# Multidrug Resistant *Stenotrophomonas maltophilia* - Changing Sensitivity Pattern with Resistance to Trimethoprim-Sulfamethoxazole - A Retrospective Study from Jaipur, India

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**ABSTRACT**

**BACKGROUND**

*Stenotrophomonas maltophilia* is a gram-negative bacillus, multidrug resistant (MDR) opportunistic pathogen, which is normally present in hospital surroundings. It has been one of the leading causes of nosocomial infections due to risk factors such as extended intensive care unit (ICU) stays and multiple invasive procedures. In this study we wanted to assess the antibiotic sensitivity pattern with various antimicrobial agents i.e. levofloxacin, minocycline, ceftazidime, chloramphenicol, & ticarcillin-clavulanic acid with special focus on trimethoprim-sulfamethoxazole (TMP-SMX).

**METHODS**

In vitro analysis was conducted on 164 *Stenotrophomonas maltophilia* strains isolated from blood and respiratory tract from January 2016 to November 2020. Antibiotic susceptibility and minimum inhibitory concentration (MIC) testing for trimethoprim-sulfamethoxazole (TMP-SMX), levofloxacin (LVX), ticarcillin - clavulanic acid (TIM), and minocycline (MIN) were performed using Vitek 2, as per clinical and laboratory standards institute (CLSI) guideline.

**RESULTS**

A total of 164 *S. maltophilia* were isolated. Out of the 164 *S. maltophilia* isolates, 26 (16 %) were isolated from blood, 114 (70 %) were isolated from respiratory samples, 20 (12 %) from pus & tissue, and 4 (2 %) from urine. Out of the 164 patients, 130 (80 %) were males and 32 (20 %) were females. Maximum patients were above 50 years of age 93 (56 %) followed by 20 - 50 years 55 (34 %). Out of the 164 patients, 67 (40 %) were admitted to wards, 92 (56 %) were in ICU and 5 (3 %) were seen in out-patient department (OPD). A total of 90 % strains were sensitive to trimethoprim-sulfamethoxazole (TMP-SMX). Total 91 % strains were sensitive to levofloxacin.

**CONCLUSIONS**

*S. maltophilia* is emerging as a significant nosocomial pathogen, with a growing rate of isolation. Trimethoprim- sulfamethoxazole (TMP-SMX) is still the drug of choice, but resistance to it has been reported.

**KEYWORDS**

Minimum Inhibitory Concentration, Trimethoprim-Sulfamethoxazole (TMP-SMX), Levofloxacin

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*DOI: 10.18410/jebmh/2021/436*

*How to Cite This Article:*
*Jain DK, Gupta YK, Sinha P, et al. Multidrug resistant Stenotrophomonas maltophilia - changing sensitivity pattern with resistance to trimethoprim-sulfamethoxazole - a retrospective study from Jaipur, India. J Evid Based Med Healthc 2021;8(26):2339-2343. DOI: 10.18410/jebmh/2021/436*

*Submission 13-01-2021,*
*Peer Review 20-01-2021,*
*Acceptance 11-05-2021,*
*Published 28-06-2021.*

## BACKGROUND

Medical microbiologists have long realized the significance of identifying infectious microbes as the cause of ailments in human beings. The emergence of novel multidrug-resistant bugs found in atmosphere, the growing incidents of community-acquired infections, and the spread of these microorganisms in the hospital setting have all emphasized the necessity to control these pathogens. The increasing incidents of multidrug-resistant organisms (MDRO) associated infections has spurred efforts to examine potential sources of these pathogens, evaluate current antimicrobial strategies used to treat these infections, and explain the mechanisms used by these microbial pathogens during infection. Gram-negative bacilli have received a lot of attention, as they are frequently associated with multidrug-resistant mechanisms which include multidrug resistance pumps, plasmids that harbour antibiotic resistance genetic materials, and various gene transfer methods involved in the acquisition of antimicrobial resistance. *Pseudomonas aeruginosa* is an example of a multidrug-resistant organism that causes respiratory infections in patients with chronic lung disease or cystic fibrosis.

*Stenotrophomonas maltophilia* was first isolated in 1943 and was named Bacterium bookeri by J. L. Edward at that time. It was later renamed in the Pseudomonas family, then family Xanthomonas, and finally, in 1993, after a very long period of debate, Palleroni and Bradbury proposed a genus Stenotrophomonas.

It is the only Stenotrophomonas species known to infect human being, while its close genetic members are only infective to plants. *Stenotrophomonas maltophilia* is an aerobic non-fermentative gram-negative bacillus that is ubiquitous in the environment in all geographic regions.[1,2]

It acquire motility via multi trichous flagella. The optimal temperature for its growth is 35°C- 37°C and no growth occurs below 5°C and above 40°C. It produces lavender-green color colonies on blood agar (BA) plates, non-pigmented small colonies on MacConkey agar (MHA) plates and tiny creamy colonies on Muller-Hinton agar (MHA) plates after 18 – 24 hours of incubation at 35°C - 37°C.

They do not ferment glucose sugar, and many isolates tend to give a positive oxidase test. These free-living micro-organisms are present in most aquatic and/or humid environment, including hospital drinking water.

*Stenotrophomonas maltophilia* has arisen as an important opportunistic nosocomial pathogen, particularly among immune-compromised hosts, patients suffering from malignancy, patients on long steroid therapy and prolonged hospitalization. *S. maltophilia* is a significant nosocomial pathogen that is now considered as a "newly emerging superbug" due to its multi-drug resistance and ability to colonise the surfaces of medical devices. *S. maltophilia* can cause a wide variety of infections, including nosocomial pneumonia, acute exacerbations of chronic obstructive pulmonary disease (COPD), urinary tract infections (UTI), bacteraemia, endocarditis, meningitis, and wound & soft tissue infections.[3]

Community-acquired *S. maltophilia* pneumonitis and acute exacerbations of chronic obstructive pulmonary disease has arisen as a serious concern in patients with no known risk factors, such as intensive critical unit stay; treatment-induced neutropenia, mechanical ventilation, long carbapenem therapy, third or fourth cephalosporins, or long hospital stays. In all geographic regions, this bacterium is most commonly associated with respiratory tract infections. Any organ may be affected by *Stenotrophomonas maltophilia.* Infections of the lungs are common. Colonization of the respiratory tract normally precedes lung infection. Pleural effusions are uncommon, but lobar or lobar consolidation is common. Infected indwelling intravascular devices cause the most of the *Stenotrophomonas maltophilia* bloodstream infections. Non–catheter-related bacteraemia is often seen in patients with prolonged neutropenia.[4,5,6]

Trimethoprim-sulfamethoxazole / Co-trimoxazole has been the primary treatment for susceptible *S. maltophilia* infections, based on in vitro activity and anecdotal reports of favourable clinical outcomes. Although levofloxacin and Minocycline have been considered as alternatives to (TMP-SMX) for the treatment of *Stenotrophomonas maltophilia* infections, more clinical data are still needed to validate their efficacies.[4,6,7,8]

Because *Stenotrophomonas maltophilia* and *B. cepacia* are present in the atmosphere, exposure to these bacteria occurs frequently. Bacterial factors contributing to colonization of the respiratory tract or prosthetic devices such as intravascular catheters and endotracheal tubes include cationic surface charge, flagella and fimbriae, which promote adhesion and biofilm formation.[9,10]

Patients at high risk for infection are those critically ill with (1) chronic obstructive pulmonary disease (2) prolonged use of broad-spectrum anti-microbial therapy with third and fourth generation cephalosporin's or carbapenem's (3) prolonged need for assisted ventilation requiring tracheotomy (4) high injury severity score (5) pulmonary contusion and (6) prior respiratory tract *S. maltophilia* colonization.[11]

In the immunosuppressed cancer and transplant population, well-recognized risk factors for invasive *S. maltophilia* disease include (1) presence of long treatment-induced neutropenia (absolute neutrophil count < 150 cells/μL) (2) recent or on-going carbapenem, higher-generation cephalosporin, or fluoroquinolone therapy (3) presence of mucositis (4) presence of indwelling medical devices such as intravascular or genitourinary catheters (5) prolonged hospital stay and (6) hyper alimentation.[12,13] *S. maltophilia* in chlorine treated water distribution hospital water supply has been identified as a source for occasional clusters of nosocomial cases.[14]

Several molecular mechanisms of *S. maltophilia* contributes to its multi-antimicrobial resistance, including, plasmids, integrons, and jumping genes. Integrons common regions and integron-like elements have been reported for *S. maltophilia* isolates across the world. Integrons are not auto-mobilizable parts but hold an integrase encoding gene that allows the insertion of antibiotic resistance gene cassettes between highly conserved deoxyribose nucleic acid (DNA) nucleotide sequences. Jumping genes (transposons) and extra-chromosomal genetic materials (plasmids) can

facilitate the integration of integrons in between bacterial cells. Trimethoprim-sulfamethoxazole has the foremost potent and good in vitro activity against isolates of *S. maltophilia*. More than 99 % were sensitive to Trimethoprim-sulfamethoxazole in early studies. However, drug resistance has risen among 30 % to 40 % of disease-associated isolates globally. The underlying mechanism (s) for Trimethoprim-sulfamethoxazole resistance is not fully known. Class 1 integrons carrying the *sul1* gene and an insertion element common region (ISCR) carrying the *sul2* gene have been demonstrated to play an important role in Trimethoprim-sulfamethoxazole resistance and the global spread of mobile *sul* genes has been linked with increased Trimethoprim-sulfamethoxazole use and noted to occur during therapy.[4,5,6,8]

Hospital associated outbreaks underline the existing practice of strict infection control measures, with a focus on minimising transmission on health-care personnel's hands and cohort segregation of infected or colonized hospitalized patients with multidrug-resistant pathogens. Continued surveillance of the hospital water supply remains critical in preventing iatrogenic and hospital-related acquisition of these free-living, nearly ubiquitous bacteria.[15,16]

### Objectives

We wanted to evaluate the antibiotic sensitivity pattern with various antimicrobial agents i.e., Levofloxacin, Minocycline, Ceftazidime, Chloramphenicol, & Ticarcillin-clavulanic acid with special focus on Trimethoprim-Sulfamethoxazole and understand in detail the clinico-microbiological profile of *S. maltophilia*.

## METHODS

This was a retrospective study done over a period of five years, Jan 2016 to Nov 2020. All non-fermenting gram-negative bacilli from any clinical specimen like blood, sputum, tracheal secretion, urine etc., were selected. The clinical samples were processed using standard microbiological procedures. Out of all the non-fermenters, *S. maltophilia* isolates were further selected. Final identification and antibiotic susceptibility of these isolates for levofloxacin, Trimethoprim-sulfamethoxazole , Minocycline, Ceftazidime, and Ticarcillin-clavulanic acid was performed by VITEK 2C system (Biomerieux, France). The VITEK 2C is an automated microbiology system utilizing growth-based technology. It has colorimetric reagent cards that are incubated and interpreted automatically.

Informed consent of patients was taken at the time of sample collection. Ethical committee clearance was not required as it was a retrospective study.

Antibiotic susceptibility testing for each isolate, the species identity was confirmed and minimal inhibitory concentrations of antibiotics were detected by VITEK 2 compact system (Biomerieux, France). Results were interpreted according to the CLSI guidelines.[17]

### Statistical Analysis

The data was analysed using statistical package for social sciences (SPSS) version 20 statistical software, and tables were generated using Microsoft Excel and Word.

## RESULTS

A total of 164 *S. maltophilia* were isolated. Out of the 164 *S. maltophilia* isolates, 26 (16 %) were isolated from blood, 114 (70 %) were isolated from respiratory samples, 20 (12 %) from pus & tissue, and 4 (2 %) from urine.

Out of the 164 patients, 130 (80 %) were males and 32 (20 %) were females. Maximum patients were above 50 years of age 93 (56 %) followed by 20 - 50 year 55 (34 %). Out of the 164 patients, 67 (40 %) were admitted to wards, 92 (56 %) were in ICU and 5 (3 %) were seen in OPD. A total of 93% isolated strains of *S. maltophilia* were sensitive to Minocycline, 91 % were to Chloramphenicol and Levofloxacin, 90 % were to Trimethoprim-sulfamethoxazole (TMP-SMX), 74 % were to Ticarcillin-clavulanic acid and 67% strains were sensitive to Ceftazidime.

| Sex | Number | Percentage |
|---|---|---|
| Male | 130 | 80 % |
| Female | 34 | 20 % |

***Table 1. Sex Wise Distribution of the S. maltophilia Isolates***

| Age | Number | Percentage |
|---|---|---|
| Less than 1 Year | 13 | 8 % |
| 1 to 5 year | 3 | 2 % |
| 21 to 30 | 21 | 13 % |
| 31 to 40 | 14 | 9 % |
| 41 to 50 | 20 | 12 % |
| 51 to 60 | 41 | 25 % |
| 61 to 70 | 19 | 11 % |
| 71 to 80 | 27 | 16 % |
| 81 to 90 | 6 | 4 % |

***Table 2. Age Wise Distribution Pattern of the S. maltophilia Isolates***

| Sample | Number | Percentage |
|---|---|---|
| Blood | 26 | 16 % |
| Respiratory sample | 114 | 70 % |
| Urine | 4 | 2 % |
| Pus & tissue | 20 | 12 % |

***Table 3. Type of Specimen – Sample Wise Distribution Pattern of the S. maltophilia Isolates***

| Area | Number | Percentage |
|---|---|---|
| Intensive care units (ICU) | 92 | 56 % |
| Patients wards | 67 | 40 % |
| Out patients departments (OPD) | 5 | 3 % |

***Table 4. Area Wise Distribution Pattern of the S. maltophilia Isolates***

| Antimicrobial Agents | Sensitivity in Percentage |
|---|---|
| Trimethoprim-sulfamethoxazole (TMP-SMX) (Co-trimoxazole) | 90 % |
| Levofloxacin | 91 % |
| Minocycline | 93 % |
| Ceftazidime | 67 % |
| Chloramphenicol | 91 % |
| Ticarcillin-clavulanic acid | 74 % |

***Table 5. Antimicrobial Susceptibility Pattern of the S. maltophilia Isolates***

## DISCUSSION

*S. maltophilia* is generally considered to be low-virulence and is becoming an emerging multi-drug resistant opportunistic microbial agent in the hospital and community settings, particularly among immunosuppressed patients. Various risk factors associated with *S. maltophilia* infection include underlying malignancy, cystic fibrosis, immunosuppressant or cortico-steroid therapy, the presence of an indwelling central venous catheter line and exposure to broad spectrum antimicrobial agents.[15]

In the present study with a sample of 164, men contributed 80% with the male : female ratio of 80 : 20. The predominance of men is likely due to behavioural and socio-economic factors in India, where men, in greater proportion than women, participate in outdoor activities and women are often not present timely in the course of the disease.

Our study reports that nearly half (N = 75, 46 %) of patients were in the age group 31 to 60 and approximately 46 (27 %) patients were in the age group 61 to 80. This can be attributed to a weekend immune system response seen in the older age group that made them more susceptible to *S. maltophilia* infections.

Our study is in close association with a study by Gopalakrishnan R which also showed that the mean age range of infected patients was 62.4 years.[18] The current study shows that the respiratory tract is the most commonly involved system (70 %) followed by bloodstream infection (16 %) and pus / soft tissue infection (12 %). Brooke et al. in their study showed the prevalence of respiratory tract involvement in their review article.[3] Bacteraemia was observed in 16 % of cases in our study, but this disagrees with a study by Jang et al. where blood culture was positive in 68.4 % of cases.[19]

Our study reports the highest susceptibility of *S. maltophilia* to Minocycline (93 %) followed by Levofloxacin (91 %), Chloramphenicol (91 %) and Trimethoprim-sulfamethoxazole / co-trimoxazole (90 %), Ticarcillin-clavulanic acid (74 %). Another study by Rutter et al.[20] also showed that 91 % of *S. maltophilia* were sensitive to co-trimoxazole. A similar recent review study found increased resistance rates of *S. maltophilia* to Cotrimoxazole and Levofloxacin. According to this review study, the susceptibility determined in different countries was 80 – 99 % for Trimethoprim-sulfamethoxazole / co-trimoxazole and 44 – 97 % for Levofloxacin.[21]

*S. maltophilia* has several characteristics that contribute to its pathogenicity. Biofilm formation is one of the main reasons for the development of antibiotic resistance.[11] The presence of fimbriae and flagella further contribute to biofilm formation. Another factor responsible for *S. maltophilia* virulence is the lipopolysaccharide in the outer membrane that causes colonization.

*S. maltophilia* was isolated predominantly from wards where there was extensive use of antibiotics, and high use of indwelling devices. This bacterium is most frequently associated with respiratory tract infections followed by bloodstream infections. According to the world health organization (WHO), *S. maltophilia* is considered one of the predominant organisms in hospitals causing pneumonia and bacteraemia (BSIs), usually resistant to most antibiotics and with the ability to rapidly change its multi-resistant phenotype.

### Future Challenges

A major difficulty facing clinicians would be to prevent *S. maltophilia´s* ability to adapt the surrounding environment of patients and modified antimicrobial strategies to keep pace with *S. maltophilia´*s evolution. In order to understand the interaction of *S. maltophilia* with the host cell surfaces and antimicrobial defences presented by the host, the improvement of the existing treatment plan must take a microbial ecological approach and analyse its effect on other possible pathogens colonised by *S. maltophilia*.

To avoid encouraging the spread of disinfectant-tolerant *S. maltophilia* strains, the use of disinfectants in hospital settings should be carefully monitored. An increase in the number of immunocompromised persons across the globe due to chemotherapy, steroid therapy, human immunodeficiency virus (HIV) infection, has been predicted.[22]

This expected increase underlines the importance of continuing to track drug resistance in emerging opportunistic pathogens like *S. maltophilia* and understanding genetic transfers between bacteria species.

The above studies can give some insight into altering antibacterial susceptibility trends. Identifying new genetic mechanisms that encourage the persistence in the community and clinical environment of opportunistic microbes can result in new ways of eliminating the survival of these pathogens.

Education and training to raise health care professionals' awareness is a key step in preventing the transmission of these opportunistic pathogens in order to tackle the increasing incidence of *S. maltophilia* infections in hospitals. Interfering with biofilm formation and lowering the risk of contamination in the hospital setting necessitates careful monitoring of aqueous-associated environments, as well as timely cleaning and disinfection of surfaces of medical instruments and equipment that come into direct or indirect contact with patients. Health care staff's hand hygienic practise must be continuously reinforced to minimise the risk of microbial transmission from tap water to patients.

Residual antibiotic/solutions, residual and potentially contaminated hand soap solutions, and patient body fluids should not be disposed of into the hospital plumbing system. A careful inspection for the observation and replacement of damaged parts of susceptible surfaces, such as corroded plumbing line systems, may help to minimize the risk of infection.

Such measures can help in minimise the number of casualties associated with infections with *S. maltophilia*. To control the trend of increasing isolation rate of this pathogen in various clinical specimen, a better understanding of the *S. maltophilia' s* epidemiology, antibiotic susceptibility profile, and clinical outcomes is required. Continuous surveillance and monitoring should be carried out for better care of patients.

## CONCLUSIONS

*S. maltophilia* is becoming an important nosocomial pathogen and its isolation rate is reported to be increasing and alarming. Trimethoprim-sulfamethoxazole (TMP-SMX) still remains the drug of choice. A better understanding of *S. maltophilia*'s epidemiology, antibiotic susceptibility profile, and clinical outcomes is needed to monitor the trend of increasing pathogen isolation rates in various clinical specimens. Continuous surveillance and monitoring should be carried out for better care of patients.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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