


RESEARCH

Open Access



Evaluation of a *Stenotrophomonas maltophilia* bacteremia cluster in hematopoietic stem cell transplantation recipients using whole genome sequencing

Stefanie Kampmeier^{1*} , Mike H. Pillukat¹, Aleksandra Pettke^{2,3}, Annelene Kossow¹, Evgeny A. Idelevich² and Alexander Mellmann¹

Abstract

Background: *Stenotrophomonas maltophilia* ubiquitously occurs in the hospital environment. This opportunistic pathogen can cause severe infections in immunocompromised hosts such as hematopoietic stem cell transplantation (HSCT) recipients. Between February and July 2016, a cluster of four patients on the HSCT unit suffered from *S. maltophilia* bloodstream infections (BSI).

Methods: For epidemiological investigation we retrospectively identified the colonization status of patients admitted to the ward during this time period and performed environmental monitoring of shower heads, shower outlets, washbasins and toilets in patient rooms. We tested antibiotic susceptibility of detected *S. maltophilia* isolates. Environmental and blood culture samples were subjected to whole genome sequence (WGS)-based typing.

Results: Of four patients with *S. maltophilia* BSI, three were found to be colonized previously. In addition, retrospective investigations revealed two patients being colonized in anal swab samples but not infected. Environmental monitoring revealed one shower outlet contaminated with *S. maltophilia*. Antibiotic susceptibility testing of seven *S. maltophilia* strains resulted in two trimethoprim/sulfamethoxazole resistant and five susceptible isolates, however, not excluding an outbreak scenario. WGS-based typing did not result in any close genotypic relationship among the patients' isolates. In contrast, one environmental isolate from a shower outlet was closely related to a single patient's isolate.

Conclusion: WGS-based typing successfully refuted an outbreak of *S. maltophilia* on a HSCT ward but uncovered that sanitary installations can be an actual source of *S. maltophilia* transmissions.

Keywords: *Stenotrophomonas maltophilia*, Outbreak investigation, Whole genome sequence-based typing, Immunocompromised hosts

Background

Stenotrophomonas maltophilia, an intrinsically multidrug resistant gram negative pathogen, is widely distributed in aqueous habitats such as, sink drains, endoscopes and hemodialysis water within clinical settings [1, 2]. Usually, this pathogen is not highly virulent in immunocompetent individuals but can cause severe infections including

bacteremia, peritonitis and meningitis in immunocompromised hosts resulting in complications, e.g. septic shock, respiratory failure, tissue necrosis and septic thrombophlebitis [3]. Recipients of hematopoietic stem cell transplantations (HSCT) are especially at risk and suffer from pulmonary hemorrhage resulting in higher mortality rates compared to non-HSCT patients [4].

In recent years, an increasing incidence of *S. maltophilia* is reported on oncologic wards [5]. Moreover, several outbreaks and pseudo-outbreaks caused by this pathogen have been reported [6, 7]. In addition to epidemiological

* Correspondence: Stefanie.Kampmeier@ukmuenster.de

¹Institute of Hygiene, University Hospital Münster, Robert-Koch-Strasse 41, 48149 Münster, Germany

Full list of author information is available at the end of the article

investigations, different typing methods, e.g. pulse field gel electrophoresis (PFGE) and multi locus sequence typing (MLST) are used to identify the genetical relationship among different *S. maltophilia* isolates [8–11]. These techniques are useful in excluding outbreak scenarios, if MLST sequence types (ST) or PFGE patterns of isolated pathogens differ. In case of identical STs or highly similar PFGE patterns both methods reach their discriminatory limits. In these cases an outbreak is assumed, although more precise methods uncover and accidental cluster. Additionally, inter-laboratory comparability is limited using PFGE. Therefore, whole genome sequence-based typing (WGS) approaches are increasingly seen as gold standard method for highly discriminatory typing [12] and the superiority of WGS in comparison to other typing methods was already demonstrated for several bacterial pathogens [13–17]. Moreover, we could recently demonstrate the high reproducibility of WGS, which is another prerequisite for the applicability in clinical routine [18].

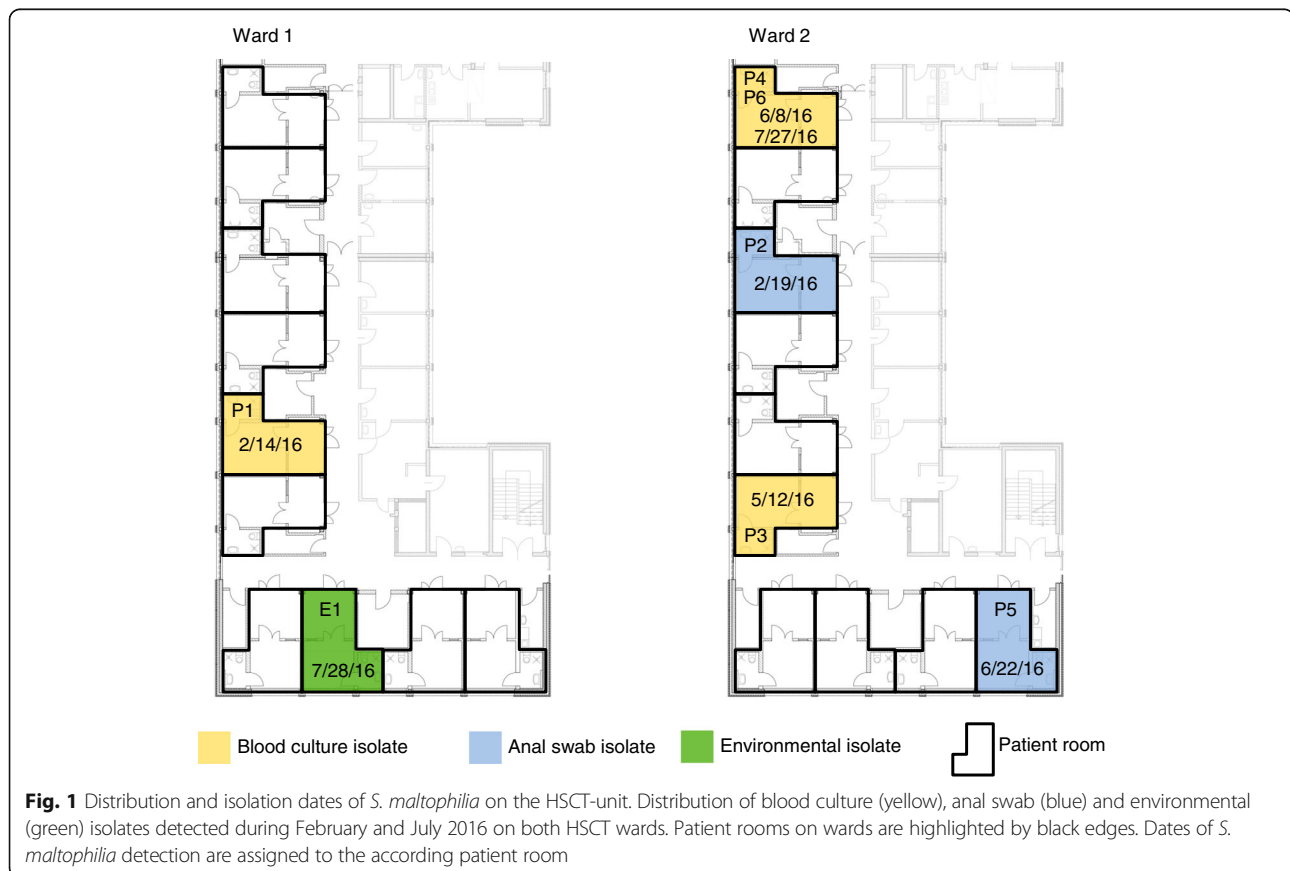
Here we evaluated a cluster of *S. maltophilia* bacteremia in recipients of allogeneic stem cell grafts using WGS-based typing.

Methods

Cluster detection, epidemiological investigations and infection control measures

The 1500-bed University Hospital Münster includes two HSCT-units each comprising 10 patient rooms, which are all HEPA-filtered and equipped with separated bathrooms for each patient (Fig. 1). In total, 193 patients were admitted to the HSCT-units during 2016. Patients usually receive an allogeneic HSCT.

S. maltophilia bloodstream infections (BSI) were defined as one or more blood cultures positive for *S. maltophilia* obtained from patients with clinical signs of infection (fever >38 °C, chills, hypotension) according to the criteria given by the European Centre for Disease Prevention and Control [19]. Four *S. maltophilia* BSI were detected in patients (P1, P3, P4, P6) in the HSCT ward between February and July 2016. As this number exceeds the average baseline of 0.5 *S. maltophilia* BSI per year detected during 2011–2015, an epidemiological investigation was initiated. Routine environmental screening of aqueous habitats (shower heads, shower outlets, washbasins and toilets) within patients' rooms in the HSCT unit, normally focusing on multidrug resistant



Pseudomonas aeruginosa, was expanded to *S. maltophilia*. Patients colonized with *S. maltophilia*, coincidentally detected in anal/rectal swabs or stool samples (as screening of HSCT-patients concentrates on multidrug resistant *Pseudomonas aeruginosa*), were retrospectively identified. Moreover, hand hygiene and surface disinfection measures were intensified.

Identification and susceptibility testing

Positive blood culture samples were detected using an automated blood culture system (BACTEC™ 9240, Becton Dickinson GmbH, Heidelberg, Germany). Environmental samples were plated on Columbia sheep blood agar (Oxoid, Wesel, Germany) and MacConkey agar (Becton Dickinson, Heidelberg, Germany) after enrichment in Tryptic Soy Broth (Becton Dickinson) for 24 h at 36 °C. Subsequent species identification was performed by Matrix-Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (MALDI-TOF MS; Bruker, Bremen, Germany). Susceptibility testing for trimethoprim-sulfamethoxazole (TMP-SMX) was performed using disk diffusion method in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards and interpreted using EUCAST clinical breakpoints (version 6).

Whole genome sequencing (WGS) based typing

To determine the clonal relationship of *S. maltophilia* strains isolated from blood cultures, the isolates were subjected to WGS using the Illumina MiSeq platform (Illumina Inc., San Diego, USA) as described previously [13]. Retrospectively identified colonization isolates were not subjected to WGS as they were no longer available in contrast to BSI isolates, that are stored for a longer time period. Using SeqSphere⁺ software version 2.0 beta (Ridom GmbH, Münster, Germany), all coding regions were extracted and compared in a gene-by-gene approach (core genome multilocus sequence typing, cgMLST) using *S. maltophilia* K279a strain (GenBank accession number AM743169.1) as a reference sequence. Instead of a published cgMLST scheme, which is not yet available, this ad hoc scheme was used to differentiate the cluster. SeqSphere⁺ software was used to display the clonal relationship in a minimum-spanning tree (MST). For backwards compatibility with classical molecular typing, i. e. MLST, the MLST sequence types (ST) were extracted from the WGS data in silico.

Results

Epidemiological investigation and susceptibility testing

Chronological order and spacial distribution of isolated *S. maltophilia* is displayed in Fig. 1. Isolates were obtained from blood culture samples of patients on both wards. Two of the four isolates (isolated from P4 and P6) were detected within short intervals in two patients both admitted to

room 1 on ward 2. Of the four patients suffering from *S. maltophilia* bacteremia, three (P1, P4 and P6) were previously tested positive for *S. maltophilia* in stool samples or anal swabs. Additionally, two colonized patients (anal swabs; P2, P5) admitted to ward 2 were uncovered by the retrospective analysis (Table 1). One environmental isolate (E1) could be detected in a shower outlet of a patient room that was a patient with *S. maltophilia* infection. Susceptibility testing revealed all patient isolates to be susceptible to TMP-SMX, except isolate P3. The environmental isolate was resistant to TMP-SMX (Table 1).

Clinical characteristics and outcome of patients with *S. maltophilia* bacteremia

All patients with *S. maltophilia* BSI suffered from acute myeloid leukemia as the underlying disease. P2 and P5, who were only colonized by *S. maltophilia*, suffered from chronic lymphatic leukemia and myeloproliferative neoplasm. Except P1, all patients had already received allogeneic SCT at the time of *S. maltophilia* detection. P1 and P6 developed an acute respiratory distress syndrome. P1, P3, and P6 died due to *S. maltophilia* bacteremia.

WGS-based typing

We analyzed four bloodstream isolates and the environmental isolate by WGS. Comparison via cgMLST, based on 1876 genes present in all isolates, revealed no genetical relationship among isolates originating from patients (Fig. 2). In contrast, *S. maltophilia* strains isolated from P3 and E1 showed a similar genotype with only four alleles difference, suggesting a relationship between these two isolates. P3 and E1 harbored the MLST ST 94. All other isolates harbored new MLST STs.

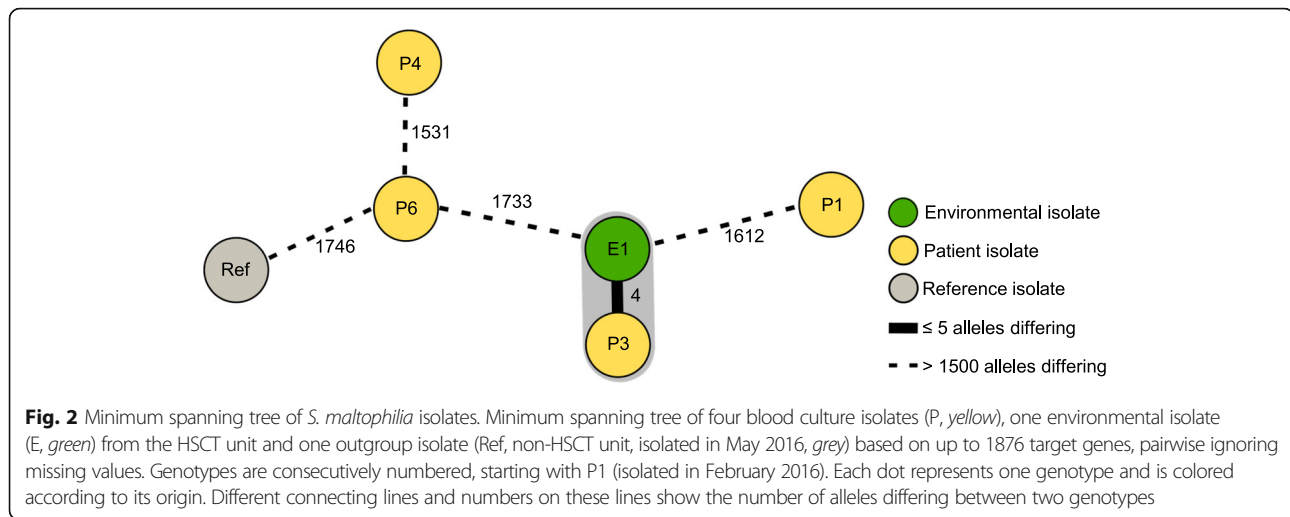
Discussion

S. maltophilia has emerged as an important nosocomial pathogen associated with increased mortality rates in patients suffering from hematological malignancies and receiving hematopoietic stem cell transplantations [20, 21]. In this study, WGS was used to evaluate a cluster of four *S. maltophilia* BSI in patients partially suffering from pulmonary dysfunction associated with the infection due to this

Table 1 Patients with *S. maltophilia* colonizations and blood stream infections during February and July 2016

Patient no.	Anal colonization	Blood stream infection	Strain susceptibility to TMP-SMX	WGS performed
P1	+	+	S	+
P2	+	-	S	-
P3	-	+	R	+
P4	+	+	S	+
P5	+	-	S	-
P6	+	+	S	+

TMP-SMX – trimethoprim-sulfamethoxazole, WGS – whole genome sequencing



pathogen [4]. Susceptibility testing, normally used as a first indication of nosocomial transmission of bacterial strains, could not exclude a spread of one single clone. WGS and subsequent cgMLST analysis, methods that have shown to provide detailed information in evaluating the epidemiology in nosocomial clusters of other pathogens [22], excluded a genetical relation and therefore an outbreak scenario.

A number of different sources are possibly responsible for the increasing number of *S. maltophilia* BSI. Due to its multidrug resistant nature, selection of this pathogen can easily occur in patients after receiving broad-spectrum antibiotics [23]. Moreover, *S. maltophilia* infections occur frequently in combination with the distinct immunocompromised status of HSCT recipients [24]. On the other hand, several environmental sites such as sink drains could be detected as sources of *S. maltophilia* within hospital settings [25].

To what extent these habitats are origins for hospital-acquired colonizations or infections of *S. maltophilia* could not yet be shown in detail. In this study we documented a genetical relation between an environmental and a patient blood stream infection isolate by analyzing WGS data. Although there was no local proximity, *S. maltophilia* could be transmitted from the environment to a patient or vice versa, at least giving the possibility of further spread via aqueous habitats in the HSCT unit.

Conclusion

WGS- analysis can be used to precisely refute an outbreak of *S. maltophilia*. Additionally, WGS- based typing documented sanitary installations in HSCT units to be an actual source or result of transmission between environmental and human habitats. Hence, in addition to classical infection control strategies, monitoring of aqueous habitats has to be established within the rooms of HSCT recipients in order to prevent transmission of these multidrug resistant organisms, as recently also shown for *P. aeruginosa* [26].

Abbreviations

BSI: Blood stream infection; cgMLST: Core genome multilocus sequence typing; HSCT: Hematopoietic stem cell transplantation; MST: Minimum spanning tree; PFGE: Pulsed field gel electrophoresis; ST: Sequence type; WGS: Whole genome sequencing

Acknowledgements

Not applicable.

Funding

None.

Availability of data and materials

Not applicable.

Authors' contributions

SK, AP, EAI were responsible for all data collection. SK, MHP, AK and AM were involved in data analysis and interpretation. All authors contributed to and approved the final draft of manuscript.

Ethics approval and consent to participate

All strategies and investigations were performed in accordance with the recommendations of the legally assigned institute for infection control and prevention (Robert Koch Institute). For the present retrospective analysis formal consent is not required.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Institute of Hygiene, University Hospital Münster, Robert-Koch-Strasse 41, 48149 Münster, Germany. ²Institute of Medical Microbiology, University Hospital Münster, Münster, Germany. ³Science for Life Laboratory, Division of Translational Medicine and Chemical Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden.

Received: 29 September 2017 Accepted: 8 November 2017

Published online: 13 November 2017

References

- Abbott IJ, Peleg AY. *Stenotrophomonas*, *Achromobacter*, and nonmelioid *Burkholderia* species: antimicrobial resistance and therapeutic strategies. *Semin Respir Crit Care Med*. 2015;36:99–110.
- Brooke JS. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clin Microbiol Rev*. 2012;25:2–41.
- Al-Anazi KA, Al-Jasser AM. Infections caused by *Stenotrophomonas maltophilia* in recipients of hematopoietic stem cell transplantation. *Front Oncol*. 2014;4:232.
- Tada K, Kurosawa S, Hiramoto N, et al. *Stenotrophomonas maltophilia* infection in hematopoietic SCT recipients: high mortality due to pulmonary hemorrhage. *Bone Marrow Transplant*. 2013;48:74–9.
- Safdar A, Rolston KV. *Stenotrophomonas maltophilia*: changing spectrum of a serious bacterial pathogen in patients with cancer. *Clin Infect Dis*. 2007;45:1602–9.
- Guyot A, Turton JF, Garner D. Outbreak of *Stenotrophomonas maltophilia* on an intensive care unit. *J Hosp Infect*. 2013;85:303–7.
- Waite TD, Georgiou A, Abrishami M, et al. Pseudo-outbreaks of *Stenotrophomonas maltophilia* on an intensive care unit in England. *J Hosp Infect*. 2016;92:392–6.
- Corlouer C, Lamy B, Desroches M, et al. *Stenotrophomonas maltophilia* healthcare-associated infections: identification of two main pathogenic genetic backgrounds. *J Hosp Infect*. 2017;96:183–8.
- Crispino M, Boccia MC, Bagattini M, et al. Molecular epidemiology of *Stenotrophomonas maltophilia* in a university hospital. *J Hosp Infect*. 2002;52:88–92.
- Gherardi G, Creti R, Pompilio A, et al. An overview of various typing methods for clinical epidemiology of the emerging pathogen *Stenotrophomonas maltophilia*. *Diagn Microbiol Infect Dis*. 2015;81:219–26.
- Tanimoto K. *Stenotrophomonas maltophilia* strains isolated from a university hospital in Japan: genomic variability and antibiotic resistance. *J Med Microbiol*. 2013;62:565–70.
- Salipante SJ, SenGupta DJ, Cummings LA, et al. Application of whole-genome sequencing for bacterial strain typing in molecular epidemiology. *J Clin Microbiol*. 2015;53:1072–9.
- Mellmann A, Bletz S, Boking T, et al. Real-time genome sequencing of resistant bacteria provides precision infection control in an institutional setting. *J Clin Microbiol*. 2016;54:2874–81.
- Joensen KG, Scheutz F, Lund O, et al. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol*. 2014;52:1501–10.
- Davis RJ, Jensen SQ, Van Hal S, et al. Whole genome sequencing in real-time investigation and Management of a *Pseudomonas aeruginosa* outbreak on a neonatal intensive care unit. *Infect Control Hosp Epidemiol*. 2015;36:1058–64.
- Kwong JC, Mercoulia K, Tomita T, et al. Prospective whole-genome sequencing enhances National Surveillance of *Listeria monocytogenes*. *J Clin Microbiol*. 2016;54:333–42.
- Fitzpatrick MA, Ozer EA, Hauser AR. Utility of whole-genome sequencing in characterizing *Acinetobacter* epidemiology and analyzing hospital outbreaks. *J Clin Microbiol*. 2016;54:593–612.
- Mellmann A, Andersen PS, Bletz S, et al. High Interlaboratory reproducibility and accuracy of next-generation-sequencing-based bacterial genotyping in a ring trial. *J Clin Microbiol*. 2017;55:908–13.
- European Centre for Disease Prevention and Control. Annual Epidemiological Report - Healthcare-associated infections acquired in intensive care units. 2016. <https://ecdc.europa.eu/en/publications-data/healthcare-associated-infections-acquired-intensive-care-units-annual>. Accessed 05 Nov 2017.
- Micozzi A, Venditti M, Monaco M, et al. Bacteremia due to *Stenotrophomonas maltophilia* in patients with hematologic malignancies. *Clin Infect Dis*. 2000;31:705–11.
- Labarca JA, Leber AL, Kern VL, et al. Outbreak of *Stenotrophomonas maltophilia* bacteremia in allogeneic bone marrow transplant patients: role of severe neutropenia and mucositis. *Clin Infect Dis*. 2000;30:195–7.
- Willems S, Kampmeier S, Bletz S, et al. Whole-genome sequencing elucidates epidemiology of nosocomial clusters of *Acinetobacter baumannii*. *J Clin Microbiol*. 2016;54:2391–4.
- Chang YT, Lin CY, Chen YH, et al. Update on infections caused by *Stenotrophomonas maltophilia* with particular attention to resistance mechanisms and therapeutic options. *Front Microbiol*. 2015;6:893.
- Paez JI, Costa SF. Risk factors associated with mortality of infections caused by *Stenotrophomonas maltophilia*: a systematic review. *J Hosp Infect*. 2008;70:101–8.
- Brooke JS. Pathogenic bacteria in sink exit drains. *J Hosp Infect*. 2008;70:198–9.
- Kossow A, Kampmeier S, Willems S, et al. Control of multidrug-resistant *Pseudomonas aeruginosa* in allogeneic hematopoietic stem cell transplant recipients by a novel bundle including remodeling of sanitary and water supply systems. *Clin Infect Dis*. 2017;65:935–42.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

