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Identification of a novel aminoglycoside nucleotidyltransferase gene in *Morganella morganii* from farm sewage

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Abstract

Background Aminoglycosides are important broad-spectrum antimicrobial agents. When combined with β-lactam drugs, these agents can be used to treat severe infections such as those causing sepsis. Identifying additional resistance mechanisms will guarantee the successful application of aminoglycoside agents in clinical practice.

Methods The isolate *Morganella morganii* A19 was obtained from a sewage sample from an animal farm by means of agar plate streaking. The agar dilution method was used to determine the minimum inhibitory concentrations (MICs) of the antimicrobial agents. Cloning of the predicted resistance gene was conducted, and its resistance function was assessed through MIC testing. The protein was expressed in *E. coli*, and the kinetic parameters were quantified. The analysis of novel resistance gene-related sequences, including their structures and evolutionary relationships, was performed using bioinformatic tools.

Results In *Morganella morganii* A19, a newly discovered chromosome-encoded aminoglycoside resistance gene named *aadA37* was identified and characterized. The protein AadA37 exhibited the highest amino acid identity (57.14%) with the functionally characterized aminoglycoside adenylyltransferase AadA33. *aadA37* confers resistance to spectinomycin, streptomycin and ribostamycin, and enzyme kinetic analysis also demonstrated that it had adenosine transfer activities against spectinomycin and streptomycin, with k_{cat}/K_m values of 0.66×10^3 M^{-1} s^{-1} and 1.63×10^3 M^{-1} s^{-1}, respectively. The *aadA*37 gene and its homologs were not related to any mobile genetic element (MGE), and they were all found to be encoded on the chromosomes of the *M. morganii* strains.

Conclusion A novel aminoglycoside resistance gene was identified from an environmental bacterium and characterized in this work. Identifying new resistance mechanisms will aid in the effective clinical use of antimicrobial agents for treating infectious diseases caused by pathogens harboring the same resistance genes.

Keywords *Morganella morganii*, Antimicrobial resistance gene, *aadA37*, Aminoglycoside *O*-nucleotidyltransferase, Kinetic parameter

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Background

Morganella morganii is a gram-negative bacterium found in the human gut and in the environment. Initially isolated in 1906 from the fecal sample of a child, it was identified as a relatively unimportant pathogen [1]. Nevertheless, beginning in the 1970s, *M. morganii* was recognized as a significant cause of human nosocomial infections, with some cases leading to elevated mortality [2, 3]. A variety of conditions are associated with *M. morganii* infections, including cellulitis, abscesses, sepsis, diarrhea, and bacteremia [4]. Furthermore, it can also cause severe infections, including fatal fibrino-hemorrhagic bronchopneumonia, deep hemorrhagic ulcers, and rot in animals [5, 6].

For treating infections caused by *M. morganii*, antimicrobials such as gentamicin ciprofloxacin, amikacin and ceftazidime were frequently used [7]. In recent decades, these aminoglycosides have been used to treat plague, tularemia, brucellosis, endocarditis and systemic infections due to the availability of other antibiotic classes and the increase in bacterial resistance [8, 9]. Advances in our understanding of the molecular processes underlying antimicrobial resistance have enabled the discovery of inhibitors and the development of innovative semisynthetic agents, such as spectinamides and arbekacin [10], which has led to increased interest in these antimicrobial agents [11]. The aminoglycosides resistance is related to a variety of mechanisms including enzyme modification of the antimicrobial agents or their targets, efflux pumps and so on. The aminoglycosides modification genes are wide-spread in Gram-negative and Gram-positive bacteria [12, 13]. At present, there were more than 50 *aadA* (*ant*) genes present in CARD, which were originated from different sources. For example, *aac(6')-Va* and *ant(9)-Id* were initialy identified and functionally characterized from *Aeromonas hydrophila* [14] and *Providencia* sp [15]., respectively. Currently, the most common resistance mechanism to aminoglycosides is deactivation by a variety of enzymes that alter aminoglycosides [16]; these enzymes are divided into three categories based on the various modification sites involved: aminoglycoside *N*-acetyltransferases (AACs), *O*-nucleotidyltransferases (ANTs), and *O*-phosphotransferases (APHs) [17]. Besides intrinsic resistance to some of spenicillins, first to third generation cephalosporins, macrolides, lincosamides and polymyxin [18, 19], *M. morganii* also demonsterated resistance to many other currently used antimicrobial agents, such as amikacin, gentamycin, tobramycin and ciprofloxacin, and so on [20]. It has been reported that an isolate designated *M. morganii* MMAS2018 is resistant to ciprofloxacin, gentamycin, streptomycin, kanamycin, azithromycin, erythromycin, tetracycline, fosfomycin, sulfamethoxazole, trimethoprim, rifampicin, amoxicillin, cefotaxime, chloramphenicol and florfenicol [18].

In our recent project investigating the antimicrobial resistance mechanisms of environmental bacteria, approximately two hundred bacteria were isolated from soil and sewage at animal farms in Wenzhou, China. The resistance profiles of these strains were examined, and their genomes were sequenced. One isolate, designated *M. morganii* A19, is resistant to several aminoglycosides, but no functionally characterized resistance gene to aminoglycoside antimicrobials was predicted from its genome. In this work, we used molecular and bioinformatic methods to elucidate the resistance mechanism of this bacterium to aminoglycosides.

Materials and methods

Bacterial strains, plasmids, culture conditions

The isolate *M. morganii* A19 was isolated from the sewage of Yongjia Animal Farm in Wenzhou, China. The owner of the farm was informed in writing of the study and provided approval for the sampling of sewage. Average nucleotide identity (ANI) assessments between the *M. morganii* A19 genome and the bacterial genomes in the NCBI nucleotide database and 16 S rRNA gene homology analysis between them were conducted to identify the bacterial species. To anlyze the function of the gene, pUCP20 was used as a vector to clone the novel resistance gene (designated *aadA37* in this work) with its promoter region and *Escherichia coli* DH5α was used as a recipient for the recombinant plasmid pUCP20-*aadA37.* For the expression of the protein AadA37, pCold I was used as a vector to clone the open reading frame (ORF) of the gene and *Escherichia coli* BL21 was used as a reciepient. Table 1 lists all the bacterial strains and plasmids utilized in this investigation. Routine cultivation of bacteria was carried out in lysogeny broth (LB) or on LB plates (LB solidified by 1.5% agar) at 37 °C.

Antibiotic susceptibility testing

The agar dilution method was utilized to determine the minimum inhibitory concentrations (MICs) of various aminoglycoside antibiotics in compliance with the recommendations established by the Clinical and Laboratory Standards Institute (CLSI) M100 (34st Edition, 2024, https://clsi.org). *E. coli* ATCC 25,922 was utilized as a quality control. The plate was incubated for 16–20 h at 37 °C, after which the MIC results were recorded.

Genome sequencing and bioinformatics analysis

The whole genome of *M. morganii* A19 was sequenced using the Illumina NovaSeq 6000 and PacBio Sequel II platforms (Shanghai Personal Biotechnology Co., Ltd., Shanghai, China). The reads from both platforms were subjected to hybrid assembly using Unicycler (v0.4.8) [21] and then polished by Pilon (v 1.23) [22]. Putative proteins were annotated against the NCBI nonredundant

Table 1 Bacteria and plasmids used in this work

Strain and plasmid	Description	Reference
Morganella morganii A19	A19, the wild-type isolate	This study
Escherichia coli DH5α	DH5α, a recipient for cloning of the *aadA37* gene	CGMCC*
Escherichia coli BL21	BL21, a recipient for expression of the *aadA37* gene	CGMCC*
Escherichia coli ATCC 25,922	ATCC 25,922, a quality control for MIC testing	CGMCC*
pUCP20-*aadA37*/DH5α	DH5α harboring pUCP20-*aadA37*, a recombinant plasmid	This study
pCold I-*aadA37*/BL21	BL21 harboring pCold I-*aadA37*, a recombinant plasmid	This study
pUCP20	a vector for cloning of *aadA37* with its promoter region, AMP^r	CGMCC*
pCold I	a vector for expression of the open reading frame (ORF) of the *aadA37* gene, AMP^r	CGMCC*

^{r}, resistance; AMP, ampicillin; CGMCC, China General Microbiological Culture Collection Center

protein database using DIAMOND (v2.0.14) [23]. The antibiotic resistance-related genes were annotated by Prokka (v.1.14.6) [24] against the CARD [25] and ResFinder databases [26]. FastANI was used to compute the ANI [27]. The molecular weight and pI of AadA37 were estimated using ProtParam (https://web.ExPASy.org/protparam/), and a neighbor-joining phylogenetic tree was constructed using MEGA X [28]. The functionally characterized AadAs (ANTs) protein sequences (Table S1) for the phylogenetic analysis with AadA37 were retrieved from the GenBank database and previous publications [16, 17]. InterProScan, which can be accessed at https://www.ebi.ac.uk/interpro/, was used to analyze conserved functional domains. Gene organization diagrams were generated using Easyfig [29]. The *aadA37* homologous genes were obtained by the BLAST program using *aadA37* as a query to search the nonredundant nucleotide database of NCBI. Genetic context analysis of *aadA37* and other related sequences was carried out by using clinker v.0.0.25 [30].

Cloning of the novel aminoglycoside resistance gene *aadA37*

To clone the *aadA37* gene, the ORF of *aadA37* with the promoter region was PCR-amplified with primers listed in Table 2. The promoter region of *aadA37* (-35 sequence CTGACT and −10 sequence AGATACCAT) was predicted with the online program BPROM (http://www.softberry.com/berry.phtml?topic=bprom%26;group=programs%26;subgroup=gfindb) and the primers were designed by SnapGene v6.0 (www.snapgene.com). The PCR product was inserted into pUCP20 utilizing T4 ligase from Takara Biomedical Technology Co., Ltd. (Takara, Dalian, China). After electroporation to introduce the ligation product (pUCP20-*aadA37*) into *E. coli* DH5α, transformants (pUCP20-*aadA37*/DH5α) were selected on LB agar plates supplemented with 100 μg/mL ampicillin. Sanger sequencing was used to verify the cloned fragment (Shanghai Sunny Biotechnology Co., Ltd., Shanghai, China).

Protein expression and purification

The ORF of the *aadA37* gene was amplified via PCR using the primers listed in Table 2, and the PCR product was subsequently ligated into the vector pCold I. pCold I-*aadA37* (the recombinant plasmid) was then introduced into BL21. Using a previously reported methodology [31], AadA37 was produced as a fusion protein with an N-terminal His6 tag and a thrombin cleavage site. The first purification stage was completed by Ni-NTA affinity chromatography, and His6 tag removal was performed with thrombin (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) at 25 °C for three hours. To remove any remaining traces of the free His6 tag, a second purification step using a Ni-NTA column was carried out. A Sartorius ultrafiltration spin column with a 10 kDa cutoff was used to produce the concentrated

Table 2 The primers used in this study

Primer[a]	Sequence (5′-3′)[b]	Restrictionendonuclease	Vector	Annealing temperature (°C)	Amplicon size (bp)
pro-*aadA37*-F	CATATGATGAAAATAAATCTGAATCATGTCCCTG		pUCP20	58	798
pro-*aadA37*-R	CTCGAGTCACGATTGTTTTCCTTCGCAT				
orf-*aadA37*-F	GCCCTCGAGGAGGATCTGTACTTTCAGAGCATGAAAATAAATCTGAATCATGTCCCTGC	*Xho*I + Thrombin	pCold I	58	832
orf-*aadA37*-R	CGGGATCCTCACGATTGTTTTCCTTCGCATAACGCC	*Bam*HI			

[a] Primers with "pro" were used for cloning *aadA37* with its promoter region, and primers with "orf " were used for cloning the *aadA37* ORF

[b] The underlined sequences represent the restriction endonuclease sites

AadA37 protein. Spectrophotometry and the BCA protein test (Beyotime Biotechnology Co., Ltd., Shanghai, China) were used to measure the protein concentration, and SDS–PAGE (12%) was utilized to evaluate the size and purity of the AadA37 protein.

Enzyme kinetic analysis

The enzyme activities of AadA37 were quantified as previously reported [32]. A continuous spectrophotometric test was used to determine the adenylation of aminoglycoside antibiotics. This test correlates the production of pyrophosphate (PPi) to the activities of glucose-6-phosphate dehydrogenase, phosphoglucomutase, and UDP-glucose pyrophosphorylase. A UV–VIS spectrophotometer (U-3900, Hitachi, Japan) was used to track the release of NADPH at 340 nm. The reaction mixture was composed of 50 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 0.2 mM UDP-glucose, 0.2 mM 4,4′-dithiodipyridine (DTDP), 5 mM ATP, 20 U/mL glucose-6-phosphate dehydrogenase, 20 U/mL phosphoglucomutase, 2 U/mL UDP-glucose pyrophosphorylase, 50–150 μmol purified AadA37 and various amounts of antimicrobials. The test was completed on a 96-well plate at 37 °C with a volume of 100 μL. The steady-state kinetic parameters (k_{cat} and K_m) were identified as reported previously [31].

Results and discussion

General genomic features and species identification and *M. morganii* A19

The *M. morganii* A19 genome consists of a 4.16 Mbp chromosome (GenBank accession number: CP135144), along with two plasmids, designated plasmid1 and plasmid2 (GenBank accession numbers: CP135145 and CP135146, respectively). The chromosome harbors 3,833 coding sequences (CDSs), while the two plasmids encode 98 and 2 CDSs, respectively (Table 3). The GC contents of the chromosome and two plasmid sequences were 50.4%, 48.6% and 41.8%, respectively. Notably, the novel resistance gene *aadA37* is located in the chromosome.

Table 3 General features of the *M. morganii* A19 genome

	Chromosome	Plasmid1	Plasmid2
Size (bp)	4,163,805	104,648	2,683
GC content (%)	50.4%	48.6%	41.8%
Predicted coding sequences (CDSs)	3,833	98	2
Known proteins	2,745	45	2
Hypothetical proteins	1,088	53	0
Protein coding (%)	87.04	80.22	40.48
Average ORF length (bp)	930.52	856.62	543
Average protein length (aa)	314.16	284.54	182
tRNAs	80	0	0
rRNA operons	(16–23 S-5 S) × 6 (16–23 S-5 S–5 S) × 1	0	0

Species identification of A19 revealed that it had the highest genome-wide ANI (98.77%) with the *Morganella morganii* type strain CTX51^T (GenBank accession number: NZ_CP076623.1), and 16 S rRNA gene homology analysis revealed that the 16 S rRNA gene of A19 had the highest similarity (95.96%) with that of *Morganella morganii* M11 (NR_028938.1) (Fig. 1). According to the criteria for classifying a bacterium as a certain species (a threshold of ≥ 95% ANI was set to classify a bacterium as a certain species) [33], isolate A19 was classified as *Morganella morganii* and was thus designated *Morganella morganii* A19.

The resistance phenotype and genotype of the *M. morganii* A19 isolate

The antimicrobial susceptibility test revealed that *M. morganii* A19 had high MICs for several antimicrobial agents tested, including spectinomycin (128 μg/mL), ribostamycin (32 μg/mL), polymyxin (> 512 μg/mL), fosfomycin (> 256 μg/mL), erythromycin (64 μg/mL), chloramphenicol (32 μg/mL) and tetracycline (32 μg/mL) (Table 4). Resistance gene annotation of the genome sequence revealed that only four resistance genes with a similarity ≥ 80.0% with the functionally characterized resistance genes were found. These genes included the β-lactam resistance gene bla_{DHA-16}, the chloramphenicol resistance gene *catII*, the tetracycline resistance gene [*tet(D)*] and the quinolone resistance gene *qnrD1* with the former three encoded in the chromosome and the last one (*qnrD1*) on the plasmid (Plasmid2) (Table 5). No gene conferring resistance to aminoglycoside, polymyxin, erythromycin or fosfomycin was annotated, even though they had high MIC values. *M. morganii* possessed intrinsic resistance to β-lactams (such as penicillins and first to third generation cephalosporins), macrolides (such as erythromycin), lincosamides and polymyxin [18, 19]; however, unidentified resistance mechanisms might exist for the aminoglycoside- and fosfomycin-resistant phenotypes.

The novel aminoglycoside nucleotidyltransferase gene *aadA37* confers resistance to spectinomycin, streptomycin and ribostamycin

To explore the mechanism underlying the resistance of the bacterium to aminoglycosides, the genome annotation results were checked, and one predicted homolog of *aadA33* (GenBank accession number of *aadA33*: UVE15953.1) was found. This *aadA33*-like gene (designated *aadA37* in this work) was subsequently cloned, and its resistance phenotype was analyzed. Compared with that of the control (*E. coli* DH5α or pUCP20/*E. coli* DH5α), the recombinant strain harboring *aadA37*

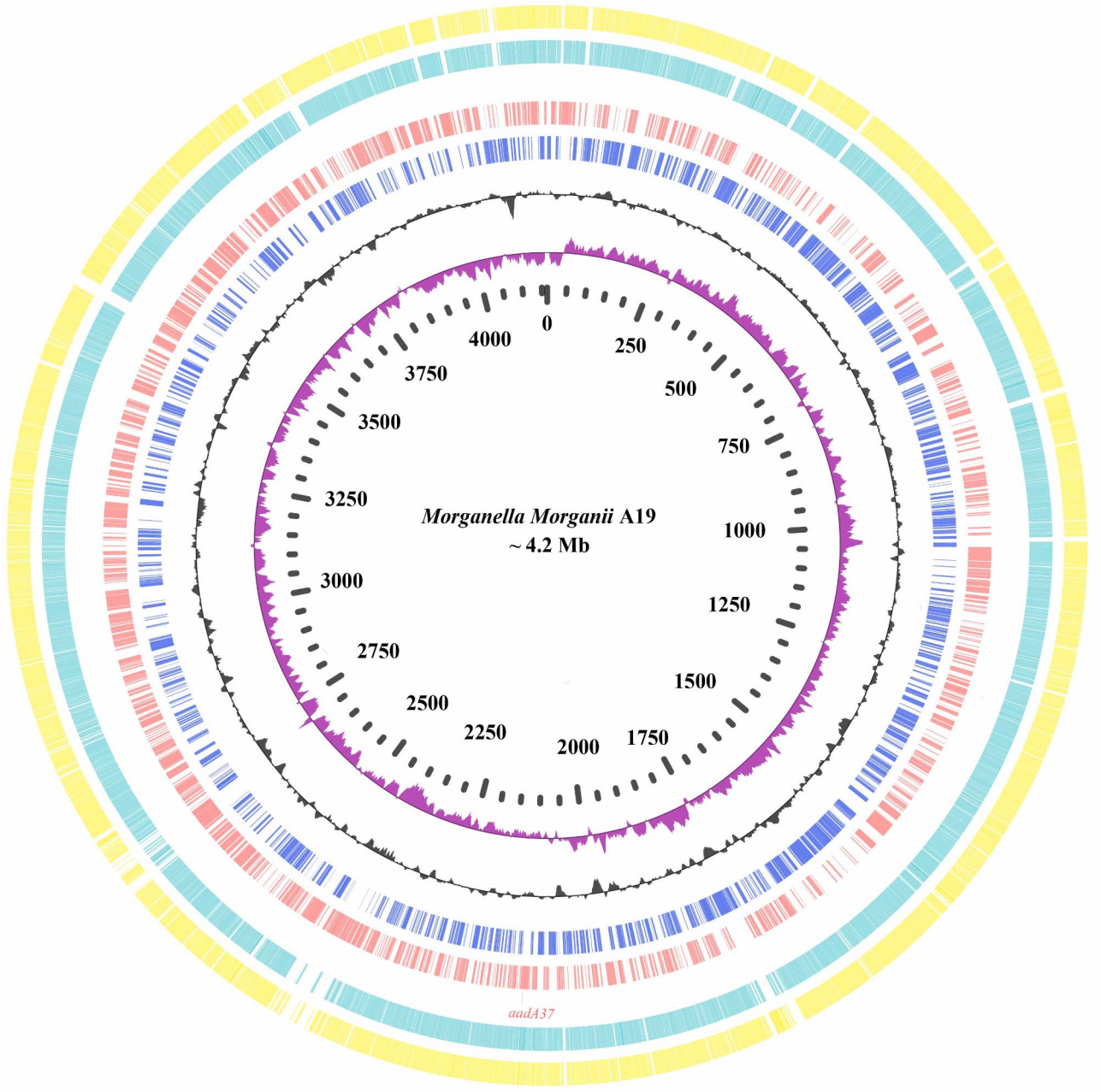

Fig. 1 Comparative analysis of the *M. morganii* A19 chromosome with other similar chromosomes of higher identity. The chromosomes of *M. morganii* CTX51T (CP076623.1) and FAM24670 (CP066140.1) are represented by circles 1 and 2, respectively, as homologous regions; the unmatched regions are left blank. Circles 3 and 4 show the predicted ORFs encoded in the forward and reverse strands, respectively; circles 5, 6, and 7 show the GC content, GC skew, and scale in kb of the *M. morganii* A19 chromosome, respectively

(pUCP20-*aadA37*/*E. coli* DH5α) demonstrated substantial increases of 64-, 16- and 4-fold in the MICs for spectinomycin, streptomycin and ribostamycin, respectively. However, no significant alteration in the MIC value was observed for micronomicin, tobramycin, or netilmicin (Table 4).

aadA37 showed a similar resistance spectrum to that of the members of the *ant(3″)-Ia* family. By analyzing the resistance phenotypes of *aadA37* and its close relatives, such as *aadA33*, *aadA36*, *aadA14* and *aadA31*, it was found that all these genes were resistant to spectinomycin and streptomycin, although the MICs of the two antimicrobial agents might vary. The MICs of spectinomycin and streptomycin for the recombinant strain harboring *aadA37* were 256 and 64 μg/mL, respectively, while the MICs for the strains harboring the other four

Table 4 Antimicrobial susceptibility for strains from this study (μg/mL)

Antibiotics	ATCC 25,922	DH5α	pUCP20/DH5α	pUCP20-aadA37/DH5α	M. morganii A19
Micronomicin	1	0.5	0.5	0.25	0.5
Ribostamycin	4	2	2	8	32
Spectinomycin	8	8	8	256	128
Streptomycin	4	4	4	256	8
Tobramycin	0.5	0.25	0.25	0.25	0.25
Netilmicin	0.5	0.25	0.25	0.25	0.25
Neomycin	2	1	1	1	1
Sisomycin	0.5	0.25	0.25	0.5	0.25
Amikacin	2	1	1	1	1
Polymyxin	0.25	0.25	0.25	0.25	> 512
Fosfomycin	4	4	4	4	> 256
Chloramphenicol	-*	-*	-*	-*	32
Erythromycin	-*	-*	-*	-*	64
Tetracycline	-*	-*	-*	-*	32
Gentamycin	0.5	0.5	0.5	0.5	0.5

*, not detected

genes (*aadA33*, *aadA36*, *aadA14* and *aadA31*) were greater than those for the strain harboring *aadA37*; these concentrations were ≥ 2048, 1024, ≥ 512 and > 512 for spectinomycin and 256, 128, 256 and 256 μg/mL for streptomycin, respectively [34–37].

Kinetic parameters and molecular characterization of the aminoglycoside adenylyltransferase AadA37

The kinetic parameters of AadA37 were generally in line with the MIC of the strain carrying the *aadA37* gene. Two aminoglycosides, spectinomycin and streptomycin, were specifically adenylated by the enzyme, with k_{cat}/K_m values of 0.66×10^3 M^{-1} s^{-1} and 1.63×10^3 M^{-1} s^{-1}, respectively. No evidence of adenosine transfer for tobramycin was observed. Table 6 shows the kinetic parameters of AadA37 for the substrates.

Table 6 Kinetic parameters of AadA37

Substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m (M^{-1} s^{-1})
Spectinomycin	5.36×10^{-3}	8.17×10^{-6}	0.66×10^3
Streptomycin	4.35×10^{-3}	2.67×10^{-6}	1.63×10^3
Tobramycin	NA	NA	NA

NA, no adenylate transfer activity was detected

AadA37 showed a similar substrate profile to that of the other close relatives, although they may have different adenosine transfer efficiencies. Similar to the MIC differences, AadA33 and AadA36 also demonstrated greater adenosine transfer efficiencies against spectinomycin or streptomycin than did AadA37. It has been reported that AadA33 and AadA36 have k_{cat}/K_m values of 3.28×10^4 M^{-1} s^{-1} and $(1.07 \pm 2.23) \times 10^4$ M^{-1} s^{-1} for spectinomycin, respectively, and 3.37×10^4 (M^{-1} s^{-1}) and $(8.96 \pm 1.01) \times 10^3$ M^{-1} s^{-1} for streptomycin, respectively [34, 35].

The aa residues W173 and D178 are critical factors determining adenylation activity toward streptomycin according to a structural study of AadA (Q8ZPX9). On the other hand, the important residues for spectinomycin have been shown to be E87, W112, D182, and either 185 H–185 N [38]. These six aa residues were shown to be conserved in the sequence alignment of AadA37 with other AadA enzymes, except for the E185 residue in AadA37 (Fig. 2).

Comparative analysis of the *aadA37* gene and its relatives

The *aadA*37 gene is 798 bp in length and encodes a 265 aa protein. AadA37 has a molecular mass of 29.18 kDa and a pI of 5.65. Currently, there are 52 AadAs (ANTs) proteins present in CARD, of which 31 termed AadAs ranging from AadA, AadA2 to AadA36. Following them, the protein encoded by the novel aminoglycoside nucleotidyltransferase gene *aadA37* was thus named AadA37. AadA37 shares aa sequence similarities ranging from 3.18 to 57.14% with the 25 of them, exhibiting the highest similarity to AadA33 (57.14%), followed by AadA36 (56.69%) (Table S1). A phylogenetic tree of the functionally characterized ANTs was constructed, which included enzymes such as ANT(9), ANT(6), ANT(4), ANT(3") and ANT(2') (Fig. 3). AadA37 constituted a branch close

Table 5 Annotation of antimicrobial resistance genes in *M. morganii* A19

Gene name	Location	Amino acid coverage (%)	Amino acid identity (%)	Similarity (%)	Reference (GenBank accession No.)	Annotation
bla_{DHA-16}	Chromosome	100	98.94	98.94	AIT76105.1	beta-lactamase DHA
catII	Chromosome	100	96.71	96.71	CAA37806.1	Chloramphenicol acetyltransferase (CAT)
tet(D)	Chromosome	100	95.94	95.94	AAL75563.1	Tetracycline efflux
qnrD1	Plasmid2	100	100	100	ACG70184.1	Quinolone resistance protein

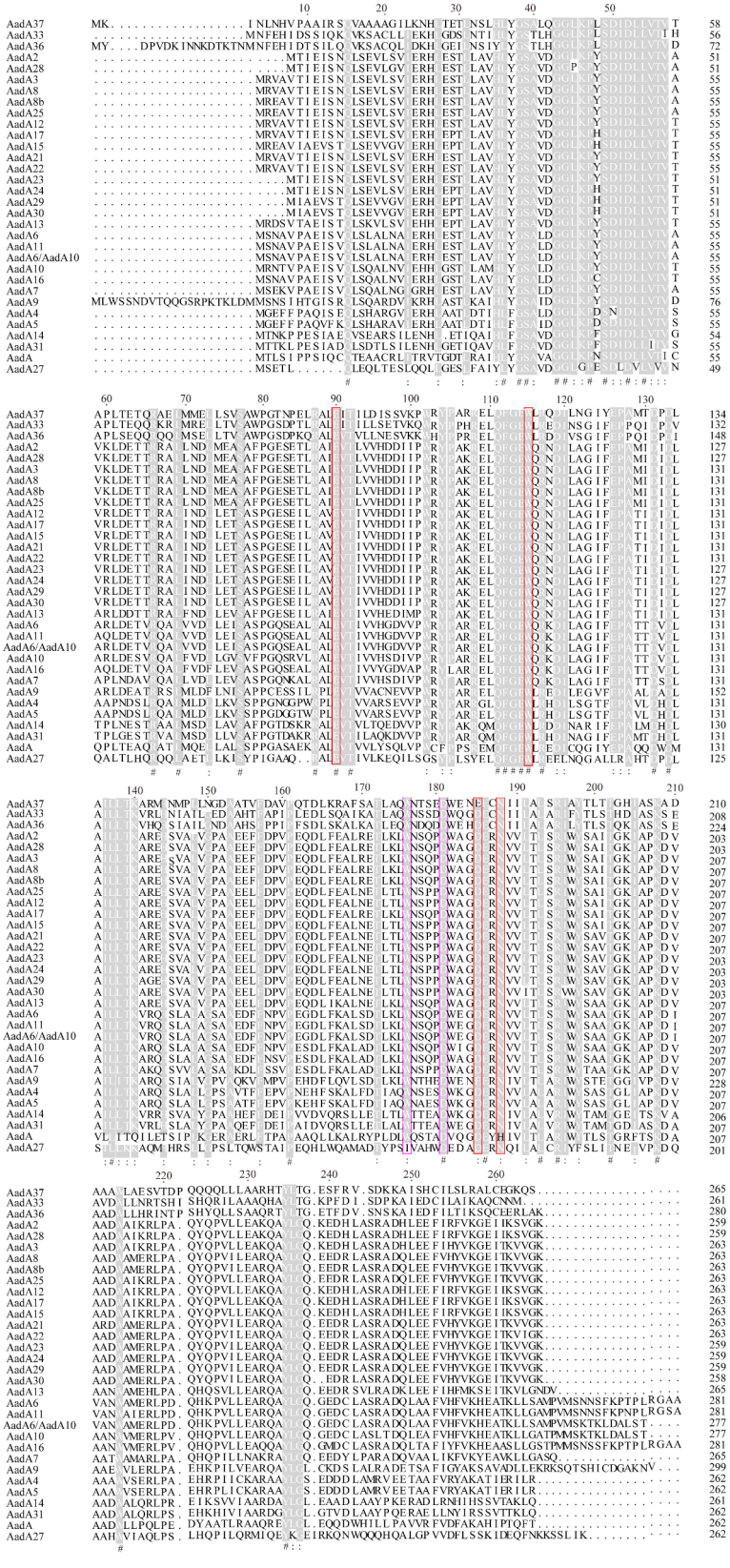

Fig. 2 Multiple sequence alignment of the aa sequences of AadA37 and its homologs. The accession numbers of the proteins are the same as those in Table S1. The pound signs (#) signify fully conserved residues, while the colons (:) denote strongly similar residues. The functional residues for spectinomycin and streptomycin are highlighted by red and purple frames, respectively. The numbers on the right side indicate the sequence lengths

Fig. 3 Phylogenetic analysis of AadA37 with all the other 51 functionally characterized ANTs. AadA37 in this study is highlighted in red. The GenBank accession numbers of the proteins are MZ241296 [ANT(9)-Ic], CAA26963 [ANT(9)-Ia], EGP12870 (Spw), AAA16527 [ANT(9)-Ib], AGW81558 (Spd), AAC64365 [ANT(2")-Ia], AAA25717 [ANT(4')-IIa], AAM76670 [ANT(4')-IIb], AAO83986 [ANT(4')-Ia], ADA62098 [ANT(4')-Ib], AAA27459 (AadS), CAB14620 (AadK), CBH51824 [ANT(6)-Ib], AHE40557 [ANT(6)-Ia], AAU10334 [Aad(6)], CAK12750 (AadA22), AAN87151 (AadA21), CAH10847 (AadA23), ABG72894 (AadA24), CAA26199 [ANT(3")-IIa], ANN23976 (AadA29), ANN23985 (AadA30), AAO49597 (AadA), ABD58917 (AadA15), ACJ47200 (AadA12), ACK43806 (AadA17), AET15272 (AadA25), ANN23979 (AadA28), AAC14728 (AadA3), AAF27727 (AadA2), CAJ13568 (AadA8b), AAN41439 (AadA8), ABW91178 (AadA13), BAD00739 (AadA7), AAL36430 (AadA10), ACF17980 (AadA16), AAL51021 [ANT(3")-Ii-AAC(6')-IId], AAV32840 (AadA11), CAJ32504 (AadA6), CAJ32491 (AadA6/AadA10), ABG49324 (AadA9), AAF17880 (AadA5), AAN34365 (AadA4), CAI57696 (AadA14), AUX81654 (AadA31), UVE15953 (AadA33), UVE15954 (AadA36), MW984426 [ANT(3")-IId], ENU91137 [ANT(3")-IIb], CTQ57092 (AadA27) and ENU37733 [ANT(3")-IIc]

to the ANT(3")-Ia proteins; consequently, it was considered an additional lineage within the ANT(3")-Ia family.

By a search for homologs of *aadA37* in the nonredundant nucleotide database of NCBI, twelve sequences with > 80.0% nucleotide sequence similarity (86.42 to 99.62%) were retrieved. Notably, these sequences were all derived from *M. morganii* isolates from different sources, including animals, human clinical specimens, marine fish and the environment (Table S2). AadA37 exhibited the highest aa similarity (99.62% identity and 100% coverage) to the hypothetical aminoglycoside adenylyltransferase family protein (WP_283623747.1) encoded by the chromosome of *M. morganii* HIS2824 (NZ_OX460951.1). These findings indicate that the *aadA37* variants are conserved in *M. morganii* of different sources, which will benifits the treatment of the infectious diseases of animals and humans caused by *M. morganii* by avoiding using spectinomycin or streptomycin to treat the infections.

To determine the genetic context of *aadA37*, a sequence approximately 22 kb in size with *aadA37* located in the middle of the sequence was used as a query to find similar sequences from the nonredundant nucleotide database of NCBI. Among the fourteen sequences

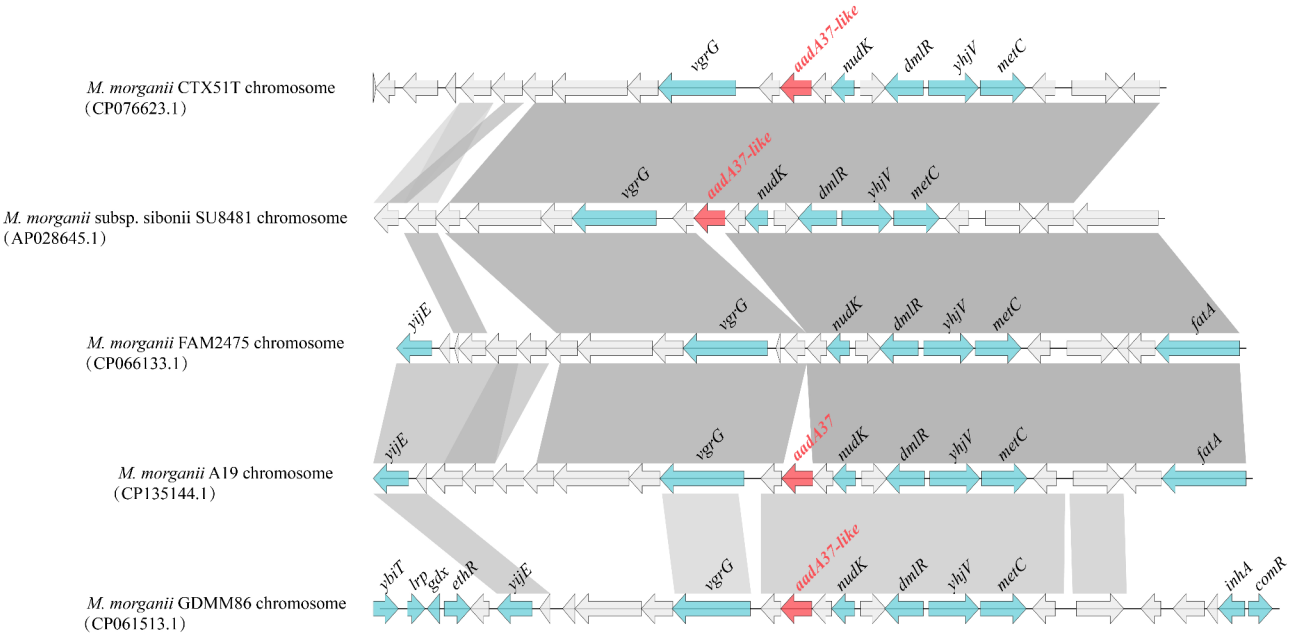

Fig. 4 Comparative analysis of the *aadA37* gene-related sequences. Genes are indicated by arrows. *aadA37* and its homolog (*aadA37*-like) are highlighted in red. Hypothetical proteins (hp) are colored gray, and areas of homology (>80% nucleotide sequence similarity) are indicated by gray shading. The sequence sources and accession numbers are *M. morganii* CTX51T (CP076623.1), *M. morganii* SU8481 (AP028645.1), *M. morganii* FAM24675 (CP066133.1) and *M. morganii* GDMM86 (CP061513.1)

with >80.0% similarity, only four sequences contained *aadA37*-like genes that showed >70.0% aa similarity with AadA37 and were all located on the chromosomes of the *M. morganii* strains. The absence of mobile genetic elements in the adjacent regions of the *aadA*37(-like) genes suggested that these elements are conserved in *M. morganii* (Fig. 4).

Conclusion

In this work, a novel resistance gene, *aadA37*, conferring resistance to streptomycin and spectinomycin, was identified on the chromosome of *M. morganii* A19, which was isolated from animal farm sewage. Identifying additional resistance genes holds promise for advancing the understanding of intrinsic resistance mechanisms against aminoglycosides in opportunistic pathogens.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03844-5.

Supplementary Material 1

Supplementary Material 2

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Author contributions
The manuscript was drafted by JWL, CY, and DW. Conceived and designed the experiments: JWL, JW, QB, CY and DW; carried out the experiments: JW, MG, JWL, KZ, CL and XH; and analyzed and interpreted the data: HF, CS, WP, JLL and CY. The manuscript was drafted by JWL, JW, and DW.

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Data availability
Sequence data that support the findings of this study have been deposited in the Nucleotide database of NCBI with the GenBank accession numbers CP135144, CP135145 and CP135146.

Declarations

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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