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RESEARCH ARTICLE

Antibiotic and metal resistance of *Stenotrophomonas maltophilia* isolates from Eboling permafrost of the Tibetan Plateau

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Abstract

Whole-genome sequencing of pathogenic bacteria *Stenotrophomonas maltophilia* from a less polluted environment of permafrost can help understand the intrinsic resistome of both antibiotics and metals. This study aimed to examine the maximum minimum inhibitory concentration (MIC) of both antibiotics and metals, as well as antibiotic resistance genes and metal resistance genes annotated from whole-genome sequences. The permafrost *S. maltophilia* was sensitive to ciprofloxacin, tetracycline, streptomycin, and bacitracin, and resistant to chloramphenicol, trimethoprim-sulfamethoxazole, erythromycin, Zn^{2+}, Ni^{2+}, Cu^{2+}, and Cr^{6+}, with a lower maximum MIC, compared with clinical *S. maltophilia*. The former strain belonged to the lower antibiotic resistance gene (ARG) and metal resistance gene (MRG) clusters compared with the latter ones. The permafrost strain contained no or only one kind of ARG or MRG on a single genomic island, which explained the aforementioned lower maximum MIC and less diversity of ARGs or MRGs. The result indicated that the co-occurrence of antibiotic and metal resistance was due to a certain innate ability of *S. maltophilia*. The continuous human use of antibiotics or metals induced selective pressure, resulting in higher MIC and more diverse ARGs and MRGs in human-impacted environments.

Keywords *Stenotrophomonas maltophilia* · Permafrost · Genome sequence · Antibiotic resistance · Metal resistance

Introduction

The World Health Organization has identified antibiotic resistance genes (ARGs) as one of the most important challenges to human health in the twenty-first century because ARGs are emerging environmental contaminants causing serious public health concerns (Sanderson et al. 2016). The annual number of human deaths due to antimicrobial resistance is expected to reach up to 10 million by 2050 (de Kraker et al. 2016). However, natural antibiotics have existed for billions of years (Barlow and Hall, 2002; Hall and Barlow, 2004; Bhullar et al. 2012; Wright and Poinar 2012). Similar to antibiotics, ARGs are also ancient, as evidenced by the studies identifying various ARGs in ancient permafrost samples (D'Costa et al. 2011; Perron et al. 2015) and isolated cave microbiomes (Bhullar et al. 2012). Then, many studies combined polluted and nonpolluted environments to study the potential sources of ARGs and the influence of human activity on ARGs (Li, et al. 2017; Yuan et al. 2019). The results showed that the nonpolluted environments contained fewer ARG subtypes than the polluted environments. However, a few studies focused on the antibiotic-resistant phenotypes and minimum inhibition concentration (MIC) to verify whether ARGs were expressed in environments with little human activity.

Whole-genome sequencing has become a powerful tool to recover ARGs from the same bacterial species from various sources, such as clinical and environmental settings. *Stenotrophomonas maltophilia*, a ubiquitous pathogen in hospitals and natural environments (Brooke 2012), has evolved as one of the multidrug-resistant bacteria causing various nosocomial infections, especially in highly debilitated patients (Patil et al. 2018). Some studies were undertaken on ARGs in *S. maltophilia* from the natural environment and clinical origin. The results showed the absence of *smeABC* in environmental *S. maltophilia* (Youenou et al. 2015). Some environmental strains carried more efflux pumps than the clinical ones (Youenou et al. 2015). However, the information on ARGs in *S. maltophilia* from natural environments with little human activity,

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such as permafrost, is limited. Comparative genomic analyses are needed to show the diversity of different ARGs among pathogenic *S. maltophilia* from different sources.

Antibiotic-resistant bacteria can transfer to other bacteria (including potential human pathogens) the ARGs they harbor through mobile genetic elements (MGEs) (Pruden et al. 2006; Zhu et al. 2013). For *S. maltophilia*, the researches about ARGs and MGEs focused on trimethoprim/sulfamethoxazole and class 1 integrons. The results showed that the most significant contribution of the class 1 integron acquisition to *S. maltophilia* was the increased resistance to trimethoprim–sulfamethoxazole through the *sulI* gene (Malekan et al. 2017; Song et al. 2010; Gallo et al. 2016). However, no other MGEs and ARGs were examined, which was important to understand the distribution of ARGs or multidrug resistance mechanism.

ARGs not only correlated with MGEs but also with metal resistance genes (MRGs), due to the potential association of antibiotic resistance and Cu, Zn, Ni, and Hg reported in various environmental settings (Baker-Austin et al. 2006; Berg et al. 2010; Mazhar et al. 2021; Hu et al. 2017; Knapp et al. 2017). MRGs, such as *merR*, *arsR*, *copG*, *cadA*, and *cadC*, existed in clinical *S. maltophilia* (Alonso et al. 2000; Kumar et al. 2020). No related research was undertaken for permafrost *S. maltophilia.* The comparative genomic analysis of *S. maltophilia* can reveal the difference in ARGs between human-impacted and less human-impacted environments due to many genomic contents with *S. maltophilia*, whose whole-genomic sequence data could be downloaded from NCBI. In addition, the widespread occurrence of metals in the environment may facilitate antibiotic resistance via co-selection of ARGs and MRGs. Thus, this study aimed to investigate the relationship between ARGs and MRGs in permafrost *S. maltophilia* to show whether this co-selection occurred in the pre-antibiotic era.

In this study, an *S. maltophilia* strain was isolated from the bottom of a ~ 11.7-m deep permafrost core (#B site: 38° 00′ 11.76″ N, 100° 54′ 24.66″ E; altitude 3615 m) of Eboling Mountain, from the Qilian Mountains of the Qinghai-Tibetan Plateau, to test its maximum MIC for both antibiotics and metals. The study then analyzed its whole-genome sequence. Considering the limited knowledge available on the *S. maltophilia* intrinsic resistome, the aims of this study were to (1) to compare the maximum MIC for antibiotics and metals, (2) the differences in ARGs and MRGs, and (3) genomic islands (GIs) between the ancient and present *S. maltophilia.*

Materials and methods

Isolation and susceptibility profile characterization

The isolation site of the *S. maltophilia* strain dates back to 5821 BP (Mu et al. 2014). One of the major features of the clinical isolates of *S. maltophilia* is their high resistance levels toward most of the currently used antimicrobial agents, including macrolides, fluoroquinolones, aminoglycosides, chloramphenicol, and tetracyclines (Brooke 2012). Thus, the MIC of the strain was determined by broth microdilution, with 40 repeats for each condition tested, in the presence or absence of seven antibiotics [ciprofloxacin, streptomycin, tetracycline, erythromycin, chloramphenicol, bacitracin, and trimethoprim–sulfamethoxazole (TMP-SMZ); Sigma, MO, USA], corresponding to fluoroquinolone, aminoglycosides, tetracycline, macrolide, phenicol, peptide, and sulfonamide, respectively. The strain was grown overnight in Mueller Hinton broth (MHB) using CLSI-recommended incubation conditions. After that, 100 µL of bacterial suspensions, with 40 repeats, and with a final optical density at 550 nm (OD550) of 0.005 were added to the wells containing the 2 × antibiotic dilutions. The clinical breakpoints for the seven antibiotics were established according to the European Society of Clinical Microbiology and Infectious Diseases (ECOFF).

A previous study assessed the levels of 11 different heavy metals Fe, Mn, Zn, Ni, Cr, Cu, As, Co, Mo, Cd, and Hg (Zhang et al. 2021). The MIC of eight metals Zn^{2+}, Mn^{2+}, Ni^{2+}, Sn^{2+}, Cu^{2+}, Cr^{6+}, Hg^{2+}, and Co^{2+} was determined. The metals were added as $ZnCl_2$, $MnCl_2.4H_2O$, $NiCl_2.6H_2O$, $SnCl_2.2H_2O$, $CuCl_2.2H_2O$, $K_2Cr_2O_7$, $HgCl_2$, and $CoCl_2$, respectively. The tubes containing R2A media were amended with increasing contents of metals (100, 200, 400, 800, and 1600 µg/mL) and incubated at 15℃ for 1 week. The MIC was defined as the lowest concentration of the metal at which the bacterial pellets remained invisible at the bottom of the tubes (Konopka and Zakharova, 1999). The cell concentration was measured using a spectrophotometer ($OD_{600} = 0.2$). *Escherichia coli* K-12, susceptible to many metals, was used as the control (Matyar et al. 2008; Akinbowale et al. 2007; Aleem et al. 2003; Malik and Jaiswal, 2000; Malik and Aleem, 2011). The strains were considered resistant if MIC values exceeded that of the control organism.

Genome sequencing and assembly

The total DNA of the bacterial colony was isolated using the Bacteria Genomic DNA Extraction Kit (TaKaRa Minibeast Ver.3.0, China) and the sample quality was ensured using NanoDrop ND-1000 microspectrophotometer (NanoDrop Technologies, DE, USA). Whole-genome sequencing was performed on an Illumina HiSeq PE150 platform (San Diego, CA, USA). A-tailed ligated paired-end adaptors with polymerase chain reaction (PCR)-amplified 350-bp inserts were used for library construction at Beijing Novogene Bioinformatics Technology Co., Ltd. From the Illumina PCR adapter reads, the low-quality reads were filtered as a quality control step by the sequencing company. All good-quality paired reads were assembled using the SOAP denovo (http://

soap.genomics.org.cn/soapdenovo.html) into several scaffolds (Li et al. 2010). The filtered reads were subjected to gap closing. The whole-genome shotgun project was deposited at GenBank (accession PRJNA504495, *S. maltophilia*).

Genome annotation

To find ARGs, the protein-coding sequences were searched against the comprehensive antibiotic resistance database (McArthur et al. 2013; Jia et al. 2017). A read was considered an ARG-like gene if the BLASTP identity was ≥ 40% (Liu et al. 2020).

MRGs in the metagenomic data were identified as previously described by Gupta et al. (2018). Experimentally confirmed MRGs were downloaded from the BacMet database (Version 2.0; Pal et al. 2014) as a reference source. Then, the clean MRG reads were matched against the reference source using BLASTX with the criteria of *e*-value < 10^{-5} and amino acid identity ≥ 90%.

The GIs were identified using Island Viewer 4 (Bertelli et al. 2017) and further analyzed using ICEfinder (Liu et al. 2019). The genes in the GIs were annotated using the Prokaryotic Genome Annotation Pipeline on NCBI3 and RASTtk server (Overbeek et al. 2014; Brettin et al. 2015). The insertion sequence transposases were detected using IS-Finder (Siguier et al. 2006). The integrons (ints) were predicted using the INTEGRALL database (Moura et al. 2009). The sequence alignment was performed with BLAST server2.

Phylogenetic analysis of 16S rRNA gene sequences

The 16S rRNA gene sequence of the permafrost *S. maltophilia* was extracted using Prokka. The 16S rRNA gene sequences from *S. maltophilia* NK-ST, BJ01, NRLFFD179, P4, and EN14ZR5 were downloaded from NCBI and used to construct a phylogenetic tree, with *S. tumulicola* T5916-2-1b, *S. humi* R-32729, and *S. pictorum* JCM 9942 as the members of the same genus. *Escherichia coli* was selected as an outgroup strain to determine the root of the tree. Multiple sequences were aligned using Clustal W 2.0 (Larkin et al. 2007) and MEGA7 (Kumar et al. 2016). The phylogenetic relationship was determined by phylogeny reconstruction analysis using the neighbor-joining method in MEGA7.

Results

Bacterial taxonomy

The permafrost *S. maltophilia* formed a cluster with *S. maltophilia* NK-ST, BJ01, NRLFFD179, P4, and EN14ZR5 (Fig. 1), with sequence similarity of 99.73%, 99.68%, 99.22%, 99.15%, and 99.06, respectively, based on pairwise alignments. This indicated that the permafrost strain was a member of the *S. maltophilia* group see Table 1.

Antibiotic and metal resistance profiles

The potential co-selections for antibiotic resistance were associated with Cu, Zn, Ni, and Hg in various environmental settings (Baker-Austin et al. 2006; Berg et al. 2010; Mazhar et al. 2021; Hu et al. 2017; Knapp et al. 2017). In addition, the MIC of Zn^{2+}, Ni^{2+}, Cu^{2+}, Cr^{6+}, and Hg^{2+} for *E. coli* K-12 was previously reported by Aleem et al. (2003), Malik and Jaiswal (2000), and Malik and Aleem (2011). Thus, the MIC of the aforementioned metals for the permafrost *S. maltophilia* was compared with that of *E. coli* K-12 to determine the metal resistance level. The MIC value for Hg^{2+} in *E. coli* K-12 was 12.5 µg/mL, while the initial concentration for Hg resistance was 100 µg/mL. Thus, the permafrost *S. maltophilia* showed resistance to Hg, as well as to other four heavy metals, in the order of $Hg^{2+} > Cr^{6+} > Zn^{2+} = Ni^{2+} > Cu^{2+}$ (Table 2). Whether the permafrost *S. maltophilia* showed resistance to Mn^{2+}, Sn^{2+}, and Co^{2+} is not clear due to the lack of MIC data for Mn^{2+}, Sn^{2+}, and Co^{2+} from *E. coli* K-12.

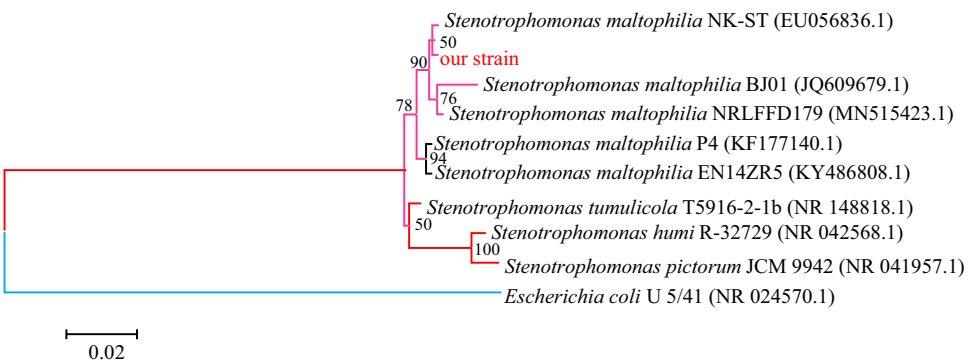

Fig. 1 Neighbor-joining phylogenetic tree obtained from 16S rRNA gene sequences. The scale bar shows the number of substitutions per site

Table 1 Antibiotic MIC profiles for permafrost *S. maltophilia*

Antibiotics	n	Number of tubes with MIC (μg/ml) of								MIC50	MIC90	ECOFF breakpoint	Number of isolates above ECOFF	Categorization
		0.5	1	2	4	8	16	32	64					
Ciprofloxacin	40	1	4	9	17	7	2*			4	8	16	0	S
Chloramphenicol	40			2	3	3	7	20*	5	32	64	2–32	5	R
TMP-SMZ	40			*			31	7	2	16	32	2	40	R
Tetracycline	40				5	32	3*			8	8	16	0	S
Streptomycin	40		1	1	1	4	4	26	3*	32	32	4–512	0	S
Erythromycin	40				5	5	2*	25	3	32	32	0.25–16	28	R
Bacitracin	40				16	19	3	3*		8	32	32	0	S

n refers to 40 repeats. Asterisk indicate the ECOFFs set by the EUCAST

ARGs

In total, 32 ARGs were identified in the genome of permafrost *S. maltophilia*, including aminoglycoside resistance genes *AAC(6')-Iz* and *APH(3')-Iic*; aminocoumarin resistance genes *alaS* and *mdtC*; fluoroquinolone resistance genes *emrR* and *mfd*; antibacterial free fatty acid resistance gene *farB*; *β*-lactam resistance gene L1 *β*-lactamase; macrolide resistance genes *macA* and *macB*; nitroimidazole resistance gene *msbA*; triclosan resistance gene *gyrA*; fosfomycin resistance gene *murA*; penam resistance gene *mecA*; peptide resistance gene *rosB*; elfamycin resistance gene *EF-Tu*; pleuromutilin resistance gene *TaeA*; triclosan resistance gene *TriC*; multidrug resistance genes *adeA*, *adeC*, and *adeG* conferring resistance to tetracycline and glycylcycline; *mexJ*, *mexK*, and *mexW* conferring resistance to tetracycline, macrolide, and triclosan; *oprN* conferring resistance to phenicol, diaminopyrimidine, and fluoroquinolone; *oqxA* conferring resistance to tetracycline, nitrofuran, glycylcycline, diaminopyrimidine, and fluoroquinolone; and *smeA*, *smeC*, *smeD*, *smeF*, *smeR*, and *smeS* conferring resistance to ciprofloxacin, tetracycline, chloramphenicol, and erythromycin. These had an amino acid sequence identity of 40.5%–99.2% (Table S1).

MRGs

A total of 36 MRGs were identified in the genome of permafrost *S. maltophilia* (Table S2), including arsenic resistance genes *arsB* and *arsC*; gold resistance genes *golS* and *golT*; chromate resistance gene *chrR*; copper resistance genes *copA*, *copC*, *pcoB*, *pcoD*, *cutA*, *cutC*, *cusA*, *cusB*, *cueR*, and *cusS*; iron resistance genes *fecA* and *fur*; mercury resistance genes *merD*, *merE*, and *merT*; manganese resistance genes *mntH* and *mntR*; molybdenum resistance genes *modA*, *modB*, *modC*, *moeA*, *moaE*, and *mobA*; tellurite resistance genes *terC*; silver resistance genes *silA and silB*; and cobalt-zinc-cadmium resistance genes *czcA*, *czcB*, *czcC*, and *czcD*. These had an amino acid sequence identity of 91.1–100.0%.

Table 2 Metal tolerance of permafrost *S. maltophilia*

Metals	*n*	Metal concentrations (μg/ml) with number of tolerant isolates					Resistant isolates	
		100	200	400	800	1600	*n*	%
Zn^{2+}	40	4*	14	20	2		36	90
Mn^{2+}	40	1	12	22	5			
Ni^{2+}	40	4*	13	22	1		36	90
Sn^{2+}	40	3	12	23	2			
Cu^{2+}	40	2	11*	24	3		27	67.5
Cr^{6+}	40	2*	10	25	3		38	95
Hg^{2+}	40	6	15	19			40	100
Co^{2+}	40	14	23	3			3	

n refers to 40 repeats. *Minimal inhibition concentration of standard strain *E. coli* K12. The MIC for Hg was 12.5 μg/mL, which was lower than the initial concentration of 100 μg/mL

Genomic islands (GIs)

A total of 12 GIs were identified from 9 scaffolds (Table S3), ranging from 7288 to 40,192 bp (average 15,108 ± 8558 bp). Among these, two GIs contained both resistance genes and MGEs. For instance, GI3 encodes for six Hg resistance genes (*merP*, *merA*, *merD, merE*, *merR*, and *merT*), a transposase, and a transposon *tnp*R. GI4 contained a tetracycline suppressor gene *tetR*, a transposon *tnp*A, and two integrases. Three GIs contained only one ARG. For instance, GI2, GI7, and GI11 contained *β*-lactamase class C gene, acrifervin resistance protein (*acr*), and aminoglycoside N-acetyltransferase AAC (6′), respectively (Fig. 2). The other 7 GIs contained no resistance genes.

Discussion

This study brings together data on both antibiotic and metal resistant genes, and antibiotic and metal resistant phenotypes, in an environmental organism. This provides mechanistic insights above studies that only consider either genes or phenotypes.

Little is known about antibiotic and metal resistance phenotypes, which is more important than studying ARGs and MRGs only in an environmental context with little human activity. This study showed that metal resistance co-occurred with antibiotic resistance in GIs.

MIC of antibiotics and ARGs

The permafrost *S. maltophilia* showed resistance to chloramphenicol, erythromycin, and TMP-SMZ, and sensitivity to ciprofloxacin, tetracycline, streptomycin, and bacitracin, which was consistent with the report showing that the culturable bacterial consortiums isolated from Antarctic soils were consistently susceptible to most of the tested antibiotics frequently used in clinical therapies (Yuan et al. 2019). However, Pankuch et al. (1994) showed that *S. maltophilia* was resistant naturally toward aminoglycosides. They recovered *S. maltophilia* from the environmental species of captive snakes. Further investigation is still needed to verify whether *S. maltophilia* from more environments with little human activity was resistant naturally toward aminoglycosides.

Previous MIC studies for *S. maltophilia* were performed on four antibiotics, including ciprofloxacin, tetracycline, chloramphenicol, and TMP-SMZ. Thus, the MIC results were compared with the findings on these four antibiotics (Table S4). TMP-SMZ has traditionally been considered the treatment of choice for *S. maltophilia* (Biagi et al. 2020), with increasing reports of resistance and adverse drug effects causing great concern for *S. maltophilia* treatment (Hand et al. 2016; Bostanghadiri et al. 2019; Gajdacs and Urban, 2019). Few studies showed the sensitivity of clinical *S. maltophilia* isolates to TMP-SMZ, with an MIC range of 0.125–2.375 μg/mL (Nakamura et al. 2021; Khan et al. 2021; Krueger et al. 2001). However, the maximum MIC of 304, 608, and 2432 μg/mL was 9.5, 19, and 76 times higher than the MIC of the permafrost strain, respectively, as reported by many researchers (Hejnar et al. 2001; Tatman-Otkun et al. 2005; Nakamura et al. 2021; Zhanel et al. 2008; Weiss et al. 2000; Fung-Tomc et al. 2002; Valdezate et al. 2001). Thus, the MIC of the permafrost *S. maltophilia* to TMP-SMZ was at the medium level. The maximum MIC of tetracycline (García-León et al. 2015) was > 256 μg/mL for *S. maltophilia* clinical isolates and 16 μg/mL for the permafrost *S. maltophilia*. For chloramphenicol, the clinical *S. maltophilia* isolates could resist up to 96 (Spierer et al. 2018), 152 (Carvalhais et al. 2021), and > 256 μg/mL (García-León et al. 2015), while the permafrost *S. maltophilia* could resist only 64 μg/mL. The maximum MIC of ciprofloxacin for clinical *S. maltophilia* isolate was > 32 μg/mL (Grillon et al. 2016; García-León et al. 2015; Spierer et al. 2018), while the permafrost *S. maltophilia* could resist only 16 μg/mL of ciprofloxacin. Overall, most clinical *S.*

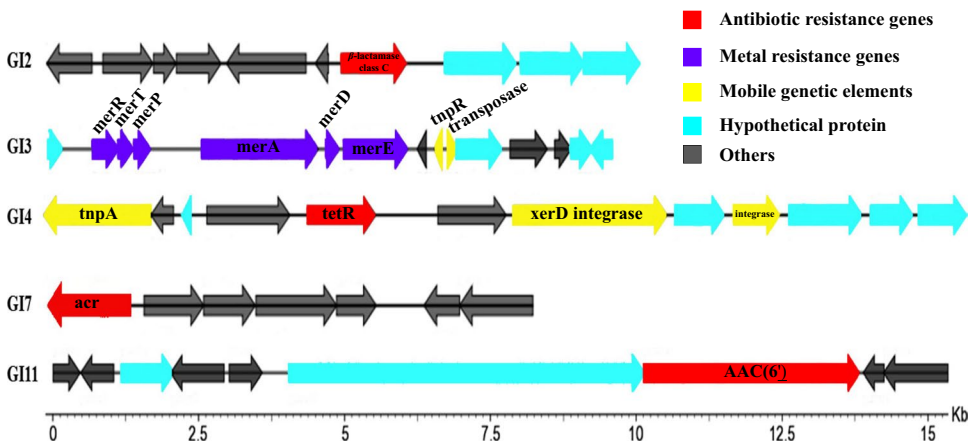

Fig. 2 Genetic structure of genomic islands from the permafrost *Stenotrophomonas maltophilia.* The orientation of transcription is indicated by arrowheads

maltophilia isolates had higher resistance (higher MICs) to ciprofloxacin, chloramphenicol, trimethoprim/sulfamethoxazole, and tetracycline compared with the permafrost strain. These results were consistent with the findings of Pankuch et al. (1994), showing that the *S. maltophilia* isolates from captive snakes were either identically or more susceptible to antibiotics than strains acquired from patients, as well as Balbin et al. (2020), showing that the urban isolates of *E. coli* showed higher resistance to chloramphenicol, ciprofloxacin, streptomycin, trimethoprim–sulfamethoxazole, and tetracycline than those from the natural area. It was speculated that antibiotic resistance was an innate ability of *S. maltophilia*. The continuous human use of antibiotics induced selective pressure on antibiotic-resistant bacteria, causing higher MIC compared with less human-impacted environments.

The aforementioned antibiotic resistance phenotypes were related to antibiotic resistance genotypes. For instance, the presence of macA and macB was consistent with the resistance of the permafrost *S. maltophilia* to erythromycin. The constitutive expression of *macABC*sm contributed to the intrinsic resistance of *S. maltophilia* to macrolides (Lin et al. 2014), which verified the result of this study. However, the *macABC*sm pump also played a physiological role in protecting *S. maltophilia* from the attack of oxidative and envelope stresses and biofilm formation (Lin et al. 2014), which was also important under permafrost conditions. Therefore, further exploration is still needed with the gene deletion method to show the exact role of *macAB* in the permafrost *S. maltophilia*. *mexVW* usually combined with *oprM* to form a tripartite multidrug efflux pump (Li et al. 2003). However, *mexV* and *oprM* were not recovered from the permafrost *S. maltophilia*. The *smeDEF* in the permafrost *S. maltophilia* was probably related to chloramphenicol resistance (Sánchez and Martínez, 2018). However, *smeE* and the regulator gene of *smeT* were not recovered from our permafrost *S. maltophilia.* In addition, it was reported that *smeDEF* was an ancient element that evolved over millions of years in *S. maltophilia*. Quinolone resistance is a recent function of *smeDEF* and that colonization of plant roots is likely one original function of this efflux pump (García-León et al. 2014). Thus, the mechanism of chloramphenicol resistance is still unexplored and needs further investigation. One way or another, the multidrug efflux systems of *macAB*, *mexW*, *smeDF* could still contribute to antibiotic resistance and their conservation even in environmental strains would cause human risk for therapeutic intervention (Poole 2001).

The aforementioned findings showed the antibiotic resistance phenotypes and the presence of corresponding ARGs. However, the present study showed antibiotic sensitivity and the absence of corresponding ARGs. The *smrA* conferring resistance to fluoroquinolones and tetracycline (Al-Hamad et al. 2009) was absent, which was consistent with its sensitivity to tetracycline and ciprofloxacin. The *qnrB* and *qnrR* conferring resistance to quinolones were absent. Furthermore, *oqxAB* is a member of the resistance–nodulation–cell division (RND) family of multidrug efflux pumps (Hansen et al. 2004), which can pump out nalidixic acid, flumequine, ciprofloxacin, and norfloxacin, causing an 8- to 64-fold increase in respective MICs (Périchon et al. 2007). The absence of *oqxB*, as well as the aforementioned *qnrB* and *qnrR*, in the permafrost *S. maltophilia* was consistent with its sensitivity to ciprofloxacin. However, *qnrB*, *qnrR*, and *smrA* were present in clinical isolates (Esposito et al. 2017; Patil et al. 2018; Zhang et al. 2020). *oqxB* was found in an isolate from the Norwegian University campus pond (Finton et al. 2020) and clinic (Esposito et al. 2017). It was reported that *smrA* was an acquired and not an intrinsic gene (Al-Hamad et al. 2009), which further demonstrated the natural origin of the permafrost strain with little human influence from antibiotic use. No *bcrABC* was recovered in the present study, which was consistent with the sensitivity of the strain to bacitracin. *strA* and *strB* were absent in the permafrost strain but present in the clinical isolate (Ma et al. 2020; Esposito et al. 2017). The result was consistent with the streptomycin sensitivity of the permafrost strain. Hence, it was speculated that the diversity of ARGs could reflect the risk caused by the human use of antibiotics. This speculation was supported by the promotion and diversification of ARGs under the release of large quantities of anthropogenic antibiotics (Liu et al. 2021, 2018; Tan et al. 2018; Chen et al. 2013, 2016; Ouyang et al. 2015; Sandner-Miranda et al. 2018).

However, a certain discrepancy between phenotype and genotype was also found. Although *adeA*, *adeC*, and *adeG* were recovered, no tetracycline resistance was reported. Also, *mfd*, *gyrA*, and *emrB* were recovered, but no fluoroquinolone resistance of ciprofloxacin was observed. For the recovery of *rosB*, the permafrost *S. maltophilia* did not show resistance to bacitracin from peptides. For the recovery of *AAC(6')-Iz*, *AAC(6')-31*, and *APH(3')-Ic*, the permafrost *S. maltophilia* did not show resistance to streptomycin from aminoglycosides. The difference between the displayed antibiotic resistance phenotypes and the associated ARGs (Smith et al. 2014; Xia et al. 2017; González-Santamarina et al. 2021; Duy et al. 2021) was probably due to the lack of function and expression of ARGs. The observed phenotypic resistance could be a product of additional resistance mechanisms such as multidrug efflux pumps or other unidentified ARGs (Smith et al. 2014).

The study then compared ARGs from the permafrost *S. maltophilia* with those from the clinical ones (Table S5). *mrcA* and *mrcB* were absent in the permafrost *S. maltophilia* but present in a patient's isolate (Ma et al. 2020). *blaL1* and *blaL2* discovered in a clinical isolate (Esposito et al. 2017; Patil et al. 2018; Crossman et al. 2008) were absent in the permafrost strain. All four ARGs were related to β-lactamase expression

(Huang et al. 2017). *sul1* and *sul2* were present in a clinical *S. maltophilia* isolate (Youenou et al. 2015; Patil et al. 2018) but not in the permafrost strain. This seemed coherent, given the fully synthetic origin of sulfonamide antibiotics (Czekalski et al. 2015). However, *sul2* was recovered from an ice core, representing the pre-antibiotic era (Okubo et al. 2019). This was probably because the authors used total DNA and PCR primers targeting sul2, which could better reflect ARG profiles in less human-impacted environments. *MacAB*, along with a member of the *tolC* family, formed a tripartite efflux pump. *macAB* was present in both the permafrost *S. maltophilia* and the clinical *S. maltophilia* (Zhang et al. 2020; Esposito et al. 2017; Patil et al. 2018), while *tolC* was present only in the clinical isolate (Zhang et al. 2020; Esposito et al. 2017; Patil et al. 2018). The absence of *tolC* in the permafrost *S. maltophilia* could have affected the function of the *macAB-TolC* efflux pump (Lu et al. 2018). *TolC* interacts with a variety of inner membrane transporters, such as *acrB*, *acrD*, *mdtABC*, and *mdtEF* (Nishino et al. 2003). Among these, only *mdtC* was present in the permafrost *S. maltophilia*, while *tolC*, *acrB*, *acrD*, *mdtB*, and *mdtC* were found in an isolate from a lung with cystic fibrosis (Esposito et al. 2017). It is known that multiple deletions of *acrB*, *acrD*, and *mdtABC* significantly decrease the export of enterobactin (Horiyama and Nishino, 2014), whether the permafrost *S. maltophilia* resists enterobactin needs further exploration. The aforementioned results further demonstrated higher ARG diversity in environments with more human activities than those with lesser activities. However, it was reported that no major variation in ARG content was observed from environmental and clinical *S. maltophilia*. Some environmental *S. maltophilia* even carried as many multidrug-resistant efflux pumps as the clinical strains or more efflux pumps than the clinical ones (Youenou et al. 2015), which was contrary to the results of this study. That is probably because of different ARGs annonation method. We used CARD, while Youenou et al. (2015) used InterPro database.

MIC of metals and MRGs

Either antibiotics or metals may select both kinds of genes. Thus, much attention has been given to metal resistance, which influences antibiotic resistance in human-impacted environments (Cesare et al. 2016; Che et al. 2019; Ma et al. 2016; Yang et al. 2019; Luo et al. 2017). This study investigated whether metal MIC was lower and MRGs were lesser in the permafrost *S. maltophilia* than in other environments, just like antibiotic MIC and ARGs.

The permafrost *S. maltophilia* showed resistance to Hg^{2+}, Cr^{6+}, Zn^{2+}, Ni^{2+}, and Cu^{2+}, which was consistent with the resistance of *S. maltophilia* to Hg^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, and Cr^{6+} (Pages et al. 2008; Naguib et al. 2019; Holmes et al. 2009; Baldiris et al. 2018; Nath et al. 2020). A previous study assessed the levels of 11 different heavy metals Fe, Mn, Zn, Ni, Cr, Cu, As, Co, Mo, Cd, and Hg, in which only the As level was higher compared with the upper continental crust (Zhang et al. 2021). The high level of As induced As-resistant bacteria, which could resist not only As but also other metals (Altimira et al. 2012). This explained the resistance of the permafrost *S. maltophilia* to Hg^{2+}, Cr^{6+}, Zn^{2+}, Ni^{2+}, and Cu^{2+}.

Next, the study compared the MIC of four metals Zn^{2+}, Ni^{2+}, Cu^{2+}, and Cr^{6+} from the permafrost *S. maltophilia* with that from other environments because previous MIC studies focused on these four metals (Table S4). The maximum MIC of Zn^{2+} for the permafrost *S. maltophilia* was 800 μg/mL, which was much lower than the MIC of those (515,200 μg/mL) recovered from metal-contaminated soil (Chien et al. 2007). The maximum MIC of Ni^{2+} for the permafrost *S. maltophilia* was 800 μg/mL, which was lower than that (1000 μg/mL) from the industrial wastewater (Aslam et al. 2018) and much lower than that (495,232 μg/mL) recovered from the metal-contaminated soil (Chien et al. 2007). The maximum MIC of Cu^{2+} for the permafrost *S. maltophilia* was 800 μg/mL, which was lower than that (1248.45 μg/mL) from East Fork Poplar Creek (Holmes et al. 2009) and much lower than that (448,000 μg/mL) recovered from the metal-contaminated soil (Chien et al. 2007). The maximum MIC of Cr^{6+} for the permafrost *S. maltophilia* was 800 μg/mL, while that recovered from the tannery effluent–contaminated soil, metal-contaminated soil, East Fork Poplar Creek, and industrial wastewater could resist up to 4854 μg/mL (Alam and Ahmad, 2012), 35,280 μg/mL (Chien et al. 2007), 2647.66 μg/mL (Holmes et al. 2009), and 1000 μg/mL of Cr^{6+} (Aslam et al. 2018), respectively. Overall, the permafrost *S. maltophilia* showed worse resistance (lower MICs) to the four metals than those from human-impacted environments. The result of this study was consistent with the report showing that the nonpolluted and metal-polluted soils had different responses for metal resistance (Schaeffer et al. 2016). A higher concentration of any metal at a particular site may lead to higher MIC values (Bhardwaj et al. 2018) due to the long-term selective pressure on microbial populations. Importantly, *S. maltophilia* with metal resistance can be used as an indicator of metal pollution. Since metal pollution exerts both metal and antibiotic resistance (Li et al. 2017; Knapp et al. 2011), special attention must be paid to increasing heavy metal levels in any kind of environment.

For the metal resistance phenotypes and genotypes, Cu^{2+} resistance was consistent with the recovery of *cueR* and *copA*. The *cueR* switch could activate the *S. maltophilia* copper transport gene of *copA* (Baya et al. 2021). The expression of both regulator gene *cueR* and structure gene *copA* was related to Cu^{2+} resistance. In the *czcCBA* operon, *czcC* was an outer membrane protein, *czcB* was a membrane fusion protein, *czcA* was responsible for Co-Zn-Cd transportation, and *czcD* was a regulatory protein. Thus, the *czcCBA* operon combined with its downstream gene of *czcD* mediated the detoxification of Zn^{2+} and was consistent with

the resistance of the permafrost *S. maltophilia* to Zn^{2+} (Sun et al. 2021). Chromate reductase, *chrR*, is significant because it not only reduces Cr^{6+} (Ackerley et al. 2004) but also provides protection against Cr^{6+} toxicity by reducing the concentration of reactive oxygen species (Ahemad 2014). The recovery of *chrR* was consistent with the resistance of the permafrost *S. maltophilia* to Cr^{6+}. Hg resistance genes *merT*, *merD*, and *merE* were recovered. The three genes probably controlled Hg resistance in the permafrost *S. maltophilia* because strains with any *mer* locus were more likely to be resistant compared with strains without *mer* (Wireman et al. 1997). This showed consistency between the metal resistance phenotypes and genotypes. Some inconsistency was also noted. For instance, the permafrost *S. maltophilia* was resistant to Ni^{2+}, but no Ni^{2+} resistance genes were found. This was probably because some efflux pumps were involved in detoxifying toxic compounds such as heavy metals and solvents, besides antibiotics naturally produced by other microorganisms (Alvarez-Ortega et al. 2013).

Some other MRGs were recovered, but the present study did not analyze the MIC of the corresponding metals, such as resistance genes *arsB* and *arsC*, Au resistance genes *gold* and *golT*, Fe resistance genes *fecA* and *fur*, Mn resistance genes *mntH* and *mntR*, Mo resistance genes *modA*, *modB*, *modC*, *moeA*, *moaE*, and *mobA*, Te resistance gene *terC*, and Ag resistance genes *silA and silB*. Much more metal MIC standards should be given to *E. coli* K-12 so that the resistance of the permafrost *S. maltophilia* to As, Au, Fe Mn, and Mo can be speculated.

Then, MRGs from the permafrost *S. maltophilia* were compared with those from other environmental isolates (Table S6). Both *chrA* and *chrR* were present in the *S. maltophilia* of wastewater (Naguib et al. 2019), while the permafrost *S. maltophilia* contained only *chrR*. Furthermore, *copA* and *copC* were present in both the clinical and permafrost *S. maltophilia*, while *copABCD* was present in the clinical *S. maltophilia* D457R (Alonso et al. 2000). The cadmium efflux determinant *cadA*, together with its transcriptional regulator gene *cadC*, was identified in the clinical *S. maltophilia* D457R (Alonso et al. 2000), while these were absent in the permafrost *S. maltophilia*. *merT* was found in both the permafrost *S. maltophilia* and clinical *S. maltophilia* 279a (Crossman et al. 2008), while *merA* and *merR* were present in the clinical *S. maltophilia* 279a (Crossman et al. 2008) and isolates from seawater, soil (Ge and Ge 2016), and wastewater (Naguib et al. 2019). Hence, it was speculated that *S. maltophilia* from human-impacted environments contained more MRGs compared with the permafrost *S. maltophilia*, which was consistent with the report that higher numbers of MGEs existed at the polluted sites compared with their control sites (Jacquiod et al. 2018; Yang et al. 2019). Some other studies reported higher MGE abundance in metal-polluted environments than in nonpolluted ones (Chen et al. 2018; Yang et al. 2019), probably due to a higher number of metal-resistant bacteria (Hemmat-Jouet al. 2021). However, whole-genome sequencing could not reflect the abundance of MRGs. Further exploration with real-time quantitative PCR as well as the standard-curve method of absolute quantification is still needed to show the difference in MRG abundance between the permafrost *S. maltophilia* and those from other environments.

Genomic islands

GIs are frequently associated with a particular microbial adaptation, such as antibiotic resistance or metal resistance (Hsiao et al. 2005). They also harbor genes coding for an integrase or transposons, contributing to the mobilization of gene clusters (AL-Jabri et al. 2018). This study aimed to investigate whether GIs in the permafrost *S. maltophilia* contained both ARGs and MRGs, and to demonstrate whether the combination of ARGs and MRGs occurred during the pre-antibiotic era.

Only one kind of resistance gene cluster was located on a single GI in the permafrost *S. maltophilia*. On the contrary, *S. maltophilia* from other environments exhibited a minimum of two kinds of antibiotics or MRG clusters on a single GI. For instance, ARGs of *aadA2*, *qacE*, *sul1*, *strA*, *strB*, *tetA*, and *tetR*, as well as two ints, were all located on a single GI of *S. maltophilia* GZP-Sm1 from porcine (He et al. 2015). In addition, *cop*, *cus* operons, and *czc* genes were all located on the GI K25 of the clinical *S. maltophilia* strain isolated from the blood of a cancer patient K279a (Rocco et al. 2009). Thus, it was speculated that *S. maltophilia* from human-impacted environments showed more multi-resistance to antibiotics or metals than those from less human-impacted environments. The result of this study also explained less diversity of ARGs and MRGs in the former than in the latter due to the possibility of horizontal gene transfer (Youenou et al. 2015). However, ARGs and MRGs present in GIs still pose a threat to human health and can not be ignored (Martinez 2009).

Conclusion

To conclude, the permafrost *S. maltophilia* exhibited lower ARG or MRG cluster components and only one kind of ARG or MRG in GIs compared with the strains from human-impacted areas, which confirmed the lower maximum MIC of antibiotics and metals. The present study suggested that the clinical *S. maltophilia* developed higher antibiotic and metal resistance due to the horizontal gene transfer of GIs. However, only one permafrost *S. maltophilia* strain was recovered and sequenced from the study site. In addition, complete genome sequences could not be generated due to the constraints inherent in using short-read Illumina sequencing data. Further analyses supplemented with long-read sequencing technology, such as PacBio sequencing,

along with more *S. maltophilia* strains from more permafrosts and a broad range of antibiotics and metals, are required to precisely determine the role and mechanism ARGs and MRGs in permafrost *S. maltophilia*.

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Declarations

Competing interest The authors declare no competing interests.

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