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ORIGINAL PAPER

Characterization of Two Macrolide Resistance-Related Genes in Multidrug-Resistant *Pseudomonas aeruginosa* Isolates

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Abstract

In analyzing the drug resistance phenotype and mechanism of resistance to macrolide antibiotics of clinical *Pseudomonas aeruginosa* isolates, the agar dilution method was used to determine the minimum inhibitory concentrations (MICs), and PCR (polymerase chain reaction) was applied to screen for macrolide antibiotics resistance genes. The macrolide antibiotics resistance genes were cloned, and their functions were identified. Of the 13 antibiotics tested, *P. aeruginosa* strains showed high resistance rates (ranging from 69.5–82.1%), and MIC levels (MIC90 > 256 μg/ml) to macrolide antibiotics. Of the 131 known macrolide resistance genes, only two genes, *mphE* and *msrE*, were identified in 262 clinical *P. aeruginosa* isolates. Four strains (1.53%, 4/262) carried both the *msrE* and *mphE* genes, and an additional three strains (1.15%, 3/262) harbored the *mphE* gene alone. The cloned *msrE* and *mphE* genes conferred higher resistance levels to three second-generation macrolides compared to two first-generation ones. Analysis of MsrE and MphE protein polymorphisms revealed that they are highly conserved, with only 1–3 amino acids differences between the proteins of the same type. It can be concluded that even though the strains showed high resistance levels to macrolides, known macrolide resistance genes are seldom present in clinical *P. aeruginosa* strains, demonstrating that a mechanism other than this warranted by the *mphE* and *msrE* genes may play a more critical role in the bacteria's resistance to macrolides.

Key words: *Pseudomonas aeruginosa*, macrolide, resistance gene, *mphE*, *msrE*

Introduction

Pseudomonas aeruginosa is a Gram-negative pathogen without saccharide fermentation capability. It is one of the mains pathogens causing nosocomial and community infections. *P. aeruginosa* has a high colonization ability and can secrete many virulence proteins. It can often cause infections of the respiratory tract, urethra, digestive tract, skin, and other parts of the human body as well as bacteremia. Moreover, it is also a common pathogenic bacterium in patients with weakened immunity, and it is frequently associated with opportunistic infections in elderly and infirm individuals (Madhusudhan et al. 2003; Paterson 2006; Tripathy et al. 2007). Due to the extensive and unreasonable use of a large number of broad-spectrum antibiotics, *P. aeruginosa* infections have become increasingly severe due to their resistance to various antibiotics, especially β-lactams, aminoglycosides, quinolones, and sulfonamides. Multidrug-resistant (MDR) or pan drug-resistant (PDR) pathogens have continuously emerged, resulting in severe problems for the clinical treatment of infectious diseases (Miyoshi-Akiyama et al. 2017).

Macrolide antibiotics not only have anti-inflammatory effects on multi-drug resistant *P. aeruginosa* infections (Kobayashi 1995), but also inhibit alginate

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production at the guanosine diphospho-D-mannose dehydrogenase (GMD) level (Mitsuya et al. 2000). Biofilm formation by *P. aeruginosa* is inhibited by macrolides, diminishing *P. aeruginosa* infection in the clinical settings. It was shown that when macrolide antibiotics combined with cefoperazone/sulbactam were used to treat children with *P. aeruginosa* pneumonia, there was a significant difference in the clinical efficacy (Huang et al. 2015). Another clinical study focused on extensive burns infected with PDR *P. aeruginosa* and demonstrated that macrolide combined with β-lactam antibiotics and β-lactamase inhibitor could effectively control this infection (Ning et al. 2011). Other studies have shown that macrolides should be used to treat *P. aeruginosa* pneumonia in children due to its potential therapeutic ability to overcome the resistance mechanisms (Huang et al. 2015). However, with increasing macrolides applications in clinical practice, the resistance against these antibiotics has started to spread worldwide (Pereyre et al. 2016). For example, comparison of the 2007 edition (Fokkens et al. 2007) and 2012 edition (Fokkens et al. 2012) of papers on rhinosinusitis and nasal polyps (EPOS) have shown that over time macrolides were significantly less effective in treating the patients with chronic sinusitis caused by *P. aeruginosa*. Macrolides, lincosamides, and streptogramins (MLSs) share overlapping binding sites on the ribosome 50S subunit, although these drugs show distinct chemical properties (Roberts 2008); however, they are usually considered together (Vester et al. 2001; Zhanel et al. 2001). The following three different mechanisms mainly confer the resistance to macrolide-lincosamide-streptogramin B (MLSB) antibiotics: ribosome modifications promoted by 23S rRNA methylases (such as ErmA, ErmB, and ErmC) together with mutations in the rRNA (Mustafa et al. 2017) and ribosomal protein genes (Wekselman et al. 2017; Golkar et al. 2018); mutations in efflux proteins (such as MefA, MsrA, and lsaA), and mutations in proteins expressed by MLS-inactivating genes. These can be further subdivided into esterases (such as EreA, EreB, and EreC), lyases (such as VgbA and VgbB), phosphotransferases (such as MphA, MphB, MphC, MphD, and MphE), and transferases (such as lnuA, lnuB, and VatA) (Roberts et al. 1999; Roberts 2008; van Hoek et al. 2011; Golkar et al. 2018). The most prominent ribosome modification is methylation of the 23S rRNA encoded by the *erm* gene, which adds one or two methyl groups to a single adenine in the 23S rRNA (Poehlsgaard et al. 2005; Golkar et al. 2018). The *mef* and *msr* subfamilies of efflux pumps, which are members of the MSF and ABC families are particularly relevant to macrolide antibiotics (Gomes et al. 2017). The *mphE* gene encodes a macrolide-2'-phospho-transferase, an intracellular enzyme with the ability to transfer the γ-phosphate of one nucleotide triphosphate to the 2'-OH group of macrolide compounds, thereby destroying the critical interaction between macrolides and A2058 (Fyfe et al. 2016). The *msrE* gene belongs to the ABC-F subfamily of ATP-binding cassette protein, which mediates a recently described new mechanism of resistance to macrolides (Janvier et al. 2017; Ero et al. 2019).

In this study, we analyzed the resistance spectrum, and the MIC levels of clinical *P. aeruginosa* isolates to commonly used in the clinic antibiotics and further analyzed the molecular mechanisms of the bacteria's resistance to macrolides. Understanding the molecular mechanisms of drug resistance will help clinicians treat infectious diseases and prevent the spread of resistance more effectively.

Experimental

Materials and Methods

Bacterial strains collection, genomic DNA extraction, and high-throughput sequencing. The 262 no duplicate clinical *P. aeruginosa* strains, isolated from clinical samples from the Fifth Affiliated Hospital of Wenzhou Medical University (Zhejiang, China) from March 2015 to October 2017, were randomly collected. They were isolated from purulent-infiltration (7/262, 2.7%), blood (5/262, 1.9%), urine (11/262, 4.2%), nasal secretions (63/262, 24.0%), and sputum (176/262, 67.2%). The strains were identified with a VITEK-60 microbial autoanalyzer (bioMerieux, Lyon, France). For the pooled genomic DNA sequencing, each strain was incubated independently in 5 ml of Luria-Bertani (LB) broth at 37°C for approximately 16 hours. All the cultures were pooled together, and genomic DNA was extracted from the mixed bacteria using an Axy-Prep Bacterial Genomic DNA Miniprep kit (Axygen Scientific, Union City, CA, USA). The genomic DNA was sequenced on a HiSeq 2500 DNA sequencer at Annoroad Gene Technology Co., Ltd. (Beijing, China). The SOAPdenovo software (https://github.com/ablab/spades) was used to assemble the HiSeq 2500 sequencing reads to acquire the genomic sequence contigs. We used the glimmer software (http://ccb.jhu.edu/software/glimmer) to predict potential open reading frames (ORFs) > 150 bp in length with BLASTX (https://blast.ncbi.nlm.nih.gov) against the NCBI non-redundant protein database, with an e-value threshold of $1\,e^{-5}$. CD-HIT (http://bioinformatics.ljcrf.edu/cd-hit) was used to cluster protein sequences to remove redundant sequences. We followed the methods of Wu and coworkers (Wu et al. 2018).

The collection and sequencing reads mapping to the reference resistance-related genes. The nucleotide

sequences of the macrolide resistance-related genes were obtained from the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/). The HiSeq 2500 sequencing reads were mapped to the macrolide resistance-related gene sequences. The relative abundance (sequencing depth) of a specific gene was calculated as the accumulated nucleotide length of the mapped short reads on the gene divided by the gene size (Wu et al. 2018).

Screening of the macrolide-resistant gene-positive strains and cloning of the *mphE* and *msrE* genes. As mentioned above, to confirm the presence of the genes related to macrolides resistance, *P. aeruginosa* strains were screened by PCR, and the positive PCR products were sequenced. The primers for cloning the complete ORFs with promoter regions and a pair of flanking restriction endonuclease adaptors (*EcoR* I for the forward primers and *Hind* III for the reverse primers) were designed using the Primer Premier 5.0 software package (Table I). The AxyPrep Bacterial Genomic DNA Miniprep kit (Axygen Scientific, Union City, CA, USA) was used to extract the template DNA from each clinical *P. aeruginosa* isolate (Wu et al. 2018). The PCR amplification was performed under the following conditions: an initial cycle of 94°C for 5 min; followed by 33 cycles of 10 s at 94°C, 1 min at a specific annealing temperature (Table I), and 1 min 45 s at an extension temperature of 72°C; and a final extension step for 10 min at 72°C. Positive amplification products were verified by sequencing with an ABI 3730 automated sequencer (Shanghai Sunny Biotechnology Co., Ltd., Shanghai, China), and the sequencing results were compared with the reference resistance gene sequences using BLAST algorithms. The amplicons of the two target genes (*mphE* and *msrE*) with its promoter regions were digested with the corresponding restriction endonucleases and ligated into pUCP18 vectors. The recombinant plasmids were transformed into competent cells (*E. coli* DH5α) using the calcium chloride method, and positive clones were selected on LB agar plates supplemented with 100 μg/ml ampicillin. The transformants were further verified by PCR, and Sanger sequencing. In this work, for the amino acid polymorphism analysis, in addition to the genes, all other the *mphE* and *msrE* gene sequences were retrieved from the NCBI nucleotide database using both genes as key search terms.

Antimicrobial susceptibility testing. In addition to other classes of antibiotics, the five macrolide antibiotics used in this work included two first-generation macrolides (erythromycin and kitasamycin), and three second-generation macrolides (roxithromycin, clarithromycin, and azithromycin). The minimum inhibitory concentrations (MICs) were determined using the agar dilution method, and the results of the antibacterial susceptibility testing were interpreted according to the CLSI breakpoint criteria and the guidelines of the European Committee on Antimicrobial Susceptibility Testing for *P. aruginosa*. Using ATCC 27853 as a quality control strain and *E. coli* DH5α with or without a pUC18 plasmid as the negative controls, the MICs were determined in triplicate from MH-broth agar plates with 2-fold serial dilutions of the antibiotics.

Sequence polymorphism analysis. The multiple sequence alignment of the MphE and MsrE amino acid sequences was performed using MAFFT (Katoh et al. 2013). Additional bioinformatics software was written using Python (https://www.python.org/), and Biopython (Cock et al. 2009).

Results

Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates. The results of the MIC tests for 262 *P. aeruginosa* strains against 13 compounds from four classes of antibiotics (β-lactams, aminoglycosides, polypeptides, and macrolides) demonstrated that the bacteria showed highest resistant rates (ranging from 69.5–82.1%) and MIC levels (MIC50 ≥ 64 μg/ml, and

Table I
Primers used in this study for the detection of macrolide resistance-related genes.

Gene	Primer	Sequence (5'→3')	Purpose	Restriction endonuclease	Vector	Amplicon size (bp)	Annealing temperature
mphE	*mphE*-SF	ATGCCCAGCATATAAATCGC	Screening			271	60°C
	mphE-SR	ATATGGACAAAGATAGCCCG					
	mphE-OF	CGGAATTCTATTCAAAAAAACTTATCCGACTTA	Cloning	*EcoR* I	pUCP18	885	60°C
	mphE-OR	CCAAGCTTTTATATAACTCCCAACTGAGCTTTT		*Hind* III			
msrE	*msrE*-SF	TATAGCGACTTTAGCGCCAA	Screening			395	62°C
	msrE-SR	GCCGTAGAATATGAGCTGAT					
	msrE-OF	CGGAATTCTTTTTGGGAGGACACTGTGATGCTA	Cloning	*EcoR* I	pUCP18	1,467	62°C
	msrE-OR	CCAAGCTTTTATATAACTCCCAACTGAGCTTTT		*Hind* III			

Table II
The MIC values for 13 antibiotics against 262 clinical *Pseudomonas aeruginosa* isolates.

Antibiotics	MIC range (μg/ml)	MIC50 (μg/ml)	MIC90 (μg/ml)	Resistance (%)
Cefuroxim	0.5–32	16	>32	44.1
Cefepime	1–64	8	64	36.7
Meropenam	0.0125–32	4	32	30.1
Ceftazidime	1–64	16	>64	52.1
Gentamicin	0.125–64	16	64	32.1
Tobramycin	0.5–32	4	>32	29.6
Amikacin	>256	2	>256	34.9
Netilmicin	0.5–512	8	>512	42.1
Colistin	0.25–16	2	>16	28.2
Azithromycin	0.5–256	64	>256	78.1
Clarithromycin	1–1024	256	>1024	69.5
Roxithromycin	0.5–1024	256	>1024	76.8
Erythromycin	1–1024	512	>1024	82.1

MIC90 > 256 μg/ml) to four macrolide antibiotics. They showed the lowest resistance rate of 26.2% to colistin, followed by resistance rates to β-lactams and aminoglycosides (below 45%, except for ceftazidime with a resistance rate of 52.1%) (Table II).

Mapping macrolide resistance genes in pooled DNA sequencing. To elucidate the molecular mechanism of macrolide resistance, the pooled genomic DNA of 262 strains was sequenced. It generated 330 million reads ranging from 100 to 110 nucleotides in length, accounting for a total of approximately 34.0 gigabases. Additionally, a total of 131 macrolide resistance gene sequences were collected from the CARD (Table SI). Bacterial resistance genes were identified by mapping the pooled genomic DNA sequencing reads onto the reference resistance gene sequences. The number of mapped reads on a specific reference was used to determine the relative abundance of the reads from the sequenced samples. The results revealed that there were only two hits related to macrolide resistance genes, including *mphE* and *msrE*, and the sequencing depths of *mphE* and *msrE* were 26 and 24, respectively (Table III).

Distribution of macrolide resistance-related genes in *P. aeruginosa* clinical isolates. The PCR screening results for the two genes revealed that among the 262 strains *msrE* was identified in four isolates (1.53%, 4/262; PAO1609, PAO1623, PAO2276, and PAO2883), and *mphE* was identified in seven isolates (2.67%, 7/262; PAO1609, PAO1623, PAO2276, PAO2883, PAO2228, PAO2231, and PAO2889). Among them, four strains (PAO1609, PAO1623, PAO2276, and PAO2883) harbored both resistance genes (*msrE* and *mphE*), while three strains (PAO2228, PAO2231, and PAO2889) harbored only the *mphE* resistance gene.

Table III
Coverage and abundance of the macrolide resistance genes in the pooled DNA from 262 *Pseudomonas aeruginosa* isolates.

Genotype	Reference sequence	Coverage[a]	Abundance[b]
mphE	AY522431	1.00	26.0
msrE	AY522431	1.00	24.0

[a] – the ratio of the number of bases that mapped to the number of bases in the reference sequence
[b] – the number of copies of the reference sequence in the genomic data

Cloning and functional determination of two resistance genes. The *msrE* genes from four strains and *mphE* genes from seven strains were sequenced. The sequencing results showed that they had identical nucleotide sequences (Tables III and IV). To identify the function of these two resistance genes, the ORFs of the genes with their promoter regions (*msrE* from PA2883 and PA1609; *mphE* from PA2883 and PA2276) were randomly selected and cloned. The MIC levels of two first-generation macrolides (erythromycin and kitasamycin) and three second-generation macrolides (roxithromycin, clarithromycin, and azithromycin) were determined for the macrolide resistance gene-positive clinical strains and recombinant strains with the cloned macrolide resistance genes (pUCP18-*mphE*/DH5α and pUCP18-*msrE*/DH5α) (Table IV). The results showed that the cloned *mphE* and *msrE* genes were functional and more effective against the three second-generation macrolides than the two first-generation macrolides. Compared with pUCP18/DH5α (a negative control), the MIC values of the recombinants with *msrE* or *mphE* increased eightfold for the three second-generation macrolides (roxithromycin, clarithromycin, and azithromycin). There was a four-fold increase in the MIC values of erythromycin (first-generation macrolide antibiotics), but

Table IV
MIC results for the recombinants, clinical strains, and controls (μg/ml).

Strain	ERY	KIT	ROX	CLR	AZM
pUCP18-*msrE*/DH5α (PAO2276)	512	256	1024	256	32
pUCP18-msrE/DH5α (PAO2883)	512	512	1024	256	32
pUCP18-*mphE*/DH5α (PAO1609)	512	256	1024	256	32
pUCP18-*mphE*/DH5α (PAO2883)	512	512	1024	256	32
PAO2883	1024	512	1024	512	>128
PAO2276	512	256	1024	512	>128
PAO1609	>1024	256	1024	512	>128
pUCP18/DH5α	128	256	128	32	4
DH5α	128	512	128	32	4
ATCC 27853	32	16	64	16	<1

ERY – erythromycin, KIT – kitasamycin, ROX – roxithromycin, CLR – clarithromycin, AZM – azithromycin

the other first-generation macrolide, kitasamycin, was not functional (Table IV).

Polymorphism analysis of the amino acid sequences from the two resistance genes. To analyze the polymorphisms in the amino acid sequences of the MsrE and MphE proteins, we collected all the protein sequences of the two genes available in the databases. A total of six and nine variants (including two MphE and two MsrE sequences from this work) were obtained. The multiple sequence alignment results showed that the two genes were highly conserved, as they only had 1–3 amino acid differences. Using the MG585957.1 sequence as a reference, among the nine MsrE proteins, eight sequences had different amino acid residues at position 183 (Glu-Asp). The two sequences from this work ($MsrE_{PAO2276}$ and $MsrE_{PAO2883}$) had the same amino acid sequences as CP032136.1, showing only one amino acid (Glu 183 Asp) difference from the reference (MG585957.1). Besides, except for CP011374.1 which had three different amino acid residues (Ser 128 Gly, Glu 183 Asp, and Glu 198 Lys), the remaining four sequences (MG585949.1, LS992184.1, CP026233.1, and CP021960.1) had two amino acid residue differences from the reference sequence (Table V). For the MphEs, the two sequences from this work (MphE1609 and MphE2883) showed the same amino acid sequences as CP035931.1 and had only one amino acid variant at position 17 (Ile-Leu) compared with the reference sequence (CP029638.1). Moreover, KX443408.1 had different amino acids at residues 17 (Ile-Leu) and 133 (Glu-Asp), while CP011374.1 differed at positions 17 (Ile-Leu), 28 (Ile-Leu), and 231 (Thr-Ile) compared with the reference (Table VI).

Discussion

Like in most bacterial species, the resistance mechanisms of *P. aeruginosa* to antibiotics are very complex. Although several macrolide-related resistance

Table V
Amino acid polymorphisms in the MsrE variants.

Accession No.	Amino acid position[a]							Reference
	45	79	80	128	183	198	444	
MG585957.1	Ser	Glu	Thr	Ser	Glu	Glu	Ile	Gonzalez-Plaza et al. 2018
MG585949.1	Ile				Asp			Gonzalez-Plaza et al. 2018
CP032136.1					Asp			
LS992184.1		Gly			Asp			
CP026233.1					Asp		Val	Weingarten et al. 2018
CP021960.1			Lys		Asp			
CP011374.1				Gly	Asp	Lys		
MsrE-2276					Asp			this study
MsrE-2883					Asp			this study

[a] – amino acid positions using the protein sequence MG585957.1 as the reference

Table VI
Amino acid polymorphisms in the MphE variants.

Accession No.	Amino acid position[a]				Reference
	17	28	133	231	
CP029638.1	Ile	Ile	Glu	Thr	Beker et al. 2018
CP035931.1	Leu				
KX443408.1	Leu		Asp		
CP011374.1	Leu	Leu		Ile	
mphE-1609	Leu				this study
mphE-2883	Leu				this study

[a] – amino acid positions using the protein sequence CP029638.1 as the reference

mechanisms, including modification of 23S rRNA (such as *ermB*), efflux pumps (*mefA*, *msrA,* and *msrD*) and inactivating genes (such as *ereA*, *ereB*, *mphA*, *mphB*, and *mp*hD) (Roberts 2008; van Hoek et al. 2011; Golkar et al. 2018) have been reported in the genus *Pseudomonas*, only a few publications have demonstrated the macrolide resistance mechanisms in this species, such as active efflux pumps (especially the RND-type efflux pump family) (Li et al. 2000; Li et al. 2003; Strateva et al. 2009; El Zowalaty et al. 2015), ABC-F subfamily of ATP-binding cassette proteins encoded by *ms*rE (Ding et al. 2018) and mutations to the 23S rRNA (Mustafa et al. 2017). In this work, using large-scale sequencing of pooled genomic DNA of 262 strains, only two macrolide-related resistance genes, *mphE* and *msrE*, were identified in seven and four *P. aeruginosa* strains, respectively. This finding indicated that the known macrolide resistance genes are not prevalent in clinical *P. aeruginosa* isolates, although this was not the first time these two genes have been detected in *P. aeruginosa* (Ding et al. 2018; Ero et al. 2019).

The *msrE* and *mphE* genes can be found on chromosomes (Kadlec et al. 2011) or plasmids (Ho et al. 2011; Dolejska et al. 2013; Zhao et al. 2015; Wang et al. 2018) in many bacterial species. The two genes often cluster together and are separated by a 55 bp spacer sequence (Kadlec et al. 2011). The *msrE-mphE* gene cluster encoded on plasmids is generally related to mobile genetic elements, and they can be transmitted between bacteria of the same or different species and cause resistance spreading (Dolejska M et al. 2013; Zhao J Y et al. 2015). However, whether the *msrE* and *mphE* genes are located on host strain chromosomes or plasmids remains to be further elucidated.

The different types of macrolide resistance mechanisms also differ in their drug resistance spectrum. Modification or mutation of the ribosome RNA subunit leads to bacterial resistance to macrolide antibiotics, lincosamides, group B streptogramins, and ketolide telithromycin (Vester et al. 2001; Tu et al. 2005; Roberts 2008). Efflux pumps mediate broad resistance to most MLSB antibiotics (Roberts 2008). Inactivating enzymes, such as esterases, phosphotransferases, transferases, and lyases; however, show different antibiotic resistance spectra (Roberts 2008; Zhu et al. 2017). A few studies have demonstrated that the resistance genes *msrE* and *mphE* mediate resistance to erythromycin and azithromycin (Schluter et al. 2007; Gonzalez-Plaza et al. 2018). In this work, both *msrE* and *mphE* facilitated resistance to three second-generation macrolides (roxithromycin, clarithromycin, and azithromycin) and one first-generation macrolide (erythromycin). It was interesting to find that the two genes showed higher resistance levels to the three second-generation macrolides than to the two first-generation macrolides. This finding may be since new generation macrolides have been more widely used in clinical practice in recent years, increasing the resistance levels of bacteria to antibiotics. Also, the *msrE* gene had the same MIC level as azithromycin, which was similar to results reported in other studies (Schluter et al. 2007).

Conclusions

In this work, two known macrolide resistance genes, *mphE* and *msrE*, were identified in a small portion (2.67% and 1.53%, respectively) of 262 clinical *P. aeruginosa* strains, even though the bacteria showed very high resistance rates and MIC levels to the five macrolide antibiotics detected. It indicated that other mechanisms aside from known resistance genes might play a role in bacterial resistance to macrolides. The cloning and functional determination of the *mphE* and *msrE* genes demonstrated that these genes warranted higher resistance levels to three second-generation macrolides (roxithromycin, clarithromycin, and azithromycin) than to two first-generation macrolides (erythromycin and kitasamycin). It may be because new generation macrolides have been more widely used in clinical practice in recent years, resulting in an increased emergence of second-generation macrolide-resistant bacteria.

Abbreviations

P. aeruginosa/PAO	– *Pseudomonas aeruginosa*
PCR	– polymerase chain reaction
MIC	– minimum inhibitory concentration
MDR	– multidrug resistant
PDR	– pandrug resistant
GMD	– guanosine diphospho-D-mannose dehydrogenase
EPOS	– European position paper on rhinosinusitis and nasal polyps edition
MLS	– macrolides, lincosamides, streptogramins
MLSB	– macrolide-lincosamide-streptogramin B
BLAST	– Basic Local Alignment Search Tool
ORF	– open reading frame
LB	– Luria-Bertani
CLSI	– Clinical and Laboratory Standards Institute
ATCC	– American Type Culture Collection

Authors' contributions

QC, WL, DZ, HL, XZ, and AL collected the strains and performed the experiments. ZS, WZ, CQ, and TX analyzed the experimental results and performed the bioinformatics analysis. QC, QB, LN, TX, and AL wrote the manuscript. LN, TX, and HX designed the experiments.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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