

Supporting Information

for

**Clinical Variants of New Delhi Metallo- β -Lactamase Are Evolving to Overcome Zinc
Scarcity**

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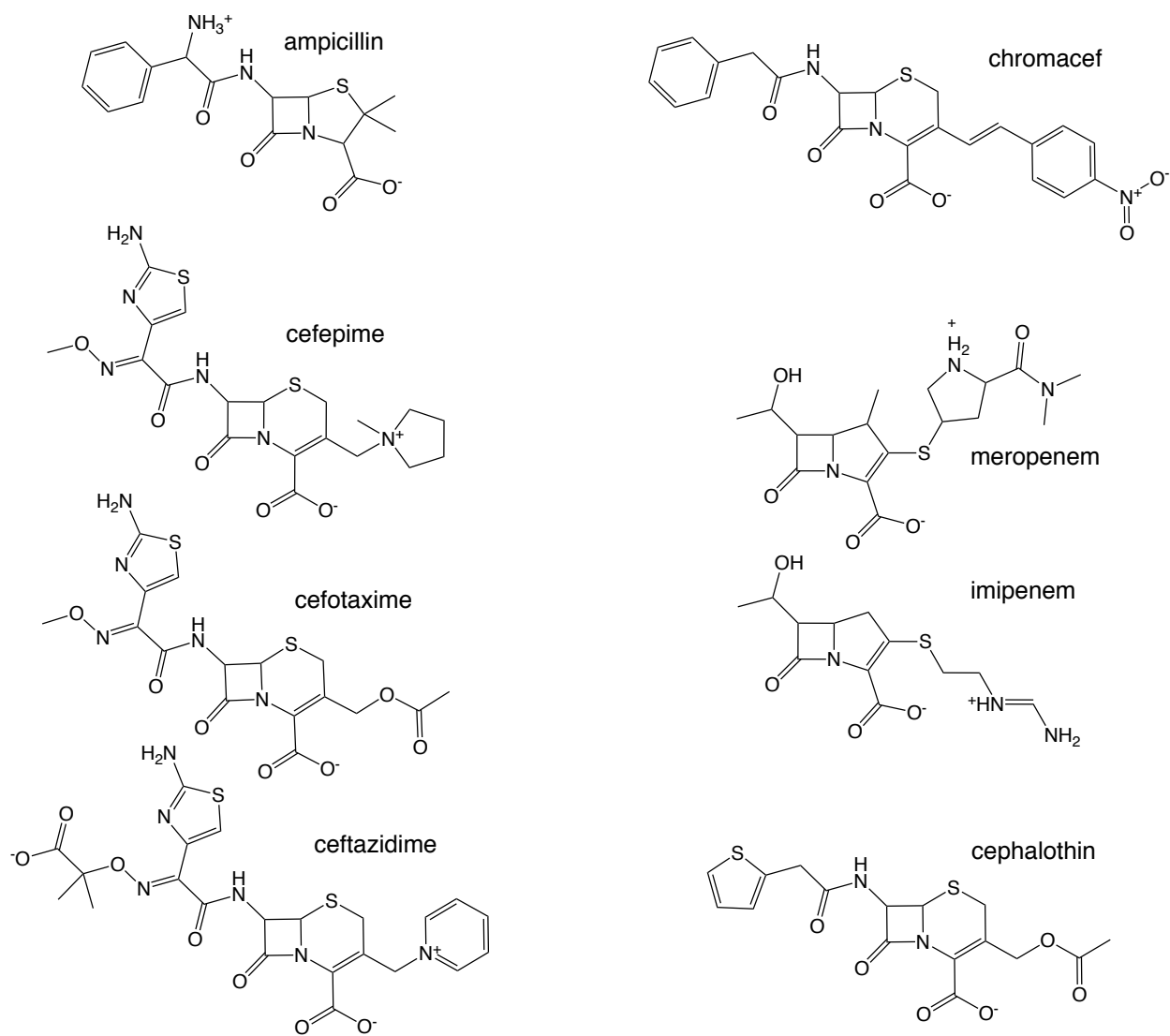


Figure S1. Structures of β -lactams used in this study

Table S1. MIC values for imipenem with NDM variants substituted at position 154 in standard and zinc(II) limited conditions

Strain	MIC (mg/L) Standard Conditions ^a	MIC (mg/L) EDTA (50 μ M) ^a
pHSG298	0.25	0.25
NDM 154M	64	0.5
NDM 154L	64	4
NDM 154I	64	0.5
NDM 154F	64	0.5
NDM 154V	64	1
NDM 154W	16	0.25
NDM 154G	8	0.25
NDM 154Q	32	0.25
NDM 154K	8	0.25
NDM 154E	2	0.25
NDM 154A	8	0.25
NDM 154D	0.5	0.25
NDM 154N	16	0.25
NDM 154P	0.5	0.25
NDM 154S	8	0.25
NDM 154T	16	0.25
NDM 154Y	32	0.5
NDM 154C	8	0.25
NDM 154H	32	0.25
NDM 154R	8	0.25
NDM-12	64	4

^aResults from three biological replicates

Table S2. MIC values for cephalothin with NDM variants substituted at position 154 in standard and zinc(II) limited conditions

Strain	MIC (mg/L) Standard Conditions ^a	MIC (mg/L) EDTA (50 μ M) ^a
pHSG298	4	4
NDM 154M	> 1024	128
NDM 154L	> 1024	512
NDM 154I	> 1024	64
NDM 154F	> 1024	64
NDM 154V	> 1024	128
NDM 154W	> 1024	32
NDM 154G	512	16
NDM 154Q	> 1024	16
NDM 154K	512	8
NDM 154E	512	8
NDM 154A	1024	8
NDM 154D	128	4
NDM 154N	1024	8
NDM 154P	128	4
NDM 154S	512	8
NDM 154T	1024	16
NDM 154Y	> 1024	32
NDM 154C	1024	8
NDM 154H	> 1024	16
NDM 154R	512	16
NDM-12	>1024	512

^aResults from three biological replicates

Table S3. MIC values for ceftazidime with NDM variants substituted at position 154 in standard and zinc(II) limited conditions

Strain	MIC (mg/L) Standard Conditions ^a	MIC (mg/L) EDTA (50 μ M) ^a
pHSG298	0.25	0.25
NDM 154M	> 512	> 512
NDM 154L	> 512	> 512
NDM 154I	> 512	512
NDM 154F	> 512	256
NDM 154V	> 512	> 512
NDM 154W	> 512	128
NDM 154G	> 512	16
NDM 154Q	> 512	128
NDM 154K	> 512	16
NDM 154E	> 512	2
NDM 154A	> 512	16
NDM 154D	512	1
NDM 154N	> 512	32
NDM 154P	512	1
NDM 154S	> 512	8
NDM 154T	> 512	32
NDM 154Y	> 512	256
NDM 154C	> 512	16
NDM 154H	> 512	128
NDM 154R	> 512	32
NDM-12	>512	>512

^aResults from three biological replicates

Table S4. MIC values for cefotaxime with NDM variants substituted at position 154 in standard and zinc(II) limited conditions

Strain	MIC (mg/L) Standard Conditions ^a	MIC (mg/L) EDTA (50 μ M) ^a
pHSG298	≤ 0.06	≤ 0.06
NDM 154M	> 128	32
NDM 154L	> 128	128
NDM 154I	> 128	16
NDM 154F	> 128	16
NDM 154V	> 128	32
NDM 154W	> 128	2
NDM 154G	128	0.5
NDM 154Q	> 128	2
NDM 154K	> 128	0.5
NDM 154E	32	≤ 0.06
NDM 154A	> 128	0.5
NDM 154D	16	≤ 0.06
NDM 154N	> 128	0.5
NDM 154P	16	≤ 0.06
NDM 154S	128	0.25
NDM 154T	> 128	1
NDM 154Y	> 128	8
NDM 154C	> 128	0.5
NDM 154H	> 128	2
NDM 154R	> 128	1
NDM-12	>128	128

^aResults from three biological replicates

Table S5. MIC values for cefepime with NDM variants substituted at position 154 in standard and zinc(II) limited conditions

Strain	MIC (mg/L) Standard Conditions ^a	MIC (mg/L) EDTA (50 μ M) ^a
pHSG298	0.06	0.06
NDM 154M	> 32	4
NDM 154L	> 32	32
NDM 154I	> 32	2
NDM 154F	> 32	0.5
NDM 154V	> 32	4
NDM 154W	> 32	0.25
NDM 154G	> 32	0.125
NDM 154Q	> 32	0.25
NDM 154K	> 32	0.125
NDM 154E	8	0.06
NDM 154A	32	0.125
NDM 154D	1	0.06
NDM 154N	> 32	0.125
NDM 154P	1	0.06
NDM 154S	32	0.125
NDM 154T	> 32	0.125
NDM 154Y	> 32	0.5
NDM 154C	16	0.125
NDM 154H	> 32	0.25
NDM 154R	32	0.125
NDM-12	> 32	32

^aResults from three biological replicates

Table S6. MIC values for meropenem with NDM variants substituted at position 154 in standard and zinc(II) limited conditions

Strain	MIC (mg/L) Standard Conditions ^a	MIC (mg/L) EDTA (50 μ M) ^a
pHSG298	≤ 0.06	≤ 0.06
NDM 154M	32	0.125
NDM 154L	32	0.5
NDM 154I	16	0.125
NDM 154F	32	0.125
NDM 154V	32	0.125
NDM 154W	16	≤ 0.06
NDM 154G	2	≤ 0.06
NDM 154Q	16	≤ 0.06
NDM 154K	4	≤ 0.06
NDM 154E	0.5	≤ 0.06
NDM 154A	4	≤ 0.06
NDM 154D	0.25	≤ 0.06
NDM 154N	8	≤ 0.06
NDM 154P	0.25	≤ 0.06
NDM 154S	4	≤ 0.06
NDM 154T	8	≤ 0.06
NDM 154Y	16	≤ 0.06
NDM 154C	2	≤ 0.06
NDM 154H	16	≤ 0.06
NDM 154R	8	≤ 0.06
NDM-12	32	2

^aResults from two biological replicates

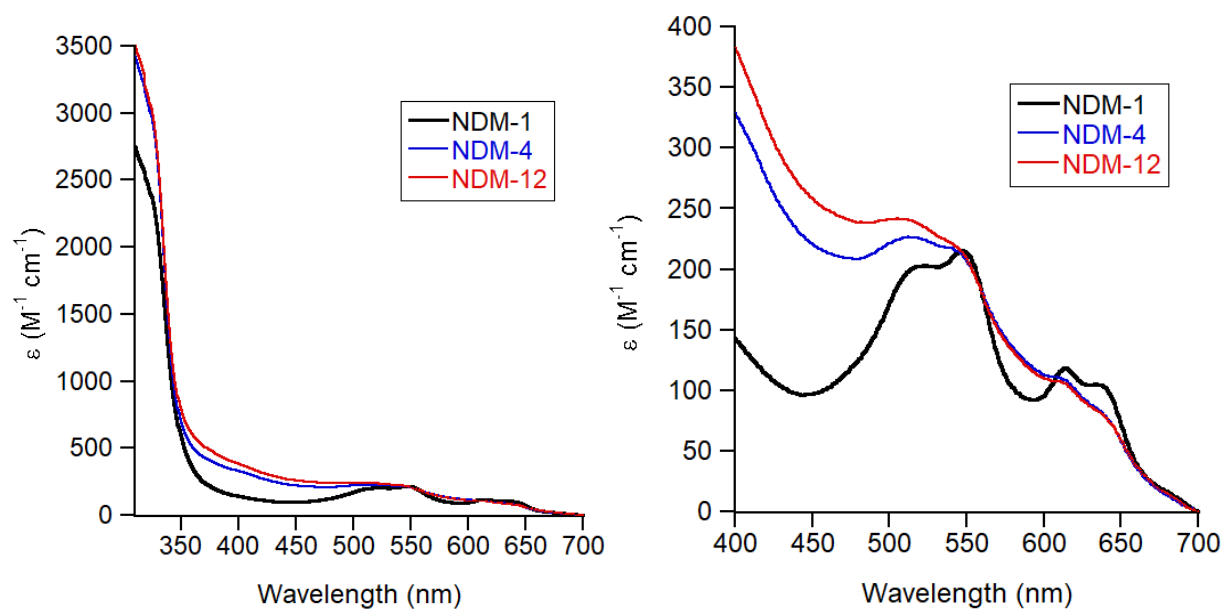


Figure S2. UV-visible spectra of dicobalt(II) NDM variants. (Left) full spectra, (Right), expansion of the ligand-field region. Each spectrum is blanked against buffer.

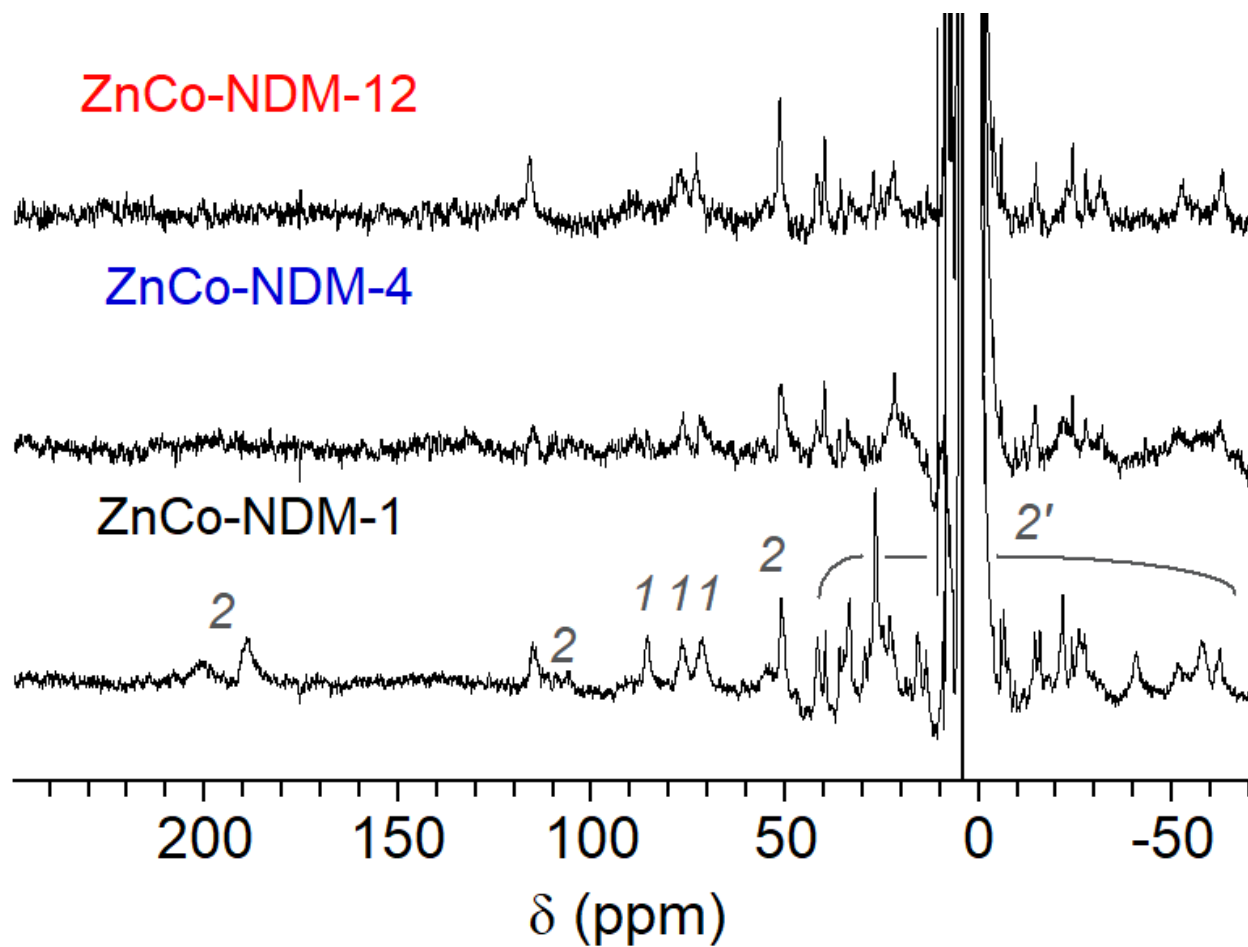


Figure S3. 300 MHz ¹H NMR spectra of the zinc(II)cobalt(II) NDM variants. The labels above the NDM-1 spectrum follow from Figure 7.

Mutagenic Primers Encoding NDM-4 and NDM-12

The template DNA used for preparation of each mutated *bla*_{NDM} gene (*bla*, β -lactamase) is noted and the mutated codons are underlined. Briefly, a plasmid encoding NDM-1 using codons optimized for expression in *E. coli* was the template for construction of NDM-4. The resulting NDM-4 encoding plasmid was used to generate a plasmid encoding NDM-12. These constructs were used to express NDM variants used for kinetic and spectroscopic experiments.

Table S7. Oligonucleotide Sequences Used for Site-Directed Mutagenesis

Construct	Primer Sequences
NDM-4 M154L (template <i>bla</i> _{NDM-1})	F: 5' -GCCCCGCAAGAGGGGCTGGTTGCGGCGCAAC-3' R: 5' -GTTGCGCCGCAACCAGCCCTCTGCGGGGC-3'
NDM-12 M154L, G222D (template <i>bla</i> _{NDM-4})	F: 5' -GCTCGGCAATCTCGATGATGCCGACACTG-3' R: 5' -CAGTGTCCGCATCATCGAGATTGCCGAGC-3'

Mutagenic Primers Encoding Variants at the M154 Position

Table S8. Primer sequences used to generate variants at the NDM154 position for use in MIC determinations. Complimentary sequences are not shown.

Primer	Sequence (5' - 3')
NDM 154S	AAGAGGGGTTCGGTTGCGGCGCAA
NDM 154P	AAGAGGGGCCGGTTGCGGCGCAA
NDM 154T	AAGAGGGGACGGTTGCGGCGCAA
NDM 154A	AAGAGGGGGCGGTTGCGGCGCAA
NDM 154Y	AAGAGGGGTACGTTGCGGCGCAA
NDM 154H	AAGAGGGGCACGTTGCGGCGCAA
NDM 154N	AAGAGGGGAACGTTGCGGCGCAA
NDM 154D	AAGAGGGGGACGTTGCGGCGCAA
NDM 154C	AAGAGGGGTGCGTTGCGGCGCAA
NDM 154R	AAGAGGGGCGCGTTGCGGCGCAA
NDM 154VTG	GCAAGAGGGGVTGGTTGCGGCGCAAC
NDM 154DTC	GCAAGAGGGGDTCGTTGCGGCGCAAC
NDM 154BGG	GCAAGAGGGGBGGTTGCGGCGCAAC
NDM 154VAG	GCAAGAGGGGVAGTTGCGGCGCAAC