



OPEN ACCESS

EDITED BY

Axel Cloeckaert,
Institut National de Recherche pour
l'Agriculture, l'Alimentation et
l'Environnement (INRAE), France

REVIEWED BY

Leonardo Gabriel Panunzi,
CEA Saclay, France
Kazuki Harada,
Tottori University, Japan
Maria Fernanda Mojica,
Case Western Reserve University,
United States
Mariana Andrea Papalia,
Universidad de Buenos Aires, Argentina

*CORRESPONDENCE

Jiyong Yang
✉ yangjy301@163.com

RECEIVED 27 April 2024

ACCEPTED 05 June 2024

PUBLISHED 14 June 2024

CITATION

Wang L, Wang Y, Ye K, Qiu X, Zhao Q,
Ye L and Yang J (2024) Molecular
epidemiology, genetic diversity, antibiotic
resistance and pathogenicity of
Stenotrophomonas maltophilia complex from
bacteremia patients in a tertiary hospital in
China for nine years.
Front. Microbiol. 15:1424241.
doi: 10.3389/fmicb.2024.1424241

COPYRIGHT

© 2024 Wang, Wang, Ye, Qiu, Zhao, Ye and
Yang. This is an open-access article
distributed under the terms of the [Creative
Commons Attribution License \(CC BY\)](#). The
use, distribution or reproduction in other
forums is permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication in
this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

Molecular epidemiology, genetic diversity, antibiotic resistance and pathogenicity of *Stenotrophomonas maltophilia* complex from bacteremia patients in a tertiary hospital in China for nine years

Lifeng Wang¹, Yu Wang², Kun Ye¹, Xuemei Qiu¹, Qiang Zhao¹,
LiYan Ye¹ and Jiyong Yang^{1*}

¹Department of Laboratory Medicine, First Medical Center of Chinese PLA General Hospital, Beijing, China, ²Department of Clinical Laboratory, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, Taiyuan, China

Background: The *Stenotrophomonas maltophilia* complex (Smc) has emerged as a significant nosocomial pathogen contributing to increased mortality rates, particularly in case of bloodstream infections.

Methods: This study employed whole-genome sequencing (WGS) to assess the genetic diversity, antimicrobial resistance profiles, molecular epidemiology and frequencies of virulence genes among 55 *S. maltophilia* isolates obtained from bacteremic cases over a 9-year period.

Results: Based on the threshold of 95% average nucleotide identity (ANI) and 70% digital DNA–DNA hybridization (dDDH) for genospecies delineation, we classified 37 isolates into 6 known species, all belonging to the Smc. The remaining 18 isolates sequenced in this study were assigned to 6 new genomospecies. Among the 55 isolates, we identified 44 different sequence types (STs), comprising 22 known and 22 novel allele combinations. The resistance rate of Smc against trimethoprim-sulfamethoxazole (TMP/SMX) was found to be 3.6%, with the *sul1* and class one integron integrase genes (*intl1*) detected in these isolates. All Smc isolates were susceptible to minocycline. Furthermore, all Smc strains harbored the *motA*, *pilU*, *smf-1* and *Stmpr2* genes. Genomospecies 1 (100%, $n = 9$), *Stenotrophomonas maltophilia* (84.21%, $n = 19$) and *Stenotrophomonas sepiolia* (71.43%, $n = 7$) demonstrated a higher percentage of the *afaD* gene, which was also associated with a higher separation rate. In addition to *motA*, *pilU*, *smf-1*, and *Stmpr2* genes, all *S. maltophilia* strains (100%) contained *entA*, *gspD*, *KatA*, and *stmPr1* genes, while all genomospecies 1 strains (100%) contained *afaD*, *entA*, *gspD*, and *KatA* genes.

Conclusion: Our study highlights the genetic diversity among Smc isolates from patients with bacteremia, revealing 22 novel ST types, 58 new alleles and 6 new genomospecies. *S. maltophilia* and *S. pavanii* were found to carry more virulence factors, emphasizing the importance of accurate strain identification. Minocycline emerged as a promising alternative antibiotic for patients who were resistant to TMP/SMX.

KEYWORDS

Stenotrophomonas maltophilia, bloodstream infection, whole-genome sequencing, virulence, antibiotic resistance, sequence type

1 Introduction

Stenotrophomonas maltophilia, previously classified as *Pseudomonas* and later as *Xanthomonas maltophilia*, is a common aerobic non-fermentative Gram-negative bacillus that exists in humid environments, water sources, soil, and plants (Brooke, 2012). The *Stenotrophomonas maltophilia* complex (Smc) has emerged as a significant nosocomial pathogen in clinical settings (Gröschel et al., 2020). Smc is capable of causing a broad spectrum of serious infections, most notably respiratory tract infections and bloodstream infection (BSI). Immunocompromised patients with underlying malignancies, indwelling devices, chronic respiratory diseases, a history of antibiotic use, or prolonged hospital or intensive care unit (ICU) stays are particularly susceptible to severe Smc infections. The mortality rate of patients with Smc bacteremia was 40.5% in a meta-analysis included 19 studies (Huang et al., 2024).

The Smc comprises seven species, including *S. maltophilia*, *S. africana*, *S. pavanii*, *P. hibiscicola*, *P. beteli*, *P. geniculata*, and *S. sepiolia* (Ochoa-Sánchez and Vinuesa, 2017; Patil et al., 2018). Due to 16S rRNA gene sequence conservation, taxonomy of Smc is complicated. Taxonogenomics and phylogenomics offer us intra-species and strain-level resolution. Commonly used genome-derived criteria include average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) with species-level cut-offs set at 95 and 70%, respectively (Anzai et al., 2000). More novel genomespecies have been identified in the post-genomic era. The multi-locus sequence typing (MLST) and whole-genome sequencing (WGS) approaches have revealed high genetic heterogeneity among Smc strains. Phylogenetic analyses of 375 nonduplicated Smc genomes have identified at least 20 genogroups (Mercier-Darty et al., 2020).

Due to the intrinsic antimicrobial resistance and acquired resistance to numerous antimicrobial drugs, the treatment of Smc infections can be challenging. Trimethoprim-sulfamethoxazole (TMP/SMX) is the first-line treatment for Smc infections. However, the resistance rate to TMP/SMX has been increasing worldwide. Studies have shown that resistance rates vary according to specimen sources, with Smc isolates from urinary tract infections demonstrating higher resistance to antibiotics compared to isolates from other specimen sources (Vidigal et al., 2014). While Smc possesses a variety of virulence factors, the genes encoding biofilm production and adherence promote their attaching to the surface of medical equipment, which is closely related to BSI (De Oliveira-Garcia et al., 2003; Trifonova and Strateva, 2019). Further study of those virulence factors in Smc causing BSI is warranted.

This study aimed to characterize the genetic diversity, phylogenetic analysis, molecular epidemiology, antimicrobial resistance and virulence genes of Smc isolates obtained from case of bacteremia in China between 2011 and 2019 using WGS.

2 Materials and methods

2.1 Bacterial strains and clinical information

We analyzed 55 nonduplicated clinical strains of Smc were collected between 2011 and 2019 from the Department of Laboratory Medicine, First Medical Center of PLA General Hospital. The isolates were obtained from blood samples and identified as Smc by Matrix-Assisted Laser Desorption/Ionization Time of Flight-Mass Spectrometry (MALDI-TOF MS) (bioMérieux SA, France). Clinical data from the 55 patients, including demographics and respective outcomes, were retrieved from medical records. The diagnosis of bacteremia was made based on both clinical and bacteriological criteria. This study was approved by the Ethics Committee of Chinese PLA General Hospital (No. S2024-349-01). All data were analyzed anonymously.

2.2 Susceptibility assays

Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method (Shanghai XingBai Biotechnology Company, Shanghai, China) and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023) interpretive criteria. Seven antibiotics/inhibitors were included in this study: TMP/SMX, ticarcillin/clavulanic acid (TIM), ceftazidime (CAZ), cefepime (FEP), ciprofloxacin (CIP), levofloxacin (LEV), and minocycline (MIN). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains for susceptibility testing.

2.3 Whole-genome sequencing

Genomic DNA from the strains was extracted using the DNeasy® UltraClean® Microbial Kit (QIAGEN GmbH, 40,724 Hilden, Germany) following the manufacturer's instructions. The purity and concentration of the DNA were determined using a spectrophotometer. WGS was performed on a HiSeq X Ten sequencer (Illumina Inc., San Diego, CA, United States) using a paired-end library with an average insert size of 350 bp. All sequences were assembled using SOAPdenovo (SOAP Version 2.21). The N_{50} , N_{90} , and scaffold number were used to identify *de novo* characteristics (Supplementary Table S2).

2.4 Genome similarity assessment and MLST analyses

Genome-based taxonomic methods, including ANI and dDDH, were used for genome similarity assessment, with species-level cut-offs

of 95 and 70%, respectively (Kim et al., 2014). *In silico* MLST analysis was performed using the web server of the Centre for Genomic Epidemiology¹ and then confirmed using the University of Oxford database.² Seven housekeeping genes were used for MLST analysis, including *atpD*, *gapA*, *guaA*, *mutM*, *nuoD*, *ppsA*, and *recA*.

New alleles of the genes were assigned based on the Smc MLST database and the specific sequence type (ST) was determined. When uploading new alleles, the following parameters were used: technology: Illumina; read length: 200–299; coverage: 20–49x; and assembly: *de novo*. The sequences were reversed with Editseq (version: 6.0) to correct star/end sites for the selected locus if necessary. The new alleles were uploaded to the pubMLST database, and the specific STs were then assigned. All genome sequences in this study were submitted to GenBank with BioProject accession number PRJNA675378.

2.5 Resistance and virulence genes analysis

The nucleotide sequences of well-characterized antibiotic resistance and virulence genes were retrieved from the complete genome of *S. maltophilia* K279a. The corresponding resistance genes were examined using ResFinder.³ The virulence genes encoding biofilm production and adherence were examined using Blast2Seq,⁴ including *afaD*, *entA*, *fliC*, *gspD*, *katA*, *motA*, *pilU*, *rmlA*, *smf-1*, *Stmpr1*, and *Stmpr2*.

2.6 Phylogenetic analysis

Single nucleotide polymorphism (SNP) analysis was conducted using the SNP-based CSI Phylogeny 1.4 tool available on the Center for Genomic Epidemiology website⁵ with default parameters for the strains in our study. In total of 59,422 SNPs were found for further analysis. The complete genome sequence of *S. maltophilia* strain K279a (GCF_000072485.1), which was isolated from the blood sample of a patient with bloodstream infection in 1998 (Looney et al., 2009) and generally used as a reference (Gröschel et al., 2020), was also used as a reference in our study. The tree file was visualized using iTOL,⁶ and annotated information was edited using iTOL editor v.1_1.

3 Results

3.1 Epidemiological data of patients infected with Smc

A total of 55 clinical isolates were obtained from 55 patients with bacteremia at the First Medical Center of Chinese PLA General Hospital between 2011 and 2019. Each isolate was recovered from a single patient. Of these isolates, 40 (72.7%) were from male patients, and 15 (27.3%)

were from female patients, with an average age of 48.6 years (range: 4–87 years). The majority of the patients were hospitalized in the Surgical Intensive Care Unit (SICU) (21.8%), followed by the Hematology Department (16.4%), Gastroenterology Department (14.6%) and Hepatobiliary Surgery (14.6%). Among the 55 patients, 12 patients died during hospitalization, resulting in an in-hospital mortality rate of 21.8%.

3.2 Genome similarity assessment and discovery of novel genomospecies

To perform genomic classification, ANI and dDDH were calculated (Supplementary Table S1). Based on the threshold of 95% ANI and 70% dDDH for genomospecies delineation, 37 isolates were classified into *S. maltophilia*, *S. pavanii*, *S. sepilia*, *P. hibiscicola* and *S. geniculata* and *S. africana*. They were all included in Smc. The remaining 18 isolates sequenced in this study were assigned to 6 novel genomospecies (Genomospecies 1–6). These 6 novel genomospecies displayed $\leq 93.9\%$ ANI and $\leq 52.1\%$ dDDH compared with the type strains of the genus *Stenotrophomonas*. Additionally, the mortality rates of patients infected with different genomospecies infection varied. The mortality rates of patients infected with *S. maltophilia*, *S. pavanii*, *P. hibiscicola*, *S. sepilia* and genomospecies 2 infected patients were 31.6% (6/19), 50% (3/6), 100% (1/1), 14.3% (1/7), and 25% (1/4) respectively. The demographic and clinical information of the patients were summarized in Supplementary Table S3.

3.3 MLST analyses and discovery of new STs

MLST analysis confirmed that the 55 strains of the Smc belonged to 44 different STs. Among these, 22 STs were identified in 30 isolates and have been previously reported in the database. However, 22 STs were reported for the first time in 25 isolates (Table 1). A total of 58 new alleles were identified and assigned new allele numbers by comparison against the pubMLST database. All new allele sequences were deposited in the public databases for molecular typing and microbial genome diversity (pubMLST). The allelic profiles of the strains are shown in Table 1. The most frequently observed ST types were ST-4 ($n = 3$), ST-233 ($n = 3$), and ST-566 ($n = 3$), followed by two strains each belonging to ST-31, ST-24, ST-138, ST-249, and ST-562. The other 36 STs only contained 1 strain.

3.4 Phylogenetic analysis

A phylogenetic tree based on WGS data is presented in Figure 1. The well-dispersed isolates in the phylogenetic tree suggested no outbreak. However, the tree included a few small clusters, such as ST-4 ($n = 3$), ST-233 ($n = 3$), and ST-566 ($n = 3$). It was noteworthy that two out of the three patients infected with the ST233 strain died.

3.5 Antimicrobial susceptibility profile and resistance genes

All Smc strains (100%) were susceptible to MIN. 3.6% (2/55) of the strains (PMA-16 and PMA-43) were resistant to TMP/

1 <https://cge.cbs.dtu.dk/services/MLST/>

2 <https://pubmlst.org/smaltophilia/>

3 <https://cge.cbs.dtu.dk/services/ResFinder/>

4 <https://blast.ncbi.nlm.nih.gov/Blast.cgi>

5 <https://cge.cbs.dtu.dk/services/CSIPhylogeny/>

6 <https://itol.embl.de>

TABLE 1 The newly discovered MLST profiles of *Smc*.

Strain number	Alleles							Sequence type
	<i>atpD</i>	<i>gapA</i>	<i>guaA</i>	<i>mutM</i>	<i>nuoD</i>	<i>ppsA</i>	<i>recA</i>	
PMA-2	181	97	398	222	182	254	213	592
PMA-4	1	1	277	46	7	19	6	561
PMA-5	5	4	282	7	25	247	208	562
PMA-13	177	215	415	100	183	130	75	570
PMA-15	1	96	307	6	6	37	6	563
PMA-17	13	172	399	50	121	121	22	564
PMA-19	14	102	400	89	68	242	214	565
PMA-20	103	171	402	217	72	251	166	577
PMA-21	74	217	403	218	87	88	215	566
PMA-23	179	218	404	219	12	250	13	594
PMA-25	74	217	403	218	87	88	215	566
PMA-26	12	27	352	151	30	29	22	567
PMA-31	13	27	410	107	30	29	22	571
PMA-32	89	167	317	223	4	200	158	572
PMA-34	74	79	405	218	87	249	217	573
PMA-35	28	8	218	33	126	200	16	568
PMA-37	3	219	406	105	186	108	99	574
PMA-39	94	220	407	119	14	252	134	582
PMA-42	2	221	277	59	2	69	5	583
PMA-50	2	222	408	45	63	168	5	584
PMA-51	5	4	282	7	25	247	208	562
PMA-52	13	28	226	151	121	140	22	569
PMA-55	13	124	409	112	6	144	80	587
PMA-56	13	223	411	83	184	255	194	595
PMA-58	74	217	403	218	87	88	215	566

In bold: the new ones found in our study.

SMX. Similarly, 3.6% (2/55) of the strains were resistant to LEV (PMA-30 and PMA-43). CAZ resistance was observed in 41.8% (23/55) of the strains. The corresponding MIC₅₀, MIC₉₀, and the ranges are shown in Table 2. The MICs of each isolate were included in Supplementary Table S4. The sulfonamide-resistance genes (*sul1* and *int1*) were present in two strains (PMA-16 and PMA-43).

3.6 Frequency of virulence genes encoding biofilm production and adherence in the *Smc* strains

The distribution of virulence genes in *Smc* strains were summarized in Table 3. 100% strains (55/55) harbored the *motA*, *pilU*, *smf-1*, and *Stmpr2* genes. In addition, we analyzed the virulence genes of different genomospecies (Figure 1). *fliC* gene was only detected in *S. maltophilia* (42.11%). In addition to *motA*, *pilU*, *smf-1*, and *Stmpr2* genes, 100% *S. maltophilia* strains (19/19) contained *entA*, *gspD*, *KatA*, and *stmPr1* genes, and 100% Genomospecies 1 strains contained *afaD*, *entA*, *gspD*, and *katA* genes.

4 Discussion

Smc has emerged as an important nosocomial pathogen, contributing to increased mortality rates (Trifonova and Strateva, 2019). In our present study, the in-hospital mortality rate of BSIs caused by *Smc* was 21.8%. WGS was employed to assess the genetic diversity, antimicrobial resistance profiles, molecular epidemiology and frequencies of virulence genes among 55 *Smc* isolates obtained from bacteremic patients over a 9-year period.

Due to the conservation of the 16S rRNA gene sequence, the taxonomy of *Smc* is intricate. Phylogenomic and taxonogenomic analyses revealed the heterogeneous structure of *Smc*. Our study suggested the presence of 6 valid species (*S. maltophilia*, *S. pavanii*, *S. seipilia*, *P. hibiscicola*, *P. geniculata*, and *S. africana*) within the *Smc*. Additionally, we identified 6 novel genomospecies. Therefore, the *Smc* comprised at least 12 distinct genomospecies in our study. Although MALDI-TOF MS is the primary identification method routinely used in most clinical laboratories, accurate identification of *Smc* should rely on ANI or dDDH. Moreover, the genomes of novel genomospecies could be used as type strain genomes to facilitate accurate species assignments and to aid in the discovery of novel species. Our results

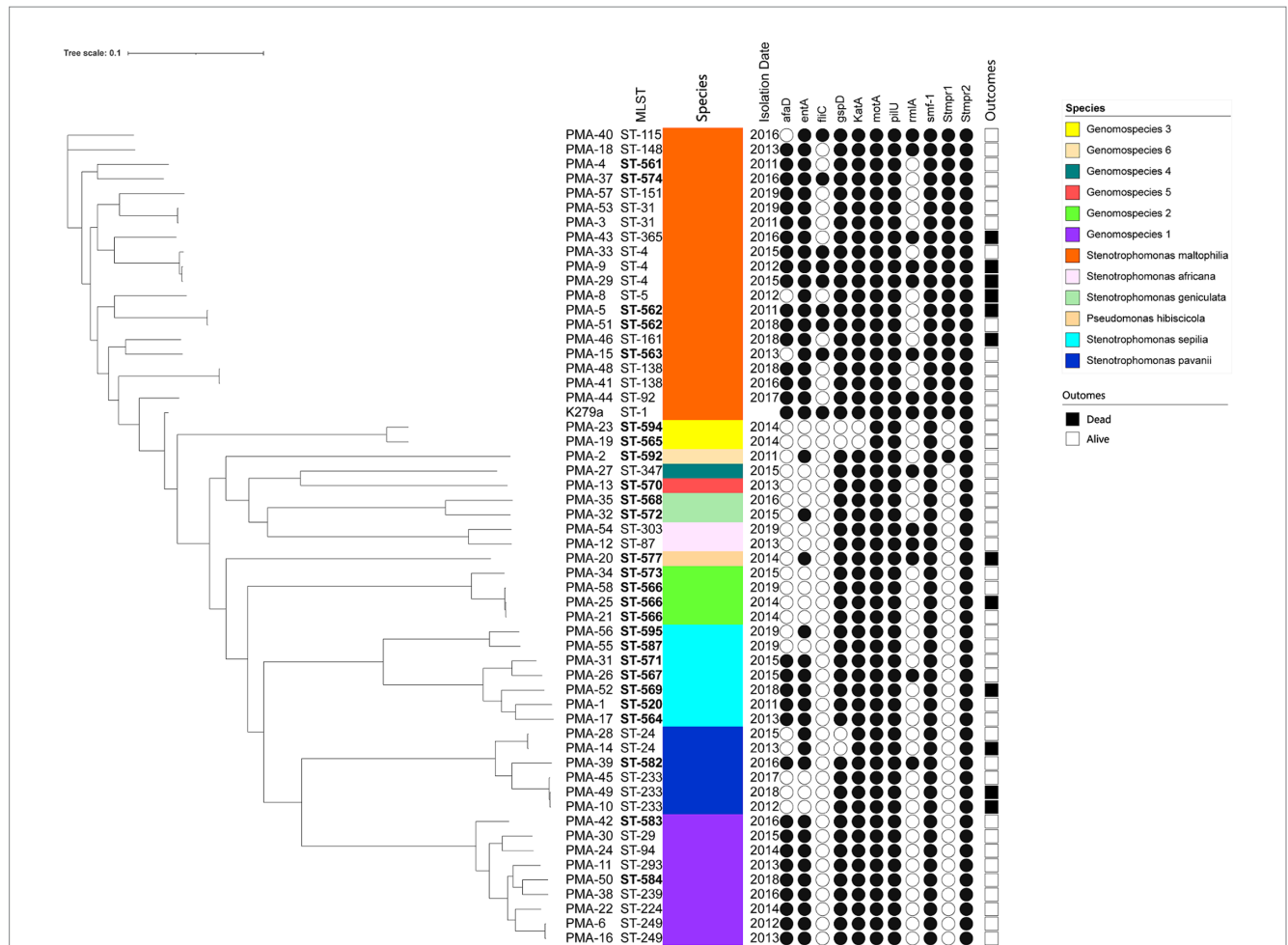


FIGURE 1
 The phylogenetic tree of clinical isolates of *Smc* ($n = 55$). The scale of the tree is shown on the top of the figure and the genetic distance is denoted by branch length. Strains number, MLST types, identification, isolation date, virulence genes and clinical outcomes are listed on the right. The newly discovered ST types are marked in bold. Black and white circles mark the presence and absence of the virulence genes, respectively. Black and white squares on the right side mark the outcomes of died and alive.

TABLE 2 Antibiotic susceptibility of the *S. maltophilia* strains ($n = 55$).

Antibiotics	MIC ($\mu\text{g/mL}$)			S	I	R
	Range	MIC ₅₀	MIC ₉₀			
FEP	4–64	32	64	–	–	–
CIP	0.5–8	2	8	–	–	–
TMP/SMX	19–152	19	19	53 (96.45%)	0	2 (3.6%)
TIM	8–128	16	64	38 (69.1%)	12 (21.8%)	5 (9.1%)
CAZ	4–64	16	64	22 (40.0%)	10 (18.2%)	23 (41.8%)
LEV	1–16	1	2	51 (92.8%)	2 (3.6%)	2 (3.6%)
MIN	2–32	2	2	55 (100.0%)	0	0

S, susceptible; I, intermediate; R, resistant; FEP, cefepime; CIP, ciprofloxacin; TMP/SMX, trimethoprim-sulfamethoxazole; TIM, ticarcillin/clavulanic; CAZ, ceftazidime; MIN, minocycline; LEV, levofloxacin.

indicated that multiple genomospecies of *Smc*, including potential novel species, were associated with BSIs. *S. maltophilia*, belonging to the core *Smc* group, was the dominant group among sequenced isolates, followed by genomospecies 1, which represents a putatively

novel species. Furthermore, we found that *S. maltophilia* was the most widespread and exhibited a high mortality rate, indicating its status as a high-risk strain. Although the mortality rate associated with *S. pavanii* infection was as high as 50%, further studies are warranted

TABLE 3 The distribution of virulence genes in Smc strains (%).

Percentage	<i>afaD</i>	<i>entA</i>	<i>fliC</i>	<i>gspD</i>	<i>KatA</i>	<i>motA</i>	<i>pilU</i>	<i>rmlA</i>	<i>smf-1</i>	<i>Stmpr1</i>	<i>Stmpr2</i>
<i>S. maltophilia</i> (<i>n</i> = 19)	84.21	100	42.11	100	100	100	100	36.84	100	100	100
<i>S. sepilia</i> (<i>n</i> = 7)	71.43	85.71	0	100	100	100	100	14.28	100	0	100
<i>S. pavanii</i> (<i>n</i> = 6)	16.67	50	0	66.67	100	100	100	16.67	100	0	100
<i>S. geniculata</i> (<i>n</i> = 2)	0	50	0	100	100	100	100	0	100	0	100
<i>P. hibiscicola</i> (<i>n</i> = 1)	0	100	0	100	100	100	100	100	100	0	100
<i>S. africana</i> (<i>n</i> = 2)	0	0	0	100	100	100	100	100	100	0	100
Genomospecies 1 (<i>n</i> = 9)	100	100	0	100	100	100	100	0	100	0	100
Genomospecies 2 (<i>n</i> = 4)	0	0	0	100	100	100	100	0	100	0	100
Genomospecies 3 (<i>n</i> = 2)	0	0	0	0	0	100	100	0	100	0	100
Genomospecies 4 (<i>n</i> = 1)	0	0	0	100	100	100	100	100	100	0	100
Genomospecies 5 (<i>n</i> = 1)	0	0	0	100	100	100	100	0	100	0	100
Genomospecies 6 (<i>n</i> = 1)	0	100	0	100	100	100	100	0	100	100	100

to expand the sample size due to the limited number of strains analyzed in this study.

To date, more than 560 STs of Smc have been deposited in the public databases for molecular typing and microbial genome diversity analysis. Numerous new STs have been reported by different countries (Esposito et al., 2017; Rizek et al., 2018; Bostanghadiri et al., 2019; Mojica et al., 2019). In our present study, we identified a total of 22 novel ST types and 58 new alleles among the isolates. Our results demonstrated a striking diversity of Smc genotype with the STs well dispersed, consistent with previous findings (Mojica et al., 2019; Yinsai et al., 2023). Genome dynamics play a crucial role in the survival and evolution of bacteria. Smc illustrates the evolutionary intricacies of an opportunistic pathogen with the emergence of novel STs posing a potential major threat in healthcare settings. While Smc outbreaks are commonly reported from respiratory specimens, BSI outbreaks are rare. In our 9-year study period, no outbreak was observed.

Smc is the third most common nosocomial non-fermenting Gram-negative bacillus and exhibits high levels of intrinsic and acquired resistance to multi-antibacterial agents (Brooke, 2014). In recent years, multidrug-resistant Smc isolates have become increasingly prevalent in certain regions, posing significant challenges to treatment (Rizek et al., 2018; Kumar et al., 2020). In our study, the resistance rate of Smc against TMP/SMX was 3.6%, with the *sulI* and *intI* detected in these isolates, mediating TMP/SMX resistance (Hu et al., 2011). However, some studies have reported higher rates of TMP/SMX resistance, ranging from 13.7 to 32.8%, with 61.3 to 100% of Smc isolates originating from the respiratory tract (Flores-Treviño et al., 2014; Adegoke et al., 2017). These discrepancies may be attributed to differences in specimen types. All Smc isolates in our study were susceptible to MIN, suggesting its potential use as a therapeutic alternative. However, Smc exhibited a high resistance rate

against CAZ, reaching up to 41.8%. Previous studies have suggested that low membrane permeability, the presence of drug efflux pumps and variations in the efflux pump genes may induce resistance expect drug hydrolyzing genes (Crossman et al., 2008).

Furthermore, we analyzed the presence of virulence genes among different genomospecies of Smc. All Smc strains harbored *motA*, *pilU*, *smf-1* and *Stmpr2* genes. Interestingly, the *fliC* (flagellin) gene was only detected in *S. maltophilia* with a prevalence of 42.11%. A previous study has reported that all *S. maltophilia* strains that originated from blood samples show 100% (17/17) positivity for *fliC* gene, it is worth noting that there was clonal transmission among these strains (Cruz-Córdova et al., 2020). Strains with a higher percentage of *afaD* gene included genomospecies 1 (100%, *n* = 9), *S. maltophilia* (84.21%, *n* = 19) and *S. sepilia* (71.43%, *n* = 7), which were also the strains with higher rates of separation. Smc strains isolated from blood samples showed a high percentage of the *fliC* and *afaD* genes which are associated with colonization (Cruz-Córdova et al., 2020). The finding suggested that these virulence factors might contribute to the persistence and dissemination of Smc strains. The *stmPr1* gene encoding extracellular protease only found in *S. maltophilia* and genomospecies 6, indicating its potential utility for the preliminary identification of strains. In addition to *motA*, *pilU*, *smf-1*, and *Stmpr2* genes, 100% of *S. maltophilia* strains also contained the *entA*, *gspD*, *katA*, and *stmPr1* genes, and 100% of genomospecies 1 strains contained the *afaD*, *entA*, *gspD*, and *katA* genes. The presence of varied virulence factors among different genomospecies underscored the importance of accurate strain identification. There are many factors affecting mortality rate, especially the immune status and underlying diseases of the patients, therefore we did not observe a correlation between virulence genes (encoding biofilm production and adherence) and mortality.

In conclusion, our study highlights the genetic diversity among Smc isolates from bacteremic patients and revealed 22 novel ST types, 58 new alleles and 6 new genomospecies. Our findings indicated that at least 12 distinct genomospecies were associated with BSIs in our hospital. MIN emerged as a potential alternative antibiotic for patients who were resistant to TMP/SMX. Both *S. maltophilia* and *S. pavonii* harbored a higher number of virulence factors and were associated with high mortality rates. Therefore, accurate strain identification is very important. Our data suggested no outbreak at our testing center. However, the limited number of collected strains in this single-center study might restricted data analysis to identify transmission relationships within the local area. Therefore, a large-scale, multicenter study should be carried out in the future.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/[Supplementary material](#).

Author contributions

LW: Conceptualization, Investigation, Validation, Writing – original draft, Writing – review & editing. YW: Investigation, Writing – original draft. KY: Data curation, Methodology, Writing – original draft. XQ: Validation, Visualization, Writing – review & editing. QZ: Methodology, Writing – review & editing. LY: Formal analysis, Writing – review & editing. JY: Conceptualization, Supervision, Writing – review & editing.

References

- Adegoke, A. A., Stenström, T. A., and Okoh, A. I. (2017). *Stenotrophomonas maltophilia* as an emerging ubiquitous pathogen: looking beyond contemporary antibiotic therapy. *Front. Microbiol.* 8:2276. doi: 10.3389/fmicb.2017.02276
- Anzai, Y., Kim, H., Park, J. Y., Wakabayashi, H., and Oyaizu, H. (2000). Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int. J. Syst. Evol. Microbiol.* 50, 1563–1589. doi: 10.1099/00207713-50-4-1563
- Bostanghadiri, N., Ghalavand, Z., Fallah, F., Yadegar, A., Ardebili, A., Tarashi, S., et al. (2019). Characterization of phenotypic and genotypic diversity of *Stenotrophomonas maltophilia* strains isolated from selected hospitals in Iran. *Front. Microbiol.* 10:1191. doi: 10.3389/fmicb.2019.01191
- Brooke, J. S. (2012). *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clin. Microbiol. Rev.* 25, 2–41. doi: 10.1128/CMR.00019-11
- Brooke, J. S. (2014). New strategies against *Stenotrophomonas maltophilia*: a serious worldwide intrinsically drug-resistant opportunistic pathogen. *Expert Rev. Anti-Infect. Ther.* 12, 1–4. doi: 10.1586/14787210.2014.864553
- Crossman, L. C., Gould, V. C., Dow, J. M., Vernikos, G. S., Okazaki, A., Sebahia, M., et al. (2008). The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. *Genome Biol.* 9:R74. doi: 10.1186/gb-2008-9-4-r74
- Cruz-Córdova, A., Mancilla-Rojano, J., Luna-Pineda, V. M., Escalona-Venegas, G., Cázares-Domínguez, V., Ormsby, C., et al. (2020). Molecular epidemiology, antibiotic resistance, and virulence traits of *Stenotrophomonas maltophilia* strains associated with an outbreak in a Mexican tertiary care hospital. *Front. Cell. Infect. Microbiol.* 10:50. doi: 10.3389/fcimb.2020.00050
- De Oliveira-Garcia, D., Dall'agnol, M., Rosales, M., Azzuz, A. C., Alcántara, N., Martínez, M. B., et al. (2003). Fimbriae and adherence of *Stenotrophomonas maltophilia* to epithelial cells and to abiotic surfaces. *Cell. Microbiol.* 5, 625–636. doi: 10.1046/j.1462-5822.2003.00306.x
- Esposito, A., Pompilio, A., Bettua, C., Crocetta, V., Giacobazzi, E., Fiscarelli, E., et al. (2017). Evolution of *Stenotrophomonas maltophilia* in cystic fibrosis lung over chronic

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank the curators of the MLST database for assigning our new sequence types.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2024.1424241/full#supplementary-material>

infection: a genomic and phenotypic population study. *Front. Microbiol.* 8:1590. doi: 10.3389/fmicb.2017.01590

Flores-Treviño, S., Gutiérrez-Ferman, J. L., Morfín-Otero, R., Rodríguez-Noriega, E., Estrada-Rivadeneira, D., Rivas-Morales, C., et al. (2014). *Stenotrophomonas maltophilia* in Mexico: antimicrobial resistance, biofilm formation and clonal diversity. *J. Med. Microbiol.* 63, 1524–1530. doi: 10.1099/jmm.0.074385-0

Gröschel, M. I., Meehan, C. J., Barilar, I., Diricks, M., Gonzaga, A., Steglich, M., et al. (2020). The phylogenetic landscape and nosocomial spread of the multidrug-resistant opportunist *Stenotrophomonas maltophilia*. *Nat. Commun.* 11:2044. doi: 10.1038/s41467-020-15123-0

Hu, L. F., Chang, X., Ye, Y., Wang, Z. X., Shao, Y. B., Shi, W., 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. doi: 10.1016/j.ijantimicag.2010.10.025

Huang, C., Lin, L., and Kuo, S. (2024). Risk factors for mortality in *Stenotrophomonas maltophilia* bacteremia - a meta-analysis. *Infect. Dis.* 56, 335–347. doi: 10.1080/23744235.2024.2324365

Kim, M., Oh, H. S., Park, S. C., and Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int. J. Syst. Evol. Microbiol.* 64, 346–351. doi: 10.1099/ijs.0.059774-0

Kumar, S., Bansal, K., Patil, P. P., Kaur, A., Kaur, S., Jaswal, V., et al. (2020). Genomic insights into evolution of extensive drug resistance in *Stenotrophomonas maltophilia* complex. *Genomics* 112, 4171–4178. doi: 10.1016/j.ygeno.2020.06.049

Looney, W. J., Narita, M., and Mühlemann, K. (2009). *Stenotrophomonas maltophilia*: an emerging opportunist human pathogen. *Lancet Infect. Dis.* 9, 312–323. doi: 10.1016/S1473-3099(09)70083-0

- Mercier-Darty, M., Royer, G., Lamy, B., Charron, C., Lemenand, O., Gomart, C., et al. (2020). Comparative whole-genome phylogeny of animal, environmental, and human strains confirms the Genogroup organization and diversity of the *Stenotrophomonas maltophilia* complex. *Appl. Environ. Microbiol.* 86:e02919-19. doi: 10.1128/AEM.02919-19
- Mojica, M. F., Rutter, J. D., Taracila, M., Abriata, L. A., Fouts, D. E., Papp-Wallace, K. M., et al. (2019). Population structure, molecular epidemiology, and β -lactamase diversity among *Stenotrophomonas maltophilia* isolates in the United States. *MBio* 10:e00405-19. doi: 10.1128/mBio.00405-19
- Ochoa-Sánchez, L. E., and Vinuesa, P. (2017). Evolutionary genetic analysis uncovers multiple species with distinct habitat preferences and antibiotic resistance phenotypes in the *Stenotrophomonas maltophilia* complex. *Front. Microbiol.* 8:1548. doi: 10.3389/fmicb.2017.01548
- Patil, P. P., Kumar, S., Midha, S., Gautam, V., and Patil, P. B. (2018). Taxonogenomics reveal multiple novel genomospecies associated with clinical isolates of *Stenotrophomonas maltophilia*. *Microb. Genom.* 4:e000207. doi: 10.1099/mgen.0.000207
- Rizek, C. F., Jonas, D., Garcia Paez, J. I., Rosa, J. F., Perdigão Neto, L. V., Martins, R. R., et al. (2018). Multidrug-resistant *Stenotrophomonas maltophilia*: description of new MLST profiles and resistance and virulence genes using whole-genome sequencing. *J. Glob. Antimicrob. Resist.* 15, 212–214. doi: 10.1016/j.jgar.2018.07.009
- Trifonova, A., and Strateva, T. (2019). *Stenotrophomonas maltophilia* - a low-grade pathogen with numerous virulence factors. *Infect. Dis. (Lond.)* 51, 168–178. doi: 10.1080/23744235.2018.1531145
- Vidigal, P. G., Dittmer, S., Steinmann, E., Buer, J., Rath, P. M., and Steinmann, J. (2014). Adaptation of *Stenotrophomonas maltophilia* in cystic fibrosis: molecular diversity, mutation frequency and antibiotic resistance. *Int. J. Med. Microbiol.* 304, 613–619. doi: 10.1016/j.ijmm.2014.04.002
- Yinsai, O., Deudom, M., and Duangsonk, K. (2023). Genotypic diversity, antibiotic resistance, and virulence phenotypes of *Stenotrophomonas maltophilia* clinical isolates from a Thai university hospital setting. *Antibiotics* 12:410. doi: 10.3390/antibiotics12020410