

Supporting Information

Molecular and functional characterization of a novel plasmid-borne *bla*_{NDM}-like gene, *bla*_{AFM-1}, in a clinical strain of *Aeromonas hydrophila*

Table S1 Primers used for functional detection of the antimicrobial resistance genes in this work

Genes	Primer sequences (5'-3')	Restriction endonucleases	Vectors	Annealing temperature	Amplicon size
<i>bla</i> _{AFM-6}	F <u>GCTCTAGAGAATACGACTCCAT</u> CCATGATTCAG R <u>CGGGATCCGATCCTTCC</u> AGCTCGTCGCAAAC	<i>Xba</i> I <i>Bam</i> HI	pUCP24	58 °C	950 bp
<i>bla</i> _{OXA-1}	F <u>GCTCTAGAAGCCCTTTA</u> CCAAACCAATAC R <u>CGGGATCCCGACTTGAT</u> TGAAGGGTTGG	<i>Xba</i> I <i>Bam</i> HI	pUCP24	62 °C	899 bp
<i>aac</i> (6')-Ib10	F <u>GCTCTAGACATAATTGCT</u> CACAGCCAAAC R <u>CGGGATCCTTTAACGGC</u> TAACAATTAATGAGG	<i>Xba</i> I <i>Bam</i> HI	pUCP20	52 °C	1036 bp
<i>aph</i> (3')-Ia	F <u>GCTCTAGAAGTTTATCAC</u> CACCGACTATTTGC R <u>CGGGATCCTGGGTCGAT</u> TCCTGGGAGG	<i>Xba</i> I <i>Bam</i> HI	pUCP20	62 °C	1036 bp
<i>msr</i> (E)	F <u>GCTCTAGACACCCTGTG</u> GATGAATAGC R <u>CGGGATCCGTGCTTCAG</u> CAAGTGATTG	<i>Xba</i> I <i>Bam</i> HI	pUCP20	62 °C	1685 bp
<i>mph</i> (E)	F <u>GCTCTAGAGGATCGGAG</u> CGAATGATATTC R <u>CGGGATCCCAAACAAG</u> GCAGTTCTCAG	<i>Xba</i> I <i>Bam</i> HI	pUCP20	62 °C	1266 bp

The underlined sequences represent the restriction endonuclease sites and their protective bases

Table S2 Antibiotic resistance genes in *Aeromonas hydrophila* SS332 detected using the CARD database

Gene ID	ARO Name	AMR Gene Family	Drug Class	Resistance Mechanism	Coverage (%)	Identity (%)
CMCFKOJJ_01136	catB3	chloramphenicol acetyltransferase (CAT)	phenicol antibiotic	antibiotic inactivation	100	100
CMCFKOJJ_01139	PER-3	PER beta-lactamase	penam; penem; carbapenem; cephalosporin; monobactam	antibiotic inactivation	100	100
CMCFKOJJ_01144	sul1	sulfonamide resistant sul	sulfonamide antibiotic	antibiotic target replacement	98.92	100
CMCFKOJJ_01151	mphA	macrolide phosphotransferase (MPH)	macrolide antibiotic	antibiotic inactivation	100	100
CMCFKOJJ_03911	tet(E)	major facilitator superfamily (MFS) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; macrolide antibiotic; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; isoniazid; glycylicycline; nucleoside antibiotic; fluoroquinolone antibiotic; phenicol antibiotic; rifamycin antibiotic	antibiotic efflux	100	99.75
CMCFKOJJ_02190	MOX-7	MOX beta-lactamase	penam; cephamycin; cephalosporin	antibiotic inactivation	100	96.61
CMCFKOJJ_01133	aadA16	ANT(3")	aminoglycoside antibiotic	antibiotic inactivation	97.86	96.36
CMCFKOJJ_04436	CRP	resistance-nodulation-cell division (RND) antibiotic efflux pump	tetracycline antibiotic; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; antibacterial free fatty acids; aminoglycoside antibiotic; acridine dye; diaminopyrimidine antibiotic; penam; triclosan; glycylicycline; monobactam; fluoroquinolone antibiotic; phenicol antibiotic	antibiotic efflux	100.95	89.15
CMCFKOJJ_03510	OXA-427	OXA beta-lactamase	penam; cephalosporin	antibiotic inactivation	100	88.26
CMCFKOJJ_01024	Salmonella enterica gyrA conferring resistance to fluoroquinolones	fluoroquinolone resistant gyrA	fluoroquinolone antibiotic	antibiotic target alteration	102.85	75.31

CMCFKOJJ_00402	ugd	pmr phosphoethanolamine transferase	peptide antibiotic	antibiotic target alteration	100	71.65
CMCFKOJJ_03289	bacA	undecaprenyl pyrophosphate related proteins	peptide antibiotic	antibiotic target alteration	100	69.6
CMCFKOJJ_02014	MCR-7.1	MCR phosphoethanolamine transferase	peptide antibiotic	antibiotic target alteration	100.19	68.7
CMCFKOJJ_04039	Escherichia coli parC conferring resistance to fluoroquinolone	fluoroquinolone self resistant parC; fluoroquinolone resistant parC	fluoroquinolone antibiotic	antibiotic target alteration	98.4	67.16
CMCFKOJJ_00834	Mycobacterium tuberculosis katG mutations conferring resistance to isoniazid	isoniazid resistant katG	isoniazid	antibiotic target alteration	96.35	63.81
CMCFKOJJ_03492	YojI	ATP-binding cassette (ABC) antibiotic efflux pump	penam; pleuromutilin antibiotic; macrolide antibiotic; peptide antibiotic; acridine dye; cephalosporin; tetracycline antibiotic; fluoroquinolone antibiotic; rifamycin antibiotic; nitroimidazole antibiotic	antibiotic efflux	99.63	63.67
CMCFKOJJ_04576	adeB	resistance-nodulation-cell division (RND) antibiotic efflux pump	penam; triclosan; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; acridine dye; antibacterial free fatty acids; diaminopyrimidine antibiotic; glycylicycline; tetracycline antibiotic; monobactam; fluoroquinolone antibiotic; aminoglycoside antibiotic; phenicol antibiotic	antibiotic efflux	97.58	62.28
CMCFKOJJ_00516	msbA	ATP-binding cassette (ABC) antibiotic efflux pump	penam; pleuromutilin antibiotic; macrolide antibiotic; peptide antibiotic; acridine dye; cephalosporin; tetracycline antibiotic; fluoroquinolone antibiotic; rifamycin antibiotic; nitroimidazole antibiotic	antibiotic efflux	99.83	61.27
CMCFKOJJ_03431	rosA	major facilitator superfamily (MFS) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; macrolide antibiotic; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; isoniazid; glycylicycline; nucleoside antibiotic; fluoroquinolone antibiotic; phenicol antibiotic; rifamycin antibiotic	antibiotic efflux	99.76	60.15

CMCFKOJJ_02738	FosC2	fosC phosphotransferase family	fosfomycin	antibiotic inactivation	100	59.09
CMCFKOJJ_02408	dfrA3	trimethoprim resistant dihydrofolate reductase dfr	diaminopyrimidine antibiotic	antibiotic target replacement	99.38	59.01
CMCFKOJJ_03186	Staphylococcus aureus fusA with mutation conferring resistance to fusidic acid	antibiotic resistant fusA	fusidic acid	antibiotic target alteration	101.01	58.86
CMCFKOJJ_02529	mdtH	major facilitator superfamily (MFS) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; macrolide antibiotic; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; isoniazid; glycylicline; nucleoside antibiotic; fluoroquinolone antibiotic; phenicol antibiotic; rifamycin antibiotic	antibiotic efflux	97.51	57.4
CMCFKOJJ_00823	H-NS	major facilitator superfamily (MFS) antibiotic efflux pump; resistance-nodulation-cell division (RND) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; aminoglycoside antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; triclosan; isoniazid; cephamycin; glycylicline; nucleoside antibiotic; monobactam; fluoroquinolone antibiotic; rifamycin antibiotic; phenicol antibiotic	antibiotic efflux	99.27	57.35
CMCFKOJJ_00335	OprM	resistance-nodulation-cell division (RND) antibiotic efflux pump	sulfonamide antibiotic; penem; tetracycline antibiotic; aminocoumarin antibiotic; cephalosporin; macrolide antibiotic; carbapenem; antibacterial free fatty acids; cephamycin; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; penam; triclosan; aminoglycoside antibiotic; phenicol antibiotic; glycylicline; monobactam; fluoroquinolone antibiotic	antibiotic efflux	96.29	56.53
CMCFKOJJ_00332	Escherichia coli acrA	resistance-nodulation-cell division (RND) antibiotic efflux pump	tetracycline antibiotic; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; cephalosporin; antibacterial free fatty acids; aminoglycoside antibiotic; acridine dye; diaminopyrimidine antibiotic; penam; triclosan; glycylicline; monobactam; fluoroquinolone antibiotic; phenicol antibiotic; rifamycin antibiotic	antibiotic efflux	97.98	55.53

CMCFKOJJ_0017 1	rosB	major facilitator superfamily (MFS) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; macrolide antibiotic; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; isoniazid; glycylyccline; nucleoside antibiotic; fluoroquinolone antibiotic; phenicol antibiotic; rifamycin antibiotic	antibiotic efflux	97.16	54.66
CMCFKOJJ_0024 9	H-NS	major facilitator superfamily (MFS) antibiotic efflux pump; resistance-nodulation-cell division (RND) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; aminoglycoside antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; triclosan; isoniazid; cephamycin; glycylyccline; nucleoside antibiotic; monobactam; fluoroquinolone antibiotic; rifamycin antibiotic; phenicol antibiotic	antibiotic efflux	102.19	54.29
CMCFKOJJ_0391 0	tetR	major facilitator superfamily (MFS) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; macrolide antibiotic; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; isoniazid; glycylyccline; nucleoside antibiotic; fluoroquinolone antibiotic; phenicol antibiotic; rifamycin antibiotic	antibiotic target alteration; antibiotic efflux	96.63	51.74
CMCFKOJJ_0256 9	macB	ATP-binding cassette (ABC) antibiotic efflux pump	penam; rifamycin antibiotic; pleuromutilin antibiotic; macrolide antibiotic; peptide antibiotic; acridine dye; cephalosporin; tetracycline antibiotic; fluoroquinolone antibiotic; nitroimidazole antibiotic	antibiotic efflux	100.47	51.16
CMCFKOJJ_0082 4	tet(35)	ATP-binding cassette (ABC) antibiotic efflux pump	penam; pleuromutilin antibiotic; macrolide antibiotic; peptide antibiotic; acridine dye; cephalosporin; tetracycline antibiotic; fluoroquinolone antibiotic; rifamycin antibiotic; nitroimidazole antibiotic	antibiotic efflux	94.85	50
CMCFKOJJ_0457 8	adeR	resistance-nodulation-cell division (RND) antibiotic efflux pump	penam; triclosan; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; acridine dye; antibacterial free fatty acids; diaminopyrimidine antibiotic; glycylyccline; tetracycline antibiotic; monobactam; fluoroquinolone antibiotic; aminoglycoside antibiotic; phenicol antibiotic	antibiotic efflux	91.9	49.78

CMCFKOJJ_0357 7	Pseudomonas aeruginosa CpxR	resistance-nodulation- cell division (RND) antibiotic efflux pump	sulfonamide antibiotic; penem; tetracycline antibiotic; aminocoumarin antibiotic; cephalosporin; macrolide antibiotic; carbapenem; antibacterial free fatty acids; cephamycin; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; penam; triclosan; aminoglycoside antibiotic; glycylicycline; monobactam; fluoroquinolone antibiotic; phenicol antibiotic	antibiotic efflux	101.33	49.56
CMCFKOJJ_0402 8	ToIC	ATP-binding cassette (ABC) antibiotic efflux pump; major facilitator superfamily (MFS) antibiotic efflux pump; resistance-nodulation- cell division (RND) antibiotic efflux pump	penem; benzalkonium chloride; tetracycline antibiotic; rhodamine; aminocoumarin antibiotic; cephalosporin; macrolide antibiotic; carbapenem; nitroimidazole antibiotic; aminoglycoside antibiotic; lincosamide antibiotic; glycylicycline; bicyclomycin; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; monobactam; oxazolidinone antibiotic; nucleoside antibiotic; penam; triclosan; antibacterial free fatty acids; rifamycin antibiotic; pleuromutilin antibiotic; isoniazid; fosfomycin; cephamycin; fluoroquinolone antibiotic; phenicol antibiotic	antibiotic efflux	85.86	48.94
CMCFKOJJ_0451 8	H-NS	major facilitator superfamily (MFS) antibiotic efflux pump; resistance-nodulation- cell division (RND) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; aminoglycoside antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; triclosan; isoniazid; cephamycin; glycylicycline; nucleoside antibiotic; monobactam; fluoroquinolone antibiotic; rifamycin antibiotic; phenicol antibiotic	antibiotic efflux	100	48.17
CMCFKOJJ_0345 9	MexW	resistance-nodulation- cell division (RND) antibiotic efflux pump	penam; triclosan; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; acridine dye; antibacterial free fatty acids; diaminopyrimidine antibiotic; glycylicycline; tetracycline antibiotic; monobactam; fluoroquinolone antibiotic; aminoglycoside antibiotic; phenicol antibiotic	antibiotic efflux	99.41	48.12
CMCFKOJJ_0387 8	H-NS	major facilitator superfamily (MFS) antibiotic efflux pump; resistance-nodulation- cell division (RND) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; aminoglycoside antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; triclosan; isoniazid; cephamycin; glycylicycline; nucleoside antibiotic; monobactam; fluoroquinolone antibiotic; rifamycin antibiotic; phenicol antibiotic	antibiotic efflux	91.24	48
CMCFKOJJ_0025 3	macB	ATP-binding cassette (ABC) antibiotic efflux pump	penam; rifamycin antibiotic; pleuromutilin antibiotic; macrolide antibiotic; peptide antibiotic; acridine dye; cephalosporin; tetracycline antibiotic; fluoroquinolone antibiotic; nitroimidazole antibiotic	antibiotic efflux	103.88	47.38

CMCFKOJJ_0133 1	hmrM	multidrug and toxic compound extrusion (MATE) transporter	acridine dye; glycylicycline; tetracycline antibiotic; fluoroquinolone antibiotic	antibiotic efflux	95.26	47.06
CMCFKOJJ_0346 6	kdpE	kdpDE	aminoglycoside antibiotic	antibiotic efflux	100	46.22
CMCFKOJJ_0457 7	adeA	resistance-nodulation-cell division (RND) antibiotic efflux pump	penam; triclosan; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; acridine dye; antibacterial free fatty acids; diaminopyrimidine antibiotic; glycylicycline; tetracycline antibiotic; monobactam; fluoroquinolone antibiotic; aminoglycoside antibiotic; phenicol antibiotic	antibiotic efflux	93.18	46.07
CMCFKOJJ_0112 8	adeG	resistance-nodulation-cell division (RND) antibiotic efflux pump	penam; triclosan; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; acridine dye; antibacterial free fatty acids; diaminopyrimidine antibiotic; glycylicycline; tetracycline antibiotic; monobactam; fluoroquinolone antibiotic; aminoglycoside antibiotic; phenicol antibiotic	antibiotic efflux	88.67	45.56
CMCFKOJJ_0351 2	smeR	resistance-nodulation-cell division (RND) antibiotic efflux pump	penam; triclosan; aminocoumarin antibiotic; macrolide antibiotic; carbapenem; acridine dye; cephalosporin; antibacterial free fatty acids; diaminopyrimidine antibiotic; glycylicycline; cephamycin; tetracycline antibiotic; monobactam; fluoroquinolone antibiotic; aminoglycoside antibiotic; phenicol antibiotic	antibiotic efflux	95.2	44.49
CMCFKOJJ_0375 2	QnrB46	quinolone resistance protein (qnr)	fluoroquinolone antibiotic	antibiotic target protection	91.59	44.44
CMCFKOJJ_0431 9	Klebsiella pneumoniae KpnF	major facilitator superfamily (MFS) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; antibacterial free fatty acids; macrolide antibiotic; cephalosporin; bicyclomycin; nitroimidazole antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; aminoglycoside antibiotic; isoniazid; glycylicycline; nucleoside antibiotic; fluoroquinolone antibiotic; rifamycin antibiotic; phenicol antibiotic	antibiotic efflux	88.99	44.33
CMCFKOJJ_0234 6	sul4	sulfonamide resistant sul	sulfonamide antibiotic	antibiotic target replacement	93.38	44.03
CMCFKOJJ_0111 0	bcr-1	major facilitator superfamily (MFS) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; macrolide antibiotic; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; isoniazid; glycylicycline; nucleoside antibiotic; fluoroquinolone antibiotic; phenicol antibiotic; rifamycin antibiotic	antibiotic efflux	91.29	43.32

CMCFKOJJ_0168 7	bcr-1	major facilitator superfamily (MFS) antibiotic efflux pump	fosfomycin; benzalkonium chloride; tetracycline antibiotic; rhodamine; macrolide antibiotic; cephalosporin; antibacterial free fatty acids; bicyclomycin; nitroimidazole antibiotic; lincosamide antibiotic; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; oxazolidinone antibiotic; penam; isoniazid; glycylicline; nucleoside antibiotic; fluoroquinolone antibiotic; phenicol antibiotic; rifamycin antibiotic	antibiotic efflux	91.29	43.05
CMCFKOJJ_0347 5	Streptomyces rishiriensis parY mutant conferring resistance to aminocoumarin	aminocoumarin self resistant parY; aminocoumarin resistant parY	aminocoumarin antibiotic	antibiotic target alteration	95.45	42.48
CMCFKOJJ_0380 2	Staphylococcus aureus fusE with mutation conferring resistance to fusidic acid	antibiotic resistant fusE	fusidic acid	antibiotic target alteration	98.31	42.29
CMCFKOJJ_0256 7	Pseudomonas aeruginosa CpxR	resistance-nodulation-cell division (RND) antibiotic efflux pump	sulfonamide antibiotic; penem; tetracycline antibiotic; aminocoumarin antibiotic; cephalosporin; macrolide antibiotic; carbapenem; antibacterial free fatty acids; cephamycin; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; penam; triclosan; aminoglycoside antibiotic; glycylicline; monobactam; fluoroquinolone antibiotic; phenicol antibiotic	antibiotic efflux	99.11	42.15
CMCFKOJJ_0364 9	Pseudomonas aeruginosa CpxR	resistance-nodulation-cell division (RND) antibiotic efflux pump	sulfonamide antibiotic; penem; tetracycline antibiotic; aminocoumarin antibiotic; cephalosporin; macrolide antibiotic; carbapenem; antibacterial free fatty acids; cephamycin; peptide antibiotic; acridine dye; diaminopyrimidine antibiotic; penam; triclosan; aminoglycoside antibiotic; glycylicline; monobactam; fluoroquinolone antibiotic; phenicol antibiotic	antibiotic efflux	100.89	40.09

Table S3 Antibiotic resistance genes in pSS332-218k of *Aeromonas hydrophila* SS332 detected using the CARD database

Gene ID	Cutoff	ARO Name	AMR Gene Family	Drug Class	Drug Class	Resistance Mechanism	Coverage (%)	Identity (%)
IBDFPOCI_00066	Perfect	msrE	ABC-F type ribosomal protection protein MsrE	pleuromutilin antibiotic; macrolide antibiotic; oxazolidinone antibiotic; tetracycline antibiotic; streptogramin antibiotic; phenicol antibiotic; lincosamide antibiotic	antibiotic target protection	ABC-F ATP-binding cassette ribosomal protection protein	100	100
IBDFPOCI_00067	Perfect	mphE		macrolide antibiotic	antibiotic inactivation	macrolide phosphotransferase (MPH)	100	100
IBDFPOCI_00083	Strict	AAC(6')-Ib7	Aminoglycoside N(6')-acetyltransferase type 1	aminoglycoside antibiotic	antibiotic inactivation	AAC(6')	75.86	98.99
IBDFPOCI_00084	Strict	OXA-1	Beta-lactamase OXA-1	penam; cephalosporin	antibiotic inactivation	OXA beta-lactamase	71.38	100
IBDFPOCI_00085	Strict	catB3	Chloramphenicol acetyltransferase	phenicol antibiotic	antibiotic inactivation	chloramphenicol acetyltransferase (CAT)	51.43	99.07
IBDFPOCI_00086	Strict	catB3	Chloramphenicol acetyltransferase	phenicol antibiotic	antibiotic inactivation	chloramphenicol acetyltransferase (CAT)	40.48	100
IBDFPOCI_00088	Strict	APH(3')-Ia	Aminoglycoside 3'-phosphotransferase	aminoglycoside antibiotic	antibiotic inactivation	APH(3')	100	98.52
IBDFPOCI_00093	Strict	sul1	Dihydropteroate synthase	sulfonamide antibiotic	antibiotic target replacement	sulfonamide resistant sul	37.63	99.05
IBDFPOCI_00102	Strict	BRP(MBL)	Bleomycin resistance protein	glycopeptide antibiotic	antibiotic inactivation	Bleomycin resistant protein	100	97.52
IBDFPOCI_00103	Loose	NDM-6	Metallo-beta-lactamase type 2	penam; carbapenem; cephalosporin; cephamicin	antibiotic inactivation	NDM beta-lactamase	96.67	86.21
IBDFPOCI_00106	Strict	sul1	Dihydropteroate synthase	sulfonamide antibiotic	antibiotic target replacement	sulfonamide resistant sul	100.72	95.37
IBDFPOCI_00108	Strict	dfrA5	Dihydrofolate reductase type 1	diaminopyrimidine antibiotic	antibiotic target replacement	trimethoprim resistant dihydrofolate reductase dfr	96.82	100

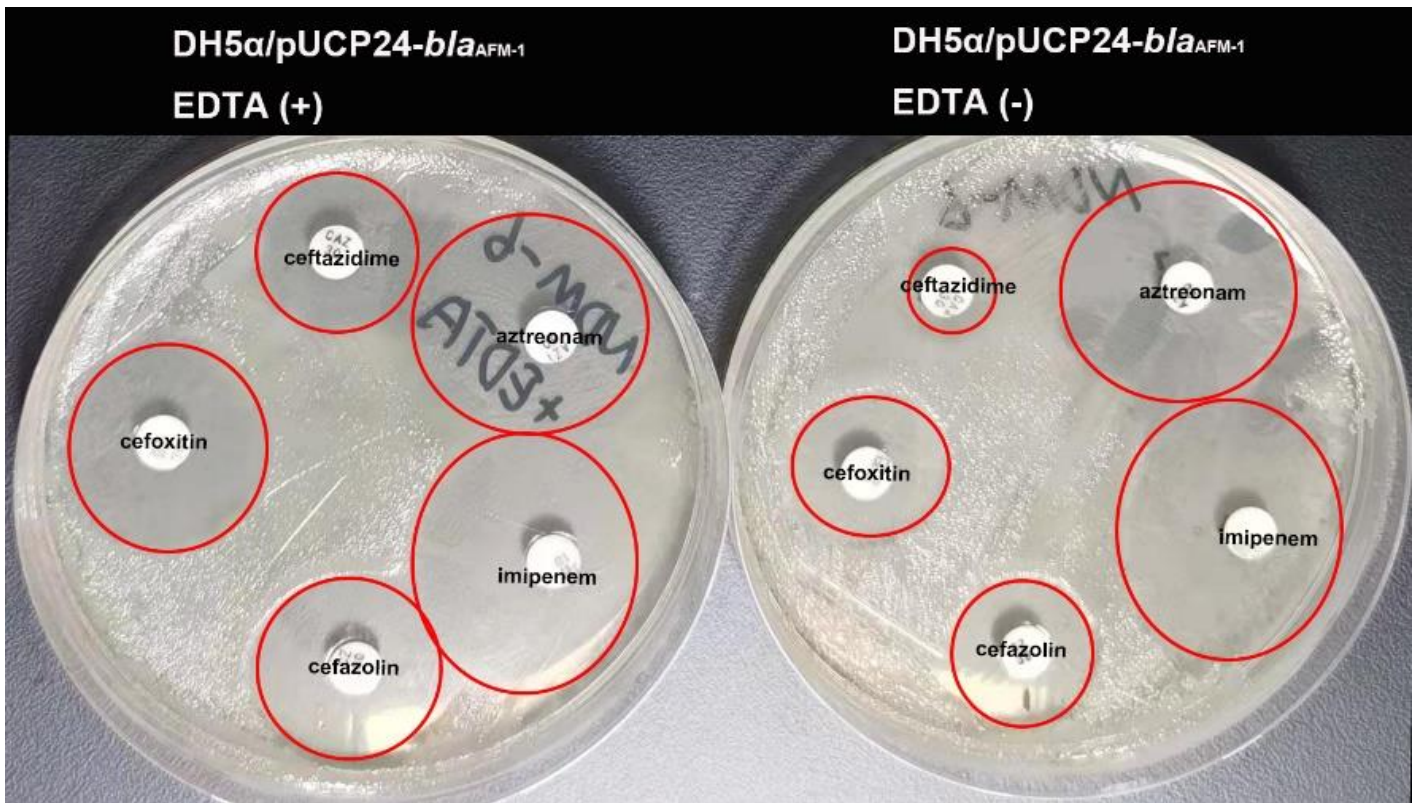


Figure S1. Antibiotic susceptibility testing by the disc diffusion method. *DH5α/pUCP24-bla_{AFM-1}* incubated with or without 0.05 mmol/L EDTA.

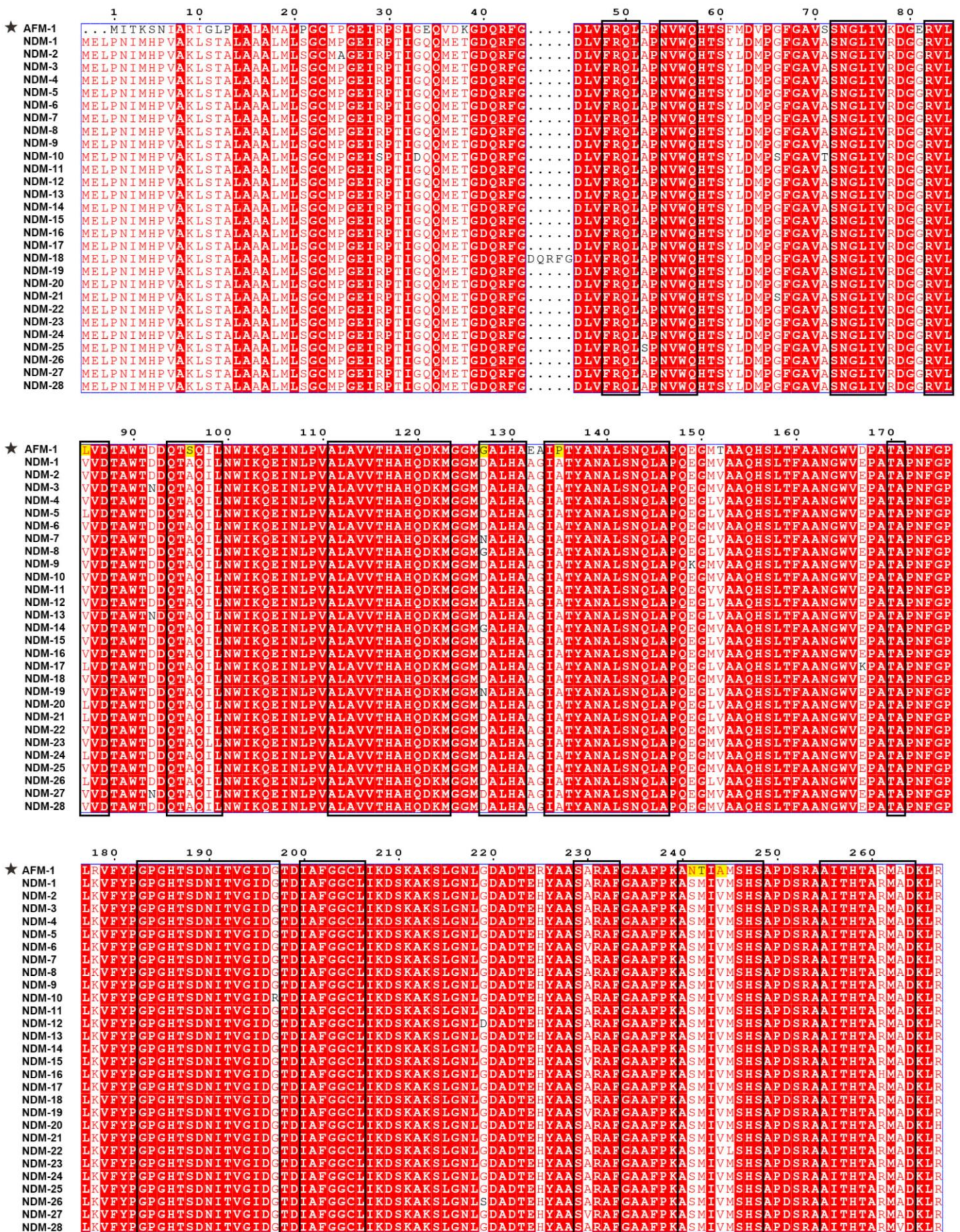


Figure S2 Sequence alignment of New Delhi metallo-beta-lactamases. Residue numbers are positioned above the sequences according to AFM-1 numbers; 14 sequence fragments (F1-F14) of structurally conserved positions are boxed in black. Amino acid variants of AFM-1 in conserved sequence fragments are boxed in yellow.