidity and mortality rates associated with *S. marcescens* outbreaks vary. During an outbreak in a French maternity hospital, only 1 newborn developed bacteraemia, and colonization was revealed in the stool samples obtained from 36 newborns [1]. In a report by Halle et al. [21], 27 infants in an NICU were involved in an outbreak; 14 of these infants developed septicemia and/or meningitis, 11 of whom died. In our study, we observed 3 cases of septicemia with fatal outcomes among 34 infected and 41 colonized neonates.

Although outbreaks are not a rare event in ICUs, consecutive outbreaks caused by genetically unrelated bacterial clones are hardly ever reported. Alferri et al. [22] investigated 2 consecutive outbreaks of *Stenotrophomonas maltophilia* infection in the medical-surgical ICU of an 800-bed tertiary care center. A prolonged outbreak of *P. aeruginosa* in an NICU was caused by 2 different genotypes. These were associated with several nurses who had long fingernails [23]. A nosocomial outbreak in a maternity hos-
hospital reported by Berthelot et al. [1] was caused by 2 different *S. marcescens* clones, but they did not appear sequentially. We documented 3 consecutive outbreaks with different clones that were sequentially introduced in the NICU.

It took >1 year to terminate the consecutive outbreaks. First, we attempted to stop the epidemic by replacement of multiple-use theophylline bottles, because some of them were identified as contaminated, and by reinforcement of strict infection-control practices. Although the milk kitchen was already considered a likely source of the outbreak after detection of *S. marcescens* in its sink during the early phase of the investigation, we were misled by the negative results of the first investigation of the different milks in use. Therefore, reorganization of suboptimal procedures in the milk kitchen was delayed.

The reported durations of outbreaks vary. Campbell et al. [24] described concurrent outbreaks of *S. marcescens* and methicillin-resistant *S. aureus* in an NICU. Infected or colonized infants were placed in isolation; all other infants were cohorn. *S. marcescens* infection was eliminated from the NICU 3 weeks after interventions were initiated. An outbreak due to *S. marcescens* in a private maternity hospital lasted for ~6 months, despite the temporary closing of the neonatal unit [1]. In a prolonged outbreak of *P. aeruginosa* in an NICU, it was postulated that long or artificial fingernails had a role in disease transmission [23]. When a policy to restrict the use of long or artificial nails was implemented, fewer bloodstream infections were noted initially, but the change in fingernail policy did not prevent additional cases from occurring. It is not rare that extensive investigations and cultures fail to identify any reservoir and source of *S. marcescens* outbreaks [19, 25]. In most incidents, the presumed source of infection has been an infected neonate, but the mode of acquisition of infection has often remained unknown [20].

In conclusion, *S. marcescens* can cause rapidly spreading outbreaks of severe and potentially fatal infections in neonatal units. Recognition of such outbreaks may be hampered if the patients are transferred to other facilities. Extended and labo-
rious epidemiologic investigations, as well as implementation of multiple appropriate infection-control measures, may be needed to contain such outbreaks. Genotyping proved to be a very useful tool for identification, tracing, and detailed analysis of consecutive outbreaks with distinct clones of the same bacterial species.

**Acknowledgments**

We thank Claudia Vanoli and the technicians of the Department of Medical Microbiology, for work in the laboratory, including microbial studies and genotyping, and Christina Maguire and Pia Beck, for logistics and laboratory work, respectively, in the prevalence studies. Moreover, we are indebted to the nursing and medical staffs of both institutions for their collaboration.

**References**


