# Title

Emergence of a quinolone resistance mutation in a case of *Stenotrophomonas maltophilia* bacteremia identified by whole genome sequencing

## Authors

Theodore R. Pak,[[1]](#footnote-1) Deena R. Altman,[[2]](#footnote-2) Oliver Attie,a Robert Sebra,a Camille L. Hamula,[[3]](#footnote-3) Martha Lewis,a Gintaras Deikus,a Leah C. Newman,a Gang Fang,a Jonathan Hand,b Gopi Patel,b Fran Wallach,b Eric E. Schadt,a Shirish Huprikarb, Harm van Bakel,a Andrew Kasarskis,a,\* and Ali Bashira

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## Running title

Sequencing cases of *S. maltophilia* bacteremia

\* Corresponding author: Andrew Kasarskis, Icahn Institute and Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1498, New York, NY 10029, USA; telephone, +1 (212) 659-8542; email [andrew.kasarskis@mssm.edu](mailto:andrew.kasarskis@mssm.edu)

Alternate corresponding author: Theodore Pak, Icahn Institute and Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1090, New York, NY 10029, USA; telephone, +1 (212) 241-6885; email [theodore.pak@mssm.edu](mailto:shirish.huprikar@mssm.edu)

# Abstract

Completely assembled genome sequences of *Stenotrophomonas maltophilia* isolates from a bacteremic patient before and after development of levofloxacin resistance differed by a single nucleotide mutation in *smeT* conferring resistance by upregulation of efflux pump *smeDEF.* Sequenced *S. maltophilia* isolates from five contemporaneous cases displayed remarkable diversity compared to current references.

# Introduction

*Stenotrophomonas maltophilia* is an aerobic, non-fermenting, and motile Gram-negative bacteria that is increasingly recognized as a cause of hospital-acquired infections with crude mortality rates of 14–69% in cases of bacteremia [1]. Treatment of *S. maltophilia* infections is challenging due to the pathogen’s intrinsic resistance to many antibiotic classes via drug efflux pumps, beta-lactamase production, and decreased membrane permeability [1]. Resistance phenotypes are known to change during the course of treatment, which complicates interpretation of automated drug susceptibility testing (DST) results [2]. A mutant strain of *S. maltophilia* with emerging resistance to tetracycline, chloramphenicol, and quinolones was previously characterized following *in vitro* tetracycline selection[3,4]. However, little is known about the genetic and molecular mechanisms underlying acquired resistance in the clinical setting. In this report, we describe the first reported use of whole genome sequencing (WGS) to identify an acquired quinolone resistance mutation in a clinical isolate of *S. maltophilia*.

# Case report

A 56 year-old man with a history of pancreatic cancer and a Whipple procedure eleven years earlier presented to The Mount Sinai Hospital with variceal bleeding at the hepaticojejunostomy site. A transjugular intrahepatic portosystemic shunt was placed, which was complicated by thrombosis. In the following weeks, he had several episodes of polymicrobial bacteremia and was treated with multiple courses of antimicrobials, including a 10-day course of levofloxacin. Two months after levofloxacin exposure, he developed another episode of polymicrobial bacteremia. Blood cultures intermittently grew *S. maltophilia*, *E. faecium*, and *Candida parapsilosis* despite appropriate antimicrobial therapy. Automated DST showed that the first *S. maltophilia* isolate acquired was susceptible to levofloxacin (minimum inhibitory concentration [MIC] 0.5µg/mL) and trimethoprim/sulfamethoxazole (TMP-SMX; MIC ≤20 µg/mL). He was treated with 400 mg intravenous ciprofloxacin every 8 hours, but blood cultures nine days later again grew *S. maltophilia*, now resistant to levofloxacin (MIC >32µg/mL) while still susceptible to TMP-SMX (MIC 1µg/mL). Ciprofloxacin therapy was stopped and intravenous TMP-SMX was given every 8 hours; subsequent cultures did not grow *S. maltophilia.*

# Methods

Standard culturing and susceptibility testing were performed by automated microbroth dilution (VITEK2, Biomérieux, France). Antimicrobial sensitivities were reported and interpreted according to Clinical Laboratory Standards Institute 2015 (CLSI) guidelines for *S. maltophilia* [5]*.* Isolates were then stocked and frozen at -80°C. Drug susceptibility for all isolates in this study were later confirmed by Etest (Biomériux, France) at 24 hours.

To prepare for sequencing, isolates were grown from single colonies in tryptic soy broth, and DNA extraction was performed as previously described [6]. WGS was performed on the PacBio RSII for the initial and subsequent *S. maltophilia* blood culture isolates from the case patient (Patient 1) and five other patients selected from a two-month period in 2013 at The Mount Sinai Hospital (Table 1). Additional details are in Supplementary Methods.

# Results

Two complete whole genome sequences were derived from the case patient isolates before and after the change in levofloxacin MIC and compared to whole genome sequences of five control *S. maltophilia* isolates. All sequences were *de novo* assembled, i.e., without regard to reference assemblies. Table 1summarizes the relative dates of collection, antimicrobial susceptibility results, and assembly statistics.

## Emergence of a point mutation conferring quinolone resistance

Assembled genome sequences for the two case patient isolates before (ISMMS2) and after (ISMMS2R) observation of levofloxacin resistance were compared directly and were identical except for one single nucleotide variant (SNV) and five one-base indels. Sanger sequencing confirmed the presence of the SNV, but identified the indels as homopolymer assembly errors. Coding domain sequence predictions for the surrounding locus (Figure 1A) revealed that the SNV was inside *smeT*, a *tetR*-like repressor upstream of the structural operon for the *smeDEF* genes, which encode a multidrug efflux pump. The SNV is an A>T substitution at position 497 of *smeT* causing a nonsynonymous L166Q mutation.

The same nonsynonymous mutation has been previously observed in an *in vitro* strain of *S. maltophilia*, D457R, created by selecting single-step tetracycline-resistant mutants from the antibiotic-susceptible clinical strain D457 [3,4]. The mutation is in the eighth α-helix of the *smeT* protein [7], which homodimerizes to repress transcription of the *smeDEF* operon [3,7]. Although the mutation is not in the DNA-binding region, it has been shown to disable the repressor activity of *SmeT* [3], leading to upregulation of *SmeDEF* and conferring an MDR phenotype [8].

Figure 1B shows an amino-acid sequence alignment comparing *SmeT* in D457 and D457R to aligned sequences from our seven isolates. Notably, while none of the remaining isolates shared the same L166Q (c.497A>T) mutation, another isolate resistant to levofloxacin, ISMMS4, displayed a C>T mutation at position 388 of *smeT* that creates a premature stop codon that likely disrupts *smeT* function (Figure 1A and 1B).

## Diverse sources of *S. maltophilia* identified with WGS

Significant diversity was observed among the *S. maltophilia* isolates from all six patients. Supplementary Figure 1 shows a maximum-likelihood phylogeny with branch lengths scaled to SNV distances. Our isolates distribute widely among all four reference assemblies for complete *S. maltophilia* genomes in GenBank. The distances of tens of thousands of SNVs seen in our phylogeny suggest that the natural diversity of pathogenic *S. maltophilia* is greater than that captured by the current set of reference assemblies, even within a single hospital setting.

Recombination is not an obvious source of diversity in our *S. maltophilia* isolates. Supplementary Figure 2 depicts whole genome alignments between the four clinical isolates where assembly produced a circularized chromosome and the four GenBank references, showing small areas of non-homology separating large regions of significant homology occurring generally in the same order for each genome. ISMMS2 and ISMMS2R are structurally identical, as expected for serial isolates, while recombination events among other strains are limited to small 1-2kb segments. Epigenetics motif analysis also suggests that the isolates are not related.Supplementary Table 1 shows different motifs in isolates from separate patients, implicating differences in type II & III restriction modification systems between the isolates more likely to be caused by inter-strain/species horizontal transfer of methyltransferases than by intra-strain mutations [9]. Together, this demonstrates that transmission did not occur among these six cases and suggests that the diversity of pathogenic *S. maltophilia* is probably greater than previously appreciated.

# Discussion

This is the first report of WGS to characterize the emergence of a resistance mutation in *S. maltophilia* during antibiotic treatment of an active infection. The mutation was a SNV that replicates a variant observed in an *in vitro* model strain created to study the MDR phenotype in 1997 [4]. This confirms that one acquired SNV is sufficient to confer quinolone resistance during the course of *S. maltophilia* infection, and underscores the need to repeat DST frequently to ensure that resistance has not emerged.

*smeT* appears to play a central role in adaptive resistance to quinolones and other antibiotics effluxed by *smeDEF*, like tetracycline, chloramphenicol, erythromycin, and aminoglycosides. Since any mutation that inactivates this protein would be able to derepress *smeDEF* and confer resistance, *smeT* is under intense selective pressure in the presence of these drugs. In this study, we observed not only a deleterious SNV in the strain that displayed resistance (ISMMS2R), but a premature stop codon in *tetR* in a strain that was already resistant at first isolation (ISMMS4). Certain nucleotide positions appear to be under greater selective pressure than others, as evidenced by our observation of the same mutation that occurred in D457R, and a relative paucity of nonsynonymous coding mutations in *smeT* observed among clinical *smeT* isolates [10]. Since sustained overexpression of *smeDEF* is physiologically unfavorable [11], it is possible that pathogenic strains of *S. maltophilia* rely on natural diversity of mutations in the *smeT* locus to activate or deactivate *smeDEF* expression, allowing for rapid adaptation to antibiotic stress, though further study is needed.

Since resistance from a single SNV emerged during a short course of ciprofloxacin, clinicians should be cautioned about using quinolone monotherapy for *S. maltophilia* bacteremia, as also highlighted in recent retrospective studies [12,13]. The wide variety of MDR phenotypes and unreliability of DST results has created uncertainty about appropriate treatment for *S. maltophilia*, but TMP-SMX remains the most common choice for monotherapy [1,12,13]. TMP-SMX resistance in *S. maltophilia* is not known to be caused by efflux systems but has been linked to Class 1 integrons and IS*CR* elements [1]. This suggests that spontaneous resistance is less likely to emerge with TMP-SMX monotherapy, although a clinical trial comparing the two antibiotics is warranted [12,13].

In conclusion, characterizing the full extent of genetic alterations that *S. maltophilia* utilizes to develop antibiotic resistance *in vivo* and improving genomic surveillance of clinical strains will help refine antibiotic selection criteria available to clinicians. This study furthermore highlights the utility of WGS for profiling the precise mutations underlying emerging antibiotic resistance in clinical cases of bacteremia.

# Notes

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## Conflict of Interest

The authors have no conflicts of interest to disclose.

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# Tables

**Table 1.** Sequenced clinical isolates and their antimicrobial susceptibilities.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patient | Time of collection[[4]](#footnote-4) (days) | Isolate name | Levo susceptibility (MIC, µg/mL) | | T-S susceptibility (MIC, µg/mL) | | Assembly quality | Depth of coverage |
| VITEK | Etest | VITEK | Etest |
| 1 | 0 | ISMMS2 | S (0.5)[[5]](#footnote-5) | S (1) | S (<20) | S (0.19) | 1 circular 4.51Mbp chromosome | 160x |
| 1 | +10 | ISMMS2R | R (>32)b | R (16) | S (1) | S (0.38) | 1 circular 4.51Mbp chromosome | 403x |
| 2 | -26 | ISMMS3 | S (0.25) | S (0.38) | U  (80, <20)[[6]](#footnote-6) | S (0.75) | 1 circular 4.80Mbp chromosome | 153x |
| 3 | +14 | ISMMS4 | R (>8) | R (>12) | U  (0.5, 80)c | S (0.75) | 3 contigs (4.73Mbp, 6.5kbp, 11.2kbp) | 303x |
| 4 | -32 | ISMMS5 | S (1) | S (1) | S (<20) | S (0.25) | 18 contigs | 270x |
| 5 | 0 | ISMMS6 | S (<0.12) | S (0.125) | S (<20) | S (1.5) | 10 contigs | 262x |
| 6 | +2 | ISMMS7 | S (1) | S (0.75) | S (<20) | S (1.5) | 1 circular 4.69Mbp chromosome, 1 additional 17.7kbp contig | 318x |

Abbreviations: Levo, levofloxacin; T-S, trimethoprim/sulfamethoxazole; MIC, minimum inhibitory concentration; S, susceptible; R, resistant; U, undeterminable; Mbp, million base pairs; kbp, thousand base pairs.

# Figure Legends

**Figure 1.** Single nucleotide variants (SNVs) observed in quinolone-resistant *S. maltophilia* clinical isolates. ***A***,assembled circular chromosome for ISMMS2, including predicted coding domain sequence (CDS) and noncoding RNA (ncRNA) features drawn with ChromoZoom [14]. Horizontal position corresponds to base pair location. The *smeDEF* operon is shown in the detail callout, which highlights both the *smeT* c.497T>A SNV that emerged in ISMMS2R and the aligned location of the *smeT* c.388C>T SNV (encoding a premature stop codon) in ISMMS4. ISMMS2 and ISMMS2R are serial isolates from a single patient before and after development of quinolone resistance, while ISMMS4 was quinolone-resistant at initial isolation from a different patient. ***B***, multiple sequence alignment of part of the predicted *smeT* product in each of the clinical isolates, the D457 reference assembly, and its quinolone resistant counterpart D457R. Predicted α-helices [7] are labeled as grey bars below the sequence. Positions identical in all sequences are shaded with a dark gray background, equivalent substitutions are typeset in red, and non-equivalent substitutions are typeset in boldface black. The L166Q and Q130\* (\*, stop codon) polymorphisms are highlighted.

1. Icahn Institute and Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY [↑](#footnote-ref-1)
2. Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY [↑](#footnote-ref-2)
3. Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY [↑](#footnote-ref-3)
4. Time of collection was defined in days relative to the date of collecting the initial *S. maltophilia* isolate in the case patient. [↑](#footnote-ref-4)
5. This is the change in levofloxacin susceptibility investigated in this study. [↑](#footnote-ref-5)
6. Inconsistent results were obtained in replicate. [↑](#footnote-ref-6)