

***Stenotrophomonas maltophilia* Outbreak in Neonatal  
Intensive Care Unit Patients.**

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Running head: *S. maltophilia* in NICU

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## **ABSTRACT**

**Introduction:** *S. maltophilia* is a nosocomial pathogen requiring strict control measures to prevent spread. The organism is multidrug resistant and can be devastating for compromised hosts such as high-risk neonates. From October 1996 to June 1997 there was an increased incidence of *S. maltophilia* in the Neonatal Intensive Care Unit (NICU) at Wolfson Children's Hospital, which prompted this epidemiologic investigation. Risk factors for *S. maltophilia* in NICU have not been studied. Identification of risk factors in NICU would be critical in establishing effective control measures.

**Methods:** Surveillance cultures from respiratory equipment, temperature probes and water faucets, as well as respiratory cultures from all patients in NICU were obtained at a time when there were two simultaneous *S. maltophilia* cases. *S. maltophilia* isolates were DNA typed by Pulsed Field Gel Electrophoresis to establish whether they belonged to the same strain. Additionally, a case-control study to identify risk factors of *S. maltophilia* in NICU was conducted. Cases of *S. maltophilia* between 10/96 and 6/97 were matched to controls with regards to gestational age and period of hospitalization. Variables compared among cases and controls included type of mechanical ventilation, type and length of antibiotics, indwelling catheters, steroids and neutropenia.

**Results:** There were six isolates of *S. maltophilia* from clinical specimens (blood: 1, peritoneal fluid: 1, tracheal aspirates: 4). PFGE done on five isolates revealed four different strains. The two patients with the same strain were admitted one week apart. Surveillance environmental and respiratory cultures were all negative. Six cases of *S. maltophilia* were matched to 12

controls. Statistical analysis of potential risk factors revealed that patients pretreated with third generation cephalosporin and ventilated using High Frequency Oscillatory Ventilation showed a trend of higher risk colonization/infection ( $p=0.15$  and  $p=0.09$  respectively). The small sample size limited our ability to find statistically significant risk factors.

**Conclusion:** Although patient-to-patient transfer of *S. maltophilia* may have occurred, most of the acquisition occurred independently. Host-centered risk factors for *S. maltophilia* in a larger NICU population deserves further investigation.

**Keywords:** *Stenotrophomonas maltophilia*, NICU, Neonate.

## **Introduction**

*Stenotrophomonas maltophilia* was first described in 1960 and was initially named *Pseudomonas maltophilia*. In 1983 the name was changed to *Xanthomonas maltophilia*. However, due to recognition of distinguishing features, the organism was re-named *Stenotrophomonas maltophilia* in 1993.<sup>1</sup>

*S. maltophilia* is an opportunistic pathogen in individuals with impaired host-defenses, such as those with malignancies and debilitating diseases (e.g. diabetes, chronic liver, renal and lung diseases), those undergoing major surgery and those admitted to intensive care units (ICU). In these settings, the incidence of life-threatening *S. maltophilia* infection appeared to have increased in the past few years.<sup>2-7</sup> *Stenotrophomonas maltophilia* is ubiquitous in nature, is commensal flora in humans and is intrinsically resistant to multiple antibiotics.<sup>8,9</sup> All of these characteristics make *S. maltophilia* an ideal nosocomial pathogen.

*S. maltophilia* was identified from five cultures of three different neonates in the 48-bed neonatal intensive care unit (NICU) at Wolfson Children's Hospital over a two-month period. Within the next seven months three more patients with *S. maltophilia* were identified. This prompted an epidemiological investigation to evaluate risk factors and possible modes of transmission. We also applied molecular typing techniques to determine relatedness of the isolates.

## **METHOD**

We defined the outbreak period as October 1996 to June 1997. The pre-outbreak period was defined as January 1994 to September 1996 and the post-outbreak period was between July 1997 and December 1998. To identify all patients with a positive culture for *S. maltophilia*, we reviewed the microbiology laboratory records for all isolates of from NICU during the three defined periods( between January 1994 and December 1998).

A culture survey of all NICU patients and environment was performed on December 12, 1996 when there were two cases in the NICU. Respiratory aspirates were collected from each patient by the same respiratory therapist. Environmental cultures were done from respiratory equipment (ventilator tubings, oxygen sensors, temperature probes) and water faucets flow restrictor. Cultures were not done on health care workers.

All environmental and patient surveillance and clinical respiratory specimens were inoculated to blood, chocolate and MacConkey agar plates. Blood and peritoneal fluid samples were inoculated onto chocolate, Center for Disease Control and Prevention (CDC) anaerobic blood, MacConkey and Colistin -Nalidixic acid agar plates. *S. maltophilia* Identification was based on VITEK® biochemical cards (bioMerieux Vitek Inc., Hazelwood, MO).

All isolates were tested and compared for *in vitro* antimicrobial susceptibility to at least eight antibiotics, including mezlocillin, imipenem, aztreonam, ceftazidime, gentamicin, tobramycin, amikacin and trimethoprin-sulfamethoxazole (TMP-SMX) by the disk diffusion Bauer-Kirby method. Although the National Committee on Clinical Laboratory Standard

(NCCLS) does not provide approved guidelines for interpretation of disc diffusion zone sizes for *S. maltophilia*, the Bauer-Kirby method was utilized to provide presumptive susceptibility results. The *S. maltophilia* isolates were molecularly typed using Pulsed-field Gel Electrophoresis (PFGE) to determine the degree of relatedness.<sup>10-12</sup>

To identify possible risk factors for *S. maltophilia* infection/colonization in NICU patients we conducted a case-control investigation. Cases were defined as patients admitted to the NICU at Wolfson Children's Hospital during the outbreak period who had a positive culture for *S. maltophilia* from any body site. Controls were defined as patients admitted to the NICU at Wolfson Children's Hospital during the same period of time who did not have a positive culture for *S. maltophilia*. Controls were matched to cases for gestational age. The records of all admissions to the NICU between October 1996 to June 1997 were reviewed to identify the controls.

The hospital records of cases and controls were reviewed by one of the investigators (MSS) and data were collected using a standard case report form. The data were collected on demographic characteristics, length of hospitalization, specific type and duration of antibiotic use, duration of endotracheal intubation, type of mechanical ventilation (conventional versus high-frequency oscillating ventilator [HFOV]), presence of indwelling catheters (central venous lines, arterial line or peripheral intravenous lines), duration of indwelling catheters, use and duration of corticosteroids treatment, lowest white blood cell count (WBC) on the peripheral blood smear, lowest absolute neutrophil count (ANC) and duration of neutropenia (defined as ANC less than or equal to 1000).

Descriptive statistics and frequencies were computed for all variables as appropriate. Chi-square procedures were used to assess differences between cases and controls for categorical variables. We used t-test to assess differences for interval variables. In addition, Z-test was used to determine differences in the incidence of *S. maltophilia* isolation in the pre-outbreak, outbreak and post-outbreak periods.

## **RESULTS**

In the 33 month pre-outbreak period, there were four cases of *S. maltophilia* and in the 18 month post-outbreak period there were two cases of *S. maltophilia* in the NICU. During the nine-month outbreak period six cases were identified. There was no significant change in the rates of admissions, discharges or patient days over the three periods of time. The incidence of *S. maltophilia* cases in the pre-outbreak and post-outbreak periods was 2/1000 admissions, and in the outbreak period 11/1000 admissions. Z-scores for the pre-outbreak and outbreak period, and the post-outbreak and the outbreak periods were 1.96 and 1.88, respectively (both *P* values were less than 0.05 by 2 tailed test).

During the outbreak period, there were eight isolates from the six cases. All cases had *S. maltophilia* isolated from their tracheal aspirates. One patient had three isolates from three different sites: tracheal aspirate, blood and peritoneal fluid. Pulsed field gel electrophoresis was done on only five isolates from five different patients (one isolate did not survive). Two isolates from two different cases (1 and 5) were identical, indicating that they were the same or a similar strain with a potential common origin (Fig 1). Although isolates 1 and 5 had an

identical PFGE pattern, they had slightly different antimicrobial susceptibility patterns. Isolate 1 was susceptible to TMP-SMX and resistant to gentamicin and amikacin, while isolate 5 was susceptible to TMP-SMX and gentamicin, but intermediately susceptible to amikacin. Both isolates were resistant to mezlocillin, tobramycin, aztreonam, ceftazidime and imipenem.

None of the surveillance cultures (respiratory and environmental) grew *S. maltophilia*.

Each case was matched to two controls for a total of twelve controls. No statistically significant differences were found between cases and controls for any of the interval variables (Table 1) or the categorical variables (Table 2) tested. Approaching significance were the use of third generation cephalosporin, the use of vancomycin , and the use of HFOV. For these three variables, statistical power was approximately 40%.

## **DISCUSSION**

During our routine NICU surveillance we felt that there was an increase in the number of *S. maltophilia* cases identified. This prompted us to take immediate steps that included cohorting of cases in a separate NICU room and placing them on contact precautions for multidrug-resistant bacteria as described in the *guidelines for isolation precautions in hospitals*.<sup>13</sup> These precautions include use of gloves, gowns, disposable patient-care equipment when feasible, and careful hand washing. The patient's medical record remained in the room or was disinfected if taken out of the NICU. There was a decrease in the number of cases after these measures were implemented. Our suspicion of an increase was confirmed by our analysis of the periods prior and after the outbreak.

Investigations of *S. maltophilia* hospital outbreaks have often found common sources which have included respiratory equipment, calibration devices, cardiopulmonary bypass pumps, water faucets, aerator and sink drains.<sup>2,3,5,14</sup> However, these findings have not been consistent and, in many instances, the mode of transmission remained unclear.<sup>6,7</sup> In a study of five preterm infants with *S. maltophilia* isolated from endotracheal tubes, tap water was found to be the source of acquisition.<sup>15</sup> We looked for potential sources for *S. maltophilia* acquisition from the environment and from other patients, but we were not able to determine the origin of this outbreak. All surveillance cultures of the environment and respiratory secretions obtained from 35 NICU patients did not grow *S. maltophilia*. We did not, however, obtain cultures from the actual HFOV that were used in *S. maltophilia* cases or from NICU healthcare providers. It is, therefore, possible that the actual HFOVs used for cases or the NICU staff caring for cases were the common source, which we could not identify.

While antimicrobial susceptibility studies provide some guidance to the epidemiologist as an inexpensive screen for possible common identity of organisms in an outbreak, they do not ensure the organism's identity. Organisms may have similar antibiotic susceptibility patterns, yet be epidemiologically unrelated and *vice versa*. Additionally, antibiotic use may result in resistance modification without other recognized biologic changes.<sup>10</sup> On the other hand, molecular techniques such as Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) and PFGE are more reliable methods for epidemiological investigations.<sup>10-12</sup> In studies of *S. maltophilia* outbreaks it has been demonstrated that, although patient-to-patient transmission can occur, many infections are caused by unrelated strains.<sup>4,6,7</sup> We used PFGE and two of the five isolates

had the same pattern. This suggests that at least these two isolates might have had the same source.

It seems logical to investigate host-centered risk factors for acquisition of *S. maltophilia* infection in addition to non-host related factors. Several investigators have looked at the host-centered risk factors in different populations and clinical settings. In association with malignancies, identified risk factors include indwelling catheters, neutropenia, prolonged hospitalization and prior therapy with broad-spectrum antibiotics.<sup>2,6</sup> One study conducted at a shock-trauma ICU concluded that mechanically ventilated patients receiving antibiotics were at increased risk of *S. maltophilia* infection.<sup>3</sup> In another study of patients with pulmonary infections, the presence of chronic respiratory disease and duration of endotracheal intubation were identified as potential risk factors.<sup>7</sup> Wilkinson and Kerr studied neutropenic patients and found that non-carbonated bottle water played a role in gastrointestinal colonization in patients with hematologic malignancies.<sup>16</sup>

Only limited information is available on risk factors for *S. maltophilia* nosocomial infection in the NICU. We were particularly interested in looking at HFOV as a potential risk factor since the first four neonates in our outbreak were all on HFOV. In our NICU, neonates are often placed on HFOV once they have failed conventional ventilation and are still critically ill. We do not offer Extracorporeal Membranous Oxygenation (ECMO) at our institution. Although we were statistically unable to establish HFOV as a risk factor, there was a trend towards increased risk. It is likely that HFOV simply serves as a surrogate marker for neonates who are severely ill, intubated and have received broad-spectrum antibiotics, and as such, more

likely to be infected with *S. maltophilia* and other nosocomial pathogens. However, we have not identified an increase in nosocomial infections in ventilated patients in our NICU.

We conclude that in the outbreak in our NICU, patient to patient transmission of *S. maltophilia* may have occurred, but most of the acquisitions occurred independently. It is important to identify the risk factors for *S. maltophilia* infection in the NICU setting; HFOV may be one such factor. This will help establish infection control practices to prevent nosocomial infections, decrease the number of infections by this difficult to treat organism and raise the clinical suspicion for *S. maltophilia* infection when encountered with these a patient with identified risk factors.

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Table 1. Comparison of Interval Variables between Controls and Cases

Variable	Control Group Mean+/-SD	Infected Group Mean+/-SD	$t$ [df]	$p$ (Significance)
Birth wight in grams	899.42/333.11	727.50/177.61	1.17[16]	0.259
Length of hospitalization	95.00/27.69	86.17/29.51	.62[16]	0.541
Gestational age	25.58/1.24	24.50/.84	1.92[16]	0.073
Days to infection	0.00/.00	34.67/31.77	NA	NA
Days on gentamicin	14.08/6.97	12.33/6.02	0.52[16]	0.608
Days on third generation cephalosporin	4.42/6.24	5.83/4.17	-0.50[16]	0.625
Days on ampicillin	10.00/3.69	10.50/4.64	-0.25[16]	0.806
Length of antibiotic therapy	24.25/14.27	20.17/8.38	0.64[16]	0.530
Day on vancomycin	3.17/5.67	8.33/4.97	-1.89[16]	0.077
Number of days on steroids	18.25/20.28	16.17/13.81	0.23[16]	0.825
Number of days intubated	30.17/17.49	32.17/26.16	-0.19[16]	0.848
Days on conventional ventilation	28.42/18.01	27.17/25.98	0.12[16]	0.906
Days on HFOV*	1.83/4.32	5.00/5.51	-1.34[16]	0.199
Number of days with CVL <sup>†</sup>	14.83/7.57	16.50/9.57	-0.40[16]	0.691
Number of days with arterial line	13.33/9.26	18.17/10.36	-1.01[16]	0.330
Number of days with peripheral line	22.25/11.13	13.67/10.13	1.59[16]	0.132

Lowest number of WBC <sup>‡</sup>	8.66/5.82	5.01/1.83	1.48[16]	0.158
Lowest ANC <sup>§</sup>	2413.08/2746.57	1592.50/1470. 56	0.68[16]	0.508
Number of days ANC <sup>§</sup> was below 1000	0.67/.99	1.50/2.74	-0.96[16]	0.351

\*HFOV: high-frequency oscillating ventilator

†CVL: Central Venous Line

‡WBC: white Blood Cells

§ANC: Absolute Neutrophil Count

Table 2. Comparison between Controls and Cases for Frequencies of Categorical Variables

Variables	Control Group (n=12)	Infected Group (n=6)	<i>Chi-square [df]</i>	<i>p</i> (Significance)
Gender: Females/Males	8/4	4/2	.000[1]	1.00
Birth weight: SGA*/AGA†	1/11	1/5	.281[1]	1.00
Gentamicin use	11	6	.529[1]	1.00
third generation cephalosporin use	5	5	2.813[1]	.152
Ampicillin use	12	6	NA	NA
Vancomycin use	5	5	2.813[1]	.152
Steroids use	7	4	.117[1]	1.00
HFOV‡	3	4	2.922[1]	.141
CVL§	11	6	.529[1]	1.00
Arterial line	12	6	NA	NA
Peripheral line	12	5	2.118[1]	.333

\*SGA: small for gestational age

†AGA: appropriate for gestational age

‡HFOV: high-frequency oscillating ventilator

§CVL: central venous line