

Resurgence of Global Opportunistic Multidrug-resistant *Stenotrophomonas maltophilia*

Pradheer Gupta, Pratibha Kale, Vikas Khillan

Department of Clinical Microbiology, Institute of Liver and Biliary Sciences, New Delhi, India

Abstract

Context: *Stenotrophomonas maltophilia* is a known nosocomial pathogen which is intrinsically resistant to multiple antibiotics. In India, *S. maltophilia* infection has only few case reports. **Aim:** To determine the incidence of *S. maltophilia* infection from clinical isolates based on the specimen type, antibiotic susceptibility pattern, and impact on outcome. **Settings and Design:** One-year retrospective study was done at a tertiary liver care center. **Methods:** Patients with *S. maltophilia* isolation in clinical samples were selected. Serial levels of serum procalcitonin and total leukocyte count were recorded. Environmental surveillance was done from the wards of *S. maltophilia* isolation as part of routine practice. **Statistical Analysis:** Continuous data were compared using Kruskal–Wallis test/Mann–Whitney test. The categorical data were compared by Chi-square/Fisher’s exact test, wherever necessary. Besides this, an appropriate analysis like survival was carried out at the time of data analysis. **Results:** One hundred isolates were obtained from eighty patients of six wards. The greatest number (44/100, 44%) were from the Liver Coma Intensive Care Unit and the lowest (3/100) from the day care. Isolation from the respiratory samples was 1.32% and bloodstream infection 0.6%. Of 100 isolates, 12 (12%) were resistant to both trimethoprim–sulfamethoxazole and levofloxacin. **Conclusion:** *S. maltophilia* was effectively isolated from the hospital environment, with two of hand impression and three of water samples’ positive. Patients with respiratory infection had most *S. maltophilia* isolates. Antibiotic susceptibility revealed more resistance than reported in this region.

Keywords: Clinical sample, environmental surveillance, *Stenotrophomonas maltophilia*

INTRODUCTION

Stenotrophomonas maltophilia is an aerobic, nonfermentative, Gram-negative bacterium. This has emerged as an important opportunistic nosocomial pathogen, especially among immunocompromised patients, and who have been hospitalized for a prolonged period. *S. maltophilia* can cause a variety of infections, including nosocomial pneumonia, urinary tract infections, bacteremia, endocarditis, and wound and soft-tissue infections.^[1]

S. maltophilia has been isolated from the medical devices, anticoagulant in blood collection tubes, disinfectants, and sterile water.^[2,3] Chlorine treated water supply in hospitals has been identified as a source for clusters of cases.^[4]

In India, the distribution of *S. maltophilia* infections has rarely been described with only few case reports of ocular infections, pyomyositis, respiratory tract infections, meningitis, osteomyelitis, and hemodialysis catheter-related bacteremia, etc.^[5-10] Therefore, the aim of this study was to determine the

incidence of *S. maltophilia* infection from various clinical isolates based on their specimen type, antibiotic susceptibility pattern, and impact on patient outcome. Serial levels of serum procalcitonin (PCT) and total leukocyte count (TLC) were also recorded to ascertain correlation of their levels in *S. maltophilia* isolates and clinical disease.

Environmental surveillance is a routine practice as a part of infection control policy, to determine if any environmental factor was associated in causation of infection.

METHODS

This retrospective study was done in a tertiary care hepatobiliary center, New Delhi, India. All patients’ records

Address for correspondence: Dr. Vikas Khillan,
Department of Clinical Microbiology, Institute of Liver and Biliary Sciences,
New Delhi, India.
E-mail: khillanv@yahoo.com

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with culture positive for *S. maltophilia* between January 2017 and December 2017 were collected. Patients admitted for >48 h were included in the study.

Patients with preexisting sepsis or expected survival of <48 h were excluded.

Clinical isolates

Details of consecutive isolates of *S. maltophilia* from various specimen types, nonbronchoscopic bronchoalveolar lavage (mini-BAL), sputum, blood, urine, body fluids (bile, ascitic, and pleural fluids), and dialysis catheter tip were collected from the patients in nephrology and hepatology wards, day care, high dependency unit (HDU), Liver Coma Intensive Care Unit (LCICU), and Transplant Intensive Care Unit (TICU).

Colonization was differentiated from infection in urine by a number of pus cells/hpf on microscopy and number of colony-forming units (CFUs)/ml on culture. In respiratory samples, infection was diagnosed by CFU/ml as per the IDSA guidelines.^[11,12]

Environmental samples

Environmental surveillance is a routine practice as a part of infection control policy, ours being tertiary liver transplant center. Any nonfermenting Gram-negative bacillus growing on routine media is subjected to automated identification. Environmental sampling included surface swabs from different equipment (ventilator, syringe pump, bed rails, injection tray, and monitor), and patient's bed and surroundings (cardiac table, electric switch, mattress, bed rails, and intravenous stand). Air samples were collected using Sampl'air™ with 12–15 air changes/h. Drinking water and tap water were collected in sterile containers, and water sterility was tested by multiple fermentation tube method to determine the presumptive coliform count/most probable number of coliforms. Any color change or turbidity produced in tubes is subjected to subculture on blood agar and MacConkey agar. Any nonlactose fermenting colony is subjected to automated identification, as described by Mahapatra *et al.*^[13-15]

Personnel hand cultures

Hand impression samples were collected from doctors and nursing staff on 90 mm, 5% sheep blood agar plate. Samples were collected from LCICU, TICU, HDU, hepatology wards, nephrology wards, and dialysis day care without prior intimation to the staff.

Identification of *Stenotrophomonas maltophilia*

Samples were first streaked on to 5% sheep blood agar and MacConkey agar and incubated at 37°C for 24–48 h. CFUs/ml were expressed by a semiquantitative method for respiratory and urine samples. *S. maltophilia* colonies were identified nonhemolytic, small, circular, raised colonies with a yellow tint. They do not ferment lactose, on MacConkey agar.

Identification of isolates and antibiotic susceptibility testing (AST) was done by VITEK-2™ (Biomérieux, France) system as per the manufacturer's instructions.

AST was done using minimum inhibitory concentrations as per the Clinical and Laboratory Standards Institute 2017 breakpoints.^[16]

Serum procalcitonin level

Serial levels of PCT were recorded for all the patients on admission and every 48 h thereafter.

Serial PCT levels were recorded for all the patients, on admission and every 48 h thereafter, by chemiluminescence method using Maglumi 1000™ (Shenzhen industries, China). Total leukocyte count (TLC) were recorded using LH-750™ hematology analyzer (Beckman Coulter, USA) every 24 h.

Patient follow-up

Patients were divided based on the wards in which they were admitted and their diagnoses. They were followed up for the period of hospital stay to access their outcome.

Statistical analysis

Data were recorded and further analyzed by IBM SPSS Statistics Version 20 in terms of median, range, and percentage.

Continuous data were compared using Kruskal–Wallis test/Mann–Whitney test. The categorical data were compared by Chi-square/Fisher's exact test, wherever necessary. Besides this, an appropriate analysis like survival was carried out at the time of data analysis. The significance was seen at 5%.

RESULTS

A total of 100 isolates of *S. maltophilia* were isolated from 80 patients: 63 male and 17 female patients. The distribution of isolates from the eight types of specimens is shown in Table 1. Sample types included blood, respiratory (mini-BAL and sputum), body fluids, urine, and dialysis catheter. The frequency of isolation is shown in Table 1. The age ranged from 1 month to 85 years with a median of 42 years. The greatest number (44/100, 44%) of isolates were from LCICU, and the least from day care (3).

Stenotrophomonas was isolated from 51 (0.6%) of 8484 samples of blood, while from respiratory samples, 27 (1.32%) of 2038 isolates belonged to *Stenotrophomonas*. Of 100 isolates, 51 (51%) belonged to blood and 27 (27%) to respiratory type, but frequency of isolation was more common in respiratory samples than blood (1.32% vs. 0.6%).

In total, 190 samples were collected from various environmental sources. We isolated *S. maltophilia* from two nursing staff, suggesting that a lack of rigorous handwashing was responsible for the spread of this infection. The colonized nurse worked in the LCICU during the study period and had not worked outside the LCICU. They were involved in patient care in LCICU, the area with most number of *Stenotrophomonas* isolates (44/100). Samples included mini-BAL (21), blood (19), ascitic fluid (2), pleural fluid (1), and bile (1).

Sequencing could not be done.

Table 1: Isolation of *Stenotrophomonas maltophilia* from clinical samples obtained from various hospital wards

Specimen, total isolates	LCICU	HDU	TICU	Hepatology ward	Nephrology ward	Day care	Total (%)
Blood (8484)	19	5	1	12	11	3	51 (0.60)
Respiratory (mini-BAL + sputum) (2038)	21	-	2	4	-	-	27 (1.32)
Body fluid (ascitic fluid + pleural fluid + bile) (3751)	4	-	-	10	-	-	14 (0.37)
Urine (8805)	-	-	-	1	1	-	2 (0.02)
Others (1099)	-	-	-	-	5	1	6 (0.54)

LCICU: Liver Coma Intensive Care Unit; HDU: High dependency unit; TICU: Transplant Intensive Care Unit; BAL: Bronchoalveolar lavage

Transmission decreased significantly once the health-care workers were decolonized in February 2017, but cases reemerged in June 2017, suggesting that other factors might be contributing to spread of bacterium. From June 2017, *S. maltophilia* infections occurred in other hospital areas, including the LCICU, HDU, TICU, hepatology ward, nephrology ward, and day care. Routine surveillance cultures of the dialysate water showed *S. maltophilia* growth in 1/6 samples. Of 12 samples from tap water of LCICU, 2 also showed *S. maltophilia* growth. Details of environmental samples are shown in Table 2.

Culture characteristics

Colony counts of *S. maltophilia* were done in mini-BAL and urine samples (26/100 isolates) which showed 100–100,000 CFU/ml, median 100 CFU/ml. Applying Kruskal–Wallis test, quantitation of *S. maltophilia* was not associated with patient outcome ($P = 0.602$).

More than one isolate other than *Stenotrophomonas* was seen in 22 isolates. Twelve of them were from respiratory sample, 4 from bile, 3 blood, and 1 each from pleural fluid, urine, and dialysis catheter tip. *Klebsiella pneumoniae* (7) was most common coisolate, followed by *Acinetobacter baumannii* (5), *Pseudomonas aeruginosa* (3), *Candida* sp. (3), *Enterobacter* sp. (2), *Staphylococcus aureus* (1), and *Aspergillus flavus* (1). Of 22 patients, 8 (36.3%) expired. Whereas, 13 (16.6%) of 78 patients with *Stenotrophomonas* mono-infection expired. Thus, mortality was significantly higher in polymicrobial infection group.

Antimicrobial susceptibilities

Antibiotic susceptibilities of all 100 clinical isolates of *S. maltophilia* isolates were determined. Resistance pattern of isolates is shown in Table 3.

Resistance to cotrimoxazole was 26 (26%) while to levofloxacin was 24 (24%) which were almost similar. Of 100 isolates, 12 (12%) were resistant to both antibiotics and 4 (33.3%) of these remained susceptible to chloramphenicol. Applying Chi-square test, *S. maltophilia* susceptibility profile did not have an impact on patient's outcome ($P = 0.77$ for cotrimoxazole and $P = 0.22$ for levofloxacin).

Patient diagnosis and outcome

In our patient group, 42 (52.5%) patients were of chronic liver disease (CLD), 14 (17.5%) were of chronic renal disease,

Table 2: Isolation of *Stenotrophomonas maltophilia* from various environmental sources

Ward	Instrument swab	Patient bed swab	Water	Air	Hand
LCICU	0/13	0/13	2/12	0/22	2/9
TICU	0/26	-	0/3	0/2	0/1
HDU	-	0/6	0/6	0/3	0/4
Nephrology ward/day care	-	-	1/6	-	-
Hepatology ward	-	0/40	0/5	0/20	-

LCICU: Liver Coma Intensive Care Unit; HDU: High dependency unit; TICU: Transplant Intensive Care Unit

8 (10%) with acute necrotizing pancreatitis, 5 (6.2%) with acute liver failure, 3 (3.7%) each with liver abscess and carcinoma gall bladder, 2 (2.5%) each were postliver transplant and postrenal transplant, and 1 baby was of biliary atresia.

Of 80 patients, 48 (60%) got discharged, while 11 (13.7%) took discharge against medical advice and 21 (26.2%) expired. Patients with CLD (19, 90.4%) and those in LCICU (20, 95.2%) had worst outcome, which was statistically significant applying Chi-square test ($P < 0.01$).

Of 21 patients, 20 (95.2%) who expired were from LCICU, i.e., they were extremely moribund, immunocompromised, and had prior antibiotic exposure.

Serum procalcitonin level and total leukocyte count

Serum PCT level ranged from 0.05 to 100 ng/ml with a median of 1.74 ng/ml; TLC ranged from 2000 to 47200/cumm with a median of 11,800/cumm. Applying Mann–Whitney test, mortality was significantly associated with raised TLC ($\geq 11,000$ /cumm) ($P = 0.038$), but not with serum PCT level (≥ 0.5 ng/ml) ($P = 0.29$) [Figures 1 and 2].

DISCUSSION

The WHO lists *S. maltophilia* as one of the leading drug-resistant pathogens in hospitals worldwide.^[17] *S. maltophilia* has become the third most common nonfermentative Gram-negative bacilli responsible for nosocomial infections, after *P. aeruginosa* and *Acinetobacter* spp. Ours being a tertiary liver care center with most of the patients immunocompromised, the incidence of *S. maltophilia* infection is higher. Earlier identification of nonfermenters, based on the biochemical tests, was cumbersome. However,

Table 3: Antibiotic resistance profiles of *Stenotrophomonas maltophilia* isolates obtained from various samples

Specimen	Number of isolates (%)	Antibiotics, number of resistant isolates, n (%)						
		Ticarcillin/ clavulanic acid	Mrp	Ceftazidime	TMP/ SMX	Cip	Chloram	Levo
Blood	51 (51)	3 (5.8)	1 (1.9)	1 (1.9)	14 (27.4)	5 (9.8)	2 (3.9)	16 (31.3)
Respiratory (mini-BAL + sputum)	27 (27)	3 (11.1)	1 (3.7)	1 (3.7)	9 (33.3)	2 (7.4)	3 (11.1)	8 (29.6)
Body fluid (ascitic fluid + pleural fluid + bile)	14 (14)	-	-	-	2 (14.2)	1 (7.1)	1 (7.1)	6 (42.8)
Urine	2 (2)	-	-	-	1 (50)	-	-	-
Other (dialysis catheter tip)	6 (6)	-	-	-	-	-	-	-
Total	100 (100)	6 (6)	2 (2)	2 (2)	26 (26)	8 (8)	6 (6)	30 (30)

Mrp: Meropenem; TMP/SMX: Trimethoprim/sulfamethoxazole; Cip: Ciprofloxacin; Chloram: Chloramphenicol; Levo: Levofloxacin; BAL: Bronchoalveolar lavage

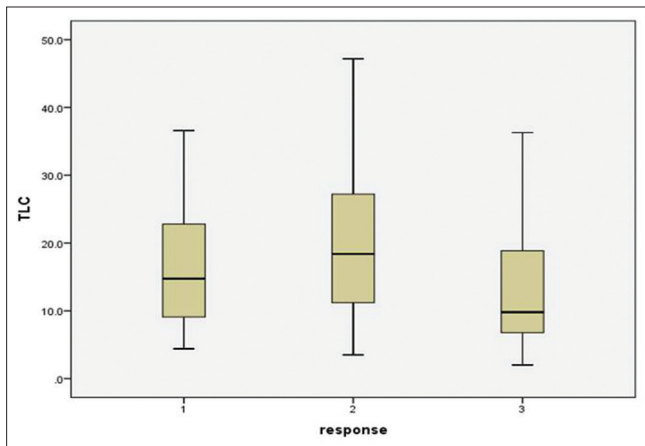


Figure 1: Mann–Whitney test showing association between total leukocyte count and patient response. 1 = patient expired, 2 = discharge against medical advice, 3 = patient discharge

now, with the advent of commercial systems such as VITEK-2 or API, this has become easier.^[8]

S. maltophilia is a known cause of nosocomial infection and for clustering of cases. It can adhere to plastic surfaces, forms biofilms, and has been identified on hospital devices.^[2,3]

In our study, this bacterium is associated with respiratory tract infection (1.32%) followed by bloodstream infections (0.6%), in contrast to the study by Batra *et al.*,^[13] which showed that most of the patients presented with bacteremia (51%), pneumonia (42%), and skin and soft-tissue infections (7%), whereas others showed it to be associated with respiratory tract infections and bloodstream infections.^[4,5]

In our study, *S. maltophilia* in clinical samples were from blood, respiratory (mini-BAL and sputum), and body fluids. A study by Paopradit *et al.* showed that isolates were most often from sputum (56.2%), blood (14%), and body fluids (14%).^[4] In our study, most isolates were from blood (51%), mini-BAL (27%), and body fluids (14%). The LCICU was the dominant ward (44%) for *S. maltophilia* isolation. Our finding correlated with the same study where ICU (31.2%) was the most common ward for isolation of *S. maltophilia*.^[4]

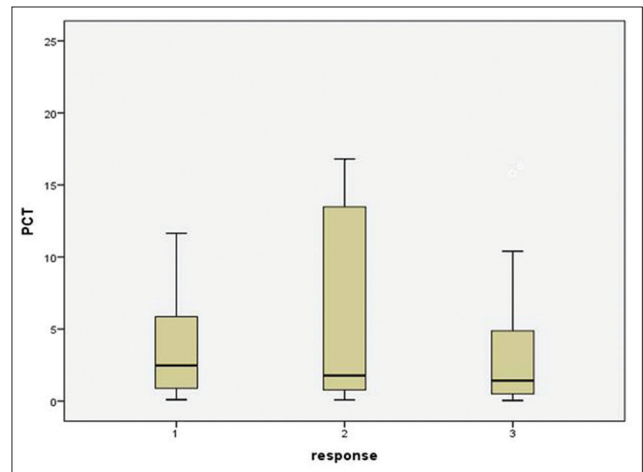


Figure 2: Mann–Whitney test showing association between serum procalcitonin, ng/ml and patient response. 1 = patient expired, 2 = discharge against medical advice, 3 = patient discharge

In our study, of 2038 respiratory samples, 27 (1.32%) *S. maltophilia* were isolated. Chawla *et al.* isolated *S. maltophilia* in 15 (0.29%) of 5056 samples, which is lesser than in our group.^[8] Odile *et al.* found concomitant presence of *Aspergillus fumigatus* and *S. maltophilia* infection in the respiratory tract of patients with liver disease in 20 (7.8%) of 257 patients.^[18] In our study, we found *S. maltophilia* and *A. flavus* coinfection in 1 (3.7%) of 27 *S. maltophilia* respiratory isolates with liver disease.

We encountered catheter-related bacteremia by *S. maltophilia* in six hemodialysis patients. In previous study, we described hemodialysis catheter-related bacteremia in three patients.^[10]

Gauna *et al.*, among 59 patients with end-stage renal disease, isolated *S. maltophilia* in 7 (10.8%) of 65 blood culture samples. In our study, 10 (19.6%) of 51 blood culture isolates were from renal disease.^[19]

Sawai *et al.* reported an intra-abdominal abscess caused by *S. maltophilia* infection in patients with colon cancer and renal cell carcinoma, sensitive to trimethoprim–sulfamethoxazole (TMP-SMX) and levofloxacin; we found *S. maltophilia* infection from bile sample of two patients with carcinoma gall bladder sensitive to TMP-SMX and levofloxacin.^[20]

Harada *et al.* identified 65 patients with *S. maltophilia* bacteremia in hematopoietic stem cell transplant (HSCT) recipients, with incidence 1.14% and median age 49 vs. 59 years in HSCT recipients and non-HSCT recipients. In our study, the incidence of bacteremia was 51 (0.6%) of 8484 patients with the median age of 42 years.^[21]

Risk factors for 90-day mortality with *S. maltophilia* isolates in allo-HSCT recipients showed that serum C-reactive protein (≥ 10.0 mg/dl), albumin (< 3.0 g/dl), creatinine (≥ 1.0 mg/dl), sepsis, and nonremoval of central venous catheter were associated with mortality. In our study, TLC count ($\geq 11,000$ /cumm) was significantly associated with mortality.^[21]

Batra *et al.* found that 23/88 *Stenotrophomonas*-infected patients had coinfection, with *A. baumannii* (12) being the most common, followed by *P. aeruginosa*, *K. pneumoniae*, *Escherichia coli*, and *Candida sp.*^[13] We found coinfection in 22/100 isolates, with *K. pneumoniae* (7) as most common isolate, followed by *A. baumannii* (5), *P. aeruginosa* (3), *Candida sp.* (3), *Enterobacter sp.* (2), *S. aureus* (1), and *A. flavus* (1).

Majority of our isolates were sensitive to TMP-SMX (75%) and levofloxacin (71%). Chawla *et al.* found isolates sensitive to ciprofloxacin (93.3%) and TMP-SMX (86.7%).^[8] The main antibiotic used to treat *S. maltophilia* infections is cotrimoxazole. However, 25% of isolates in our study were resistant to it, which is higher than reported in this region.^[13]

Paopradit *et al.* studied 360 environmental samples and found *S. maltophilia* in 121 isolates with 22 (61.1%) of 36 in drinking and tap water.^[4] Gallo *et al.* studied for surveillance of *S. maltophilia* in 936 nosocomial samples. *S. maltophilia* was found in 3% of bed rail samples.^[22] We collected 190 samples, 59 bed rails, and 32 water samples with three positive for *S. maltophilia*. Sah *et al.* evaluated the isolation of *S. maltophilia* from the seven blood cultures in pediatric patients, hand of one health-care provider found harboring similar organism.^[23] We isolated *S. maltophilia* from two nursing staff, suggesting that a lack of rigorous handwashing was responsible for the spread of this infection. Although this finding is consistent with the resident nurses disseminating the infection within the LCICU, we cannot exclude other scenarios, including transmission by other medical personnel who may have been transiently colonized and thus would not have been detected. The colonized nurses worked in the LCICU during the study period and had not worked outside the LCICU.

CONCLUSION

Nonbronchoscopic mini-BAL followed by blood samples were the clinical specimens with the most *S. maltophilia* isolates. Antibiotic susceptibility revealed more frequent resistance in clinical samples than reported in this region. In conclusion, *S. maltophilia* was effectively isolated from hospital environments, with three of water samples and two of hand impression samples in nursing staff positive.

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Conflicts of interest

There are no conflicts of interest.

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