Communication

Clonal dissemination and resistance genes among *Stenotrophomonas maltophilia* in a Greek University Hospital during a four-year period

Matthaios Papadimitriou-Olivgeris\(^1,2\), Fevronia Kolonitsiou\(^3\), Maria Militsopoulou\(^3\), Iris Spiliopoulou\(^3\) and Nikolaos Giormezis\(^3\)*

\(^1\) Division of Infectious Diseases, School of Medicine, University of Patras, Patras, Greece
\(^2\) Infectious Diseases Service, Lausanne University Hospital, Lausanne, Switzerland
\(^3\) Department of Microbiology, School of Medicine, University of Patras, Patras, Greece

* Correspondence: Email: giormenik@yahoo.gr; Tel: +00302610996111.

Abstract: Treatment of *Stenotrophomonas maltophilia* infections comprises of sulfamethoxazole/tripethoprim (SXT) or fluoroquinolones. We investigated antimicrobial resistance, presence of resistance genes (*sul1, smqnr*) and clonal dissemination in *S. maltophilia* from a university hospital. Among 62 isolates, 45 (73%) represented infection. Two isolates (3%) were resistant to SXT and three (5%) to levofloxacin. Twenty-nine isolates (47%), including two out of three levofloxacin-resistant, carried *smqnr*. Resistance of *S. maltophilia* was low and was not associated with *sul1* or *smqnr* carriage. Although high degree of genetic diversity was identified (29 pulsotypes), 22/62 (35.5%) strains were classified into four clones; clone b was associated with bacteraemias.

Keywords: *Stenotrophomonas maltophilia*; resistance; infections; clones; bacteraemia

1. Introduction

*Stenotrophomonas maltophilia* is an emerging pathogen which can be found in the environment but is also able to cause infections in immunocompromised patients, critically ill patients and those suffering from cystic fibrosis. Despite its susceptibility to antimicrobials, it has emerged in the last decades as an important nosocomial pathogen with mortality rates between 14 and 69% in bacteraemic patients [1]. Risk factors for infection include prolonged hospitalization, especially in Intensive Care Units, previous antibiotic treatment, chronic respiratory disease, prolonged endotracheal intubation
and the presence of a central venous catheter [1,2].

Sulphamethoxazole/trimethoprim (SXT) remains the treatment of choice for *Stenotrophomonas* infections, whereas, fluoroquinolones are the second-line drug. Treatment can be complicate by the transfer and acquisition of antimicrobial resistance, since mobile genetic elements, such as transposons and plasmids, carry resistance genes [3]. Carriage of *sul1* and *sul2* genes as part on integron I has been associated with resistance to SXT [4]. Another gene family, known as *smqnr* encodes proteins associated with resistance to fluoroquinolones [5].

The aim of this study was to investigate possible clonal dissemination, antimicrobial resistance patterns and the presence of resistance genes among *S. maltophilia* in a Greek University Hospital.

2. Materials and methods

The retrospective study was conducted in the University General Hospital of Patras (UGHP), Greece during a four-year period (2014–2017). UGHP is a 800-bed tertiary hospital in Southwestern Greece. The Hospital Ethics Committee approved the study and waived the need for informed consent (HEC No: 785).

All *S. maltophilia* strains isolated from various clinical specimens (blood, bronchial aspirations, intravenous catheters and wounds) were identified to species level by the Vitek 2 Advanced Expert System (bioMerieux, Marcy l’Etoile, France). Infection or colonization was distinguished according to clinical diagnoses. Colonization was defined as the presence of *S. maltophilia* on the respiratory system without causing adverse clinical signs or symptoms and no specific antimicrobial treatment was initiated by the treating physician. Minimum inhibitory concentration (MIC) of SXT was determined by E-test (bioMerieux) and susceptibility against levofloxacin was tested by the disk diffusion method according to CLSI guidelines [6]. Amplification of the resistance genes *sul1*, *sul2* and *smqnr* was performed by PCRs with specific primers, as published [7].

Strains were classified into pulsotypes by pulsed-field gel electrophoresis (PFGE) of chromosomal DNA *XbaI* digests (Promega Corporation) performed in a CHEF DR III apparatus (Bio-Rad, Richmond, CA). PFGE was performed under the following conditions: initial switch time 5 s, final switch time 5 s, voltage 6V/cm, included angle 120°, run time 23 hours. A dendrogram comparing molecular weights of strains’ DNA fragments was performed by FPQuest software version 4.5 (Bio-Rad Laboratories Inc). Patterns differing by less than 79% (corresponding to a difference of less than seven bands) were considered to belong to the same PFGE type [8].

Risk factors for *S. maltophilia* infection as compared to colonization were studied in patients that medical records were available. Epidemiological data, comorbidities, antimicrobial administration, and mortality prediction were obtained from patients’ chart reviews.

SPSS version 23.0 (SPSS, Chicago, IL) software was used for data analysis. Categorical variables were analyzed by using the chi-square or Fisher exact test. All statistic tests were 2-tailed and *P* < 0.05 was considered statistically significant.

3. Results

In total, sixty-two isolates were included (one per-patient) deriving from bloodstream infections (BSIs, *n* = 26; 42%), surgical site infections (SSIs, *n* = 13; 21%), catheter-related infections (CRIs, *n* = 6; 10%) or colonization of the respiratory tract (17; 27%). The majority was recovered from patients
hospitalized in medical wards (n = 23; 37%), followed by adult ICU (19; 31%), surgical wards (11; 18%), emergency department (5; 8%) and paediatric ICU (4; 6%).

Two strains (3.2%) were resistant to SXT (MIC: 32 mg/L) and three (4.8%) to levofloxacin. Five strains carried sul1 including both SXT-resistant and three SXT-susceptible ones, whereas all isolates were negative for sul2. Twenty-nine strains (46.8%), including two out of three levofloxacin-resistant, carried smqnr. Four S. maltophilia strains carried both sul1 and smqnr (Table 1). No significant difference among infective and colonizing isolates was identified regarding antimicrobial resistance or genes’ carriage.

**Table 1.** Clonal distribution in relation to infection type/colonization and resistant determinants of studied isolates.

| Clones | Infection type/Colonization | SXT-Resistant | Levofloxacin-Resistant | sul1 | smqnr |
|--------|-----------------------------|---------------|------------------------|------|-------|
| a (6)  | BSI (2)                     | -             | -                      | -    | 2     |
|        | SSI (2)                     | -             | -                      | -    | 2     |
|        | Colonization (2)            | 1             | -                      | 1    | 1     |
| b (6)  | BSI (6)                     | -             | -                      | -    | -     |
| c (5)  | BSI (1)                     | -             | -                      | -    | -     |
|        | SSI (1)                     | -             | -                      | -    | -     |
|        | Colonization (3)            | -             | -                      | -    | 3     |
| d (6)  | BSI (1)                     | -             | -                      | -    | -     |
|        | SSI (1)                     | -             | -                      | -    | 1     |
|        | CRI (2)                     | -             | -                      | -    | -     |
|        | Colonization (2)            | -             | -                      | -    | 2     |
| Others (39) | BSI (16) | -             | -                      | 1    | 6     |
|         | SSI (9)                     | 1             | 2                      | 2    | 5     |
|         | CRI (4)                     | -             | -                      | -    | 2     |
|         | Colonization (10)           | -             | 1                      | 1    | 5     |
| Total (62) |                   | 2             | 3                      | 5    | 29    |

*Note: Infection or colonization was distinguished according to clinical diagnoses. Colonization was defined as the presence of S. maltophilia on the respiratory system without causing adverse clinical signs or symptoms. Number of isolates are presented in parentheses. BSI: Bloodstream infection, SSI: Surgical site infection, CRI: Catheter-related infection.*

Twenty-nine pulsotypes were identified by PFGE, with 23 out of 62 (37.1%) strains classified into four main clones, consisting of five or six strains each (Figure 1). The remaining 39 strains were classified into 25 PFGE types, including one or two strains each. No clonal relationship was identified regarding antimicrobial resistance patterns, genes’ carriage, or hospital wards. However, a statistically significant association was found for strains of pulsotype b that were exclusively recovered from bacteraemic patients (P = 0.004).

Medical records were available for 45 patients (27 infected and 18 colonized). No statistical difference was observed among comorbidities, immunosuppression or presence of resistant genes. Patients with S. maltophilia infection were more frequent exposed to ceftazidime/avibactam (48% vs 6%);
No difference in 30-day mortality was observed among patients with infection vs colonization (Table 2).

Figure 1. Dendrogram of representative S. maltophilia strains.

4. Discussion

Resistance to the two main antibiotics used for the treatment of S. maltophilia infections, SXT and levofloxacin, was low (3.2% and 4.8%, respectively) [9]. In our study presence of the studied resistance genes (sul1, smqnr) did not confer phenotypic resistance to SXT or levofloxacin, since some phenotypically susceptible strains carried those genes. This finding is in accordance to the literature, since sul1 and/or smqnr were commonly detected in both resistant and susceptible isolates [7,10–12]. Treatment of infected patients with S. maltophilia was successful, even for strains carrying resistance genes. Infective and colonizing isolates were both highly susceptible to SXT and levofloxacin, in accordance to a study by Juhász et al, where a low resistance rate was also identified in both groups [13]. Carriage of sul1 was low (8.06%) and all our strains tested negative for sul2, whereas in other studies worldwide both genes have been detected in clinical isolates [14]. sul2 has even been reported as commoner than sul1 in one study from 106 strains in India [15].
Table 2. Univariate analysis of risk factors for infection by *S. maltophilia* as compared to colonization.

| Characteristics                          | *S. maltophilia* colonization (n = 18) | *S. maltophilia* infection (n = 27) | P   |
|-----------------------------------------|---------------------------------------|------------------------------------|-----|
| Days at riska                           | 36.8 ± 33.9                           | 31.8 ± 21.0                        | 0.613|
| Demographics                            |                                       |                                    |     |
| Age (years)                             | 62.6 ± 13.0                           | 58.2 ± 13.2                        | 0.338|
| Male gender                             | 10 (63%)                              | 10 (59%)                           | 1.000|
| Chronic diseases                        |                                       |                                    |     |
| Diabetes Mellitus                       | 3 (19%)                               | 1 (6%)                             | 0.335|
| Chronic Obstructive Pulmonary Disease   | 0 (0%)                                | 0 (0%)                             | -    |
| Chronic Heart Failure                   | 1 (6%)                                | 1 (6%)                             | 1.000|
| Chronic Renal Failure                   | 0 (0%)                                | 3 (18%)                            | 0.227|
| Malignancy                              | 1 (6%)                                | 2 (12%)                            | 1.000|
| Immunosuppression                       | 1 (6%)                                | 2 (12%)                            | 1.000|
| Obesity (BMI ≥ 30kg/m²)                 | 3 (19%)                               | 5 (29%)                            | 0.688|
| Charlson Comorbidity Index              | 3.8 ± 1.6                             | 3.3 ± 2.5                          | 0.492|
| Admission data                          |                                       |                                    |     |
| APACHE II Score upon admission          | 20.0 ± 4.4                            | 18.0 ± 4.6                         | 0.271|
| Prior surgery (prior month)             | 7 (44%)                               | 9 (53%)                            | 0.732|
| Antibiotic administration (prior month) |                                       |                                    |     |
| Penicillin/beta-lactamase inhibitors    | 9 (56%)                               | 4 (24%)                            | 0.080|
| 3rd- and 4th-generation cephalosporins  | 2 (13%)                               | 4 (24%)                            | 0.656|
| Ceftazidime/avibactam                   | 1 (6%)                                | 13 (48%)                           | 0.003|
| Carbapenems                             | 13 (81%)                              | 16 (94%)                           | 0.335|
| Quinolones                              | 2 (13%)                               | 4 (24%)                            | 0.656|
| Colistin                                | 8 (50%)                               | 14 (82%)                           | 0.071|
| Aminoglycosides                         | 5 (31%)                               | 9 (53%)                            | 0.296|
| Tigecycline                             | 4 (25%)                               | 7 (41%)                            | 0.465|
| Glucopeptides                           | 14 (88%)                              | 10 (59%)                           | 0.118|
| Linezolid                               | 5 (31%)                               | 7 (41%)                            | 0.721|
| ICU procedures                          |                                       |                                    |     |
| Corticosteroid administration           | 10 (63%)                              | 13 (77%)                           | 0.465|
| Parenteral nutrition                    | 8 (50%)                               | 6 (35%)                            | 0.491|
| Enteral nutrition                       | 9 (56%)                               | 11 (65%)                           | 0.728|
| Microbiologic data                      |                                       |                                    |     |
| Presence of *smqnr* gene (among 21 patients) | 5 (56%) | 7 (58%) | 1.000|
| Presence of *sul* gene (among 21 patients) | 2 (22%) | 3 (25%) | 1.000|
| Outcome                                 |                                       |                                    |     |
| 30-day mortality                        | 7 (44%)                               | 4 (24%)                            | 0.282|

*Note: Data are number (%) of patients or mean ± standard deviation. APACHE II: Acute Physiology and Chronic Health Evaluation II. a Length of stay until infection or colonization.

*S. maltophilia* strains isolated from UGHP patients showed major genetic diversity. (29
pulsotypes), as previously reported [9]. This genetic diversity could be attributed to the colonization of patients before or after their admission to the hospital. No correlation between specific clones and resistance to aforementioned antibiotics or carriage of resistance genes was found. The isolation of genetically similar strains, belonging to four main clones, from different patients hospitalized in various wards raised the possibility of transmission within the hospital, but no specific link could be established among personnel or patients’ transfer. One of the major clones, pulsotype b, consisted of six strains isolated exclusively from blood infections ($P = 0.004$). Another pulsotype comprised of three isolates derived also from BSIs, but no other statistically significant correlation between clones and infection type was identified. In a previous study of bacteraemias at the UGHP, *S. maltophilia* represented 0.8% of all bloodstream infections, percentage comparable to that from a point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals (1%) [16,17]. In another study by Valdezade et al., 139 *S. maltophilia* isolates recovered from hospitalized non-cystic fibrosis patients were classified in 99 distinct PFGE profiles, but despite this genetic diversity a few clones were transmitted among different patients causing outbreaks [18].

At the exception of ceftazidime/avibactam, no difference was observed among patients with infection and colonization. The low number of patients might explain the absence of association of typical risk factors, such as immunosuppression, prior carbapenem treatment, and *S. maltophilia* infection [19]. The fact that prior ceftazidime/avibactam use was associated with *S. maltophilia* infection was striking, since ceftazidime/avibactam retains some activity against *S. maltophilia* strains [20].

Our study has limitations since it was performed in a single center, the number of *S. maltophilia* strains was relatively low and PCR was performed only for the main, and not all, genetic determinants conferring resistance to SXT and levofloxacin. Another limitation was the low number of patients for which clinical data were available.

5. Conclusions

*S. maltophilia* isolates presented low rates of resistance to SXT or levofloxacin that was not associated with the presence of *sul1*, *sul2* or *smqnr* genes. Even though a high degree of genetic diversity was found, a statistically important correlation of a specific clone with blood infections was detected.

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. Brooke JS (2012) *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clin Microbiol Rev* 25: 2–41. https://doi.org/10.1128/CMR.00019-11
2. Lai CH, Chi CY, Chen HP, et al. (2004) Clinical characteristics and prognostic factors of patients with *Stenotrophomonas maltophilia* bacteremia. *J Microbiol Immunol Infect* 37: 350–358. Available from: https://pubmed.ncbi.nlm.nih.gov/15599467/
3. Chang LL, Chen HF, Chang CY, et al. (2004) Contribution of integrons, and SmeABC and SmeDEF efflux pumps to multidrug resistance in clinical isolates of *Stenotrophomonas maltophilia*. *J Antimicrob Chemother* 53: 518–521. https://doi.org/10.1093/jac/dkh094

4. Hu LF, Chang X, Ye Y, et al. (2011) *Stenotrophomonas maltophilia* resistance to trimethoprim/sulfamethoxazole mediated by acquisition of sul and dfrA genes in a plasmid-mediated class 1 integron. *Int J Antimicrob Agents* 37: 230–234. https://doi.org/10.1016/j.ijantimicag.2010.10.025

5. Malekan M, Tabaraie B, Akhoundtabar L, et al. (2017) Distribution of class I integron and smqnr resistance gene among *stenotrophomonas maltophilia* isolated from clinical samples in Iran. *Avicenna J Med Biotechnol* 9: 138–141. Available from: https://pubmed.ncbi.nlm.nih.gov/28706609/

6. Clinical and Laboratory Standards Institute (2020) Performance standards for antimicrobial susceptibility testing. In: CLSI supplement M100, 30 Eds., Wayne: PA. Available from: https://www.clsi.org/standards/products/microbiology/documents/m100/

7. Bostanghadiri N, Ghalavand Z, Fallah F, et al. (2019) Characterization of phenotypic and genotypic diversity of *Stenotrophomonas maltophilia* strains isolated from selected hospitals in Iran. *Front Microbiol* 10: 1191. https://doi.org/10.3389/fmicb.2019.01191

8. Tenover FC, Arbeit RD, Goering RV, et al. (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33: 2233–2239. https://doi.org/10.1128/jcm.33.9.2233-2239.1995

9. Neela V, Rankouhi SZ, van Belkum A, et al. (2012) *Stenotrophomonas maltophilia* in Malaysia: molecular epidemiology and trimethoprim-sulfamethoxazole resistance. *Int J Infect Dis* 16: e603–e607. https://doi.org/10.1016/j.ijid.2012.04.004

10. Song JH, Sung JY, Kwon KC, et al. (2010) Analysis of acquired resistance genes in *Stenotrophomonas maltophilia*. *Korean J Lab Med* 30: 295–300. https://doi.org/10.3343/kjlm.2010.30.3.295

11. Hu LF, Chen GS, Kong QX, et al. (2016) Increase in the prevalence of resistance determinants to trimethoprim/sulfamethoxazole in clinical *Stenotrophomonas maltophilia* isolates in China. *PloS One* 11: e0157693. https://doi.org/10.1371/journal.pone.0157693

12. Kanamori H, Yano H, Tanouchi A, et al. (2015) Prevalence of smqnr and plasmid-mediated quinolone resistance determinants in clinical isolates of *Stenotrophomonas maltophilia* from Japan: novel variants of Smqnr. *New Microbes New Infect* 7: 8–14. https://doi.org/10.1016/j.nmni.2015.04.009

13. Juhász E, Krizsán G, Lengyel G, et al. (2014) Infection and colonization by *Stenotrophomonas maltophilia*: antimicrobial susceptibility and clinical background of strains isolated at a tertiary care centre in Hungary. *Ann Clin Microbiol Antimicrob* 13: 333. https://doi.org/10.1186/s12941-014-0058-9

14. Ebrahim-Saraie HS, Heidari H, Soltani B, et al. (2019) Prevalence of antibiotic resistance and integrons, sul and Smqnr genes in clinical isolates of *Stenotrophomonas maltophilia* from a tertiary care hospital in Southwest Iran. *Iran J Basic Med Sc* 22: 872–877. https://doi.org/10.22038/ijbms.2019.31291.7540

15. Kaur P, Gautam V, Tewari R (2015) Distribution of class 1 integrons, sul1 and sul2 genes among clinical isolates of *Stenotrophomonas maltophilia* from a tertiary care hospital in North India. *Microbial drug resistance (Larchmont,N.Y.)* 21: 380–385. https://doi.org/10.1089/mdr.2014.0176
16. Kolonitsiou F, Papadimitriou-Olivgeris M, Spiliopoulou A, et al. (2017) Trends of bloodstream infections in a university greek hospital during a three-year period: Incidence of multidrug-resistant bacteria and seasonality in gram-negative predominant. Pol J Microbiol 66:171–180. https://doi.org/10.5604/01.3001.0010.7834

17. European Centre for Disease Prevention and Control (2013) Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals. https://doi.org/10.2807/ese.17.46.20316-en

18. Valdezate S, Vindel A, Martín-Dávila P, et al. (2004) High genetic diversity among Stenotrophomonas maltophilia strains despite their originating at a single hospital. J Clin Microbiol 42: 693–699. https://doi.org/10.1128/JCM.42.2.693-699.2003

19. Wang N, Tang C, Wang L (2022) Risk factors for acquired Stenotrophomonas maltophilia pneumonia in intensive care unit: A systematic review and meta-analysis. Front Med 8: 808391. https://doi.org/10.3389/fmed.2021.808391

20. Lin Q, Zou H, Chen X, et al. (2021) Avibactam potentiated the activity of both ceftazidime and aztreonam against S. maltophilia clinical isolates in vitro. BMC Microbiol 21: 60. https://doi.org/10.1186/s12866-021-02108-2

© 2022 the Author(s), licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0).