Molecular and Functional Characterization of a Novel Plasmid-Borne blaNDM variant, blaAFM-1, in a Clinical Strain of *Aeromonas Hydrophila*

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Research

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Abstract

Background

Aeromonas hydrophila is a zoonotic and human opportunistic pathogen. Increasing antibiotic resistance profiles have been discovered in both clinical and environmental *A. hydrophila* isolates in recent years. However, there are still very few in-depth studies regarding the role of plasmids in antibiotic resistance of *A. hydrophila*. Hence, we investigate the molecular characterization and functional characterization of a multidrug resistant plasmid which encoding a novel NDM variant, AFM-1, in clinical *A. hydrophila* SS332.

Methods

The minimum inhibitory concentrations (MICs) of 24 antibiotics for *A. hydrophila* SS332 were measured by the agar dilution method. The genome of *A. hydrophila* SS332 was sequenced with Pacific and Illumina platforms. Six plasmid-borne antimicrobial resistance genes were chosen for cloning, including *bla*_{AFM-1}, *bla*_{OXA-1}, *msr*(E), *mph*(E), *aac*(6')-Ib10, and *aph*(3')-Ia. Phylogenetic analysis, amino acid sequence alignment, and comparative genomic analysis were performed to elucidate the active site requirements and genetic context of *bla*_{AFM-1} gene.

Results

A. hydrophila SS332 showed high levels of resistance to 15 antibiotics, especially those with MIC levels up to or higher than 1024 µg/mL, including ampicillin, cefazolin, ceftriaxone, aztreonam, spectinomycin, roxithromycin. Six plasmid-borne resistance genes were verified to be functional in *E. coli* DH5α. The *bla*_{AFM-1} gene, a novel *bla*_{NDM} variant, encodes a subclass B1 metallo-β-lactamase AFM-1 which shares 86% amino acid identity with NDM-1. AFM-1 showed resistance to penicillins, cephalosporins and carbapenems. Besides, *bla*_{AFM-1} gene was associated with three different novel *ISCR19*-like elements, designated *ISCR19-1*, *ISCR19-2* and *ΔISCR19-3*, which may be involved in the acquisition and mobilization of *bla*_{AFM-1} gene.

Conclusions

Our investigation showed that plasmid-borne resistance genes played a major role in the resistance profiles of *A. hydrophila* SS332. A novel *bla*_{NDM} variant, *bla*_{AFM-1}, was verified to be functional and disseminated among different bacteria species mediated by novel *ISCR19*-like elements. The fact indicated that the risk of spread of novel resistance genes and novel *ISCR* elements.

Introduction

Aeromonas hydrophila is a gram-negative bacterium and ubiquitous in aquatic environments that can cause infections in fish [1], amphibians [2], reptiles [3], and humans [4]. It has been identified as an antibiotic-resistant and virulent etiologic agent in an increasing list of human diseases, including gastroenteritis [5], diarrhea [6], necrotizing fasciitis [7], septicemia [8], meningitis [9], and hemolytic uremic syndrome [10]. Unfortunately, increasing antibiotic resistance profiles, including colistin and carbapenem, that are considered as the last line of defense against multi-resistant infections, have been discovered in both clinical and environmental *A. hydrophila* isolates in recent years. Hughes et al. reported that *A. hydrophila* AHNIH1 carrying *bla*_{KPC-2} gene was resistance to ertapenem which was isolated from perirectal surveillance culture [11]. Similarly, *A. hydrophila* GSH8-2 carrying *bla*_{KPC-2} genes reduced susceptibility to all tested β-lactams which was isolated from a waste water treatment plant effluent in Japan [12]. Interestingly, the above-mentioned antimicrobial resistance genes (ARGs) are all located on the plasmids of *A. hydrophila* [12].

Antimicrobial resistance is a global public health challenge that threatens human health. The increasing incidence of phenotypic resistance to antibiotics is mainly attributed to abused use of antimicrobials and acquisition of ARGs by bacterial pathogens [13]. Several phenotypic properties of bacteria have been proven to be encoded on the plasmid, such as antimicrobial resistance and virulence factors [14]. Besides, the dissemination of ARGs is closely associated with mobile genetic elements, including plasmids, which increase their ability to replicate and contribute to self-transmission or horizontal gene transfer among different bacterial species. It has been reported that *A. hydrophila* is able to harbor one or more plasmids. However, there are still very few in-depth studies regarding the role of plasmids in antibiotic resistance of *A. hydrophila*.

In this study, a multi-resistant clinical *A. hydrophila* strain SS332 was isolated from fecal sample of a male gastric carcinoma patient in Lishui Central Hospital, Zhejiang, China. Genome sequencing showed that a plasmid pSS332-218k carried by SS332 encodes 12 putative ARGs, including *bla*_{AFM-1}, which was a *bla*_{NDM}-like gene with 86% identity to *bla*_{NDM-14}. The β-lactamase gene *bla*_{AFM-1} was first

cloned and its antibiotic resistance profile was verified. Phylogenetic analysis, amino acid sequence alignment, and comparative genomic analysis were performed to elucidate the active site requirements and genetic context of *bla*_{AFM-1} gene.

Materials And Methods

Bacterial strains and plasmids

A. hydrophila SS332 was isolated from fecal specimen of a 76-year-old male patient in Lishui Central Hospital, Zhejiang, China in 2013. It was identified by a microorganism auto-analysis system (VITEK®2, BioMerieux, France) and homologous comparisons of the 16S rRNA gene sequence to the GenBank database by using the BLAST program (www.ncbi.nlm.nih.gov/BLAST/). *Escherichia coli* ATCC 25922 was used as the quality control strain in antimicrobial susceptibility testing. *E. coli* strain DH5α was used as a recipient for cloning experiments. pUCP20 and pUCP24 were used as vectors in cloning experiments.

Antimicrobial susceptibility testing

The agar dilution method was employed for measurement of the minimum inhibitory concentration (MIC) of all tested antibiotics against *A. hydrophila* SS332 according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, M45 3rd edition (2015). Twelve β-lactam antibiotics (ampicillin, cefminox, cefazolin, ceftazidime, cefotaxime, ceftriaxone, cefoselis, cefepime, aztreonam, imipenem, and meropenem), four aminoglycoside antibiotics (spectinomycin, gentamicin, kanamycin, and amikacin), two macrolide antibiotics (erythromycin and roxithromycin), two phenicols antibiotics (chloramphenicol and florfenicol), three quinolone antibiotics (nalidixic, ciprofloxacin and levofloxacin) and polymyxin B were tested. *E. coli* ATCC 25922 was used as the quality control strain. The results were interpreted according to the standards of the CLSI.

Whole-genome sequencing and bioinformatic analysis

Genomic DNA of *A. hydrophila* SS332 was extracted by using Bacterial Genomic DNA Miniprep kit (Generay, Shanghai, China) following the manufacturer's instructions. The whole-genome sequencing was performed with Illumina (HiSeq 2500, Illumina, United States) and Pacific (PacBio RS II/Pacific Biosciences/United States) platforms. Initially complete genome was assembled from PacBio long reads by Canu v1.7, and then corrected by pilon with the Illumina short reads. Potential open reading frames (ORFs) were predicted using Prodigal and annotated against the UniProt/Swiss-Prot and nonredundant protein databases using the BLASTX program. Annotation of ARGs and mobile genetic elements was performed using online databases including Comprehensive Antibiotic Resistance Database (CARD) (<https://card.mcmaster.ca/>) and ISfinder (<https://www-is.biotoul.fr/>), respectively. Similar sequences of pSS332-218k showing >50% query coverage were chosen for comparative genomic analysis in the nr/nt database with BLASTN. Amino acid alignment for AFM-1 and representative subclass B1 MBLs was performed using the program Clustal W (<https://www.genome.jp/tools-bin/clustalw>) and the final output was produced upon processing with the program ESPript 3.0 (<http://esprict.ibcp.fr/ESPript/cgi-bin/ESPript.cgi>). The maximum likelihood phylogenetic tree of AFM-1 was constructed by MEGA-X software with 1000 bootstrap replications. The molecular weight and pI value of AFM-1 was predicted using ProtParam2. The putative signal peptide cleavage site of *bla*_{AFM-1} was identified by SignalP 5.0. Comparisons of the nucleotide sequences were performed using BLASTN. Plasmid map was generated using GView. Other bioinformatics tools were written using Python and Biopython.

Cloning experiments

Six ARGs related to antibiotic resistance profiles of *A. hydrophila* SS332 were chosen for cloning, including *bla*_{AFM-1}, *bla*_{OXA-1}, *msr*(E), *mph*(E), *aac*(6')-Ib10, and *aph*(3')-Ia. The primers of the ARGs with the predicted promoter regions were designed then synthesized by TSINGKE biological technology (Beijing, China) (**Table S1**). For cloning, *Xba*I (Takara) and *Bam*HI (Takara) restriction endonuclease sites and their protective bases were incorporated into primers (**Table S1, underlined letters**). The ARG sequences were PCR amplified from *A. hydrophila* SS332 genomic DNA with the primers. The PCR products were digested with restriction endonucleases *Xba*I and *Bam*HI, and then ligated into expression vector (pUCP20 or pUCP24) with T₄ DNA ligase (Takara). The recombinant plasmids were transformed into *E. coli* DH5α and grown on Luria-Bertani agar plates supplemented with ampicillin (100 mg/L) or gentamicin (20 mg/L), then further verified by colony PCR and sequencing. The antimicrobial susceptibility testing for the transformants were performed using agar dilution method to verify the function of ARGs. Strain DH5α/pUCP20 or DH5α/pUCP24 were used as controls.

Results

Antibiotic resistance phenotypes and genome analysis of *A. hydrophila* SS332

Antimicrobial susceptibility results revealed that of 24 antibiotics detected, *A. hydrophila* SS332 showed high levels of resistance to 15 antibiotics, especially those with MIC levels up to or higher than 1024 µg/mL, including ampicillin, cefazolin, ceftriaxone, aztreonam, spectinomycin, roxithromycin. However, SS332 was susceptible to imipenem, meropenem, gentamicin, amikacin, chloramphenicol, florfenicol, levofloxacin, ciprofloxacin and polymyxin B (**Table 1**).

To better understand the molecular resistance mechanism of SS332, the complete genome sequence of this strain was determined. The genome of SS332 consists of a chromosome (GenBank accession number) and two circular plasmids designated pSS332-218k (No.XXX) and pSS332-5k (No.XXX). The chromosome comprises 4,792,137 bp with 60.77% G+C content (**Table 2**). It contains 4474 coding sequences (CDSs), of which 85.09% are protein coding genes with an average ORF length of 911 bp. A total of 122 tRNAs and 10 rRNA operons were predicted. The genome of pSS332-218k is 218,315 bp with 55.30% G+C content and a total of 243 CDSs were identified with an average length of 749 bp (**Table 2**).

In addition, a total of 5 known (100% identity) and 59 putative ARGs (40% identity) were predicted to be encoded over the whole genome, of which 52 ARGs (such as *bla*_{PER-3}, *mphA*, *bla*_{MOX-7}, *bla*_{OXA-427}, *aadA16*, and *mcr-7.1*, etc) were located in the chromosome (**Table S2**), 12 ARGs (including *bla*_{AFM-1}, *bla*_{OXA-1}, *msr(E)*, *mph(E)*, *aac(6')-Ib10*, and *aph(3')-Ia*) were encoded on the plasmid pSS332-218k (**Table S3**), and no ARGs were founded on plasmid pSS332-5k. Notably, *bla*_{AFM-1}, a *bla*_{NDM}-like gene (804 bp), displayed the highest identity (85%) with the function known resistance gene *bla*_{NDM-6}. This gene was first discovered in plasmid pAN70-1 of *Alcaligenes faecalis* strain AN70 (GenBank: MK757441.1) in NCBI database, but its functional and genetic characterization hasn't been studied so far.

ARGs in pSS332-218k reduced antimicrobial susceptibility

To clarify the role of pSS332-218k in the multiple antibiotic resistance phenotypes of SS332, 6 ARGs carried by pSS332-218k were successfully cloned, including *bla*_{AFM-1}, *bla*_{OXA-1}, *aac(6')-Ib10*, *aph(3')-Ia*, *msr(E)* and *mph(E)*. The MICs of antibiotics against the recombinant strains were shown in **Table 1**. DH5α/pUCP24-*bla*_{AFM-1} exhibited more than 8-fold increase in the MIC level to ampicillin (16 µg/mL), cefazolin (16 µg/mL), ceftazidime (8 µg/mL) compared with DH5α/pUCP24, suggesting that AFM-1 was functional in *E. coli* DH5α. Expression of OXA-1 in *E. coli* DH5α conferred increased MIC of ampicillin compared to the control strain. Expression of AAC(6')-Ib10 and APH(3')-Ia conferred resistance to kanamycin, of which MICs were 128 and 512 µg/mL, respectively. Meanwhile, *msr(E)* and *mph(E)* conferred resistant to erythromycin and roxithromycin. These transformants exhibited reduced susceptibility to several tested antibiotics, indicating that 6 ARGs on pSS332-218k could be involved in the antibiotic resistance phenotypes of SS332.

Comparative genomics analysis of the pSS332-218k

To further investigate the molecular characteristics of pSS332-218k, comparative genomic analysis was conducted. Only one sequence with >50% query coverage (75% coverage) with pSS332-218k sequence was searched in NCBI database by BLASTN program, which is a complete sequence of plasmid pMCR5-045096 (NZ_CP028567) of an *A. hydrophila* strain WCHAH045096. WCHAH045096 was isolated from sewage in China in 2015. Comparative genomic analysis showed pSS332-218k and pMCR5_045096 shared a conserved backbone. However, *msr(E)*, *mph(E)*, *bla*_{OXA-1}, and *bla*_{AFM-1} genes are missing in pMCR5_045096 (**Figure 1**). Comparative genomics analysis suggested that these ARGs might be acquired by horizontal gene transfer.

Phylogenetic Analysis

AFM-1, encoded by *bla*_{AFM-1}, was 267 amino acids in length and displayed the highest level of identity with NDM β-lactamases (84-86%) followed by ElBla2 (55.79%, ABC63608.1) and FIM-1 (42%, AFV91534.1). A phylogenetic tree was created using non-redundant MBLs of subclass B1. Phylogenetic analysis showed that the closest relatives of AFM-1 were the NDM-type enzymes (**Figure 2A**), and the obvious changes in the amino acid sequences suggested AFM-1 is a new variant of NDM family.

Sequence alignment analysis of AFM-1 and representative MBLs of subclass B1 showed that AFM-1 contains the conserved zinc-binding residues, namely, H117, H119, D121, H186, C205 and H247 (**black spots, Figure 2B**) [15]. The requirement for metal ions (usually Zn²⁺) is the key feature of catalytic activity of MBLs [16]. The D87 and D189 residues are essential for structure and stability of NDM-1 by forming a hydrogen bond around Zn2 and Zn1, respectively (**green spots, Figure 2B**) [17]. A type II signal peptide cleavage site (**yellow**

box, Figure 2B) was predicted between amino acid residues 22 and 23 and yielding a mature protein of 244 amino acids (theoretical mass, 25,991.23 g/mol; predicted pI, 5.7). AFM-1 has seven amino acid substitutions (V85L, A96S, D127G, A135P, S241N, M242T, and V244A) (**yellow box, Figure S1**) compared with NDM-1 in structurally conserved sequences fragments (**black box, Figure S1**) according to the sequence alignment of NDM-beta-lactamases (**Figure S1**) [15].

Genetic context of *bla*_{AFM-1}

Using BLASTN against the GenBank nr database with the *bla*_{AFM-1} of SS332 as a query, four sequences identical to *bla*_{AFM-1}, including pAN70-1(MK757441.1), plasmid pNFYY023-1 (MT011984.1), *Bordetella trematum* E202 chromosome (CP049957.1) and *Stenotrophomonas maltophilia* NCTC10498 chromosome (CP049956.1) were retrieved. In order to fully elucidate the genetic context of *bla*_{AFM-1}, comparative genomic analysis was performed on the *bla*_{AFM-1}-flanking regions of approximately 30 kb in the five *bla*_{AFM-1}-carrying sequences including the one of this work (**Figure 3A**).

The *bla*_{AFM-1} gene in pSS332-218k was located upstream of a Tn3-type transposon carrying *dfrA5* gene and downstream of an *aph(3')-Ia* gene surrounded by two copies of IS26. A conserved fragment (*bla*_{AFM-1}-*ble*_{MBL}-*trpF*-*ISCR19-2*-*msrB*-*msrA*-*yfcG*-*corA*) was found around the *bla*_{AFM-1}. Furthermore, the sequence between *bla*_{AFM-1} and *ISCR19-2* were identical in these five *bla*_{AFM-1}-carrying sequences, but the sequence between *msrB* and *corA* of NCTC10498 share only 97% identity to that of the other four. The *ble*_{MBL} gene encoded a bleomycin resistance protein and was widely found downstream of *bla*_{NDM} gene [18]. However, the *ble*_{MBL} gene beside *bla*_{AFM-1} was 89% identical to that beside *bla*_{NDM-1}. *MsrA/B* belong to the multidrug efflux pumps and play a positive role in the protection of the cellular proteins from oxidative stress damage [19]. Similar structure (*msrB*-*msrA*-*ygcG*-*corA*) has also been found around other β -lactamases, such as *bla*_{NDM-1} from *Pseudomonas asiatica* [20]. The conserved fragment containing *bla*_{AFM-1} may improve the adaptability of host bacteria to the environment and might aggravate its spread among different bacteria.

*bla*_{AFM-1} gene in pSS332 was associated with three novel different *ISCR19*-like elements or their truncated gene named *ISCR19-1*, *ISCR19-2* and Δ *ISCR19-3* in this study (**Figure 3B**). *ISCR19-1* and *ISCR19-2* contain the intact transposase gene but *ISCR19-3* was interrupted at the 5' end (within the transposase gene). The lengths of transposase of these three elements were 1542 bp, 1254 bp and 846 bp in length, respectively, and shared 91.0%, 94.0% and 89.1% nucleotide identity to that of *ISCR19*, respectively. The *oriS* site were identified 245 bp downstream of the stop codon of the transposase gene of *ISCR19-1*, *ISCR19-2* and *ISCR19-3*. These sequences were conserved in *ISCR19*-like element (Fig.3B). The *terS* site was identified 135 bp upstream of the beginning of the transposase gene of *ISCR19-1* but was absent around the corresponding regions of *ISCR19-2* and Δ *ISCR19-3*.

ISCR19-2 was found in all *bla*_{AFM-1}-carrying sequences, but *ISCR19-1* was not identified in pNFYY023-1 and Δ *ISCR19-3* was not found in NCTC10498. Interestingly, *ISCR19-1*, *ISCR19-2* and Δ *ISCR19-3* were associated only with the *bla*_{AFM-1} in Genbank. The result suggested that *ISCR19*-like elements were involved in the capture and mobilization of *bla*_{AFM-1} which were similar to other *ISCR*-related β -lactamases like *bla*_{SPM-1}, *bla*_{AIM-1} and *bla*_{FIM-1}, et al. According to the direction of these *ISCR19*-like elements, we infer that *ISCR19-1* plays a major role in the dissemination of the conserved fragments, while *ISCR19-2* was initially involved in the capture of *msrB*-*msrA*-*ygcG*-*corA* regions.

Discussion

In this study, a clinical isolate of *A. hydrophila* was highly resistant to most tested β -lactams, aminoglycosides, and macrolide antibiotics, including 3rd and 4th generation cephalosporins, as well as carbapenems (aztreonam), which are considered as the last-resort antibiotics for the treatment of multidrug-resistant (**Table 1**). According to the whole-genome sequencing, more than one resistance genes encoding extended-spectrum- β -lactamases are chromosome-mediated, including *bla*_{PER-3}, *bla*_{MOX-7}, and *bla*_{OXA-427}, which may be one reason for the high-level resistance of *A. hydrophila* SS332 to β -lactam antibiotics (**Table 1, S2**). In addition, *A. hydrophila* SS332 had ARGs from almost all the classes, and multidrug efflux genes are no exception (**Table S2**). A previous seven-year surveillance study demonstrated that *A. hydrophila* (65.6%, 745/1135) was the most predominant species from clinical *Aeromonas* spp. in Southwest China from 2011 to 2017 [21]. It also reported that *A. hydrophila* has a resistance rates of 30.2% to ceftriaxone, followed by 20% to ceftazidime, cefepime, aztreonam, and ciprofloxacin, and less than 10% to imipenem and meropenem. These findings suggest that drug resistance of *A. hydrophila* is an increasingly serious problem in clinical infection. However, the role of plasmids in the antibiotic resistance of *A. hydrophila* is poorly investigated. pSS332-218k, a multi-drug resistance plasmid carried by *A. hydrophila* SS332, encodes antibiotic resistance genes of *bla*_{AFM-1}, *bla*_{OXA-1}, *msr*(E), *mph*(E), *aac*(6')-Ib10, and *aph*(3')-Ia, which conferred resistance

to many tested antibiotics when expressed in *E. coli*, suggesting these genes are functional (**Table S3**). Another multi-drug resistance plasmid, pR148, was also obtained from *A. hydrophila* and encoded entirely different ARGs *qacH*, *bla*_{OXA-10}, *aadA1*, *sul1*, *tetA*, *tetR*, *catA2* [22]. Plasmid is one of mobile genetic elements that can transfer ARGs to the bacterial chromosome or gain ARGs through horizontal gene transfer. So, finding out the way of ARGs transmission may be helpful for deceleration the spread of ARGs.

A conserved genomic DNA fragment (*bla*_{AFM-1}-*ble*_{MBL}-*trpF*-*ISCR19-2-msrB-msrA-yfcG-corA*) containing *bla*_{AFM-1} was founded in all five different AFM-1-producing strain, two of which are located on chromosome and the other three are located on the plasmids (**Figure 3A**). These results indicated that the *bla*_{AFM-1} gene spread widely among different strains. Novel *ISCR19*-like elements were found in the near region of AFM-1 (**Figure 3B**), suggesting that AFM-1 might be transferred from one to another strain mediated by *ISCR* through rolling-circle transposition [23]. The acquisition and spread of ARGs is the predominant factor for the escalation of antibiotic resistance [24]. *ISCR* elements are peculiar mobile elements and can transpose adjacent DNA sequences with a single copy of the element [23]. The most worrying thing is that *ISCR* elements are increasingly associated with resistance genes encoding extended-spectrum β -lactamases, such as *bla*_{PER-3} and *bla*_{OXA-18} [25, 26].

NDM-1 has become a great public health concern for its high carbapenem resistance and global dissemination since it was first reported in 2009 [27]. Until now, 28 variants of NDM β -lactamase with 1~5 amino acid substitutions have been reported, except for NDM-18 where an insertion of five amino acids have been found [28]. Among the 28 NDM variants, substitutions of amino acids were identified at 20 different positions. Compared to other NDM variants, AFM-1 has more changes (42 amino acid substitutions) in amino acid residues and shares similarity of 86% with NDM-1 (**Figure 2, S1**). However, the critical position that plays a critical role in the enzymatic activity of NDM remained unclear. Further studies like site-directed mutagenesis are needed to clarify this issue. Nevertheless, AFM-1 possesses essential residue class which are indispensable for catalytic activity and substrate-specific residue class which are alternative for ampicillin, cefotaxime, or imipenem hydrolysis [17] (**Figure S2**). The functions of essential residues and substrate-specific residues for NDM-1 β -lactamase hydrolysis have been well proved [18]. As AFM-1 exhibited greatly reduced resistance to all β -lactam antibiotics, it is possible that the substituted residue(s) are responsible for attenuated β -lactam resistance activity of AFM-1 compare with NDM-1.

Conclusion

In this work, the complete genome sequence of the multi-drug resistant *A. hydrophila* SS332 was determined. Six ARGs (*bla*_{AFM-1}, *msr*(E), *mph*(E), *aac*(6')-*lb10*, and *aph*(3')-*la*) encoded by pSS332-218k were proved to contribute to resistance against ampicillin, cefazolin, ceftazidime, gentamicin, kanamycin, amikacin, erythromycin and roxithromycin. Most importantly, a novel *bla*_{NDM} variant, *bla*_{AFM-1}, was first cloned and AFM-1 confers resistance to ampicillin, cefazolin, ceftazidime and ceftazidime. However, AFM-1 exhibited a reduced substrate profile and increased MIC value which may be caused by amino acid substitutions in structurally conserved sequences fragments. Further analysis of genetic environment of *bla*_{AFM-1} suggested that the *bla*_{AFM-1} gene spread widely among different strains which was associated with novel *ISCR19*-like elements.

Abbreviations

ARGs: antimicrobial resistance genes; MIC: minimum inhibitory concentration; CLSI: Clinical and Laboratory Standards Institute; ORFs: open reading frames; CARD: Comprehensive Antibiotic Resistance Database.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XL and JWL performed the experiments, analyzed data, wrote the manuscript; CRQ, HLL, QLL and XYZ analyzed data and implemented the computer code and algorithms; HML, ZWS, DYZ and WL participated in the design of the study, helped to perform the experiments; MZ, HLZ and TX contributed to the visualization and data presentation of the work; KWL, QYB and LL conceived and designed experiments, revised the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Antibiotic susceptibility profiles of *A. hydrophila* SS332 and transformants

Strain	MIC (µg/mL)											
	AMP	CFZ	FEP	CTX	FOX	CAZ	CTRX	CSL	CMN	ATM	IMP	MEM
SS332	>1024	>1024	128	512	512	>1024	>1024	512	64	>1024	1	1
ATCC 25922	8	4	0.125	0.06	2	0.25	≤0.03	0.06	0.5	0.03	0.25	0.125
<i>E. coli</i> DH5α	2	1	0.06	0.06	2	0.125	≤0.03	0.06	0.25	0.125	0.25	0.125
DH5α/pUCP24	2	1	0.06	≤0.03	2	0.125	≤0.03	0.06	0.25	0.03	0.25	0.125
DH5α/pUCP24- <i>bla</i> _{AFM-1}	16	16	0.125	0.25	16	8	0.25	0.25	0.25	0.03	0.25	0.125
DH5α/pUCP24- <i>bla</i> _{OXA-1}	64	1	0.06	≤0.03	2	1	≤0.03	0.06	0.25	0.03	0.25	0.125
	SPT	GEN	KAN	AMK	ERY	ROX	CHL	FFC	NAL	LEV	CIP	PB
SS332	>1024	4	256	8	256	1024	8	2	256	4	≤0.25	4
ATCC 25922	8	0.5	4	2	64	64	4	4	8	2	≤0.25	≤1
<i>E. coli</i> DH5α	8	0.25	1	1	-	-	-	-	-	-	-	-
DH5α/pUCP20	8	0.25	1	1	64	256			-	-	-	-
DH5α/pUCP20- <i>aac</i> (6')- <i>lb</i> 7	8	0.5	128	16	-	-	-	-	-	-	-	-
DH5α/pUCP20- <i>aph</i> (3')- <i>la</i>	8	0.125	512	2	-	-	-	-	-	-	-	-
DH5α/pUCP20- <i>msr</i> (E)	-	-	-	-	256	512	-	-	-	-	-	-
DH5α/pUCP20- <i>mph</i> (E)	-	-	-	-	128	512	-	-	-	-	-	-

Notes: -, no detected.

Abbreviations: ampicillin, AMP; cefazolin, CFZ; cefepime, FEP; cefotaxime, CTX; ceftazidime, CAZ; ceftriaxone, CTRX; cefoselis, CSL; cefminox, CMN; aztreonam, ATM; imipenem, IMP; meropenem, MEM; spectinomycin, SPT; gentamicin, GEN; kanamycin, KAN; amikacin, AMK; erythromycin, ERY; roxithromycin, ROX; chloramphenicol, CHL; florfenicol, FFC; nalidixic, NAL; levofloxacin, LEV; ciprofloxacin, CIP; and polymyxin B, PB.

Table 2 General features of *Aeromonas hydrophila* SS332 genome

Characteristics	Chromosome	pSS332-218k	pSS332-5k
size (bp)	4,792,137	218,315	5,213
GC content (%)	60.77	55.30	55.02
CDSs	4474	243	6
hypothetical proteins	1717	176	3
protein coding (%)	85.09	83.41	73.21
average ORF length (bp)	911	749	646
tRNAs	122	-	-
rRNAs	10*(5S+16S+23S) 1*5S	-	-

Figures

Figure 1

The circular map of complete genome sequences of pSS332-218k and comparison with pMCR5-045096. Circles 1-5 (from the inside to outside) represent the information as follows: (1) GC content; (2) GC skew; (3) negative strand CDS; (4) positive stranded CDS, and (5) pMCR5-045096. Functional features of pSS332-218k are highlighted in different colors (mobile genetic elements are in red; antimicrobial resistance genes are in yellow; the other genes are in indigo blue; and hypothetical protein genes are in grey).

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Figure 2

Phylogenetic analysis and sequence alignment of AFM-1. (A) Phylogenetic analysis of the protein sequence using the maximum likelihood method. (B) Sequence alignment analysis of AFM-1 and representative MBLs of subclass B1. Residue numbers are positioned above the sequences according to the class B β -lactamase standard numbering scheme; Black spots indicate the conserved zinc-binding residues at H117, H119, D121, H186, C205 and H247. Green spots represent the essential residues at D88 and D189 for the stable expression of NDM β -lactamase.

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Figure 3

BLAST homology search results. (A) The homology of the whole nucleotide sequence of blaAFM-1 was generated against the nr/nt database using BLASTN. The GenBank accession numbers for the sequences are as follows: *Alcaligenes faecalis* AN70 plasmid pAN70-1, MK757441.1; *Comamonas testosteroni* pNFYY023-1, MT011984.1; *Bordetella trematum* E202, CP049957.1; *Stenotrophomonas maltophilia* NCTC10498, CP049956.1. (B) Three novel ISCR19-like elements, ISCR19-1, ISCR19-2 and Δ ISCR19-3.

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