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ORIGINAL ARTICLE

Investigation of an outbreak of *Serratia marcescens* in a neonatal intensive care unit

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KEYWORDS

Neonatal intensive care unit;
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Background: *Serratia marcescens* is a well-known but relatively uncommon cause of nosocomial infections, particularly in neonatal intensive care unit (NICU) patients. We investigated an outbreak of *S marcescens* in the NICU at the Farabi Hospital of Karadeniz Technical University in Trabzon, Turkey.

Methods: Between March 21 and May 27, 2006, nine of the neonates were identified with cultures of *S marcescens*, and there were three deaths because of septicemia. For the purpose of identifying the source of infection, 85 environmental samples, two breast milk samples from two babies' mothers, and 38 hand-washing samples from the health care workers (HCWs) were collected for the detection of *S marcescens*. All the *S marcescens* isolates were genotyped by pulsed-field gel electrophoresis with endonuclease *SpeI* restriction enzyme.

Results: *Serratia marcescens* was identified from one hand-washing sample and two breast milk samples. None of the environmental samples yielded *S marcescens*. Of the 13 isolates analyzed, four belonged to one major genotype, whereas eight (6 from neonates and 2 from breast milk) were indistinguishable; two isolates (2 from neonates) were closely related; and three isolates (2 other neonates and 1 from HCW's hand) were different. Our intensive efforts failed to determine the source of the outbreak despite the finding that *S marcescens* strain was isolated from a HCW's hand.

Conclusion: Present investigation suggested that an outbreak of *S marcescens* infection was caused by a major clone in our NICU, possibly transmitted through the hands of HCWs,

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emphasizing that strict hand washing before and after contact with patients must be the rule of the thumb.

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Introduction

Serratia is a genus of gram-negative rod belonging to the Enterobacteriaceae family. *Serratia marcescens* is the species of *Serratia* most commonly isolated from human infections.¹ *Serratia marcescens*, although initially thought to be nonpathogenic and relatively uncommon in the health care system, appears to emerge as a cause of serious nosocomial infections, especially in neonatal intensive care units (NICUs).^{2,3} Urinary tract infections are not uncommon, followed by pneumonias and skin and soft tissue infections.¹

Management of *S marcescens*-associated infection is problematic because many strains of the bacterium manifest resistance to multiple antibiotics, including β -lactams, aminoglycosides, and quinolones.^{1,4} Therefore, it is important to identify the sources of the outbreak for better management of the spread of the organism. In our NICU, an increase in the number of cases of *S marcescens* was observed over the past few months, and thus, an epidemiologic investigation was launched on the clinical features of a *S marcescens* outbreak in the NICU with a view to prevent further cases.

Methods

Background

The study was carried out in a 720-bed, tertiary care, teaching hospital of Farabi in Trabzon, Turkey. The NICU has 20 beds admitting critically ill neonates. The unit admits between 500 and 600 patients annually. Three sinks with sensor-operated automatic faucets are available in the NICU, and gloves are used routinely. Hand disinfectants containing alcohol are kept near each bed. Active surveillance for nosocomial infections are performed by the infection control nurses and infectious-disease specialists, using the criteria proposed by the Centers for Disease Control and Prevention and National Nosocomial Infections Surveillance System methodology.

Between March 21 and May 27, 2006, an increase in the number of cases of *S marcescens* infection was observed in the NICU; nine neonates became infected and one was identified to be colonized with *S marcescens*, three of whom died with septicemia (Table 1). Seven of these neonates were born prematurely.

The study was initiated when a female infant was found to have *S marcescens* sepsis and died on March 23, 2006. She (gestational week 40) was delivered vaginally in another hospital in Trabzon. The 5-day-old neonate was transferred to our NICU because of neonatal convulsion and generalized hypotonia for further management. On the 7th day of admission, *S marcescens* was grown in a blood culture. Within a week, microbiological samples yielded *S marcescens* from

two more neonates in the NICU. The neonates infected with *S marcescens* were treated with antibiotic regimens, including meropenem (60 mg/kg per day divided into 3 doses) and amikacin (15 mg/kg per day divided into 2 doses).

Bacterial isolates

Serratia marcescens was isolated from clinical specimens using routine bacteriological procedures. All clinical samples except blood were inoculated onto trypticase soy agar with 5% sheep's blood (Becton Dickinson Bioscience, Sparks, MD, USA) and eosin methylene blue agar (Hi - Media Laboratories, Mumbai, India) plates, whereas the blood samples were inoculated first into an aerobic BACTEC 9240 Peds Plus bottle (Becton Dickinson Bioscience). Specimens for surveillance cultures from hospitalized neonates were obtained as described.⁵ Eighty-five environmental and infant formula samples (using swabs and direct culturing of fluids) were taken from incubators, humidifiers, antiseptics, soaps, and sinks, in an attempt to identify any possible source of infection. Thirty-eight hand cultures were taken from health care workers (HCWs) by a standard broth bag technique.⁶ All the samples were incubated overnight in brain heart infusion broth at 37°C and subcultured on eosin methylene blue agar. Additionally, two breast milk samples were also taken from two mothers.

The isolates from clinical or screening specimens were identified by standard laboratory methods, including the Phoenix automatic system for the identification and susceptibility testing (Becton Dickinson Bioscience). Antimicrobial susceptibility tests were performed using the NMIC/ID-55 panels of the Phoenix automatic system, according to the manufacturer's instructions, and the results were interpreted according to the breakpoints established by the Clinical and Laboratory Standards Institute.⁷ The antimicrobial agents included were amoxicillin/clavulanate, ampicillin, ampicillin/sulbactam, aztreonam, cefazolin, cefepime, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, amikacin, gentamicin, imipenem, levofloxacin, meropenem, piperacillin, piperacillin/tazobactam, tetracycline, and trimethoprim/sulphamethoxazole. Extended spectrum beta-lactamase (ESBL) production was initially screened by Phoenix automatic system. ESBL production was also investigated by a double-disk test. The isolates were stored at -70°C for further studies.

Pulsed-field gel electrophoresis typing

Molecular typing with pulse-field gel electrophoresis (PFGE) was used to compare 13 *S marcescens* isolates, including the samples from 10 patients, one hand wash sample, and two breast milk samples. Of the 10 isolates, four were derived from urine, three from blood, three from tracheal aspirates, and one from an abscess. For PFGE analysis, bacterial DNA was prepared as previously described with

some modifications.^{8,9} Chromosomal DNA was digested using 30 U per sample *SpeI* (Promega Corp., Madison, WI, USA). Lambda phage concatemers (Bio-Rad Laboratories, Richmond, CA, USA) were run simultaneously as a size marker. The electrophoresis conditions using the CHEF-DR III system (Bio-Rad) had an initial switch time of 5 seconds and final switch time of 20 seconds (gradient of 6 V/cm and an included angle of 120°) for a 20-hour run. The gels were stained for 20 minutes with ethidium bromide and destained in distilled water for 45 minutes. The results were analyzed with a Bio-Rad Gel Doc System (Bio-Rad) and Molecular Analyst Software (Bio-Rad). The genotypes were defined on the basis of DNA banding patterns as described elsewhere.¹⁰ Isolates with identical patterns were considered genetically "indistinguishable", whereas those that differed by one to three bands were defined as "closely related", four to six bands as "possibly related", and greater than or equal to seven bands as "unrelated".

Results

The epidemiological data, antimicrobial profiles, and the PFGE patterns, corresponding to all isolates, are summarized in Tables 1 and 2. Environmental samples and hand swabs from HCWs and breast milk samples from mothers were obtained between April and June 2006 for *S marcescens* isolation. Although no growth was obtained from 27 of the environmental samples, there was one growth from the hand samples of 14 HCWs and one from the milk samples taken from the mothers of Patients 2 and 5 on April 4, 2006. Additional hand cultures grew methicillin-resistant *Staphylococcus aureus*, coagulase-negative staphylococci, *Bacillus* spp., *Escherichia coli*, *Enterobacter aerogenes*, and *Acinetobacter lwoffii*. There was *S marcescens* growth in the milk sample of Patient 5's mother and in the urine sample taken on the same day. No *S marcescens* growth was obtained from any of the 17 environmental samples taken on May 30, 2006, and 12 of the hand samples, including those hand samples previously showing growth. Furthermore, there was no growth of *S marcescens* in 41 of the environmental samples and 14 of the HCW samples taken on June 3. In total, there was one case of *S marcescens* growth from 38 hand samples taken from HCWs and two of the breast milk samples taken from the mothers. Milk storage is not a routine procedure in

the NICU; instead, mothers are called to provide breast milk for their babies who are fed by nurses. For this reason, breast milk was not available from other mothers. None of the mothers had evidence of mastitis.

PFGE with *SpeI* yielded 16 or more visible bands for each isolate. They were sufficiently distinct to allow the division of the strains into three patterns (A, B, and C) with two subtypes (A1 and A2) (Fig. 1). Genomic typing identified one major pattern, PFGE-designated Pattern A (Patients 1, 2, 4, 5, 8, and 10) shared by 6 of the 10 neonates, suggesting some cross-transmission of this bacterial strain between patients in the NICU. The PFGE patterns of isolates from Patients 6 and 7 showed a one-fragment difference. Hence, they were considered closely related (Pattern A1). The isolates from the other two neonates were genetically distinct. Two of the isolates from breast milk were indistinguishable with PFGE Pattern A. The isolate obtained from one hand-washing sample was unrelated to the outbreak strain, which differed by seven bands (Pattern D). With the exception of Clone D, all isolates belonging to Clones A, A1, B, and C, were susceptible to trimethoprim/sulfamethoxazole, amikacin, imipenem, meropenem, and ciprofloxacin, whereas susceptibility to aztreonam, cefepime, and piperacillin/tazobactam, varied among strains. The Clone D strain was susceptible to cefotaxime, ceftazidime, and gentamicin. No correlation was found between PFGE and antibiotic patterns.

The outbreak was stopped by reinforcing standard infection control procedures, including hand washing, cohorting infected/colonized neonates, and disinfecting environmental surfaces. No other infections by *S marcescens* were diagnosed, and the colonization rate returned to the pre-epidemic value in another 12 months of follow-up in the NICU.

Discussion

Serratia marcescens is a well-known causative agent of hospital infections and can be the source of epidemic outbreak in NICU.⁹ Recently, there have been several reports of *S marcescens* outbreaks in NICUs^{3,9,11–15} where the newborns known to be immature in the sense of proper immune response are taken care of for various health problems.¹¹

Table 1 Characteristics of the neonates infected with *Serratia marcescens* in neonatal intensive care unit

Neonate no.	Gestational age (wk)	Weight at birth (g)	Date of admission (mm/dd/yr)	Date of first isolation (mm/dd/yr)	Source of isolate	Type of infection
1	40	3,000	3/15/2006	3/21/2006	Blood	Septicemia
2	33	2,300	3/24/2006	3/26/2006	Urine	Urinary tract infection
3	28	2,000	3/27/2006	3/27/2006	Blood	Septicemia
4	40	3,250	3/19/2006	3/30/2006	Right wrist abscess	Soft tissue infection
5	36	2,020	3/28/2006	4/1/2006	Urine	Urinary tract infection
6	34	3,100	4/8/2006	4/10/2006	Urine	Urinary tract infection
7	34	2,050	4/19/2006	4/21/2006	Tracheal aspirates	Pneumonia
8	38	3,860	5/1/2006	5/1/2006	Urine	Colonization
9	27	1,000	5/21/2006	5/22/2006	Tracheal aspirates	Pneumonia
10	22	630	5/20/2006	5/27/2006	Blood	Septicemia

Table 2 Phenotypic and genotypic characterization of *Serratia marcescens* isolates recovered from the neonatal intensive care unit

Isolate no.	Source of isolate	Antibiogram ^a	PFGE genotype
1	Blood (Neonate 1)	Am, Azt, Cft, Caz, Pi, PTc, Gm, C, Cpe, Cfz	A
2	Urine (Neonate 2)	Am, Azt, Cft, Caz, Pi, PTc, Gm, C, Cpe, Cfz	A
3	Blood (Neonate 3)	Am, Azt, Cft, Caz, Pi, Gm, C, Cpe, Cfz	B
4	Right wrist abscess (Neonate 4)	Am, Azt, Cft, Caz, Pi, Gm, C, Cpe, Cfz	A
5	Urine (Neonate 5)	Am, Azt, Cft, Caz, Pi, PTc, Gm, C, Cpe, Cfz	A
6	Urine (Neonate 6)	Am, Azt, Cft, Caz, Pi, PTc, Gm, C, Cfz	A1
7	Tracheal aspirates (Neonate 7)	Am, Azt, Cft, Caz, Pi, PTc, Gm, C, Cpe, Cfz	A1
8	Urine (Neonate 8)	Am, Azt, Cft, Caz, Pi, PTc, Gm, C, Cpe, Cfz	A
9	Tracheal aspirates (Neonate 9)	Am, Azt, Cft, Caz, Pi, PTc, Gm, C, Cpe, Cfz	C
10	Blood (Neonate 10)	Am, Cft, Caz, Pi, PTc, Gm, C, Cpe, Cfz	A
11	Hand of an HCW	Am, Pi, PTc, Cfx, Cfz	D
12	Breast milk for Neonate 2	Am, Azt, Cft, Caz, Pi, PTc, Gm, C, Cpe, Cfz	A
13	Breast milk for Neonate 5	Am, Azt, Cft, Caz, Pi, PTc, Gm, C, Cpe, Cfz	A

^a Antibiograms are expressed as the antimicrobial resistance profiles.

Am = ampicillin; Azt = aztreonam; C = chloramphenicol; Caz = ceftazidime; Cft = cefotaxime; Cfz = cefazolin; Cpe = cefepime; Gm = gentamicin; HCW = health care worker; Pi = piperacillin; PTc = piperacillin/tazobactam; PFGE = pulsed-field gel electrophoresis.

The environmental surfaces, invasive medical devices, intravenous and topical solutions, or soap may seem to be the pathogen's source,^{12,16,17} whereas the hands of HCWs can serve as an important source of infection.¹ Colonization rates of *S marcescens* on the hands of HCWs have been described to range from 15.4% to 24%.¹⁷ However, it has been reported that infected or colonized neonates are the primary source in most *S marcescens* outbreaks.¹⁴ In our study, environmental cultures from NICU were all negative for *S marcescens*. Our intensive efforts failed to determine the source of the outbreak despite the finding that *S marcescens* strain was isolated from an HCW's hand. The bacterial isolate obtained from the staff member was determined by

PFGE to be unrelated to the outbreak strain, which differed by seven bands. Despite our negative results, we believe that cross-transmission through transient contamination of hands played a major role in this outbreak. Because breast milk has also been suggested to be a source of infection,¹⁸ we also tested the breast milk samples of the mothers of Patients 2 and 5 and detected *S marcescens*. The contaminated breast milk in our case could be implicated as a source of infection in two neonates, but we were unable to conclude definitely whether it is the case because no milk samples could be taken from their mothers before the infection.

The PFGE patterns of isolates from Patients 3 and 9 were genetically distinct from that of the outbreak-causing strain. These neonates were transferred from another hospital to our NICU, and infections occurred before or within 48 hours of admission to our NICU, suggesting that they had acquired the infection from the hospital where they were first admitted.

Outbreaks of *S marcescens* have been reported to spread very rapidly with significant morbidity and mortality.^{9,12,14} Therefore, the rapid typing methods are essential in terms of prompt and sensitive evaluation of *S marcescens* outbreaks in hospitals and, thus, implementing effective preventive measures when the sources are identified.¹⁵ A number of epidemiological typing methods, including antibiotyping, serotyping, phage typing, plasmid typing, polymerase chain reaction-based typing, and analysis of genomic DNA by PFGE have been described. Antibiograms present little value in most epidemiological studies because isolates with unrelated genetic and epidemiological features have been shown to present the same susceptibility pattern because of the acquisition of the same plasmid by multiple species, named as plasmid outbreak.¹⁹ In the present study, the antibiograms revealed quite variable results as compared with those of PFGE typing, and unrelated isolates had similar antibiotypes. PFGE is the method of choice for outbreaks of *S marcescens*,^{3,12} and particularly, the method used in this study is considerably rapid, taking about 28 hours to complete, to allow prompt acting in the case of an outbreak and to implement effective preventive measures.

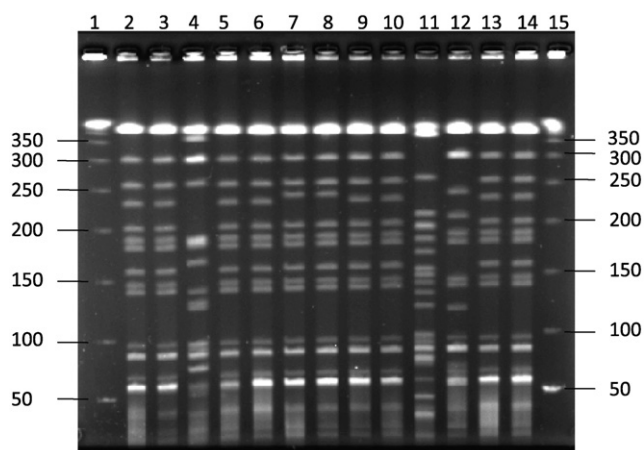


Figure 1. Pulse-field gel electrophoresis (PFGE) of *SpeI*-digested genomic DNA extracted from *Serratia marcescens* isolates. Lanes 1 and 15 show molecular weight standards of lambda in kilobase pairs. Lanes 2, 3, 5, 6, 9, 10, 13, and 14 show identical PFGE patterns responsible for the outbreak (Pattern A) in Patients 1, 2, 4, 5, 8, and 10, and the breast milk sample. Lanes 7 and 8 show the PFGE Pattern A1 from Patients 6 and 7, respectively. Lanes 4, 11, and 12 show distinct PFGE patterns (B, C, and D) from Patients 3 and 9, and the hand culture of a health care worker, respectively.

Although the source of the outbreak could not be definitely identified in the present study, it was concluded that the successful growth of *S marcescens* from the hand samples of one HCW may indicate that transient contamination from working staff may be more important than environmental contamination. One of the interesting findings in our study was to be able to grow *S marcescens* from a breast milk sample, suggesting that contamination from mother's milk could be possible with a caveat that no correlation can be established regarding contamination among patients. The PFGE protocol used in this study is a simple, highly discriminatory, and cost-effective method for the thorough epidemiological investigation of an outbreak of *S marcescens*.

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