Comparative genomics analysis of Stenotrophomonas maltophilia strains from a community

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Abstract

**Background** *Stenotrophomonas maltophilia* is a multidrug resistant (MDR) opportunistic pathogen with high resistance to most clinically used antimicrobials. The dissemination of MDR *S. maltophilia* and the difficult treatment of its infection in clinical settings are global issues.

**Methods** To provide more genetic information of *S. maltophilia* and find a better treatment strategy, we isolated five *S. maltophilia*, SMYN41-SMYN45, from community in China which were subjected to antibiotic sensitivity testing, biofilm formation assay, and whole-genome sequencing. And the whole genome sequences have been compared with other twenty-five *S. maltophilia* sequences.

**Results** These five *S. maltophilia* strains had similar antibiotic resistance profiles that were resistant to β-lactams, aminoglycosides, and macrolides. They conferred similar antimicrobial resistance (AMR) genes, including various efflux pumps, β-lactamase resistant genes (*bla*1/2), aminoglycosides resistant genes [*(aac(6'/3))*], and macrolide resistant gene (*MacB*). Genome sequencing analysis revealed that SMYN41-SMYN45 belonged to sequence type 925 (ST925), ST926, ST926, ST31, and ST928, separately, and three new STs were identified (ST925, ST926 and ST928).

**Conclusions** This study provides genetic information by making a comparison of genome sequences of several *S. maltophilia* isolates from community and various origins, expecting to optimize antibiotic use for patient treatment and contributing to the worldwide efforts of tackling antibiotic resistance.

**Background**

The evolution and dissemination of antibiotic resistance has become one of the significant threats to the public health in the world nowadays, contributing to difficulties in treatment and being associated with high morbidity and mortality [1]. *Stenotrophomonas maltophilia* is an emerging new multidrug resistant (MDR) opportunistic human pathogen, which often can cause nosocomial infections with high resistance to most clinically used antimicrobials [2–6]. *S. maltophilia* is an ubiquitous microorganism in the environment, predominantly colonizing the skin, the respiratory tract, urinary catheters, and breathing tubes [7]. Infections generally result in pneumonia, bacteremia, urinary tract infection, or meningitis, particularly associated with cystic fibrosis or chronic lung diseases [2,8]. The crude mortality rates of bacteremia is 14 to 69% [2].

To the best of knowledge, the major resistance molecular mechanisms include the intrinsic and acquired antibiotic resistance mechanisms in *S. maltophilia*. While the major intrinsic resistance mechanism responsible for its MDR phenotype can be attributed to the activity of the chromosomally encoded multidrug efflux pumps, the low membrane permeability, and antibiotic modifying enzymes, such as β-lactamases and other aminoglycoside phospho- and acetyl-transferases [2,8–10]. The genome of *S. maltophilia* encodes various multidrug efflux pumps, including ATP-binding cassette (ABC)-transporter family, major facilitator superfamily (MFS)-type, resistance nodulation cell division (RND) efflux systems, the small multidrug resistance (SMR) family, and the fusaric acid resistance efflux pump family [11–17]. Furthermore, more and more studies have extensively described the biofilm formation of *S. maltophilia* which can lead to infections and antimicrobial resistance [18]. Biofilms are products of bacterial adherence to a natural or living surface. The starting point of various infections is often the formation of a biofilm by the infecting organism [19]. Thus, anti-infective therapy targeting biofilm phase of the organism is an important consideration for effective treatment.

The treatment of infection caused by *S. maltophilia* is controversial and difficult due to its genotypic and phenotypic variability. Pak, T. R. et al reported that a patient whose *S. maltophilia* associated bacteremia had broken out four times, the sensitive antibiotic got lesser and finally it is resistant to fluoroquinolones while still susceptible to trimethoprim-sulfamethoxazole (SXT) [20]. However, research has shown that the susceptibility of bacterial isolates to SXT for *S. maltophilia* infection, decreased from 97.2% in 2001-2004 to 95.7% in 2013-2016, and varied according to the geographic region [21].

The spread of MDR *S. maltophilia* is a global public health issue. Understanding the genetic makeup of such opportunistic pathogen will enable us to optimize antibiotic use for patient treatment and contribute to the worldwide efforts of tackling antibiotic resistance. Whole-genome sequencing (WGS) is gaining importance in the analysis of bacterial pathogens to provide information about genomic determinants and antimicrobial resistance (AMR) genes. WGS studies allow comparative genomic analysis of bacterial populations, providing new insights into their genetic diversity and evolution. What’s more, global genome-based collections are missing for *S. maltophilia*, which listed as one of the leading drug-resistant nosocomial pathogens worldwide [22–24]. Therefore, the present study was conducted with the aim of providing genetic information by making a comparison of genome sequences of several *S. maltophilia* isolates from community in China and expecting a better treatment strategy.
Methods

2.1 Bacterial isolation and identification

The strains are isolated from 63 sputum or urine samples collected from a community in Luzhou, Sichuan Province, China, in 2017. Samples were plated on TSA medium with vancomycin 16mg/L and meropenem 6mg/L to select the resistant bacteria. Bacterial species identification was carried out by 16s rRNA sequencing. All of the procedures were approved by the Regional Committee of Ethics for Human Research of Southwest Medical University.

2.2 Antimicrobial susceptibility testing and biofilm formation assay

The antibiotics, including SXT, cefoperazone/sulbactam (SCF), levofloxacin (LVX), norfloxacin (NOR), ciprofloxacin (CIP), minocycline (MIN), ampicillin (AM), gentamicin (GM), cefotaxime (CTX), piperacillin (PIP), aztreonam (ATM), imipenem (IPM), and erythromycin (EM), were determined using the K-B diffusion technique interpreted in accordance with the recommendations of the Clinical and Laboratory Standard Institute (CLSI) guidelines [25].

Biofilm formation assay was performed by crystal violet staining assay. The isolates were cultured overnight in TSB broth for 72 hours at 37°C. Each isolate was diluted in fresh TSB broth to achieve a cell density equivalent of $10^8$ CFU/ml. A total of 100ul of each diluted culture was transferred into the microtiter plates (96-well plates, round-bottom) and incubated at 37°C for 72 hours. Culture supernatants were discarded, and added 200ul aseptic saline to all wells, cleaned them three times by distilled water, and plates were allowed to dry at 37°C for one hour. Then add 100ul 1% crystal violet to every well for 20 minutes at room temperature to stain the samples for the quantification of biofilms, and carefully washed them three times with water to remove excess dye and make them dry at room temperature. Finally, dissolve the dye with 100μl of 30% acetic acid for 30 minutes. The absorbance of solubilized crystal violet was measured at 595 nm optical density (OD$_{595}$) using 30% acetic acid as a reference. All experiments were performed in triplicate and were repeated three times. The optical density cut-off value (ODc) was considered to be the average optical density value (OD) of the negative control. The strength of biofilm formation was categorized as follows [26]: no biofilm production (OD ≤ ODc); weak biofilm formation (ODc < OD ≤ 2×ODc); moderate biofilm formation (2×ODc < OD < 4×ODc); and strong biofilm formation (OD > 4×ODc).

1. Whole genome sequencing, annotation, and analysis

Genomic DNA of Stenotrophomonas maltophilia strains were extracted using the DNA Kit (QIAGEN, Germany). A 300 bp paired-end library were constructed using the standard Illumina DNA sample preparation instructions, and then sequenced on MiSeq systems sequencing platforms (Novogene, China). Sequence reads were assembled using SPAdes version 3.12 [27]. The whole genome sequence was automatically annotated by the National Center for Biotechnology Information (NCBI) Prokaryote Genome Annotation Pipeline (PGAP) [28]. Functional annotation of genes in the genomes was carried out by COG (Clusters of Orthologous Group) (ncbi.nlm.nih.gov/research/cog) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/) databases. And the GO annotation of protein-coding genes was performed by BLAST2GO [29,30]. Annotation of antibiotic resistance determinants was performed using the Resistance Gene Identifier (RGI) in the Comprehensive Antibiotic Resistance Database (CARD; card.mcmaster.ca) [31]. Acquired resistance genes were predicted using ResFinder 4.0 at the Center for Genomic Epidemiology (CGE) [32,33]. The sequences were analyzed with the DNASTAR and Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4 Genome data, phylogenetic analysis, and multi-locus sequence typing (MLST)

The genomes of S. maltophilia were obtained from the publicly available genome database in NCBI. A total of twenty-five genomic sequences were analyzed in a comparative fashion throughout this study and statistically classified by the source, including clinical, animal, and environmental origin.

For phylogenetic analysis and comparative genome, our isolated S. maltophilia strains sequencing genomes and twenty-five S. maltophilia genome sequences from the GenBank were compared using PGAP, with which the genes shared by all genomes were collected, concatenated, and aligned. The alignment of the conserved genes was used for the construction of neighbor joining tree using
PGAP. A phylogenetic tree was reconstructed with the maximum-likelihood method by implementation in RAxML V7.9.5 with 1,000 bootstraps replicates [34]. Based on seven housekeeping genes (atpD, gapA, guaA, mutM, nuoD, ppsA, and recA), the MLST of whole-genome sequence data of the isolates was performed according to the PubMLST.org website (PubMLST - Public databases for molecular typing and microbial genome diversity) [35]. New alleles and sequence types (STs) were confirmed by the University of Oxford database. The MLST minimum spanning tree has been constructed on PHYLOViZ Online (PHYLOViZ Online).

2.5 Nucleotide sequence accession numbers

The whole genome sequences have been deposited in GenBank under the accessions SRZW00000000, SRVN00000000, SRVO00000000, SRVQ00000000, and SRVP00000000 for Stenotrophomonas maltophilia strains SMYN41, SMYN42, SMYN43, SMYN44, and SMYN45, respectively.

Results

3.1 The phenotype, antimicrobial susceptibilities, and biofilm formation

There are five \textit{S. maltophilia} isolates identified from the 63 samples collected from community in China, named SMYN41, SMYN42, SMYN43, SMYN44, and SMYN45, respectively. Almost all of the five \textit{S. maltophilia} strains were resistant to AM, GM, PIP, CTX, ATM, IPM, and EM, whereas they are almost sensitive to SXT, SCF, LVX, NOR, CIP, and MIN according to CLSI breakpoints (M100-S23) (Table 1, at the end of this text).

The OD values of biofilm formation of \textit{S. maltophilia} SMYN41-SMYN45 in OD\textsubscript{595} were ranged from 0.101 to 0.218, whereas the OD\textsubscript{c} is 0.051 (Table 1). The \textit{S. maltophilia} SMYN41 has produced weak biofilm, while SMYN42, SMYN43 and SMYN45 have had moderate biofilm. And SMYN44 is a strong biofilm-producer.

3.2 Characteristics of the whole genome

The general genomic features of the five isolates sequenced in this study are summarized in the Additional file 1. Whole genome sequences of strain SMYN41, SMYN42, SMYN43, SMYN44, and SMYN45 have total sized ranging from 4,371,193 to 4,897,474 bp with no plasmid. The genomes consist of 72, 18, 17, 62, and 32 contigs with a G+C content 66.60%, 66.72%, 66.31%, 66.72%, and 66.59%, respectively. The predicted genes were then annotated by the COG, KEGG, and GO gene databases (Additional file 2-16). Specifically, a total of 3070 (67.94%), 2828 (72.36%), 2828(72.46%), 2986(70.31%), and 2938(71.78%) genes that were functionally annotated according to GO were classified into 3 categories (biological process, cellular component, and molecular function). A total of 3825 (84.64%), 3419 (87.49%), 3419(87.60%), 3608(84.95%) and 35.47(86.66%) genes belonging to 24 categories were annotated from the COG database. Moreover, based on searches against the KEGG database, 1952(43.20%), 1865(47.72%), 1863(47.73%), 1983(44.57%), and 1886(46.08%) genes were predicted.

3.3 Biofilm-forming relative genes

There are forty genes associated with different mechanisms of biofilm formation in \textit{S. maltophilia} SMYN41-SMYN45 that annotated based on the NCBI PGAP (Figure 1). All these genes were located on the chromosome. The genes responsible for polysaccharide production (spgM, rmlA, and rmlC) [26,36–38], quorum sensing (QS) (rpfF, ax21, and smoR) [39–45], and flagella (fleQ, flgE/G/G/K/I, fha, filF/I/K/M/N/O/A, and fimV) [46–50] were identified in \textit{S. maltophilia} SMYN41-SMYN45 [18]. Several other biofilm related genes (purE/D/C/I, guaA, and ravS) were annotated in the five strains [46]. Moreover, flID gene was annotated in SMYN42-SMYN45 [46]. SMYN44 and SMYN45 contain fimbiae gene smf-1[51], polysaccharide production gene xanA[46], and other gene purK [46].

3.4 Antimicrobial resistance analysis

A total of thirty-four genes involved in different mechanisms of drug resistance were annotated and identified based on the NCBI PGAP and CARD (Figure 2). All these genes were located on the chromosome. Several genes encoding RDN family (smeABC, smeDEF, adeF, MntP, and MacB), SMR family (qacJ), and MFS efflux pumps (bcr/CfA, emrCBAsm, and TolC protein family) were identified in SMYN41-SMYN45. The five genome sequences also contain a variety of AMR genes, including those conferring
aminoglycoside resistance (aph(3')-II and aph(6)), β-lactam resistance (blaL1 and blaL2), macrolide resistance (MacB) and fluoroquinolone resistance (qnr, gyrA, and parC). The presence of point mutation in gyrA and parC gene may be responsible for fluoroquinolone resistance in strains. Furthermore, SMYN44 and SMYN45 contain smeS, and another aminoglycoside resistance gene aac(3')-Ia was identified in SMYN41. Notably, aminoglycoside resistance genes (aph(3')-Ila and aac(6')-Iz) as well as β-lactamaseblaIa were confirmed as acquired resistance genes based on ResFinder4.0 at CGE. However, none of these 5 genomes encoding SXT resistance related genes (suI1, suI2, and dfrA).

The AMR gene profiles of SMYN41-SMYN45 were compared to those of the twenty-five S. maltophilia isolates obtained from the GenBank database (Figure 2). According to the origin of the strains, S. maltophilia SMYN41-SMYN45 conferred similar AMR genes in all human derived strains. SMYN41 shares almost identical antibiotic genes compared to S. maltophilia k279a, and S. maltophilia SMYN44-SMYN45 possessed similar antibiotic genes. Moreover, animal origin isolates contained the fewest AMR genes and did not contain SXT and fluoroquinolone related resistance genes. Environment derived strains contained different kinds of AMR genes and the genes fewer than that in human origin strains. Overall, 28 out of 30 S. maltophilia isolates conferred three or more classes of antibiotic resistant genes. All isolates were harbored sme-related efflux pumps. Most S. maltophilia isolates (25/30) contained SMR family (qacJ or qacG) associated with resistance to disinfecting agents and antiseptics. Almost all strains contained macrolide resistance gene macB except S. maltophilia JV3. β-lactam resistance genes (28/30) and aminoglycoside resistance genes (27/30) were present in most of S. maltophilia isolates. Sm32COP and ZBG7B did not confer the former, as well as the latter were not identified in D457, W18, and ZBG7B. A part of strains (17/30) conferred quinolones resistance genes qnr and gyrA/parC. But no mutations were found in the latter. Only S. maltophilia W18 conferred MFS efflux pumps arlR and norA/C, β-lactamaseblaZ, and SXT resistance gene dfrA, while none of other genomes conferred.

### 3.5 Phylogenetic analysis

We have downloaded the complete sequences of twenty-five S. maltophilia strains from GenBank and the characteristics are summarized in Table 2. These strains are from human (n = 5), animal (n = 5), or environment (n = 15). The complete genomes of these sequences have total sizes ranging from 4,065,399 to 4,949,420 bp. Most antibiotic phenotypes of these strains are unknown that strains for which the antibiotic resistance profile is not described in the references.

The phylogenetic tree is in Figure 2. Most of strains are not grouped within clusters from the origins. The environmental derived strain CSM2 from Mexican laboratory sink clustered with human strain SMYN41 from China. Human derived strains SMYN42 and SMYN43 clustered with animal origin strains Sm32COP and SmSOFb1, environmental origin isolate mecca, and human isolate SM16975. Moreover, strain k297a from human origin and BurE1 from Burkina environment clustered with SMYN44 and SMYN45 from Chinese human origin and SmF3 from France cattle manure. These confirmed that the phylogeny does not cluster these strains by their geography and origin (human, environmental or animal origin).

In the same way, this phylogeny is not grouped within clusters by the multidrug resistant and antibiotic sensitive strains. In spite of the lack of information for many sequenced strains, according to the known drug-resistant phenotype, for instance, the MDR strain k297a, BurE1, BurA1 and sensitive strain Sm32COP, R551-3, and PierC1 seem to group within different clusters. Although BurE1 is genetically close to k297a, it cannot confirm MDR strains are grouped within clusters. The number of resistance genes or putative resistance genes is not related to resistant phenotype. For example, strain BurE1 are multi-resistant bacteria (Table 2).

### 3.6 MLST results

Based on the housekeeping genes under the Oxford MLST scheme, we searched and submitted the sequences of these thirty strains on pubMLST (Additional file 17). MLST sequencing showed that S. maltophilia SMYN42, SMYN43 and SMYN45 are new STs, and SMYN41-SMYN45 belong to ST325 (101, 264, 563, 271, 211, 294, and 157 for atpD, gapA, guaA, mutM, nuoD, ppsA, and recA), ST926 (13, 28, 564, 116, 212, 143, and 22), ST926 (13, 28, 564, 116, 212, 143, and 22), ST31 (3, 4, 24, 7, 22, and 27), and ST928 (1, 1, 326, 3, 25, 295, and 1), respectively. Isolates SMYN42 and SMYN43 possess the same housekeeping gene alleles and both belong to ST926. Among these twenty-five sequences downloaded from GenBank, fourteen types are the new STs (ST929-ST942). Eight isolates consisted of existing types in the database without any duplicate sequence types. And we could not find complete trusted alleles or hits in the other five isolates, including Sm46PAILV, SmSOFb1, W18, and ZBG7B. The MLST minimum spanning tree has further confirmed that this phylogeny is not grouped within clusters by the source or antibiotic resistance (Figure 3).
Discussion

In the present work, we characterized the whole-genome sequencing features of five human origin *Stenotrophomonas maltophilia* MDR isolates SMYN41-SMYN45 from a community and performed comparative analysis of biofilm-formatting genes in the five isolates as well as AMR genes in a total of thirty *S. maltophilia* isolates.

Based on the antibiotic sensitivity test, it can be concluded that *S. maltophilia* SMYN41-SMYN45 were indeed the MDR bacteria with resistance to aminoglycosides, carbapenems, macrolides, and glycopeptide antibiotics, although separated from community. The phenotypic antibiotic susceptibility results were basically consistent with the antibiotic resistance predicted by AMR genes. Furthermore, multidrug resistance was preserved throughout *S. maltophilia* strains, with most isolates (28/30) harboring multiple AMR genes predicted to be resistant to three or more classes of antibiotics. Noteworthy, it is reported that the resistance of *S. maltophilia* to quinolones may be mainly caused by the mutations at the target sites of DNA gyrase and topoisomerase (mainly gyrA and parC), plasmid or chromosomemediated mutations of drug resistance genes (like Qnr family) and drug efflux pumps (mainly smeDEF, which is the major determinant of quinolones resistance in *S. maltophilia*) [52–56]. SMYN41-SMYN45 conferred the latter two mechanisms but are not resistant to quinolones. The possible reasons are the unknown gene expression and that *S. maltophilia* contains a chromosomally encoded qnr gene which just confers low-level resistance to quinolones upon its expression in a heterologous host [57,58]. Treatment options are limited by resistance to a variety of antimicrobials. What's more, several studies have reported that antibiotic resistance of *S. maltophilia* in clinical is associated with previous antibiotic treatment [59–61]. Therefore, although these *S. maltophilia* isolates were sensitive to quinolones antibiotics at that time, the predicted quinolone resistance genes indicate the possible resistance to quinolones in the future. Additionally, the use of quinolones may lead to SXT resistance [60]. And these multidrug-resistant *S. maltophilia* strains from community are sensitive to SXT and do not possess SXT-related resistance gene. And, studies found that fluoroquinolone-containing regimens may be better options than SXT-containing regimens in treating *S. maltophilia*-related infections [62,63]. Therefore, treatment choice for *S. maltophilia* infections remains unclear when the strain is sensitive to both SXT and quinolones.

In addition to the inherent resistance genes of *S. maltophilia*, biofilm formation also exhibits greater resistance to antimicrobial drugs, which also is an important virulence feature. And biofilms are more difficult to treat clinically [64]. SMYN41-SMYN45 all can form biofilms. From several major classes of genes known to be associated with biofilm formation, weak biofilm-producer SMYN41 possesses the fewest biofilm-associated genes. Among them, the crucial role played by bacterial flagella in biofilm development has been well recognized [65]. Inactivation of some flagellar genes, such as *fliA* and an orphan response regulator *FsnR*, can be shown to result in deficiencies in biofilm formation [51]. These genes are present in SMYN41-SMYN45 which can contribute to biofilm-forming. Furthermore, it has been shown that almost all harbored *smf-1* isolates can form biofilm [51]. But it can be shown from our results that *smf-1* is not indispensable for biofilm production. Actually, there was a strong correlation between the results of the ability of biofilm production and biofilm-forming genes. Additionally, according to the antimicrobial sensitivity test results, the strong biofilm-producer SMYN44 and SMYN45 with the same biofilm-forming gene cluster showed relatively high antibiotic resistance. Notably, the use of fluoroquinolones in the early stages of *S. maltophilia* infection may possibly inhibit biofilm formation [64]. However, previous results have suggested that the use of quinolones may lead to subsequent SXT resistance. Therefore, the timing of quinolone use and whether to use it remains controversial. In hospital-acquired infections, the increased *S. maltophilia*-related infections are mainly due to inadequate use of antibiotics in treatment, which may also be further complicated by biofilm production [66]. Therefore, the use of biofilm-based antibiotic sensitivity testing for the selection of antimicrobials for *S. maltophilia* infections may provide an accurate and effective guide for appropriate treatment [19].

The five isolates from community had a high degree of similarity in COG, KEGG, and GO classifications, and similar KEGG pathways, which indicates that genes related to essential processes were mostly conserved in evolution for the clinical and environmental settings. According to phylogenetic results, there was no evolutionary correlation between phenotypic profiles, the genomes, and the origins. This is consistent with the results of other studies [67–69]. We found that the phylogenetic clades of *S. maltophilia* isolates SMYN44 and SMYN45 are highly closer to K279a and they have similar antibiotic resistant genotypes. But strain SMYN41 homologs of strain CSM2 have widely varying drug-resistant genotypes. And this also exists among many strains. As demonstrated by Zhao et al. that tiny differences in nucleic acid levels could lead to various significant phenotypes in close genetic relationship strains [67]. These results both underlined the genetic diversity and conservation compared with the other Stenotrophomonas representatives.

Three new STs (ST925, ST926 and ST928) and one exciting ST were identified from our primary *S. maltophilia* isolates SMYN41-SMYN45 that are similar in AMR and biofilm-formatting genes. Isolates SMYN42 and SMYN43 we collected are the exact same sequence types, which indicated the possible clonal transmission of *S. maltophilia* infections in community. And the new STs in this study indicated that isolates from community in China were different from those from other sources. The sequence types of the total of twenty-six
isolates were quite scattered that indicated loose associations. The MLST spanning tree were not consistent with PGAP phylogenetic tree, which also indicates the genetic diversity in *S. maltophilia* isolates. These inconsistencies were consistent with other relevant studies [70,71].

To conclude, *Stenotrophomonas maltophilia* strains isolated from community are MDR strains that play fundamental roles in hospital-acquired infections with high resistance to most clinically used antibiotics. They are sensitive to quinolones and SXT antibiotics with relatively conserved gene expression, but do not possess the resistance gene of the latter. Different isolates possess similar AMR genes classes. The strong adhesion of the biofilm also confirmed that it is highly susceptible to infections and difficult to treat clinically. It has been shown that the antibiotic treatment is also responsible for its acquisition of resistance. These data somewhat support current treatment regimens (SXT or fluoroquinolones) and suggest a risk of nosocomial infection with *S. maltophilia* [72–75]. But more data are still needed. Furthermore, the relevance and risk of MDR transmission amongst humans, animals, and the environment also should be taken into considerations, although the phenotypic profiles, genomes, origins, and geographical features cannot discriminate *S. maltophilia* isolates. Continuous monitoring of *S. maltophilia* strains is needed and may be able to find some variations of the antibiotic resistant phenotypes and AMR genes, and further reveal a high risk of this MDR pathogen.

**Conclusions**

Overall, understanding the genetic determinants, virulence factors, and AMR genes of these human derived *S. maltophilia* strains will help optimize antibiotic treatment options, improve surveillance and prediction of hospital outbreaks of *S. maltophilia* and contribute to global implementation of more effective infection prevention and control strategies. In this study, our in vitro results have provided more meaningful genetic information and an experimental basis for a possible optimal treatment strategy for *S. maltophilia* infections.

**Abbreviations**

MDR: multidrug resistant; MFS: major facilitator superfamily; SMR: small multidrug resistance; SXT: trimethoprim-sulfamethoxazole; WGS: Whole-genome sequencing; AMR: antimicrobial resistance; SCF: cefoperazone/sulbactam; LVX: levofloxacin; NOR: norfloxacin; CIP: ciprofloxacin; MIN: minocycline; AM: ampicillin; GM: gentamicin; CTX: cefotaxime; PIP: piperacillin; ATM: aztreonam; IPM: imipenem; EM: erythromycin; CLSI: Clinical and Laboratory Standard Institute; NCBI: National Center for Biotechnology Information; PGAP: Prokaryote Genome Annotation Pipeline; COG: Clusters of Orthologous Group; KEGG: Kyoto Encyclopedia of Genes and Genomes

**Declarations**

**Funding**

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**Authors’ contributions**

ZY and FL contributed to the conception and design of this study. LY drafted the manuscript and ZY was a major contributor in writing the manuscript. LY, SX, WH, GR, and LX collected the strains and performed the experiments. LY, LX, and CL analyzed the experimental results. All authors read and approved the final manuscript.

**Availability of data and materials**

The data used to support the findings of this study are available from the corresponding author on reasonable request.

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**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**
Not applicable.

**Competing Interests**

The authors declare no competing interests.

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**References**


### Tables

**Table 1. Antibiotic susceptibility and the optical density value of biofilm in Stenotrophomonas maltophilia SMYN41-45**

<table>
<thead>
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<td>R</td>
<td>0.218</td>
</tr>
<tr>
<td>SMYN45</td>
<td>S</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>0.170</td>
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<table>
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<th>Susceptibility rate</th>
<th>100%</th>
<th>86%</th>
<th>100%</th>
<th>100%</th>
<th>86%</th>
<th>100%</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
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<tbody>
<tr>
<td>Intermediary rate</td>
<td>0</td>
<td>14%</td>
<td>0</td>
<td>0</td>
<td>14%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14%</td>
<td>29%</td>
<td>29%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Resistance rate</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
<td>86%</td>
<td>71%</td>
<td>71%</td>
<td>100%</td>
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</table>
*: the average optical density (OD) value of biofilm formation of S. maltophilia SMYN41-45 and the mean value of the negative control wells is 0.051.

Abbreviations: SXT, trimethoprim/sulfamethoxazole; SCF, cefoperazone/sulbactam; LVX, levofloxacin; NOR, norfloxacin; CIP, ciprofloxacin; MIN, minocycline; AM, ampicillin; GM, gentamicin; PIP, piperacillin; CTX, cefotaxime; ATM, aztreonam; IPM, imipenem; EM, Erythromycin; S: susceptibility; R: Resistance; I: Intermediary.

Table 2. General features of 5 new Stenotrophomonas maltophilia strains and 25 S. maltophilia isolates available in NCBI
<table>
<thead>
<tr>
<th>Stenotrophomonas maltophilia strain</th>
<th>source</th>
<th>location</th>
<th>Genome size (bp)</th>
<th>Accession no.</th>
<th>Antibiotic phenotype</th>
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<td>D457</td>
<td>Clinical</td>
<td>Spain: Mostoles</td>
<td>4,769,156</td>
<td>NC_017671</td>
<td>TET, ERY, NAL, NOR, OFX</td>
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<td>K279a</td>
<td>blood</td>
<td>UK: Bristol</td>
<td>4,851,126</td>
<td>NC_010943</td>
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<td>PG 157</td>
<td>perineum</td>
<td>Slovenia: Golnik (2011)</td>
<td>4,949,420</td>
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<td>SanG_2012</td>
<td>lung</td>
<td>Italy: Rome (2012)</td>
<td>4,909,273</td>
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<td>Unknown</td>
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<td>SM-16975</td>
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<td>India (2012)</td>
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<td>SMYN41</td>
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<td>China: Sichuan (2017)</td>
<td>4,371,193</td>
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<td>Animal origin</td>
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<td>Sm32COP</td>
<td>horse manure</td>
<td>France: Feucherolles (2010)</td>
<td>4,548,960</td>
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<td>SmSOFb1</td>
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<td>AB550</td>
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<td>BurE1</td>
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<td>Burkina Faso (2008)</td>
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<td>CSM2</td>
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<td>Mexico: Morelos (2016)</td>
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<td>D5.1</td>
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<td>HPCN19</td>
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<td>India: Nagpur (2016)</td>
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<td>JV3</td>
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<td>Brazil (2013)</td>
<td>4,544,477</td>
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<td>Mecca</td>
<td>public</td>
<td>Saudi Arabia (2015)</td>
<td>4,386,843</td>
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<td>OUC_Est10</td>
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<td>PierC1</td>
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<td>4,638,575</td>
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<td>R551-3</td>
<td>poplar tree</td>
<td>USA: Washington (2011)</td>
<td>4,573,969</td>
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<td>SJTH1</td>
<td>waste</td>
<td>China: Shanghai</td>
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</table>
Unknown: Strains for which the antibiotic resistance profile is not described in the references.

Abbreviations: TET, tetractcline; ERY, erythromycin; NAL, nalidixic acid; NOR, norfloxacin; OFX, ofloxacin; AM, ampicillin; GM, gentamicin; PIP, piperacillin; CTX, cefotaxime; ATM, aztreonam; IPM, imipenem; EM, erythromycin.

**Figures**

**Figure 1**

Genes associated with biofilm formation in five *S. maltophilia* strains.

The isolates are shown at left, and the biofilm-formatting genes are shown at top. The dark blue color indicates the presence of the gene; the light blue indicates the absence of it.
Figure 2

The phylogenetic tree and antimicrobial resistance genes in 30 Stenotrophomonas maltophilia strains.

The isolates are shown at right, and the resistance genes are shown at bottom.

Figure 3

MLST minimum spanning tree.

The color indicates strains source. The number in circle is the sequence type of strains, and the number on line is the alleles that differed between two isolates.

Supplementary Files

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• Additionalfile16.jpg
• Additionalfile17.rtf