Alcaligenes faecalis metallo-β-lactamase in extensively drug-resistant Pseudomonas aeruginosa isolates

Li, Yue; Zhu, Yiwei; Zhou, Wanqing; Chen, Zhongju; Moran, Robert A; Ke, Huanhuan; Feng, Yu; van Schaik, Willem; Shen, Han; Ji, Jingshu; Ruan, Zhi; Hua, Xiaoting; Yu, Yunsong

DOI:
10.1016/j.cmi.2021.11.012

Alcaligenes faecalis metallo-β-lactamase in extensively drug-resistant *Pseudomonas aeruginosa* isolates

Running Title: *Alcaligenes faecalis* metallo-β-lactamases

Yue Li[1,2,3#], Yiwei Zhu[1,2,3#], Wanqing Zhou[4#], Zhongju Chen[5#], Robert A. Moran[6], Huanhuan Ke[7], Yu Feng[7], Willem van Schaik[6], Han Shen[4], Jingshu Ji[2,3,8], Zhi Ruan[8], Xiaoting Hua[1,2,3*], Yunsong Yu[1,2,3*]

[1]Department of Infectious Diseases, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China

[2]Key Laboratory of Microbial Technology and Bioinformatics of Zhejiang Province, Hangzhou, China

[3]Regional Medical Center for National Institute of Respiratory Diseases, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China

[4]Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, China

[5]Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[6]Institute of Microbiology and Infection, University of Birmingham, Birmingham, UK

[7]Department of Biophysics, Zhejiang University School of Medicine, Hangzhou, China.

[8]Department of Clinical Laboratory, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China.

[#]These authors contributed equally to this work.

*Correspondence:

Xiaoting Hua

address: No. 3, East Qingchun Rd, Jianggan District, Hangzhou, China, 310016

email: xiaotinghua@zju.edu.cn

tel: +86-571-86006660

Yunsong Yu

address: No. 3, East Qingchun Rd, Jianggan District, Hangzhou, China, 310016

email: yvys119@zju.edu.cn

tel: +86-571-86006660

Abstract

Objectives: This study aimed to characterize *Alcaligenes faecalis* metallo-β-lactamases AFM-2 and AFM-3 from clinical *P. aeruginosa* isolates NDTH10366, NDTH9845 and WTJH17.

Methods: Clinical isolates were whole-genome sequenced using the Illumina and Oxford Nanopore platforms. Minimum inhibitory concentrations (MICs) of clinical isolates and transformants containing MBL genes were determined using broth microdilution methods. Kinetic parameters of purified AFM and NDM-1 were measured using a spectrophotometer. The AFM structure was modelled with SWISS-MODEL.

Results: NDTH10366 and NDTH9845 were extensively drug-resistant (XDR) isolates carrying $bla_{\text{AFM-2}}$ and multiple copies of $bla_{\text{KPC-2}}$, while WTJH17 was an XDR isolate carrying $bla_{\text{AFM-3}}$. The plasmid-borne $bla_{\text{AFM-2}}$ and $bla_{\text{AFM-3}}$ genes are associated with a novel IS*CR* element, IS*CR29*. AFM-2 and AFM-3, differing from AFM-1 by one amino acid substitution each, shared 86.2% and 86.6% amino acid sequence identity with NDM-1, respectively. Phylogenetic analysis confirmed the close relationship between AFM and NDM. Expression of AFM and NDM-1 under their native promoters in DH5α and PAO1 led to elevated MICs for all tested β-lactams except aztreonam. Comparable catalytic abilities were observed for AFM and NDM-1 when hydrolyzing nitrocefin, cefepime, imipenem and biapenem, while for other tested β-lactams AFM displayed weaker enzymatic activities. Modelling AFM structure revealed a characteristic αβ/βα fold with two zinc-binding active sites.

Conclusions: AFM from clinical *P. aeruginosa* isolates demonstrated β-lactamase activity comparable to NDM-1. Co-carriage of bla_{AFM} and bla_{KPC} renders clinical *P. aeruginosa* isolates non-susceptible to all antipseudomonal β-lactams. The association of bla_{AFM} genes with translocatable genetic elements and plasmids highlights their concerning potential for dissemination.

Keywords: *Pseudomonas aeruginosa*, bla_{AFM-2}, bla_{AFM-3}, extensively drug-resistant, metallo-β-lactamase

Introduction

Listed as a critical pathogen for which new antibiotics are urgently required [1], carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) has become a major global health challenge. Metallo-β-lactamases (MBLs) are by far the most common carbapenemases in CRPA, and can be further divided into subclasses B1a, B1b, B2 and B3 [2]. Among them, New Delhi metallo-β-lactamase (NDM) is of particular concern because it is often carried by mobile genetic elements that also carry resistance determinants for other antibiotic classes [3].

For much of the past decade, NDM was considered the only member of MBL subclass B1b. In 2018 another subclass B1b MBL was recognized in an *Alcaligenes faecalis* strain and named *Alcaligenes faecalis* metallo-β-lactamase-1 (AFM-1) (GenBank accession: MK143105). Subsequently, AFM-1 has been found in *Comamonas testosteroni*, *Stenotrophomonas maltophilia* and *Bordetella trematum* (GenBank

accessions: MT011984, CP049956, CP049957). However, the biochemical properties of the AFM enzyme have not been described in detail.

Besides MBLs, class A carbapenemases are increasingly being identified in CRPA, especially *Klebsiella pneumoniae* carbapenemase (KPC). The co-occurrence of KPC and MBL in *P. aeruginosa* can inactivate all β-lactams including monobactams, ceftazidime/avibactam and imipenem/relebactam, making clinical treatment extremely difficult.

Here, we report two extensively drug-resistant *P. aeruginosa* (XDR-PA) isolates carrying $bla_{\text{AFM-2}}$ and multiple copies of $bla_{\text{KPC-2}}$, and one XDR-PA isolate carrying $bla_{\text{AFM-3}}$. We characterized AFM-2 and AFM-3 by comparing their catalytic abilities and their impacts on antimicrobial susceptibility with AFM-1 and NDM-1.

Methods

Bacterial strains and whole genome sequencing

All bacterial strains and plasmids used are listed in Table S1. Three clinical *P. aeruginosa* isolates NDTH10366, NDTH9845 and WTJH17 (Table S2) were isolated and whole-genome sequenced. A hybrid assembly of Nanopore and Illumina sequencing reads was generated using Unicycler v0.4.8 (https://github.com/rrwick/Unicycler). Prokka v1.14.6 (https://github.com/tseemann/prokka) was used for annotation with *P. aeruginosa* PAO1 (GenBank accession: NC_002516) as the reference. Multi-locus sequence typing was performed using mlst v2.19.0 (https://github.com/tseemann/mlst). Antibiotic resistance genes were recognized by ABRicate v1.0.1

(https://github.com/tseemann/abricate) using the NCBI AMRFinderPlus database [4]. Insertion sequences were identified with ISfinder (https://www-is.biotoul.fr).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted using broth microdilution methods according to CLSI performance standards [5].

Plasmid transfer experiments

NDTH10366, NDTH9845 and WTJH17 were mated with a rifampin-resistant derivative of *P. aeruginosa* PAO1 as described previously [6]. Putative transconjugants were confirmed using PCR with primers listed in Table S3.

Sequence alignment and phylogenetic tree

All amino acid sequences were aligned with ClustalW [7]. The phylogenetic tree was generated using FastTree v2.1.10 and visualized using iTOL v6.1.1 [8]. Alignment of AFM-1/2/3 with NDM-1 was visualized using ESPript 3.0 [9].

Cloning of MBL genes

Plasmid pGK1900 was constructed to express MBL genes. Briefly, the region of plasmid pCasPA [10] containing *oriT*, *traJ* and plasmid pEX18Gm [11] containing GmR were amplified and recombined into the region of broad-host-range plasmid pACRISPR

[10] containing pRO1600 *oriV* and T7 promoter. Eventually, the pGK1900 plasmid was obtained.

The $bla_{\text{NDM-1}}$, $bla_{\text{AFM-2}}$, $bla_{\text{AFM-3}}$ genes were amplified from clinical isolates, while $bla_{\text{AFM-1}}$ was obtained from $bla_{\text{AFM-2}}$ by site-directed mutagenesis using PCR. Both full-length *bla* genes, and *bla* genes with their upstream promoter regions were amplified and cloned into pGK1900. The resulting plasmids were transformed into *Escherichia coli* DH5α and *P. aeruginosa* PAO1. Minimum inhibitory concentrations (MICs) of transformants to various antibiotics were then evaluated.

Expression and purification of MBLs

Because the mature AFM-1/2/3 proteins are identical, only the $bla_{\text{NDM-1}}$ and $bla_{\text{AFM-2}}$ genes, without their signal peptide sequences, were amplified and cloned into pET28a, introducing an N-terminal His_6-Tag. The constructed plasmids were transformed into *E. coli* BL21(DE3). Protein expression and purification were carried out as previously described with some modifications [12]. Briefly, IPTG was added to bacterial culture reaching an OD_{600} of 0.6-0.8, which was then shaken at 18℃ overnight. Cells were harvested and resuspended, then homogenized by a French press. The supernatant was collected and loaded onto a Ni-column, then the bound protein was eluted. The extract was loaded onto a Superdex 200 column for further purification. Protein purity (>95%) was confirmed by SDS-PAGE.

Measurement of enzyme kinetic parameters

Enzymatic activities were measured by a D8 UV-visible spectrophotometer (Runqee, Shanghai, China) in the assay buffer (10 mM HEPES, 200 mM NaCl, 20 μM $ZnCl_2$, pH 7.5) at room temperature. The enzyme kinetic parameters were calculated using the Michaelis-Menten Equation by GraphPad Prism v9.0.0.

Modelling of AFM

Structure homology-modelling of AFM was achieved using SWISS-MODEL [13]. NDM-1 (Protein Data Bank ID: 4EY2) was adopted as the modelling template. The resulting model was further analyzed and visualized with PyMOL v2.4.1.

Ethics statement

Approval was obtained from the Ethics Committee of Sir Run Run Shaw Hospital (approval/reference number: 20201118-49).

Results

Complete genome sequences of three XDR-PA isolates

Of the antibiotics tested, NDTH10366, NDTH9845 and WTJH17 were only susceptible to colistin. These isolates were resistant or exhibited reduced susceptibility to aminoglycosides and fluoroquinolones (Table S4), and were resistant to all tested β-lactams, even when combined with avibactam (Table 1). Thus, NDTH10366, NDTH9845

and WTJH17 were defined as XDR-PA according to the criteria of Magiorakos *et al.* [14].

The complete genomes of NDTH10366, NDTH9845 and WTJH17 consisted of a circular chromosome and plasmid each (Table S5). NDTH10366 and NDTH9845 belong to sequence type 463, while WTJH17 belongs to sequence type 260. The replication initiation genes of pNDTH10366 and pNDTH9845 are identical to that of pBT2101 (GenBank accession CP039991) while pWTJH17's differs by 5 SNPs, indicating that all three belong to the same globally-disseminated family of megaplasmids that has been associated with antibiotic resistance genes in *Pseudomonas* since at least the 1970s [15].

The acquired antibiotic resistance genes in all three isolates are listed in Table S5. Notably, NDTH10366 and NDTH9845 contain multiple copies of $bla_{\text{KPC-2}}$. In both, three copies of $bla_{\text{KPC-2}}$ were found in the chromosome, in segments flanked by directly-oriented copies of IS*26*. The segments are derived from the $bla_{\text{KPC-2}}$ context in *K. pneumoniae* plasmid pKP048 (GenBank accession: FJ628167), relatives of which are the predominant $bla_{\text{KPC-2}}$-harboring plasmids in China [16]. A further two copies of $bla_{\text{KPC-2}}$ are found in pNDTH10366. These are in adjacent 5,270 bp and 5,659 bp segments in a 13,389 bp region that contains three IS*26*. In addition, pNDTH10366 and pNDTH9845 contain $bla_{\text{OXA-246}}$, a $bla_{\text{OXA-10}}$-like non-extended-spectrum β-lactamase [17].

Genetic context of metallo-β-lactamase genes $bla_{\text{AFM-2}}$ and $bla_{\text{AFM-3}}$

Each of the plasmids in the XDR-PA isolates contained an open reading frame (ORF) that encodes a putative metallo-β-lactamase. The ORFs in NDTH10366 and NDTH9845 were identical, and the ORF in WTJH17 differed at two nucleotide positions.

The two different protein sequences were most similar to AFM-1 (GenBank accession: NG_063835), differing by just one amino acid each - A15V in the case of NDTH10366 and NDTH9845, and P13S in the case of WTJH17. These two genes were designated *bla*$_{\text{AFM-2}}$ and *bla*$_{\text{AFM-3}}$, respectively.

The *bla*$_{\text{AFM-2}}$ and *bla*$_{\text{AFM-3}}$ genes are found in 5,316 bp segments that differ only by the SNPs that distinguish the genes. The *bla*$_{\text{AFM}}$-containing segment is bounded on the right by a novel IS*CR* element that we have named IS*CR29* and on the left by a 644 bp fragment from the *ori* end of another IS*CR* element (Figure 1A). The 644 bp remnant is 97.8% identical to the corresponding part of IS*CR29*. The IS*CR*-flanked 3,005 bp sequence that includes *bla*$_{\text{AFM-2}}$ resembles the *bla*$_{\text{NDM-1}}$-containing passenger segment of Tn*125* (GenBank accession JN872329) [18] and contains a putative *ble*$_{\text{MBL}}$ bleomycin resistance gene (Figure 1A). The region also includes a Δ*trpF* gene downstream of *ble*$_{\text{MBL}}$ that is truncated by the ΔIS*CR* and a 312 bp Δ*groEL* upstream of *bla*$_{\text{AFM-2}}$ that is 83.7% identical to the 3' end of *groEL* of Tn*125*.

The *bla*$_{\text{AFM}}$-IS*CR29* unit of pNDTH10366 was compared to sequences containing *bla*$_{\text{AFM-1}}$ (Figure 1A). This revealed that *bla*$_{\text{AFM-1}}$ is also associated with IS*CR29*. In the *bla*$_{\text{AFM-1}}$-containing sequences, the 3,005 bp segment that includes *bla*$_{\text{AFM}}$ and *ble*$_{\text{MBL}}$ differed from the sequence in pNDTH10366 by just the SNP that distinguishes *bla*$_{\text{AFM-1}}$ from *bla*$_{\text{AFM-2}}$. In the *A. faecalis* plasmid pAN70-1, this region has been interrupted by a 10,768 bp insertion comprised of a putative mercury resistance transposon that contains copies of IS*26* and IS*6100*. The major difference between *bla*$_{\text{AFM-1}}$ and *bla*$_{\text{AFM-2}}$-bearing sequences was the presence of a ΔIS*CR* in *bla*$_{\text{AFM-1}}$-associated sequences that is just 96.3% identical to the ΔIS*CR* associated with *bla*$_{\text{AFM-2}}$ in pNDTH10366.

The IS*CR29-bla*$_{AFM}$ unit is associated with class 1 integrons in XDR-PA

The IS*CR29-bla*$_{AFM}$ unit in pNDTH10366 lies between two truncated class 1 integrons (Figure 1B). The integron to the left contains the cassette array *aacA7-aadB-cmlA1-aadA24* and a 3'-conserved segment (3'-CS; yellow in Figure 1B) that ends in an In4-like configuration that includes a copy of the inverted repeat IR_t and IS*6100* (Figure 1B). The integron to the right is in the same context as In28 [19] adjacent to a complete Tn*1403* transposition module, but contains the cassette array *aac(6')-IIa-cmlA8-bla*$_{OXA-246}$*-arr-3-dfrA27* (Figure 1B). The *intI1* gene of the In4-like integron and the 3'-CS of the Tn*1403*-derived integron have been truncated by the IS*CR29-bla*$_{AFM}$ unit.

The integrons flanking the IS*CR29-bla*$_{AFM}$ unit in pNDTH9845 and pWTJH17 have been modified by recombination and the actions of translocatable elements. In both, the region downstream of *sul1* in the In4-like class 1 integron has been replaced (Figure 1C). The new segment in pNDTH9845 is identical to part of the *Acinetobacter baumannii* chromosomal resistance island AbGRI3 [20] and contains the aminoglycoside resistance gene *armA* and macrolide resistance genes *msr*(E)-*mph*(E). The *aadA24* gene in this integron has been interrupted by insertion of IS*1394* in pNDTH9845, and the *aadB* gene cassette has been lost in pWTJH17 (Figure 1C).

The Tn*1403*-derived integrons in pNDTH10366 and pNDTH9845 are identical, but the one in pWTJH17 has been truncated by an IS*26* that is part of a pseudo-compound transposon containing *msr*(E)-*mph*(E) (Figure 1C). The *bla*$_{OXA-246}$ gene in the pWTJH17 cassette array has been interrupted by insertion of IS*Pre2*.

Transfer of *bla*$_{AFM}$-bearing plasmids

As pBT2101-like plasmids have been reported to be conjugative [15], the transferability of pNDTH10366, pNDTH9845 and pWTJH17 was tested. Transconjugants were only obtained from WTJH17-PAO1 mating experiments, suggesting that only pWTJH17 is conjugative (Figure S1).

Effects of AFM and NDM on antibiotic susceptibility

Alignment revealed that AFM-2 and AFM-3 share 86.2% and 86.6% amino acid sequence identity with NDM-1, respectively. Major differences were found in the signal peptide region (Figure 2). Phylogenetic analysis of AFM and other MBLs revealed that AFM-2 and AFM-3 are subclass B1b MBLs most closely related to the NDM subfamily (Figure 3).

To compare the drug resistance conferred by MBL genes *bla*$_{AFM-1/2/3}$ and *bla*$_{NDM-1}$, they were cloned into pGK1900 and transformed into *E. coli* DH5α and *P. aeruginosa* PAO1. The MICs of various antibiotics against transformants were assessed (Table 1). When expressing MBLs from the T7 promoter, all transformants showed no or low-level resistance to β-lactams and β-lactam + inhibitor combination agents. A significant increase in MICs was observed when MBLs were expressed from native promoters. Interestingly, in *E. coli* DH5α, resistance levels conferred by *bla*$_{AFM-1/2/3}$ under their native promoters were much lower than that of *bla*$_{NDM-1}$. In contrast, *P. aeruginosa* PAO1 transformants carrying *bla*$_{AFM-1/2/3}$ with their native promoters exhibited much higher MICs than *bla*$_{NDM-1}$ to all tested β-lactams except aztreonam.

Comparison of AFM and NDM kinetic parameters

Hydrolyzing abilities of AFM and NDM-1 were tested for aztreonam and other β-lactams (Table 2). As predicted, both AFM and NDM-1 had no catalytic effect on aztreonam. For all tested β-lactams except nitrocefin and ertapenem, AFM exhibited higher affinities but lower turnover numbers than NDM-1, which was indicated by K_M and k_{cat} values. Similar catalytic efficiencies between AFM and NDM-1 were observed for nitrocefin, cefepime, imipenem and biapenem, as indicated by k_{cat}/K_M values. However, compared with NDM-1, AFM displayed lower catalytic efficiencies for penicillin G, ampicillin, carbenicillin, cefuroxime, cefotaxime, ceftazidime, meropenem, ertapenem.

Structural modelling of AFM

The structure of AFM was modelled using NDM-1 as the reference homology template. The overall fold of AFM was predicted to be the classic αβ/βα sandwich, which is common to all MBLs (Figure 4). Two zinc ions bound in the active sites of AFM, with Zn1 interacted with histidine residues 117, 119 and 186 at coordination distances of 2.07Å, 1.88 Å, 2.04 Å, Zn2 interacted with residues Asp121, Cys205 and His247 at coordination distances of 2.05 Å, 2.42 Å, 1.92 Å (Figure 4).

Discussion

Clinical *P. aeruginosa* isolates NDTH10366, NDTH9845 and WTJH17 are XDR and sensitive only to colistin. Our analysis of $bla_{AFM-2/3}$ from clinical isolates and bla_{AFM-}

$_1$-bearing sequences in GenBank allowed us to characterize the contexts of AFM determinants and assess their potential for horizontal dissemination. These genes are associated with a novel IS*CR* element, IS*CR29*, which is likely responsible for their mobility. IS*CR* elements have been responsible for the dissemination of multiple resistance determinants, including NDM gene variants [21]. Concerningly, the *bla*$_{\text{AFM}}$ genes in pNDTH10366, pNDTH9845 and pWTJH17 are already found in close proximity to multiple copies of IS*26*, which is arguably the most important translocatable element associated with antibiotic resistance genes [22]. It appears that there is significant potential for IS*26* to capture *bla*$_{\text{AFM-2}}$ from its current context and contribute to its wider dissemination. This appears to have occurred in the case of *bla*$_{\text{NDM-1}}$ [23] which, like the *bla*$_{\text{AFM}}$ genes, is thought to have originally been mobilized by an IS*CR* element [24].

pNDTH10366, pNDTH9845 and pWTJH17 are all members of the pBT2101-like megaplasmid family [15]. Plasmids in this family can carry diverse antimicrobial resistance genes and are widely distributed, both geographically and amongst *Pseudomonas* hosts in which they are stable and do not impose a measurable fitness cost [15]. The association of *bla*$_{\text{AFM}}$ genes with pBT2101-like plasmids in *P. aeruginosa* may potentiate their rapid spread within the genus, which warrants continuous surveillance.

To the best of our knowledge, this is the first report of an MBL-producing *P. aeruginosa* co-harboring five copies of the *bla*$_{\text{KPC-2}}$ gene. Co-expression of *bla*$_{\text{KPC-2}}$ and *bla*$_{\text{AFM-2}}$ in NDTH10366 and NDTH9845 conferred high-level and broad-range resistance to all antipseudomonal β-lactams, including aztreonam/avibactam. This could be explained by two factors. First, high-level expression of KPC-2 could not be totally inhibited by avibactam due to its high copy number, as previously reported [25]. Second,

mechanisms other than β-lactamases may be involved in aztreonam resistance, such as the upregulation of efflux pump systems [26].

From our evaluation, the ability of AFM to hydrolyze some antibiotics was slightly weaker than NDM-1. This could be explained by certain amino acid differences within AFM and NDM-1. For example, residues Leu65 and Met67 in NDM-1 are changed to Met and Val in AFM, respectively. Residues Leu65 and Met67 of NDM-1 have been reported to form strong hydrophobic interactions with the phenyl ring of penicillin or ampicillin [27]. These substitutions could lead to the alteration of loop conformation and hydrophobicity, thus indirectly influencing the enzymatic activities of AFM.

In conclusion, we have described the genetic contexts of bla_{AFM} MBL genes, and demonstrated that their products exhibit β-lactamase activity comparable to that of NDM-1. The association of bla_{AFM} genes with translocatable genetic elements and plasmids highlights their potential for dissemination. That $bla_{\text{AFM-2}}$ and $bla_{\text{AFM-3}}$ have been found in organisms as clinically relevant as XDR-PA is of great concern. Genomic surveillance will be required to track the spread of AFM determinants in bacterial populations globally.

Conflicts of interests

The authors declare that they have no conflict of interest.

Funding

This study is supported by the National Natural Science Foundation of China (grant no. 81830069). R.M was supported by the Medical Research Council-National Natural Science Foundation of China DETECTIVE project (MR/S013660/1).

Acknowledgements

We are very grateful to Dr. Quanjiang Ji (ShanghaiTech University, Shanghai, China) for providing plasmids pCasPA and pACRISPR.

Access to data

The nucleotide sequences reported in this study have been submitted to the EMBL/GenBank databases under accession numbers CP064401 (NDTH10366 chromosome), CP064402 (NDTH10366 plasmid), CP073080 (NDTH9845 chromosome), CP073081 (NDTH9845 plasmid), CP073082 (WTJH17 chromosome) and CP073083 (WTJH17 plasmid), respectively.

Author's contributions

X.H and Y.Y conceived, designed, and coordinated this study. W.Z, Z.C, and H.S collected the isolates from respective hospitals. Y.L and Y.Z constructed the plasmids and performed the antimicrobial susceptibility tests. Y.Z, R.M, J.J, and X.H analyzed the

genome sequencing data. Y.L, H.K, and Y.F purified the proteins, measured their kinetic parameters, and analyzed the modelled protein structure. Y.L and Y.Z wrote the initial version of the manuscript. R.M, W.S, Z.R, X.H, and Y.Y revised the manuscript.

Table 1. MICs for *Pseudomonas aeruginosa* clinical isolates carrying bla_{AFM} and transformants containing different MBLs

Strains	Plasmid [a]	MICs (mg/L) determined by broth microdilution methods										
		AMP	PIP	FEP	CAZ	PTZ	CZA	ETP	IPM	MEM	AZT	AZA
NDTH10366	pNDTH10366	/	>1024	>1024	>1024	>256/4	>1024/4	/	1024	>1024	>1024	256/4
NDTH9845	pNDTH9845	/	1024	>1024	>1024	>256/4	>1024/4	/	1024	>1024	1024	32/4
WTJH17	pWTJH17	/	512	>1024	>1024	>256/4	>1024/4	/	1024	>1024	64	32/4
DH5α	pGK1900	2	1	0.03	0.03	1/4	0.06/4	0.008	0.12	0.016	0.06	/
DH5α	pGK1900_NDM-1	4	2	0.03	0.12	2/4	0.12/4	0.008	0.06	0.03	0.25	/
DH5α	pGK1900_NDM-1 P+	>1024	512	128	>1024	256/4	>1024/4	32	16	32	0.06	/
DH5α	pGK1900_AFM-1	4	2	0.03	4	2/4	2/4	0.008	0.12	0.03	0.12	/
DH5α	pGK1900_AFM-1 P+	256	16	2	256	16/4	256/4	0.25	2	0.5	0.06	/
DH5α	pGK1900_AFM-2	2	1	0.12	2	2/4	1/4	0.008	0.06	0.03	0.06	/
DH5α	pGK1900_AFM-2 P+	512	32	8	512	32/4	512/4	0.25	2	1	0.06	/
DH5α	pGK1900_AFM-3	8	2	0.06	4	1/4	2/4	0.008	0.25	0.03	0.12	/
DH5α	pGK1900_AFM-3 P+	512	32	4	256	32/4	512/4	0.5	2	0.5	0.06	/
PAO1	pGK1900	/	4	2	2	8/4	2/4	/	1	1	4	/
PAO1	pGK1900_NDM-1	/	4	4	8	8/4	8/4	/	1	1	4	/
PAO1	pGK1900_NDM-1 P+	/	64	512	>1024	128/4	>1024/4	/	256	128	4	/
PAO1	pGK1900_AFM-1	/	32	16	64	8/4	64/4	/	1	2	4	/
PAO1	pGK1900_AFM-1 P+	/	1024	>1024	>1024	>256/4	>1024/4	/	1024	256	4	/
PAO1	pGK1900_AFM-2	/	8	8	64	16/4	64/4	/	1	2	4	/

PAO1	pGK1900_AFM-2 P+	/	1024	>1024	>1024	>256/4	>1024/4	/	1024	256	4	/
PAO1	pGK1900_AFM-3	/	4	16	128	4/4	128/4	/	2	2	4	/
PAO1	pGK1900_AFM-3 P+	/	1024	>1024	>1024	>256/4	>1024/4	/	1024	256	4	/

AMP, ampicillin; PIP, piperacillin; FEP, cefepime; CAZ, ceftazidime; PTZ, piperacillin/tazobactam; CZA, ceftazidime/avibactam; ETP, ertapenem; IPM, imipenem; MEM, meropenem; AZT, aztreonam; AZA, aztreonam/avibactam.

[a] P+ indicates MBLs were expressed from their native promoters.

Table 2. Kinetic parameters of AFM and NDM-1

	AFM			NDM-1		
	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} μM^{-1})	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} μM^{-1})
PG	19.25±1.06	18.05±0.08	0.94	54.72±9.30	63.05±4.63	1.17
AMP	21.55±2.58	23.43±0.82	1.10	114.90±6.01	228.18±2.36	1.99
CB	75.81±4.63	19.88±0.54	0.26	149.73±14.14	54.91±3.52	0.37
NCF	3.67±0.57	7.05±0.36	1.95	2.22±0.44	3.97±0.44	1.82
CXM	2.11±0.11	9.40±0.19	4.46	6.54±0.34	46.72±0.91	7.16
CTX	5.45±0.04	9.54±0.07	1.75	14.76±0.14	62.69±0.99	4.25
CAZ	44.74±1.14	20.42±0.14	0.46	79.90±3.05	88.69±2.09	1.11
FEP	28.53±0.49	5.54±0.02	0.19	135.67±5.60	26.58±0.82	0.20
IPM	51.11±3.55	30.47±1.84	0.60	117.86±24.04	70.35±12.28	0.60
MEM	8.88±0.55	3.42±0.11	0.39	27.64±1.09	23.89±0.65	0.86
ETP	13.20±1.50	2.13±0.08	0.16	10.06±0.72	8.67±0.36	0.86
BPM	42.34±1.60	7.60±0.20	0.18	499.70±44.19	59.40±5.30	0.12
AZT	ND	ND	ND	ND	ND	ND

K_M and k_{cat} values are shown as means ± standard deviation from three independent experiments.

ND, not detectable.

PG, penicillin G; AMP, ampicillin; CB, carbenicillin; NCF, nitrocefin; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; ETP, ertapenem; BPM, biapenem; AZT, aztreonam.

References

[1] E. Tacconelli, E. Carrara, A. Savoldi, S. Harbarth, M. Mendelson, D.L. Monnet, et al., Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis, The Lancet. Infectious diseases 18(3) (2018) 318-27.

[2] K. Bush, The ABCD's of β-lactamase nomenclature, Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy 19(4) (2013) 549-59.

[3] D.M.P. De Oliveira, B.M. Forde, T.J. Kidd, P.N.A. Harris, M.A. Schembri, S.A. Beatson, et al., Antimicrobial Resistance in ESKAPE Pathogens, Clinical microbiology reviews 33(3) (2020).

[4] M. Feldgarden, V. Brover, D.H. Haft, A.B. Prasad, D.J. Slotta, I. Tolstoy, et al., Validating the AMRFinder Tool and Resistance Gene Database by Using Antimicrobial Resistance Genotype-Phenotype Correlations in a Collection of Isolates, Antimicrobial agents and chemotherapy 63(11) (2019).

[5] CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

[6] H. Cai, Y. Zhu, D. Hu, Y. Li, S. Leptihn, B. Loh, et al., Co-harboring of Novel bla (KPC-2) Plasmid and Integrative and Conjugative Element Carrying Tn6203 in Multidrug-Resistant Pseudomonas aeruginosa, Frontiers in microbiology 12 (2021) 674974.

361 [7] M.A. Larkin, G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H.
362 McWilliam, et al., Clustal W and Clustal X version 2.0, Bioinformatics (Oxford,
363 England) 23(21) (2007) 2947-8.
364 [8] I. Letunic, P. Bork, Interactive Tree Of Life (iTOL) v4: recent updates and new
365 developments, Nucleic acids research 47(W1) (2019) W256-W59.
366 [9] X. Robert, P. Gouet, Deciphering key features in protein structures with the new
367 ENDscript server, Nucleic acids research 42(Web Server issue) (2014) W320-4.
368 [10] W. Chen, Y. Zhang, Y. Zhang, Y. Pi, T. Gu, L. Song, et al., CRISPR/Cas9-based
369 Genome Editing in Pseudomonas aeruginosa and Cytidine Deaminase-Mediated Base
370 Editing in Pseudomonas Species, iScience 6 (2018) 222-31.
371 [11] T.T. Hoang, R.R. Karkhoff-Schweizer, A.J. Kutchma, H.P. Schweizer, A broad-
372 host-range Flp-FRT recombination system for site-specific excision of chromosomally-
373 located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa
374 mutants, Gene 212(1) (1998) 77-86.
375 [12] Z. Liang, L. Li, Y. Wang, L. Chen, X. Kong, Y. Hong, et al., Molecular basis of
376 NDM-1, a new antibiotic resistance determinant, PloS one 6(8) (2011) e23606.
377 [13] A. Waterhouse, M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny, et al.,
378 SWISS-MODEL: homology modelling of protein structures and complexes, Nucleic
379 acids research 46(W1) (2018) W296-W303.
380 [14] A.P. Magiorakos, A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske,
381 et al., Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an
382 international expert proposal for interim standard definitions for acquired resistance,

Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 18(3) (2012) 268-81.

[15] A. Cazares, M.P. Moore, J.P.J. Hall, L.L. Wright, M. Grimes, J.G. Emond-Rhéault, et al., A megaplasmid family driving dissemination of multidrug resistance in Pseudomonas, Nat Commun 11(1) (2020) 1370.

[16] K.D. Chavda, L. Chen, M.R. Jacobs, R.A. Bonomo, B.N. Kreiswirth, Molecular Diversity and Plasmid Analysis of KPC-Producing Escherichia coli, Antimicrobial agents and chemotherapy 60(7) (2016) 4073-81.

[17] Y. Qing, K.Y. Cao, Z.L. Fang, Y.M. Huang, X.F. Zhang, G.B. Tian, et al., Outbreak of PER-1 and diversity of β-lactamases among ceftazidime-resistant Pseudomonas aeruginosa clinical isolates, Journal of medical microbiology 63(Pt 3) (2014) 386-92.

[18] S. Bontron, P. Nordmann, L. Poirel, Transposition of Tn125 Encoding the NDM-1 Carbapenemase in Acinetobacter baumannii, Antimicrobial agents and chemotherapy 60(12) (2016) 7245-51.

[19] H.W. Stokes, L.D. Elbourne, R.M. Hall, Tn1403, a multiple-antibiotic resistance transposon made up of three distinct transposons, Antimicrobial agents and chemotherapy 51(5) (2007) 1827-9.

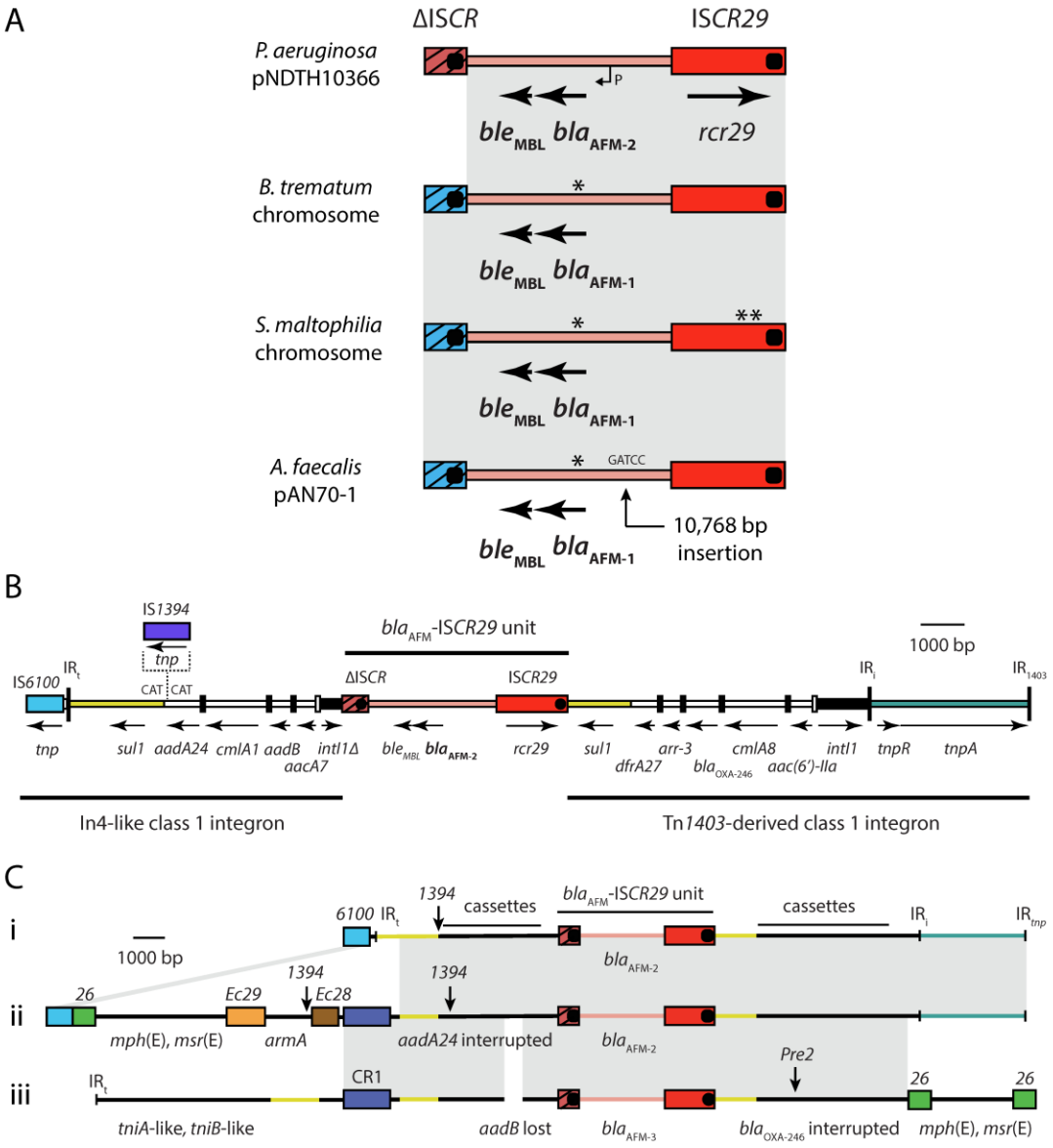
[20] G.A. Blackwell, K.E. Holt, S.D. Bentley, L.Y. Hsu, R.M. Hall, Variants of AbGRI3 carrying the armA gene in extensively antibiotic-resistant Acinetobacter baumannii from Singapore, The Journal of antimicrobial chemotherapy 72(4) (2017) 1031-39.

[21] A.M. Wailan, H.E. Sidjabat, W.K. Yam, N.F. Alikhan, N.K. Petty, A.L. Sartor, et al., Mechanisms Involved in Acquisition of blaNDM Genes by IncA/C2 and IncFIIY Plasmids, Antimicrobial agents and chemotherapy 60(7) (2016) 4082-8.

406 [22] C.J. Harmer, C.H. Pong, R.M. Hall, Structures bounded by directly-oriented
407 members of the IS26 family are pseudo-compound transposons, Plasmid 111 (2020)
408 102530.
409 [23] R.E. Weber, M. Pietsch, A. Frühauf, Y. Pfeifer, M. Martin, D. Luft, et al., IS26-
410 Mediated Transfer of bla (NDM-1) as the Main Route of Resistance Transmission During
411 a Polyclonal, Multispecies Outbreak in a German Hospital, Frontiers in microbiology 10
412 (2019) 2817.
413 [24] S.R. Partridge, J.R. Iredell, Genetic contexts of blaNDM-1, Antimicrobial agents and
414 chemotherapy 56(11) (2012) 6065-7; author reply 71.
415 [25] P. Gaibani, M.C. Re, C. Campoli, P.L. Viale, S. Ambretti, Bloodstream infection
416 caused by KPC-producing Klebsiella pneumoniae resistant to ceftazidime/avibactam:
417 epidemiology and genomic characterization, Clinical microbiology and infection : the
418 official publication of the European Society of Clinical Microbiology and Infectious
419 Diseases 26(4) (2020) 516 e1-16 e4.
420 [26] N. Masuda, E. Sakagawa, S. Ohya, N. Gotoh, H. Tsujimoto, T. Nishino, Substrate
421 specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in
422 Pseudomonas aeruginosa, Antimicrobial agents and chemotherapy 44(12) (2000) 3322-7.
423 [27] H. Zhang, Q. Hao, Crystal structure of NDM-1 reveals a common β-lactam
424 hydrolysis mechanism, FASEB journal : official publication of the Federation of
425 American Societies for Experimental Biology 25(8) (2011) 2574-82.
426
427

Table 1. MICs for *Pseudomonas aeruginosa* clinical isolates carrying bla_{AFM} and transformants containing different MBLs

Table 2. Kinetic parameters of AFM and NDM-1



433

434 **Figure 1. Contexts of *bla*$_{AFM}$ metallo-β-lactamase genes.** A) Comparison of *bla*$_{AFM}$-

435 IS*CR29* units associated with *bla*$_{AFM-1}$ and *bla*$_{AFM-2}$. Sequences are shown as coloured

436 boxes, with IS*CR* elements labelled and grey shading linking sequences that are

437 essentially identical. Black circles in IS*CR* elements represent the location of *ori*IS. The

438 location of a putative promoter for *bla*$_{AFM}$ is indicated by an arrow labelled 'P'. Asterisks

439 show the locations of single nucleotide polymorphisms and the position of a transposon is

marked by a labelled arrow. The source of each structure is indicated to the left. Drawn to scale from GenBank accessions CP064402, CP049957, CP049956 and MK757441. B) Context of the *bla*$_{\text{AFM-2}}$ gene in pNDTH10366. DNA sequence is shown as coloured boxes with labelled arrows below indicating the location and orientation of genes. IS and IS*CR* elements are labelled above. Sequences derived from In4-like and Tn*1403*-derived integrons are marked by labelled horizontal lines below. Small rectangles within integron regions show the positions of *attI* (open rectangles) and *attC* (filled rectangles) sites, respectively. Inverted repeats are shown as labelled vertical lines. Drawn to scale from GenBank accession CP064402. C) Comparison of IS*CR29*-*bla*$_{\text{AFM}}$ unit contexts in pNDTH10366 (i), pNDTH9845 (ii) and pWTJH17 (iii). Genetic elements are drawn and coloured as in panel (B). Grey shading links identical sequences. The identities of IS shown as coloured boxes are labelled above, and the insertion positions of IS removed to generate this comparison are shown as labelled vertical arrows. Additional, interrupted or lost antibiotic resistance genes are labelled. Drawn to scale from GenBank accessions CP064402, CP073081 and CP073083.

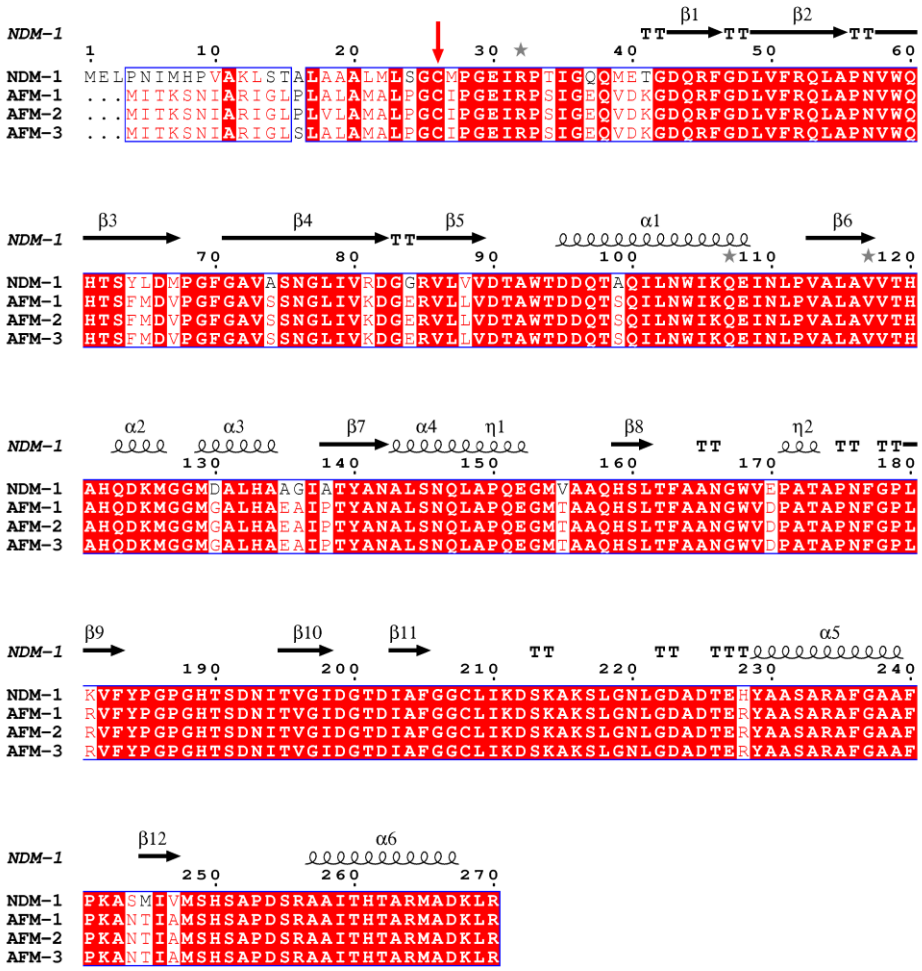
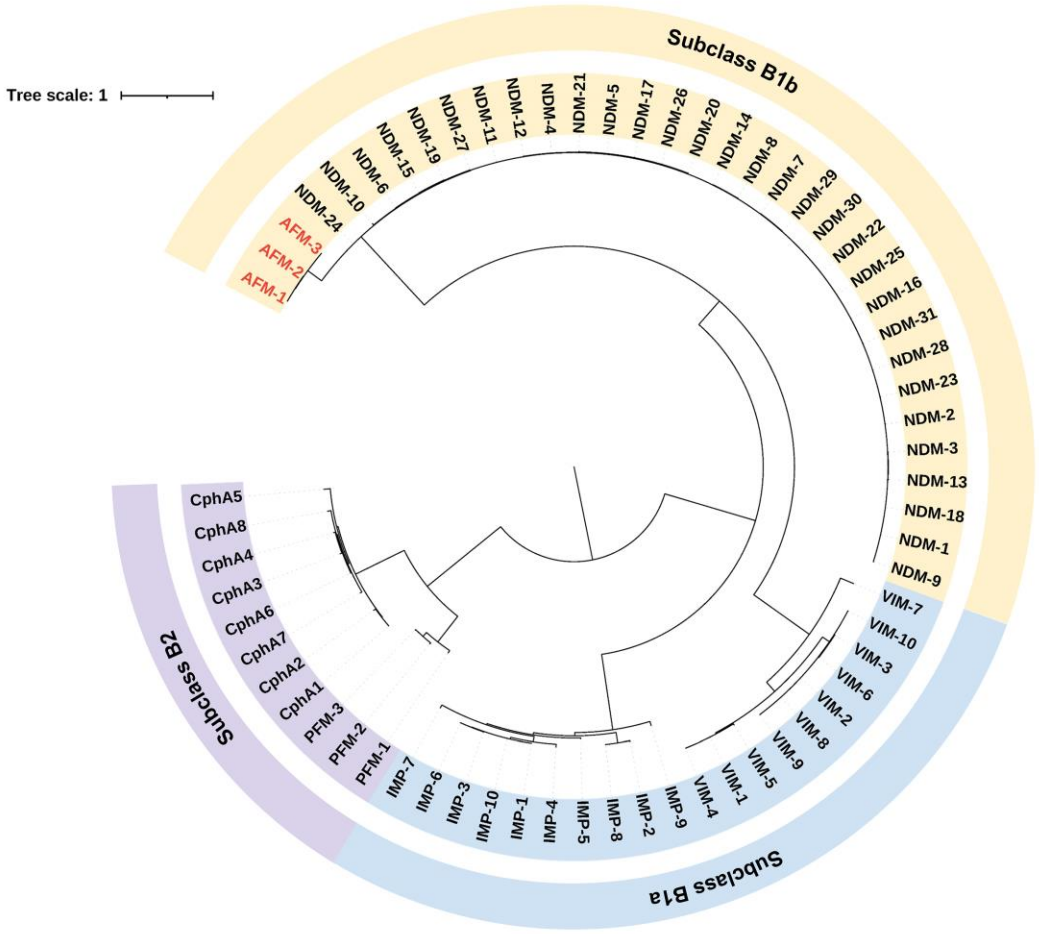


Figure 2. Amino acid sequence alignment of AFM-1, AFM-2, AFM-3 with NDM-1. Sequence alignment was generated by ClustalW and ESPript 3.0. The secondary structure of NDM-1 was depicted above the sequence. Residues with alternate conformations are indicated by grey stars. The red arrow represents the head amino acid positions of the mature proteins.



464

465

466 **Figure 3. Phylogenetic relationships between AFM and other MBLs.** The tree was

467 generated by FastTree v2.1.10 and visualized by iTOL v6.1.1 using midpoint rooting

468 method. Labels of the phylogenetic tree are highlighted in different colours based on

469 MBL subclasses. AFM-1, AFM-2 and AFM-3 are marked in red. Amino acid sequences

470 were obtained from NCBI Bacterial Antimicrobial Resistance Reference Gene Database.

471

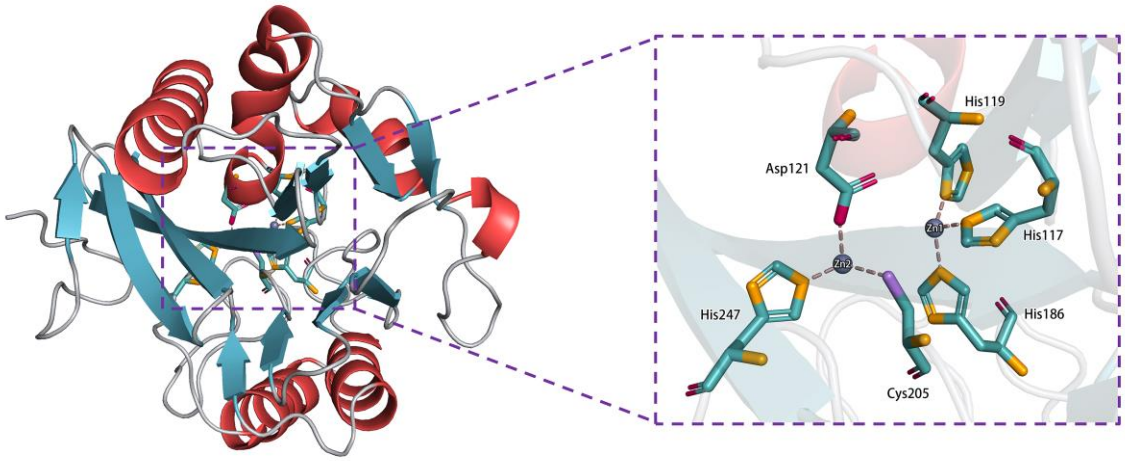


Figure 4. The overall modelling structure of AFM and its active sites bounded with zinc ions. AFM was made up of four layers, α/β/β/α. Two zinc ions interacted with residues His117, His119, His186 and residues Asp121, Cys205, His247 in the active sites, respectively. The metal-binding residues within the active sites are displayed as sticks. The zinc ions are coloured in grey. Interactions are shown as dashes.

Supporting Information

Alcaligenes faecalis metallo-β-lactamase in extensively drug-resistant *Pseudomonas aeruginosa* isolates

Running Title: *Alcaligenes faecalis* metallo-β-lactamases

Yue Li[1,2,3#], Yiwei Zhu[1,2,3#], Wanqing Zhou[4#], Zhongju Chen[5#], Robert A. Moran[6], Huanhuan Ke[7], Yu Feng[7], Willem van Schaik[6], Han Shen[4], Jingshu Ji[2,3,8], Zhi Ruan[8], Xiaoting Hua[1,2,3*], Yunsong Yu[1,2,3*]

[1]Department of Infectious Diseases, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China

[2]Key Laboratory of Microbial Technology and Bioinformatics of Zhejiang Province, Hangzhou, China

[3]Regional Medical Center for National Institute of Respiratory Diseases, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China

[4]Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, China

[5]Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[6]Institute of Microbiology and Infection, University of Birmingham, Birmingham, UK

[7]Department of Biophysics, Zhejiang University School of Medicine, Hangzhou, China.

[8]Department of Clinical Laboratory, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China.

[#]These authors contributed equally to this work.

*Correspondence:

Xiaoting Hua

address: No. 3, East Qingchun Rd, Jianggan District, Hangzhou, China, 310016

email: xiaotinghua@zju.edu.cn

tel: +86-571-86006660

Yunsong Yu

address: No. 3, East Qingchun Rd, Jianggan District, Hangzhou, China, 310016

email: yvys119@zju.edu.cn

tel: +86-571-86006660

Table S1. Bacterial strains and plasmids used in this study

Name	Description	Source/Reference
Strains		
Escherichia coli		
DH5α	Strain for general cloning and plasmid maintenance	TaKaRa
BL21(DE3)	Strain for protein expression	Biomed
ATCC 25922	Quality control strain for antimicrobial susceptibility testing	Lab stock
Klebsiella pneumoniae		
ATCC 700603	Quality control strain for antimicrobial susceptibility testing	Lab stock
Pseudomonas aeruginosa		
PAO1	Standard laboratory *P. aeruginosa* strain	Lab Stock
ATCC 27853	Quality control strain for antimicrobial susceptibility testing	Lab stock
NDTH10366	Clinical *P. aeruginosa* isolate carrying $bla_{\text{AFM-2}}$ and five $bla_{\text{KPC-2}}$ genes	This study
NDTH9845	Clinical *P. aeruginosa* isolate carrying $bla_{\text{AFM-2}}$ and three $bla_{\text{KPC-2}}$ genes	This study
WTJH17	Clinical *P. aeruginosa* isolate carrying $bla_{\text{AFM-3}}$	This study
Plasmids		
pNDTH10366	Plasmid of NDTH10366	This study
pNDTH9845	Plasmid of NDTH9845	This study
pWTJH17	Plasmid of WTJH17	This study
pACRISPR	Guide RNA expression vector containing pRO1600 *oriV* and T7 promoter	[1]

pCasPA	Cas9 nuclease expression vector containing *oriT*, *traJ*	[1]
pEX18Gm	Suicide vector; Gm^R	[2]
pGK1900	Broad-host-range cloning vector with T7 promoter; Gm^R	This study
pGK1900_AFM-1	pGK1900 carrying bla_{AFM-1} inserted downstream of T7 promoter	This study
pGK1900_AFM-1 P+	pGK1900 carrying bla_{AFM-1} and its upstream promoter regions inserted downstream of T7 promoter	This study
pGK1900_AFM-2	pGK1900 carrying bla_{AFM-2} inserted downstream of T7 promoter	This study
pGK1900_AFM-2 P+	pGK1900 carrying bla_{AFM-2} and its upstream promoter regions inserted downstream of T7 promoter	This study
pGK1900_AFM-3	pGK1900 carrying bla_{AFM-3} inserted downstream of T7 promoter	This study
pGK1900_AFM-3 P+	pGK1900 carrying bla_{AFM-3} and its upstream promoter regions inserted downstream of T7 promoter	This study
pGK1900_NDM-1	pGK1900 carrying bla_{NDM-1} inserted downstream of T7 promoter	This study
pGK1900_NDM-1 P+	pGK1900 carrying bla_{NDM-1} and its upstream promoter regions inserted downstream of T7 promoter	This study
pET28a	Bacterial vector for protein expression; Km^R	Novagen
pET28a_NDM-1	pET28a carrying a N-terminally His_6-tagged NDM-1 without signal peptide	This study
pET28a_AFM-2	pET28a carrying a N-terminally His_6-tagged AFM-2 without signal peptide	This study

References

1. Chen W, Zhang Y, Zhang Y, et al. CRISPR/Cas9-based Genome Editing in Pseudomonas aeruginosa and Cytidine Deaminase-Mediated Base Editing in Pseudomonas Species. *iScience* 2018; **6**: 222-31.

519 2. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP. A broad-host-range Flp-FRT recombination system for site-specific
520 excision of chromosomally-located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa mutants. *Gene* 1998;
521 **212**(1): 77-86.

Table S2. Clinical information of *Pseudomonas aeruginosa* strains NDTH10366, NDTH9845 and WTJH17 harboring *bla*$_{AFM}$

Strains	Area	Hospital	Department	Collection Date	Patient Age (yrs)	Patient Gender	Sample Source	Infection Site	Anti-PA Treatment	Outcome
NDTH10366	Eastern China	NDTH	ICU	Jan-2019	55	Male	Urine	Urinary tract colonization	/	Recovered
NDTH9845	Eastern China	NDTH	NICU	Nov-2018	63	Female	Urine	Urinary tract colonization	/	Recovered
WTJH17	Central China	WTJH	ICU	Feb-2018	44	Male	Blood	Bloodstream infection	Colistin	Dead

NDTH, Nanjing Drum Tower Hospital; WTJH, Wuhan Tongji Hospital; ICU, intensive care unit; NICU, neurosurgery intensive care unit.

Table S3. Primers used for putative transconjugants confirmation in this work

Genes	Primer Sequences (5'→3')
bla_{AFM}	**F:** TGCTAATGGCACCCTTTGACATC **R:** GCGTTGCAGGATCATCCAGC
PA1935	**F:** CCACGTCATAGTCGCTCGATTTCTTCCGCCCTG **R:** GGATGACTTCGCAGTGATGGCGCAGC
Replication initiation genes of pNDTH10366, pNDTH9845 and pWTJH17	**F:** ATGGATGTAATCGAATCACAGAACGACTTGC **R:** CTGCCGGCAGACGACAGGTC

Table S4. MICs of aminoglycosides, fluoroquinolones and colistin for *Pseudomonas aeruginosa* clinical isolates carrying *bla*$_{AFM}$

	MICs (mg/L)					
Strains	**AK**	**GM**	**TM**	**CIP**	**LEV**	**COL**
NDTH10366	>64	>64	>64	16	>32	0.25
NDTH9845	>64	>64	>64	16	>32	1
WTJH17	32	64	64	>16	32	1

AK, amikacin; GM, gentamicin; TM, tobramycin; CIP, ciprofloxacin; LEV, levofloxacin; COL, colistin.

Table S5. Genome characteristics of XDR *Pseudomonas aeruginosa*

Isolate	Molecule	Size (bp)	Acquired antibiotic resistance genes
NDTH10366	chromosome	6,974,425	*bla*$_{KPC-2}$ (x3), *sul1*, *aadB*, *aac(6')-IIa*, *catB7*, *crpP* (x2)
	pNDTH10366	392,244	*bla*$_{KPC-2}$ (x2), *sul1* (x2), *aadA24*, *cmlA1*, *aadB*, *aacA7*, *dfrA27*, *arr-3*, *bla*$_{OXA-246}$, *cmlA8*, *aac(6')-IIa*, *ble*$_{MBL}$, *bla*$_{AFM-2}$
NDTH9845	chromosome	7,137,026	*bla*$_{KPC-2}$ (x3), *sul1*, *aadB*, *aac(6')-IIa*, *catB7*, *bla*$_{CARB-2}$, *aph(3')-VI*, *crpP* (x3)
	pNDTH9845	463,517	*sul1* (x3), *cmlA1*, *aadB*, *aacA7*, *dfrA27*, *arr-3*, *bla*$_{OXA-246}$, *cmlA8*, *aac(6')-IIa*, *ble*$_{MBL}$, *bla*$_{AFM-2}$, *msr*(E)-*mph*(E), *armA*, *qnrVC1*, *dfrA22*
WTJH17	chromosome	6,389,938	*catB7*, *bla*$_{CARB-4}$
	pWTJH17	436,486	*sul1* (x3), *aadA24*, *cmlA1*, *aacA7*, *dfrA27*, *arr-3*, *cmlA8*, *aac(6')-IIa*, *ble*$_{MBL}$, *bla*$_{AFM-3}$, *msr*(E)-*mph*(E), *qnrVC1*, *aadA1*, *catB2*-like

Antibiotic resistance genes were detected with abricate using the ResFinder database. Only complete, uninterrupted genes are listed. Chromosomal *bla*$_{PAO}$, *bla*$_{OXA-396/486}$, *fosA* and *aph(3')-IIb* were considered intrinsic *P. aeruginosa* genes. *aacA7* is also called *aac(6')-Il*. *aadB* is also called *ant(2'')-Ia*.

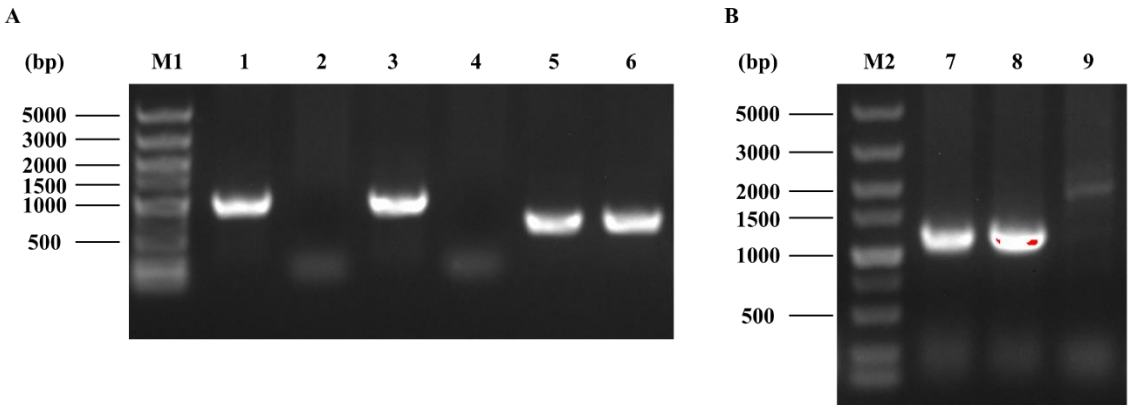


Figure S1. Agarose gel electrophoresis of PCR-amplified products from WTJH17, PAO1 and their transconjugant. (A) Lane M1 = 5000 bp DNA marker; lane 1 = WTJH17 (amplified for $bla_{\text{AFM-3}}$); lane 2 = PAO1 (amplified for $bla_{\text{AFM-3}}$); lane 3 = transconjugant (amplified for $bla_{\text{AFM-3}}$); lane 4 = WTJH17 (amplified for PAO1-specific PA1935); lane 5 = PAO1 (amplified for PAO1-specific PA1935); lane 6 = transconjugant (amplified for PAO1-specific PA1935). (B) Lane M2 = 5000 bp DNA marker; lane 7 = WTJH17 (amplified for pWTJH17 replication initiation gene); lane 8 = transconjugant (amplified for pWTJH17 replication initiation gene); lane 9 = PAO1 (amplified for pWTJH17 replication initiation gene). Primers used were listed in Table S3.