Clinical and microbiological characteristics of patients colonized or infected by *Stenotrophomonas maltophilia*: is resistance to sulfamethoxazole/trimethoprim a problem?

Elisa Teixeira Mendes¹, Jorge Isaac Garcia Paez², Juliana Rosa Ferraz², Ana Paula Marchi², Ivan Leonardo Avelino França e Silva³, Marjorie Vieira Batista³, Ana Lucia Munhoz de Lima⁴, Flávia Rossi⁵, Anna Sara Levin⁶,⁷, Silvia Figueiredo Costa⁶,⁷,⁸

ABSTRACT

*Stenotrophomonas maltophilia* has emerged as an important opportunistic pathogen in the last decade. Increased resistance to sulfamethoxazole/trimethoprim (SMX/TMP) has been reported in *S. maltophilia* strains in the past few years, leading to few therapeutic options. We conducted a prospective multicenter study at two Brazilian teaching hospitals that identified *S. maltophilia* isolates and evaluated their antimicrobial susceptibility profile, SMX/TMP resistance genes and their clonality profile. A total of 106 non-repeated clinical samples of *S. maltophilia* were evaluated. Resistance to SMX/TMP was identified in 21.6% of the samples, and previous use of SMX/TMP occurred in 19 (82.6%). PCR detected the *sul1* gene in 14 of 106 strains (13.2%). Of these isolates, nine displayed resistance to SMX/TMP. The resistant strains presented a polyclonal profile. This opportunistic pathogen has emerged in immunocompromised hosts, with few therapeutic options, which is aggravated by the description of emerging resistance mechanisms, although with a polyclonal distribution profile.

KEYWORDS: *Stenotrophomonas maltophilia*. Immunocompromised patients. Sulfamethoxazole/trimethoprim resistance. Gram-negative infection.

INTRODUCTION

The management of *Stenotrophomonas maltophilia* infection is usually challenging as a result of its intrinsic resistance profile to the various classes of antimicrobials¹. *S. maltophilia* produces chromosomal β-lactamases (L1 and L2), which present an impermeable membrane that expresses efflux pumps and acquires additional resistance genes in class 1 integrons². To date, the treatment of choice for infections caused by *S. maltophilia* has been sulfamethoxazole/trimethoprim (SMX/TMP)³. However, resistance to this drug has increased in recent years, mainly due to the spread of *sul1*, *sul2*, and *dfrA* genes⁴.

The aim of this study was to describe the clinical and microbiological characteristics of *S. maltophilia* isolates and to study its antimicrobial susceptibility, presence of resistance genes and clonality profile.

MATERIALS AND METHODS

Study design

A prospective multicenter study was conducted over a 2-year period...
(January 2009 to December 2010) in two Brazilian teaching hospitals: Hospital das Clinicas, Faculty of Medicine, Sao Paulo University (2,000 beds) and an oncology center at Hospital A.C. Camargo (450 beds). We collected clinical samples and evaluated demographic and clinical data of patients colonized or infected by *S. maltophilia*. Patients were classified as colonized by *S. maltophilia* if the isolates were identified from the tip of a central venous catheter (CVC) and/or CVC blood samples and infected based on the Centers for Diseases Control and Prevention (CDC) criteria. The following variables were evaluated: sex, age, hospital unit (nursery, intensive care unit, transplantation unit etc.), underlying diseases, presence of CVC, site of colonization or infection, previous use of SMX/TMP and drug used to treat the *S. maltophilia* infection. A database was created using Epi Info software version 3.5.1 (Centers for Disease Control and Prevention, Atlanta, USA). Variables were evaluated as frequencies, means and medians.

### Microbiology

Isolates were identified by API 20 NE (bioMérieux, Craponne, France). The microdilution method was used to evaluate susceptibility to SMX/TMP, ciprofloxacin, levofloxacin, minocycline, ceftazidime, chloramphenicol and ticarcillin/clavulanate according to the Clinical & Laboratory Standards Institute. The minimum inhibitory concentration (MIC) of tigecycline was determined following the US Food and Drug Administration recommendation for Enterobacteriaceae. Endonuclease-digested genomic DNAs were separated by pulsed-field gel electrophoresis using a CHEF-DR III system (Bio-Rad, Hercules, CA, USA). Genomic DNA was digested with 10 U of SpeI (Fermentas, Waltham, MA, USA). Running conditions were 21 h at 14 °C, with an initial switching time of 1 s and final time of 30 s at 6 V/cm.

A polymerase chain reaction (PCR) was performed to evaluate resistance to SMX/TMP of the 106 samples and to detect the genes *sul1*, *sul2*, and *dfrA1*. The presence of mobile genetic elements with the *int1* and *iscr2* genes was also investigated. The presence of *sul1*, *sul2*, *dfrA1*, *int1*, *iscr2* genes in each strain was assessed using the primers described below (Table 1). After amplification of the genes by PCR, one of the products of each reaction was used to perform a new PCR with the oligonucleotides chosen for gene sequencing. The amplified gene was purified using a GFXTM PCR DNA and Gel Band Purification kit (GE Healthcare, Chicago, IL, USA) according to the manufacturer’s instructions. The DNA quantification was estimated by using the Low DNA Mass Ladder (Invitrogen, Carlsbad, CA, USA) in 2% agarose gel electrophoresis.

Sequencing of three different strains were performed at the human USP genome Institute, using an ABI 3730 DNA Analyzer DNA analysis system (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) and the BigDye Terminator v3.1 Cycle Sequencing Kit (NimaGem, Nijmegen, The Netherlands). The BioEdit software version 7.0.9 (Nucleics Pty Ltd., Woollahra, Australia) was used to perform the analyses. The genetic sequence was compared with BLAST<sup>®</sup>. This study was approved by the Ethics Committee of the two hospitals.

## RESULTS

We evaluated data from 106 patients with *S. maltophilia* during the study period. The mean age was 57 years and 58% were male. There were 24 cases (22.6%) of colonization and 82 cases (77.6%) of infection.

### Clinical samples

A total of 106 non-repeated clinical samples of *S. maltophilia* were evaluated, as described in Table 2. Forty-nine were isolated from blood cultures (46.2%). Table 2 also shows the distribution of *S. maltophilia* isolates according to the site of infection; bloodstream infection was the main site (44.2%) followed by the respiratory tract (34.9%). The patients’ comorbidities suggest some degree of immunosuppression (25.3%) or chronic respiratory disease (37.7%) (Table 2). The antimicrobial susceptibility profile of the strains is shown in Table 3. The strains displayed remarkable resistance to SMX/TMP 23 (21.6%), and 13.3% were resistant to levofloxacin. The MIC50 and MIC90 of the strains were 4.56 μg/mL and 9.12 μg/mL, respectively. The MIC50 and MIC90 of the strains were 4.56 μg/mL and 9.12 μg/mL, respectively. The MIC50 and MIC90 of the strains were 4.56 μg/mL and 9.12 μg/mL, respectively.

### Table 1 - Sequence of primers to amplify int1, sul1, sul2, dfrA and Iscr2 genes and the corresponding molecular weight of the targets.

| Primer | Sequence 5’-3’ | Target |
|--------|----------------|--------|
| Int1   | CGAATTCTTGGCGTTCTTTCGACGTTCCAATGTCCTAACCGCC | 457 |
| Sul1   | ATGTTGACGGGTTGCGATTCTGTACGAGCCATGACGCGC | 840 |
| Sul2   | CTAGGCTATCCTAACCCTCGGCTCTGAATAAACTGCTCATATTTCGG | 810 |
| dfrA   | CTTTGAGCAGCGAGTTGACTCAGCAGGCTACCGTCACCTTGGC | 425 |
| Iscr2  | CGAGGCATAGACTGATACGCTTACCTTCTTACATTGGC | 425 |

*Table 1 - Sequence of primers to amplify int1, sul1, sul2, dfrA and Iscr2 genes and the corresponding molecular weight of the targets.*
method (Table 2). The characteristics of the 23 SMX/TMP-resistant samples are shown in Table 4. Most were isolated at Hospital das Clinicas (91.3%). Nine (39.2%) samples were from tracheal aspirates and 10 were from blood cultures (43.5%). Previous use of SMX/TMP was reported in 19 (82.6%) of the 23 patients, and the main comorbidity was cancer (39.1%). Fourteen (60.8%) patients were admitted to the intensive care unit. The sul-1 gene was found in only 9 of 23 strains (39.1%). The antimicrobial susceptibility of the 23 SMX/TMP-resistant strains are shown in Table 4. All of them showed resistance to other antimicrobials, and 12 (52%) also displayed resistance to levofloxacin. PCR detected the sul1 gene in 14 of 106 strains (13.2%) (Table 2). Of these isolates, nine displayed resistance to SMX/TMP, with MICs ranging from 8 to 128 μg/mL.

Twenty-one strains (19.8%) carried the integrase1 (int1) gene, of which nine displayed resistance to SMX/TMP. The sul1 gene was detected in seven cases (MIC50, 8 μg/mL; MIC90, 128 μg/mL). On the other hand, 14 samples harboring int1 and negative for the sul1 gene presented lower SMX/TMP MICs (MIC50, 2 μg/mL; MIC90, 16 μg/mL). The sul2 gene was detected in only one SMX/TMP-resistant

| Characteristics                  | Total 106, n (%) | SMX/TMT resistance, n (%) |
|----------------------------------|-----------------|---------------------------|
| **Clinical isolates**            |                 |                           |
| Blood                            | 46 (44.3)       | 6 (26)                    |
| Catheter tip                     | 4 (3.8)         | 4 (17.4)                  |
| Tracheal aspirate/bronchoalveolar lavage | 31 (29.2)   | 10 (43.5)                 |
| Pleural fluid                    | 1 (0.9)         | 0                         |
| Urine                            | 11 (10.3)       | 1 (4.3)                   |
| Bone                             | 2 (1.8)         | 1 (4.3)                   |
| Others                           | 9 (8.5)         | 1 (4.3)                   |
| **Site of infection**            |                 |                           |
| Primary bloodstream infection    | 15 (14.1)       | 6 (20)                    |
| Central venous catheter infection| 32 (30.1)       | 4 (12.5)                  |
| Respiratory tract infection      | 37 (34.1)       | 10 (27.7)                 |
| Urinary tract infection          | 11 (10.3)       | 1 (9)                     |
| Osteomyelitis                    | 2 (1.8)         | 1 (50)                    |
| Others                           | 9 (8.5)         | 1 (12.5)                  |
| **Resistance genes**             |                 |                           |
| sul1                             | 14 (13.2)       | 8 (34.8)                  |
| sul2                             | 1 (0.94)        | 1 (4.3)                   |
| dfrA1                            | 1 (0.94)        | 0                         |
| **Mobile genetic elements**      |                 |                           |
| Integron                         | 21 (19.8)       | 9 (39.1)                  |
| Iscr2                            | 1 (0.94)        | 1 (4.3)                   |
| **Underlying disease**           |                 |                           |
| Chronic respiratory disease      | 40 (37.7)       |                           |
| Hematologic malignancy           | 15 (14.1)       |                           |
| Solid tumor                      | 11 (10.3)       |                           |
| Chronic renal insufficiency      | 10 (9.4)        |                           |
| Cardiovascular disease           | 7 (6.6)         |                           |
| Trauma                           | 6 (5.6)         |                           |
| AIDS                             | 1 (0.9)         |                           |
| Others                           | 16 (15.6)       |                           |
strain (MIC, 16 μg/mL), which was sul1 negative. No SMX/TMP susceptible strain was positive for sul2 (Table 4). Of all 106 strains tested, only one that was susceptible to SMX/TMP was positive for the dfra1 gene (MIC, 0.25 μg/mL) (Table 2). ISCR2 was evaluated in the SMX/TMP-resistant strains and was identified only in the strain harboring the sul2 gene.

Figure 1 shows a graphical representation of the genes from a S. maltophilia strain (number 24) carrying sul2 and iscr2 genes. It shows a sequence of 1,713 bp corresponding
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...to the *iscr2* gene, followed by a region of 184 bp representing the phosphoglucomutase (*glmM*) mutase pseudogene and the adjacent 730 bp *sul2* gene. The integron-1 sequence of the sample (number 15) with MIC >128 μg/mL showed an approximate size of 4,000 bp containing the *aac4* and *aadA1* cassette genes and the *qac/sul1* region (Figure 2). A schematic representation of integron class 1 in SMX/TMP-resistant *S. maltophilia* sample (number 13) with MIC of 8 μg/mL is shown in Figure 3. The strain presented in Figure 4 was sequenced (2,000 bp) and contains the *aadA1* cassette gene and the *qac/sul1* terminal region (SMX/TMP MIC, 8 μg/mL). Isolates were assigned the same pulse type if the Dice coefficient value of similarity was 80%8.

**DISCUSSION**

This study described the epidemiological profile, antimicrobial susceptibility, and mechanisms of resistance of 106 strains of *S. maltophilia* in two teaching, tertiary hospitals in Brazil. This opportunistic pathogen has emerged in debilitated hosts, who are often hospitalized for prolonged periods, using invasive devices, and are on broad-spectrum antibiotic therapy3. This profile was confirmed in our series; 63.2% of the infected patients were immunocompromised or had a chronic respiratory disease and 60.8% were admitted to the intensive care unit.

Although *S. maltophilia* is not a highly virulent pathogen9,10, it has a unique ability to colonize the respiratory tract and invasive devices. The main sites of infection in this study were CVC-related infection and lower respiratory tract infection as seen in many other studies9-11.

SMX/TMP is the treatment of choice for this infection, presenting near 90% susceptibility in most centers12,13. However, SMX/TMP resistance has increased in the last decade2,12,14. In our case series, 78.3% of the *S. maltophilia* isolates showed susceptibility to SMX/TMP. Another concern is that, in our study, all strains resistant to SMX/TMP also displayed resistance to other antimicrobials, such as ceftazidime (100%), ticarcicline/clavulanate (87%)...
and levofloxacin (52%). On the other hand, minocycline and tigecycline exhibited good activity in vitro in our study. However, there is a lack of evidence evaluating the clinical efficacy of these antimicrobials to treat severe infections caused by S. maltophilia. Shohaib et al. described broad-spectrum antimicrobial use and previous intensive care unit admission as risk factors for multidrug-resistant S. maltophilia infections. We observed that, among the patients with SMX/TMP-resistant strains, 82.7% had already used this antibiotic previously.

The sul1 gene is the main mechanism of resistance to SMX in S. maltophilia described to date. This gene is in the conserved 3’ region of class 1 integrons, which are located in plasmids ranging in size from 2.1 to 54.2 kbp. Another study described 55 genetically unrelated strains of which 25 were resistant to SMX/TMP, and 17 had the sul1 gene located in the 3’ region of the class-1 integron. The authors asserted that susceptible strains did not contain the sul1 gene. In our series, the sul1 gene was found in 39.1% of SMX/TMP-resistant strains and in only 6% of the 83 susceptible ones, suggesting an association of this gene with resistance to SMX/TMP. The sul1 gene was found in 39.1% of SMX/TMP-resistant strains. Intrinsic mechanisms such as inducible efflux pumps have not been evaluated.

CONCLUSION

In conclusion, this study showed that S. maltophilia infection was observed mostly in severe immunocompromised patients. The most frequent SMX/TMP resistance mechanism was the sul1 gene associated with previous use of this antibiotic. These findings warn on the potential spread of the resistance to SMX/TMP in hospital settings.
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