Stenotrophomonas maltophilia in Malaysia: molecular epidemiology and trimethoprim–sulfamethoxazole resistance

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SUMMARY

Objectives: Stenotrophomonas maltophilia is a recently identified nosocomial pathogen in Malaysia. Despite limited pathogenicity, its rate of isolation has increased in recent years. The aim of this study was to investigate the antibiotic susceptibility patterns, antibiotic resistance determinants, and the epidemiology of S. maltophilia at the largest tertiary care hospital in Malaysia.

Methods: This study was carried out from January to December 2008. Sixty-four S. maltophilia isolates were investigated for their antibiotic susceptibility patterns by disk diffusion test and E-test. The antibiotic resistance mechanism for trimethoprim–sulfamethoxazole (TMP–SMX) was assessed by PCR for sul1, sul2, qac/smr, and class 1 integrons in general. Epidemiological relatedness among isolates was determined by pulsed-field gel electrophoresis (PFGE).

Results: The highest number of S. maltophilia infections was observed in the intensive care unit (ICU) (n = 13; 20.3%), while the lowest number of infections was seen in the neurology, psychiatric, and dermatology wards (each n = 1; 1.6%). All isolates were susceptible to minocycline. One isolate was resistant to TMP–SMX with a minimum inhibitory concentration (E-test) >32 mg/l. The strain carried the sul1 gene and class 1 integron. None of the isolates were positive for the qac/smr genes. Although the data suggest the potential for patient to patient transmission, most of the S. maltophilia strains showed unrelated PFGE patterns and were considered to be genetically diverse.

Conclusion: The increasing number of S. maltophilia isolates seen in the ICU, their resistance to mainstay antibiotics, their genetically diverse nature, and possible cross-transmission within the hospital, strongly underscores the need for continuous surveillance for S. maltophilia in the hospital setting.

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1. Introduction

Stenotrophomonas maltophilia, previously known as Pseudomonas maltophilia or Xanthomonas maltophilia, is found ubiquitously in nature. This opportunistic bacterial species is now an emerging nosocomial pathogen. Its intrinsic or acquired resistance to most antibiotics and its ability to colonize the surfaces of medical devices make S. maltophilia a potentially dangerous pathogen, especially among patients with prolonged hospitalization, malignancy, immune suppression, and breakdown of the mucocutaneous defense barriers (e.g., following catheterization, artificial implantation, tracheotomy, or peritoneal dialysis).1 In severely ill patients, S. maltophilia causes a wide range of infections, including nosocomial pneumonia, bacteremia, pulmonary infections, urinary tract infections, wound infections, skin and soft tissue infections, meningitis, and endocarditis.1–3 Thus S. maltophilia is a highly versatile pathogen.

The management of S. maltophilia infections represents a great challenge to clinicians due to problems with in vitro susceptibility testing, a lack of clinical trials to determine optimal therapy, and its intrinsic resistance to a plethora of antimicrobial agents, which severely limits the effectiveness of commonly used empiric antimicrobial therapies.4,5 Trimethoprim–sulfamethoxazole (TMP–SMX) is the recommended drug for the treatment of S. maltophilia infections based on in vitro susceptibility data, and a favorable outcome has been observed in patients treated with these agents.1,4 TMP–SMX is reported to be more effective than the new fluoroquinolones such as levofloxacin and gatifloxacin.6 However, during recent years,
there have been several reports of the emergence of TMP–SMX-resistant *S. maltophilia*, with a prevalence ranging from 3.8% in Latin America, North America, and Europe to 28.3% in Turkey.7,8 Several studies investigating the molecular epidemiology of *S. maltophilia* have shown clinical isolates to be genetically diverse.7,9

In Malaysia, the occurrence of *S. maltophilia* infection has increased over recent years, from less than 50 cases in 2003 to more than 150 in 2007 at the largest tertiary care hospital. Unfortunately, no published data are available on the antibiotic susceptibility patterns, antibiotic resistance mechanisms, or the genetic diversity and epidemiology of Malaysian *S. maltophilia* strains. As the management of *S. maltophilia* infections is challenging, understanding the characteristics of local strains is necessary for the development of new strategies for the prevention or prophylaxis of such infections. Therefore the aim of the present study was to investigate the antibiotic resistance patterns and genotypes of *S. maltophilia* isolates from clinical specimens in Malaysia.

2. Methods

2.1. Clinical setting and bacterial strains

*S. maltophilia* isolates were collected between January and December 2008 at the largest tertiary care hospital in Malaysia, where an average of 100 *S. maltophilia* isolates are isolated annually. Specimen types and the ward of isolation were recorded. Among isolates collected from January to December 2008, 64 positive cultures from 56 patients (isolate numbers 2 and 3 from patient 2; 6 and 7 from patient 7; 14, 15, 18, and 23 from patient 14; 47 and 48 from patient 47; 41, 42, and 46 from patient 41) were available for investigation at our laboratory (Medical Microbiology, Universiti Putra Malaysia, Serdang). The isolates were phenotypically confirmed as *S. maltophilia* by API 20 NE (bioMérieux, Marcy l’Etoile, France), the presence of lavender green colonies on blood agar plates, and the production of DNase. All isolates previously identified to the species level by phenotypic methods were reconfirmed molecularly by species-specific PCR10 and stored at −80°C in Luria–Bertani broth supplemented with 20% glycerol. *S. maltophilia* ATCC 13637 was used as the reference strain.

2.2. Antibiotic susceptibility testing

Susceptibility to antimicrobial agents was determined by the disk diffusion method. Data obtained for isolates sensitive or resistant to tigecycline, clavulanic acid, ceftazidime, minocycline, and levofloxacin were interpreted in accordance with the Clinical and Laboratory Standard Institute guidelines (CLSI).11 Minimum inhibitory concentration (MIC) values for TMP–SMX were determined by E-test (bioMérieux, Marcy L’Etoile) as per CLSI guidelines.11

2.3. Integrons and sulfonamide and quaternary ammonium compound (QAC) resistance genes PCR

The presence of class 1 integrons, the associated sulfonamide resistance gene (*sul1*), and QAC (antiseptic) resistance genes *qac/smr* were screened in the *S. maltophilia* isolates with primers and PCR conditions described previously.12–14 Template DNA for PCR was prepared by boiling 5–10 colonies of *S. maltophilia* in 25 μl of sterile water for 10–12 min. The boiled suspension was snap-cooled and centrifuged at 10 000 g for 5 min; the supernatant or boiled cell lysate was used as the PCR template. Amplification reactions of 20 μl were prepared with 0.5 μl of template DNA, 5 pmol of forward and reverse primers, and 5 μl of PCR master mix (i-DNA Biotechnology (M) Sdn Bhd, Malaysia). The amplified products were sequenced and further analyzed by GenBank BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4. Molecular typing of *S. maltophilia* isolates by pulsed-field gel electrophoresis (PFGE)

PFGE macro-restriction analysis of isolates was performed essentially by the method of Denton and Kerr.3 *S. maltophilia* DNA was digested with SpeI restriction endonuclease (Fermentas, Axon Scientific, Malaysia) using Xbal-digested Salmonella Braenderup H2812 DNA as a size and gel normalization standard. Electrophoresis was performed in 1% agarose (Seakem Gold, Lonza, Rockland, ME, USA) on a CHEF DRII apparatus (BioRad Laboratories) at 6 V/cm, with switching linearly ramped from 5 to 35 s for 22 h at 14°C. Images of ethidium bromide-stained gels were captured electronically and compared (UPGMA, Dice coefficient) using BioNumerics v6.6 software (Applied Maths, Sint-Martens-Latem, Belgium). PFGE analysis was repeated in triplicate to confirm reproducibility of the banding patterns.

3. Results

3.1. Distribution of *S. maltophilia* among the different wards and clinical sources

*S. maltophilia* strains were isolated from patients admitted to various wards, as listed in Table 1. The highest number of *S. maltophilia* infections was observed in the ICU (n = 13; 20.3%), and the lowest number was seen in the neurology, psychiatric, and dermatology wards (each n = 1; 1.6%).

The largest number of isolates were obtained from tracheal aspirates (n = 25, 39.1%), while only one isolate (1.6%) was obtained from peritoneal fluid and one (1.6%) from bronchoalveolar lavage (Table 2).

**Table 1** Distribution of *Stenotrophomonas maltophilia* isolates based on the ward of isolation (N = 64)

<table>
<thead>
<tr>
<th>Ward</th>
<th>Number of isolates (%)</th>
</tr>
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<tbody>
<tr>
<td>Neurosurgery</td>
<td>9 (14.1%)</td>
</tr>
<tr>
<td>Anesthesiology/ICU</td>
<td>13 (20.3%)</td>
</tr>
<tr>
<td>General medicine</td>
<td>9 (14.1%)</td>
</tr>
<tr>
<td>Pediatric</td>
<td>10 (15.6%)</td>
</tr>
<tr>
<td>Orthopedic</td>
<td>3 (4.7%)</td>
</tr>
<tr>
<td>Urology and nephrology</td>
<td>12 (18.8%)</td>
</tr>
<tr>
<td>Surgery</td>
<td>3 (4.7%)</td>
</tr>
<tr>
<td>Respiratory institute</td>
<td>2 (3.1%)</td>
</tr>
<tr>
<td>Neurology</td>
<td>1 (1.6%)</td>
</tr>
<tr>
<td>Psychiatric</td>
<td>1 (1.6%)</td>
</tr>
<tr>
<td>Radiotherapy and oncology</td>
<td>1 (1.6%)</td>
</tr>
</tbody>
</table>

ICU, intensive care unit.

**Table 2** Distribution of *Stenotrophomonas maltophilia* isolates based on the specimen type (N = 64)

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>Number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal aspirate</td>
<td>25 (39.1%)</td>
</tr>
<tr>
<td>Pus</td>
<td>10 (15.6%)</td>
</tr>
<tr>
<td>CSF</td>
<td>9 (14.1%)</td>
</tr>
<tr>
<td>Sputum</td>
<td>7 (10.9%)</td>
</tr>
<tr>
<td>Blood</td>
<td>6 (9.4%)</td>
</tr>
<tr>
<td>Urine</td>
<td>4 (6.3%)</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>1 (1.6%)</td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td>1 (1.6%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1.6%)</td>
</tr>
</tbody>
</table>

CSF, cerebrospinal fluid.
3.2. Antibiotic resistance

All isolates were susceptible to minocycline. Resistance was observed for ceftazidime (56.3%), ticarcillin–clavulanic acid (31.3%), and levofloxacin (32.8%). One isolate was resistant to TMP–SMX, with an E-test MIC value >32 mg/l; this isolate possessed the sul1 gene and class 1 integrons and was also resistant to ceftazidime. None of the isolates carried qac/smr genes.

3.3. Integrons and sulfonamide and quaternary ammonium compound (QAC) resistance

Among the 64 isolates screened for class 1 integrons and sul1 and qac/smr genes, one isolate (isolate 4) phenotypically resistant to TMP–SMX was positive for integron and sul1 genes. All of the isolates were negative for antiseptic resistance genes.

Figure 1. The PFGE profile of 63 Stenotrophomonas maltophilia isolates isolated in different wards and from different clinical sources, showing 59 distinct patterns.
3.4. Molecular typing of *S. maltophilia* isolates

In general, the PFGE analysis (Figure 1) revealed the *S. maltophilia* isolates to be unrelated; 63 isolates yielded 59 distinct patterns. Multiple isolates from the same patient (i.e., isolates 14, 15, 18, and 23 from patient 14; 47 and 48 from patient 47), the same body site, or recovered at different times over the course of the same admission, were found to be genetically diverse, implicating more than one strain in the infection. However, there were instances where indistinguishable isolate pairs were recovered from the same or different body sites of patients over the course of the same admission (e.g., isolates 2 [peritoneal fluid] and 3 [blood] from patient 2; isolates 6 and 7 [both from pus] from patient 6). Isolates that were homologous (e.g. isolates 14 and 19) were also cultured from different patients on different wards.

4. Discussion

In the current study, *S. maltophilia* strains isolated over a 1-year period at the largest public hospital of Kuala Lumpur were examined. As noted by others,15,16 *S. maltophilia* was found as a high-risk nosocomial pathogen in the ICU (Table 1). This unfortunate trend is reinforced by the generally weak and immune-compromised state of ICU patients and other factors including increased length of stay, mechanical ventilator support, and inadequate empiric antibiotic therapy.

With regard to antibiotic susceptibility, all isolates were found to be susceptible to minocycline. However, for the other antibiotics tested the susceptibility varied from 20% to 68%. An extensive review by Nicodemo and Paez17 demonstrated *S. maltophilia* susceptibility to minocycline to be >80%, while the susceptibility to ticarcillin–clavulanic acid, the second therapeutic option, was >70%. Gesu et al.18 in an in vitro study reported *S. maltophilia* susceptibility to levofloxacin at 85.5%. Since *S. maltophilia* isolates in Malaysia are susceptible to minocycline and resistance to levofloxacin and ticarcillin–clavulanic acid is low, these antibiotics are considered good therapeutic agents against infection in this region, as also suggested by Nicodemo and Paez.17

With less than 5% resistance, TMP–SMX remains the therapy of choice against *S. maltophilia* worldwide.19 However, a 6-year study has reported increasing (84%) resistance to TMP–SMX.20 In the current study, only one of the 64 isolates tested (1.6%), a strain isolated from a CSF sample, showed resistance to TMP–SMX, with a MIC >32 mg/l. This result is in accordance with that of Barbolla et al.21 who reported less than 1% resistance to TMP–SMX. Although TMP–SMX is the first choice for the treatment of *S. maltophilia* infections, the combination of TMP–SMX with ticarcillin–clavulanate, or TMP–SMX plus tobramycin, or TMP–SMX plus ciprofloxacin is reported to be more effective with a more pronounced bactericidal activity.19,21–23

Several studies performed on *S. maltophilia* isolates have shown that sul1 genes associated with class 1 integrons are the major mechanism of TMP–SMX resistance. In a survey of 55 *S. maltophilia* isolates (30 sensitive and 25 resistant) by PCR, Toleman et al.20 found that 17 of 25 resistant isolates possessed the sul1 gene and class 1 integrons. Similarly Chang et al.24 reported that 26 out of 100 (26%) *S. maltophilia* isolates were resistant to TMP–SMX, with 81% sul1-positive and carrying class 1 integron. In another study in Taiwan,25 an increased class 1 integron presence in *S. maltophilia* isolates (15 out of 17, 88%) was demonstrated, with 73% (n = 11) carrying the sul1 gene. These data underscore the high prevalence of class 1 integrons in TMP–SMX-resistant clinical isolates of *S. maltophilia*.

Resistance to TMP–SMX has also been be associated with the presence of QAC resistance genes such as smr, qacE, and qacH, which are also harbored by class 1 integrons.24 Chang et al.24 have shown that QAC resistance genes (including qac/smr carried on a class 1 integron) are significantly associated with resistance to TMP–SMX in Taiwan. A high incidence of QAC resistance is favored when biocides containing QACs are used above recommended concentrations for treatment and decontamination in hospitals.26 Despite the routine use of antiseptics such as chlorhexidine (4%) for patients and 70% alcohol for surface disinfection at the large tertiary hospital in the current study, no resistance to QAC compounds was detected in *S. maltophilia* isolates.

In the current study, 63 *S. maltophilia* isolates were available for epidemiological analysis by PFGE (Figure 1) with results demonstrating a high level of diversity among isolates despite similar body sites and wards of isolation. These results are in accordance with earlier studies demonstrating an elevated genetic diversity in *S. maltophilia* isolates even when recovered from the same hospital.27,28 For example, Valdezate et al.29 found five phylogenic clusters with diversity ranging from low (28.0%) to high similarity (80.0%). Although the isolates examined here exhibited high heterogeneity, the recovery of homologous isolates from different patients on different wards (e.g., isolates 14 and 19, 21 and 23, and 35 and 38) shows the possibility of transmission within the hospital, which in the future could certainly include TMP–SMX-resistant strains. A study by Junna et al.30 found that 16 out of 21 *S. maltophilia* isolates had distinct PFGE patterns, although three clusters with more than 95% similarity were detected, two isolated from the same patient while the other contained two strains isolated from two different patients from different wards and at different time points. These findings further reinforce the conclusion that while multiple sites (routes) of *S. maltophilia* acquisition are more common, cross-transmission is also possible.

In conclusion, the results of the current study show that minocycline and TMP–SMX continue to be the best therapeutic options for the treatment of *S. maltophilia* infections in the Malaysian setting, although resistance to the mainstay antibiotic TMP–SMX was observed in one isolate. Epidemiologically, *S. maltophilia* strains are genetically diverse and their emergence is common. However, the isolation of homologous isolates from different patients and different wards demonstrates that cross-transmission of strains across wards is also possible. Hence, management of these infections is problematic as strains may not behave uniformly and transmission may include multiple drug-resistant isolates. These results support efforts directed towards continuous surveillance for antimicrobial drug resistance and epidemiological monitoring, which may act as early warning systems for predicting resistance and preventing outbreaks.

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Conflict of interest: None to declare.

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