Nosocomial outbreak of colonization and infection with *Stenotrophomonas maltophilia* in preterm infants associated with contaminated tap water

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SUMMARY

Between March and May 1996 *Stenotrophomonas maltophilia* was cultured from endotracheal aspirate samples from five preterm infants in a neonatal intensive care unit (NICU). Four infants were superficially colonized, but a fifth died due to *S. maltophilia* septicaemia. *S. maltophilia* was cultured from tap water from three outlets in the NICU including one with a previously unnoticed defective sink drain. Water from these outlets was used to wash the preterm infants. Environmental and clinical *S. maltophilia* isolates yielded identical banding patterns on random arbitrary polymorphic DNA (RAPD) PCR analysis. The outbreak was controlled by reinforcement of hand disinfection, limitation of the use of tap water for hand washing and by using sterile water to wash the preterm infants. We conclude that tap water should not be used for washing preterm infants in the NICU, unless steps are taken to prevent microbial growth in the outlets.

INTRODUCTION

Stenotrophomonas maltophilia is a non-fermentative Gram-negative bacillus that is emerging as an important nosocomial pathogen. S. maltophilia can be isolated from environmental sources including water, soil, sewage, raw milk and human faeces [1]. It has also been cultured from various locations in the hospital environment such as sinks [2], respirators [2] and chlorhexidine-cetrimide disinfectants [3]. However, although S. maltophilia is being isolated with increasing frequency in the hospital setting, the reservoirs and modes of transmission remain largely unknown. Clinically significant infection with S. maltophilia is uncommon among healthy individuals and there is evidence that this bacterium has limited pathogenicity for humans in the absence of underlying deficiencies in immune function [4]. S. maltophilia infection has been documental among debilitated patients, including those with endocarditis [5], bloodstream infections [6–8] and malignancy [7–9]. Several risk factors for *S. maltophilia* infection have been identified including malignancy, neutropenia [8], prior treatment with broad-spectrum antimicrobial therapy [2, 8–10], indwelling vascular catheters [9], stay in the intensive care unit [2, 4, 10] and mechanical ventilation [2, 10].

Though adult patients over 40 years of age are at highest risk of disease [4], preterm infants, especially those admitted to neonatal intensive care units (NICU) may also have a high risk of colonization or infection. Perhaps surprisingly, although preterm infants have several risk factors for acquisition of *S. maltophilia*, including immaturity of the immune system, respiratory support and treatment with broadspectrum antibacterial agents, infections by this microorganism have rarely been reported in this

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patient population. We describe an outbreak of *S. maltophilia* colonization and infection among five preterm infants cared for in the NICU of the University Hospital Nijmegen. We also describe the results of an epidemiological investigation which included extensive environmental sampling and genotyping by RAPD-PCR of clinical and environmental isolates.

MATERIALS AND METHODS

The NICU studied had 16 incubators equally divided between two Units (I and II). Standard infection control measures were implemented in the care of all patients. Routine surveillance cultures of endotracheal aspirates were collected twice weekly. In addition, if an infant developed symptoms of infection, appropriate clinical specimens were obtained for analysis. Standard methods and media were used for isolation and identification of Gram-negative bacilli including *S. maltophilia*, and antibiotic susceptibility testing was performed by broth microdilution.

Epidemiological investigations included observation of health care workers, review of microbiological records and hospital charts of patients, and sampling of hospital environmental sources. Cultures were obtained from respirator tubes, incubators, soaps, stethoscopes, disinfectants, tap water and a sink drain. The microbiological quality of the tap water was assessed by culturing water obtained directly from the outlet and by culturing water drawn from the outlet after the aerator screen was dismantled and the faucet heat-sterilized by open flame. From each outlet 50 ml samples of water were drawn and centrifuged at 2500 rpm for 10 min in conical plastic tubes. Supernatants were discarded and the residue was plated on blood agar plates and Levine plates, a selective medium for Gram-negative bacteria, and incubated at 35 °C for 24 h.

RAPD-PCR as described previously was used for genotypic characterization of the bacterial isolates cultured from hospital environmental and clinical sources [11]. For each bacterial species a short oligonucleotide primer of arbitrary chosen sequence was selected based on published data of the discriminatory power of the primer with DNA of that specific micro-organism. Oligonucleotide primers ERIC1 and ERIC2 [12] were used for genotyping of *S. maltophilia* [13], *Acinetobacter* species [14], and *Klebsiella pneumoniae* [15]; primer A05 (5'-AGCAG-CGCCTCA-3') for *Aeromonas hydrophila* [16]; and primer D8635 (5'-GAGCGGCCAAAGGGAGCA-GAC-3') for *Pseudomonas aeruginosa* [17]. Unrelated control isolates of each species were included in each PCR analysis. After PCR, amplified DNA fragments were separated by aragose gel electrophoresis. Gels were photographed and banding patterns were interpreted by visual inspection. The genotypes were characterized as identical (identical banding pattern), highly related (one mismatch in banding pattern) or unrelated.

Statistical process control (SPS) charts were applied to survey the number of new patients colonized or infected with *S. maltophilia* in the NICU. The upper control limits (UCL) and upper action limits (UAL) were set at 2 and 3 standard deviations respectively, oriented at the mean values of the number of new patients per month, during the 12 months before the outbreak period [18].

RESULTS

Description of the outbreak

The prevalence rate of respiratory tract colonization with S. maltophilia was 0.2/1000 patient days in the 12 months prior to the outbreak compared with 2.9/1000 patients days during the outbreak period (16 March-28 May). According to the U-charts statistics the UCL of 2.59 and UAL of 3.42 were exceeded in May 1996, indicating a statistically significant increase in cases. The first case (case 1) was a preterm male infant born at a gestational age of 31 weeks by vaginal delivery after prolonged rupture of membranes. He needed assisted ventilation and was treated with intravenous amoxycillin-clavulanic acid and gentamicin for 5 days. On day 8 he appeared septicaemic and treatment with vancomycin and ceftazidime was started. He responded clinically although blood cultures remained sterile. On 16 March (day 25) S. maltophilia was cultured from a routine endotracheal aspirate, but cultures from four successive aspirates remained sterile.

A second case was identified in a preterm male infant cared for in the incubator adjacent to that of case 1. He was born on 25 March 1996 at 33 weeks of gestation and received assisted ventilation for respiratory insufficiency. *S. maltophilia* was cultured from an endotracheal aspirate on 1 April. No signs or symptoms consistent with infection were apparent although four consecutive aspirates remained culture positive until he was relocated to another hospital on 12 April.

Table 1. Cultured microorganisms, sites of positive culture, and results of comparisons between environmental isolates and those cultured from clinical specimens from patients involved in the outbreak (cases 1–5) and those admitted to unit I of the NICU between 1 January and 1 June 1996 (cases 6–9).

Case no.	Culture result	Culture site	RAPD pattern identical to environmental strain
1	S. maltophilia	Endotracheal aspirate	N.A.*
	A. hydrophila	Endotracheal aspirate	N.A.
2	S. maltophilia	Endotracheal aspirate Endotracheal aspirate	Yes
3	S. maltophilia	Endotracheal aspirate, blood, urine	Yes
4	S. maltophilia	Endotracheal aspirate, axilliary swab	Yes
5	S. maltophilia	Endotracheal aspirate	Yes
6	K. pneumoniae	Blood, endotracheal aspirate, insertion site CVC	No
7	K. pneumoniae	Endotracheal aspirate, urine	No
8	A. hydrophila	Endotracheal aspirate	No
9	A. baumanii	Endotracheal aspirate	Yes

* N.A., isolate not available for RAPD genotyping.

The third case was a male preterm infant of 25 weeks gestation, weighing 800 g, who was born on 2 April and cared for in the same incubator as case 1. He developed respiratory distress syndrome (grade III) and was ventilated. Treatment with intravenous amoxycillin and gentamicin was initiated at birth and erythromycin was added after *Ureaplasma urealyticum* was cultured from the sputum. On 12 April (day 10 after birth) he became septicaemic, deteriorated rapidly and died within 24 h. *S. maltophilia* grew from antemortem cultures from endotracheal aspirate, urine and blood, but these culture results became available only after the infant had died.

Cross-infection transmitted by hands of health care workers was suspected, and consistent hand disinfection before and after patient contact was reinforced. However, 2 weeks later another two newborn infants became colonized with *S. maltophilia*. Case 4, a male preterm infant, was born on 22 March by caesarean section at 29 weeks of gestation with multiple congenital abnormalities, including cerebral atrophy. He was cared for in an incubator opposite that of case 2. He required respiratory support and received multiple courses of antibacterial agents for presumed and documented infection before becoming colonized with *S. maltophilia* on 29 April. Although he also suffered from severe bronchopulmonary dysplasia antibacterial treatment was not started. Endotracheal aspirate cultures remained positive until he died on 21 May, although his death was not thought to be related to *S. maltophilia*.

The fifth case was a male infant weighing 1700 g born at 29 weeks of gestation, by caesarean section on 15 April. He was cared for in the same incubator as cases 1 and 3 and required ventilation for respiratory distress syndrome (grade III). S. maltophilia was cultured from an endotracheal aspirate on 6 May, but antibacterial treatment was withheld. He received trimethoprim-sulphamethoxazole prophylaxis during surgery for paralysis of the diaphragm and his recovery was uneventful. Cultures of his endotracheal aspirates remained positive for S. maltophilia until he was relocated to another hospital on 28 May. None of the implicated neonates received bottle feeding. All S. *maltophilia* isolates were resistant *in vitro* to β -lactam and carbapenem antibiotics and aminoglycosides but susceptible to trimethoprim-sulphamethoxazole and tetracycline and variably susceptible to ciprofloxacin.

Epidemiological investigation

An outbreak was recognized after three patients had become colonized or infected within 2 months. Since reinforcement of hand washing and disinfection had

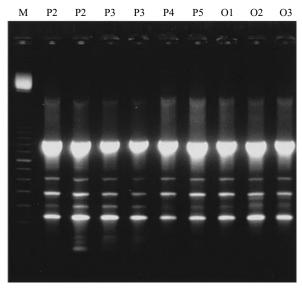


Fig. 1. RAPD-PCR fingerprinting of DNA isolated from *S. maltophilia* from case 2 (lanes P2; endotracheal isolates), case 3 (lanes P3; endotracheal aspirate and blood), case 4 (lane P4; endotracheal isolate) and case 5 (lane P5; endotracheal isolate) and from *S. maltophilia* cultured from water samples from three outlets in the NICU (O1–O3). The lane marked M contains length markers (1-kilobase ladder; Gibco BRL).

failed to control the outbreak, an environmental source of infection was suspected. After the fifth case an extensive programme of environmental sampling was initiated, including incubators, respirator tubes, disinfectants, soaps, stethoscopes and water from three elbow-activated faucets in units I and II. Though axillary swabs and endotracheal aspirates were obtained from all patients in the NICU, no additional cases were identified. All cultures were negative for S. maltophilia except those from the water samples from all three outlets. Further investigation revealed that tap water was used routinely to wash preterm infants and that the drain pipe of one sink had been defective, allowing water to stagnate. Health care workers had continued to use water from this outlet, and all cases involved in the outbreak had been washed with water from the tap above the defective drain. There was no apparent relation between the level of colonization and the distance to the faucet. Water from each outlet was cultured again after the aerator screens were dismantled and the faucet was heat sterilized but S. maltophilia was not recovered. Culture of the defective sink drain grew S. maltophilia, Aeromonas hydrophila (two phenotypes), Klebsiella pneumoniae (two phenotypes), Acinetobacter baumanii, an unspeciated Acinetobacter, and Pseudomonas aeruginosa. Microbiological records of patients admitted to the NICU

between 1 January 1996, and 1 June 1996, were reviewed to establish if these microorganisms had been recovered from clinical specimens. This retrospective search resulted in identification of an additional five cases (cases 1, 6-9; Table 1). RAPD genotyping of the environmental isolates showed that the S. maltophilia and A. baumanii isolates cultured from the three outlets and the sink drain were genotypically identical (Table 1). The banding pattern for S. maltophilia isolates is shown in Figure 1. The outlet with the defective sink was closed immediately until repairs had taken place and the aerator screens had been replaced. The use of tap water for hand washing from the other outlets was restricted. Health care workers were instructed to disinfect their hands solely with an alcoholic hand scrub and preterm infants were washed with sterile water. After implementation of these infection control measures no further cases were observed although sporadic cases with genotypically unrelated S. maltophilia isolates remain present in the NICU.

DISCUSSION

S. maltophilia is increasingly recognized as a nosocomial pathogen which may cause infections in hospitalized, especially debilitated patients. Widespread use of broad-spectrum antibacterial agents and the inherent resistance of the microorganism to aminoglycoside and β -lactam antibiotics may have opened an ecological niche in modern hospitals [1, 4]. However, although S. maltophilia is being isolated with increasing frequency in the hospital setting, the reservoirs and modes of transmission remain largely unknown. As far as we know, this is only the second report of an outbreak associated with contaminated water. In the previously reported outbreak, storage tanks for deionized water were found to be the source of infection [3]. In the present outbreak contaminated aerator screens of tap water outlets in the NICU were the source of contamination. It remains unclear how the microorganism was originally introduced into the aerator screen, although sporadic cases of S. maltophilia were observed in the NICU before the outbreak. S. maltophilia was probably transmitted by the use of contaminated water from the outlet with the defective sink drain to wash preterm infants. The fact that most colonized infants were localized in the area of the NICU closest to the defective sink and the finding of identical genotypes among S. maltophilia and A. baumanii isolates cultured from clinical specimens and

the sink drain, support this finding. Furthermore, bacteria which persist in water, such as *Aeromonas hydrophila*, were recovered from both clinical specimens and the sink drain, although they were not genotypically identical.

An alternative mode of transmission might be the hands of health care workers harbouring the outbreak microorganism after hand-washing with contaminated water. The successful control of the outbreak by prohibiting handwashing, reinforcement of alcoholic hand disinfection, and using sterile water to wash infants also support our hypothesis that contaminated water was the mode of transmission of S. maltophilia. Although all aerator screens were contaminated with S. maltophilia, only infants washed with water obtained from the outlet with the defective sink drain became colonized which suggests that additional factors were required to cause the outbreak. The practice of washing preterm infants cared for in NICUs with tap water with or without the addition of a disinfectant is common in many countries. The hazard of contamination of tap water with nonfermentative bacilli like Pseudomonas species has been recognized, and S. maltophilia may come to play an important role in this respect in hospitals. The routine testing of environmental water, however, is generally unwarranted although it may be useful in hospitals with a history of recognized outbreaks. The addition of disinfectants to tap water does not guarantee sterility as has been demonstrated for chlorhexidinecetrimide disinfectant [3]. Alternatively, aerator screens can be removed or replaced frequently in order to prevent contamination. Aerator screens with wider gratings have been developed, especially for use in the hospital setting, which may reduce the risk for contamination.

Infections caused by *S. maltophilia* in preterm infants have been reported only rarely. Among 99 patients with nosocomial *S. maltophilia* infection at the University of Virginia Hospital, 12 cases were identified in patients under 1 year of age [4]. Ten of these occurred in patients born prematurely, and four patients from whom *S. maltophilia* was isolated from the sputum died. However, none of the four deaths was attributed to *S. maltophilia*. *S. maltophilia* caused infection of the umbilicus in 2 of 21 neonates who had become colonized by use of contaminated disinfectant, but both infected infants responded to local treatment [3]. Our study showed that 4 or 5 infants involved in the outbreak remained colonized by *S. maltophilia* and that in all 5 infants the organism was cultured from respiratory secretions. This is consistent with findings in adults among whom most were found to have a pulmonary source for *S. maltophilia* [1, 2, 4, 10]. Patients with underlying respiratory illnesses were found to be prone to acquiring *S. maltophilia* [10]. Indeed, all infants involved in the outbreak were on assisted ventilation for respiratory insufficiency. Furthermore, all infants had received prior broadspectrum antimicrobial therapy, a known risk factor for *S. maltophilia* colonization and infection [2, 10].

One infant (case 3) developed septicaemia and died within 24 h after deteriorating rapidly. To our knowledge this is the first reported fatal case of *S. maltophilia* infection in a preterm infant. In addition to other known risk factors this infant weighed only 800 g at birth. Very low birth weight infants may be at increased risk for becoming infected with *S. maltophilia*.

This investigation highlighted how *S. maltophilia* infection may be associated with contamination of an environmental source, and the importance of environmental sampling. The use of tap water to wash preterm infants should be avoided unless microbiological quality can be guaranteed. Contamination of health care workers' hands during handwashing was another possible mechanism of transmission. This study shows that preterm infants, especially those with very low birth weight, are at risk for colonization and infection with *S. maltophilia* and expands the spectrum of disease caused by this organism.

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