Supplementary Materials

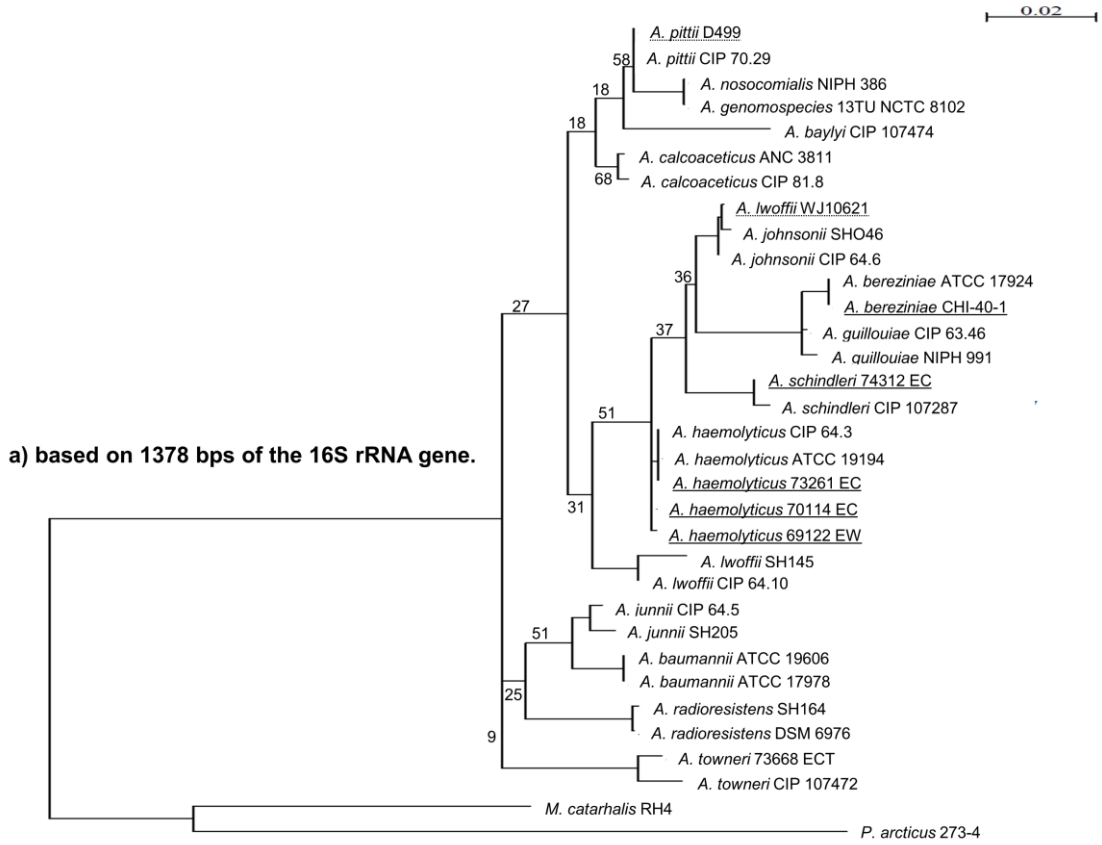
Supplementary Material S1 – 16S rRNA gene and rMLST phylogenetic analysis

Methods

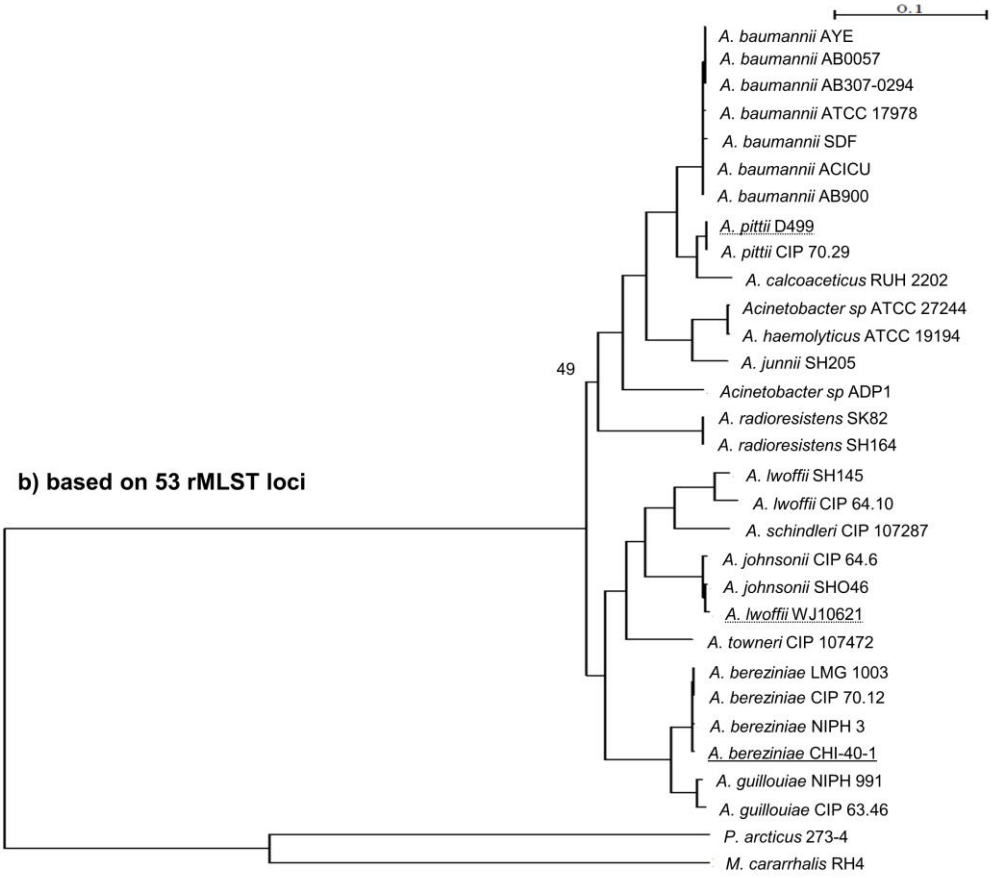
Phylogenetic comparison was performed using 1378 bps of the 16S rRNA gene. For Pakistan isolates, the 16S rRNA gene sequences were obtained by sequencing PCR amplicons and from illumina whole genome sequence (WGS) data for CHI-40-1. CHI-40-1 was also analysed by ribosomal MLST (1). Loci were identified from WGS contigs by comparison with loci from *Acinetobacter johnsonnii* deposited in the rMLST database at http://rmlst.org/. Loci were also identified, extracted and concatenated for the remaining 15 *Acinetobacter* spp. sequences currently in the rMLST database, additional *Acinetobacter* spp. WGS available in GenBank, *Moraxella catarrhalis* RH4 and *Psychrobacter arcticus* 273-4. All phylogenetic trees were built by aligning sequences of interest using the MUSCLE alignment tool(2) at http://www.ebi.ac.uk/Tools/msa/muscle/. Phylogenetic trees were built using phyML 3.0,(3) with 100 replicate bootstraps.

Figure S1 – Phylogenetic trees of *Acinetobacter* spp. a) Based on 1378 bps of the 16S rRNA gene; b) Based on 53 rMLST loci.

Performed with 100 bootstraps. Only bootstrap values of < 70 are shown. Isolates with a solid underline are *bla*$_{\text{NDM-1}}$ *Acinetobacter* spp. characterised for this study. Isolates with a dotted underline are *bla*$_{\text{NDM-1}}$ *Acinetobacter* spp. for which whole genome sequences are publically available from the NCBI database. All other sequences are from *Acinetobacter* spp. isolates which do not harbour *bla*$_{\text{NDM-1}}$ available from NCBI nucleotide, draft genome or complete genome databases or in the rMLST database.



a) based on 1378 bps of the 16S rRNA gene.



b) based on 53 rMLST loci

Supplementary Material S2 – PCR conditions and primers.

PCR conditions

All primers used for PCR and sequencing are listed in Table S2. Standard PCR reactions were performed on a G-Storm GS1 Thermal Cycler (G-storm, Somerton, UK). PCRs used for sequence closure and screening for the presence of pNDM-BJ01-like plasmids were performed using the conditions 95 °C for 5 min, then 35 cycles of 95 °C for 1 min, 55 °C for 1 min and 68 °C for 1 min per kb of expected product size, followed by 68 °C for 5min. All PCRs used as template 1 µl of genomic DNA prepared using the Wizard® Genomic DNA Purification Kit (Promega, Madison USA). PCR mastermixes were composed of 12.5 µL of ReddyMix Extensor PCR Master Mix 1 (Thermo Scientific), 1.25 µL of each primer and 9 µL of molecular grade water. After separation by electrophoresis and ethidium bromide staining, bands were purified using the QIAquick Gel Extraction Kit, (Qiagen, Limburg, Netherlands) as per manufacturer's instructions.

Real-time quantitative PCR

PCRs were optimised for annealing temperature (50-70 °C), $MgCl_2$ concentration (2-5 mM), primer concentration (0.25-0.75 µM) and probe concentration (0.2-0.4 µM). qPCR for *bla*$_{NDM-1}$ and *traA* were run as duplex reactions at 95 °C for 15 min, then 35 cycles of 95 °C for 10 s and 60 °C for 30 s. The qPCR for the *rpoB* references was different for each strain background. For CHI-40-1 and AG3528$_{NDMP1}$ the same primer pair was used (rpoB Ac F1 and rpoB Ac R1) but the probes differed (*rpoB* 40-1 and *rpoB* AG3, respectively). For UAB190$_{NDMP2}$ passaged isolates the primers *rpoB* Ac F3 and *rpoB* Ac R3 were used with probe *rpoB* Ec. All *rpoB* qPCR runs were performed using the conditions 95 °C for 15 min, then 40 cycles of 95 °C for 10 s and 58 °C for 30 s. All qPCR reactions were performed on Rotorgene Q HRM (Qiagen, Manchester, UK) with a final volume of 20 µL, with 2 µL of Lightcycler FastStart DNA Master HybProbe (Roche, Penzberg, Germany) and 5 µL of template. Final concentrations of primers, probes and $MgCl_2$ were as indicated in Table S2.

Table S2 – List of primers and probes used for PCR and sequencing of PCR products

Primer	Sequence	Use	Primer/ probe conc. (μM)	Mg^{2+} conc. (mM)
27F(4)	AGAGTTTTGATCCTGGCTCAG	PCR and sequencing of 16sRNA locus	0.2	2.25
1492R(4)	GGTTACCTTGTTACGACTT	PCR and sequencing of 16sRNA locus	0.2	2.25
800R(5)	CTACCAGGGTATCTAAT	Sequencing 16s rRNA locus	0.2	2.25
ndm-1F(6)	GAAGCTGAGCACCGCATTAG	*bla*$_{NDM-1}$ detection and sequencing	0.2	2.25
ndm-1R(6)	TGCGGGCCGTATGAGTGATT	*bla*$_{NDM-1}$ detection and sequencing	0.2	2.25
aphA6-5F	AATTGGTCAGTCGCCATCGG	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
IS125-5R	TGTGACCACGTCTACGTCTAGC	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
IS125gapF	GCAAAGGCAGAATCAGTGCG	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
NDM_5R	CTCAGCTTCGCGACCGGGTG	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
ndm-p1	CAGTTGCGGAGCTTTGAAGC	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
ndm-3f	GCCATTCCGCCCCCGATAGC	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
trpF-R	CACGGCAAGGCACCGCGATA	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
tat-3R	GGCACCGCACCTCGGTCAAG	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
tat-gapR1	GTACCAGGGCTGCGCCGATG	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
groEL-5F2	GCGCAGGCGATGGACAAGGT	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
groEL-MR	GCCTTCACCGCGCAGACCTT	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
ISCR27-gap2F	GGCAAGGTCGGCGGCTTCTC	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
ISCR27-gap2R	ATTGCGCCACGGCGTCTTGA	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
resF	AAAGACTGCCAAACGCCCTG	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
PN2F(7)	TAGATTCGATTCACGGCATA	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
PN5R(7)	CGTCTTTGTAGCCTTTATCTC	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25
ble3F	CATGGTGGCATTGGTGAACGC	PCR and sequencing *bla*$_{NDM-1}$ context	0.2	2.25

Primer	Sequence	Use	Primer/ probe conc. (μM)	Mg^{2+} conc. (mM)
res3F	TGCAAAACAAATTAACGCCCAGTCTGA	PCR and sequencing bla_{NDM-1} context	0.2	2.25
res-gapR	AGAAGGCGAGGATGAGGGACT	PCR and sequencing bla_{NDM-1} context	0.2	2.25
ISAba7like-FF	GCCAGTAACCATACGTAAAGAAAGACG	PCR and sequencing bla_{NDM-1} context	0.2	2.25
ISAba7like-RR	ATGCAACAAAGCCGTCGGGA	PCR and sequencing bla_{NDM-1} context	0.2	2.25
69122gapF	TGGTGATATAAAACGGCGAATTCAAACA	PCR and sequencing bla_{NDM-1} context	0.2	2.25
45c143R	ACGCTCCGCCATAATCGTTC	PCR and sequencing bla_{NDM-1} context	0.2	2.25
IS125 3R	CGCATGTGCCTTTTTGCCAGGG	PCR and sequencing bla_{NDM-1} context	0.2	2.25
aphA6-3R	TCAGCATTAAAAACCCCGCAAA	PCR and sequencing bla_{NDM-1} context	0.2	2.25
aphA6-5R	AGTCATGATGAGTTCAGGCACC	PCR and sequencing bla_{NDM-1} context	0.2	2.25
5PgapF1	TCAGCACTCAATTCAGCAAGTGT	PCR and sequencing bla_{NDM-1} context	0.2	2.25
5PgapF4	GTTGGTGGGTTGGTGTCTGT	PCR and sequencing bla_{NDM-1} context	0.2	2.25
5PgapF5	TCTGCCCCCATCAAAACGTG	PCR and sequencing bla_{NDM-1} context	0.2	2.25
5PgapR1	TAAACCGCCACCAACCGAAC	PCR and sequencing bla_{NDM-1} context	0.2	2.25
5PgapR3	TGGGACTTTTGGATTTGCGGA	PCR and sequencing bla_{NDM-1} context	0.2	2.25
orfA F	ACTGGGCCGCTTCAACCACA	Gap closure pNDM-40-1	0.2	2.25
p40-1gap1 F	ACGCTTTCCACGTTGCCCTGA	Gap closure pNDM-40-1	0.2	2.25
p40-1gap2F	TGCGGTTCTGCGGTCAGCTC	Gap closure pNDM-40-1	0.2	2.25
p40-1gap3F	TCAGAGCGACACCGCACGAA	Gap closure pNDM-40-1	0.2	2.25
p40-1gap4F	ACGGGGGAGTATGGGAAACT	Gap closure pNDM-40-1	0.2	2.25
p40-1gap5F	CTTGTAGGAATGTTGGCAGGGT	Gap closure pNDM-40-1	0.2	2.25
AphA6gap5R	AGTCATGATGAGTTCAGGCACC	Gap closure pNDM-40-1	0.2	2.25
p40-1gap2R	TTCGTGCGGTGTCGCTCTGA	Gap closure pNDM-40-1	0.2	2.25
p40-1gap3R	TCCCATACTCCCCCGTCATAGC	Gap closure pNDM-40-1	0.2	2.25
p40-1gap5R	AGGTACGCCAACGAAACAGC	Gap closure pNDM-40-1	0.2	2.25
PN11F(7)	AATGTGGTCTGCGGTGTA	Detection of pNDM-BJ01-like plasmids	0.2	2.25
PN11R(7)	GCCTGCTGTAACTTCTCAA	Detection of pNDM-BJ01-like plasmids	0.2	2.25
PN6F(7)	TCAGGATTCACCCACCAT	Detection of pNDM-BJ01-like plasmids	0.2	2.25
PN6R(7)	GGCTCAAGACTACAACGATA	Detection of pNDM-BJ01-like plasmids	0.2	2.25

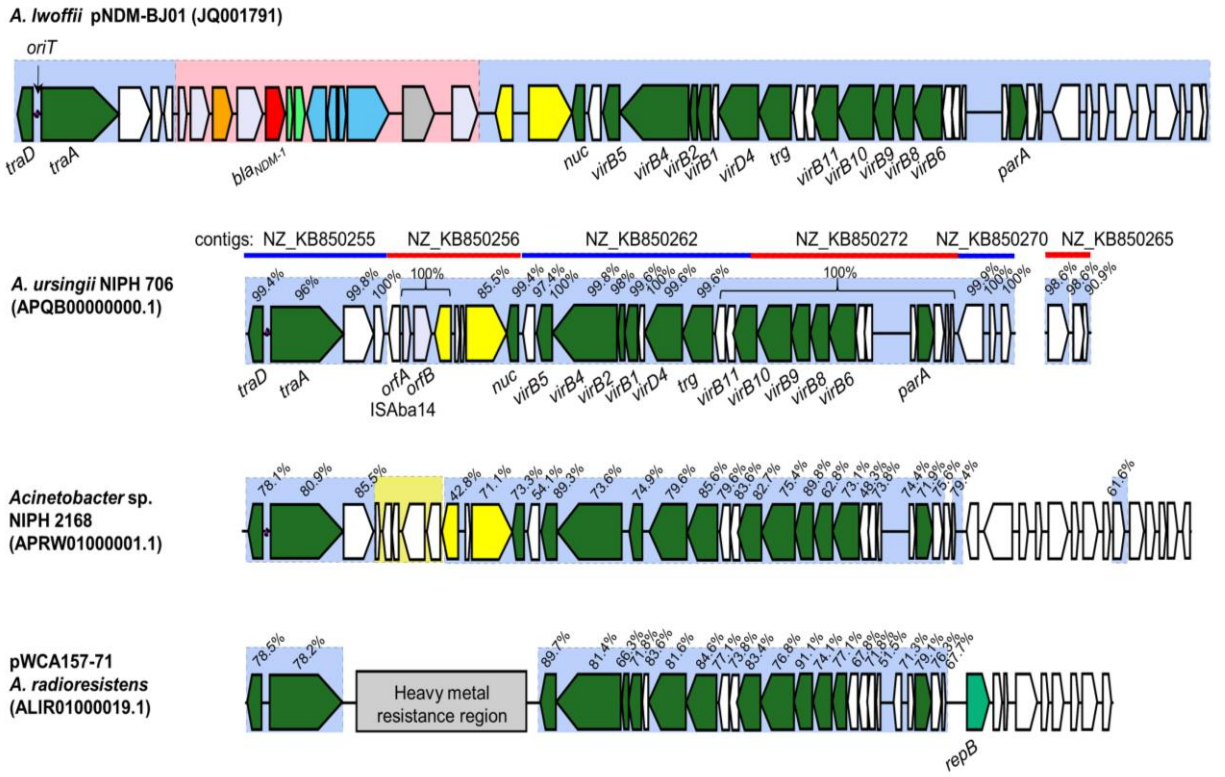
Primer	Sequence	Use	Primer/ probe conc. (μM)	Mg^{2+} conc. (mM)
PN9F(7)	ATCTACGATCTTGCCTTGTT	Detection of pNDM-BJ01-like plasmids	0.2	2.25
PN9R(7)	CTTGTTCTGACGAGCCTAA	Detection of pNDM-BJ01-like plasmids	0.2	2.25
TraA F1	TGGTCAGCAAAACCCGCATGT	TraA quantification by qPCR	0.5	4
TraA R3	GGTTAGCCCATTCTAGGCGGGT	TraA quantification by qPCR	0.5	4
Tra Probe	TCCAGTAAACCCTGAAAAGGGCGGTGCGGGT	TraA quantification by qPCR	0.2	4
NDM RT F1	TGGGTCGAACCAGCAACCGC	NDM quantification by qPCR	0.25	4
ndm RT R1	TGCCGAGCGACTTGGCCTTG	NDM quantification by qPCR	0.25	4
NDM probe	ACCCCGGCCCCGGCCACACCAGT	NDM quantification by qPCR	0.2	4
rpoB Ac RT F1	ATGGCATACTCATATACCGA	*Acinetobacter* rpoB reference for qPCR	0.75 (40-1 probe) 0.5 (AG3 probe)	3 (40-1 probe) 4 (AG3 probe)
rpoB Ac RT R1	TGGAGACCGATATCTTCGCG	*Acinetobacter* rpoB reference for qPCR	0.75 (40-1 probe) 0.5(AG3 probe)	3 (40-1 probe) 4 (AG3 probe)
rpoB 40-1 probe	TGCCCCAAGTCATGCATGCTCCGTACTTGC	*A. bereziniae* rpoB reference for qPCR	0.2	3
RpoB AG3 probe	TGCCCCAAGTAATGGATGCACCGTACTTAC	*A. pittii* rpoB reference for qPCR	0.2	4
rpoB Ec F3	TCCTTTCTATCCAGCTTGACTCGT	*E. coli* rpoB reference for qPCR	0.25	4
rpoB Ec R3	CGCAGTTTAACGCGCAGCGG	*E. coli* rpoB reference for qPCR	0.25	4
RpoB Ec Probe	ACGTCAGCTACCGCCTTGGCGAACCGGTGT	*E. coli* rpoB reference for qPCR	0.2	4

Supplementary Material S3 – pNDM-BJ01-like plasmids and related sequences.
Table S3 – Strain details and sequence differences for fully sequenced or published reports of pNDM-BJ01-like plasmids.

Species/ strain	Plasmid	Accession No.	Backbone compared to pNDM-BJ01	Resistance region compared to pNDM-BJ01	Country of isolation	Travel History	Reference No.
A.lwoffii WJ10621	pNDM-BJ01	JQ001791	NA	NA	China		7
A. bereziniae CHI-40-1	pNDM-40-1	KF702385	Identical	17,688C>T, 17,760T>C in 3' IS*Aba125*, 10,121_11,420del including 3' end of *ble* to 5' end of *tat*, 15,761_15,912del within IS*CR27*.	India		This work
A. calcoaceticus XM1570	pXM1	AMXH01000087	Identical	17,688C>T, 17,760T>C in 3' IS*Aba125*.	China		8
A.lwoffii WJ10659	pNDM-BJ02	JQ060896	Identical	16,859_17,969del including most of 3' IS*Aba125*, excluding only 3' 18 bp.	China		7
A. baumannii GF216	pNDM-AB	KC503911	47,274_1ins – 3,530bp long containing part of *traD*, *insB*, methyltransferase	12,036-18,237 from *cutA* to 3' IS*Aba125* replaced by sequence including *msr*(E) and *mph*(E).	China		9
A. pittii D499	*pAB_D499*	AGFH01000030	32,541T>A in *virB10*, 46,541_46,712del.	8,364A>G in 5' IS*Aba125,* 10,531C>G in *trpF*, 16,866-18,101 containing 3' IS*Aba125*, IS*Aba11*-like insertion 3' end, 18,123T>A.	China		10
A. baumannii ZW85-1	pAbNDM-1	JN377410	Identical	17,132A>G, 17,151T>C, 17,154T>C, 17,340C>A, 17,688C>T, 17,760T>C and 17,984_17,985insCC in 3' IS*Aba125*.	China		-
Acinetobacter sp. M131	pM131_NDM-1	JX072963	47,200T>C in hypothetical protein coding sequence.	16,866-18,101 containing 3' IS*Aba125*, IS*Aba11*-like insertion 3' end.			-
A. lwoffii Iz4b	pNDM-Iz4b	KJ547696	504G>A in *traA*, 31,694T>G in virB11, 40,341_40,342insC, 43,781_44,487del	8,328A>C in 5' IS*Aba125*, 17,688C>T and 17,760T>C in 3' IS*Aba125*.			-
A. soli TCM341	Unnamed (contig 5)	JAPY01000005	20,767G>T and 20,977_21,019del in putative zeta-toxin coding sequence.	8,174A>G in 5' IS*Aba125*, 16,859_17,969del including most of 3' IS*Aba125*, excluding only 3' 18 bp.			-
A. schindleri MRSN 10319	Unnamed	Not applicable	> 99.9% identity	Unclear from report	USA	Afghanistan	11
A. pittii 2012276	Unnamed	Not applicable	Similar based on partial sequencing	Full Tn*125* as in JQ001791	Belgium	India and Egypt	12

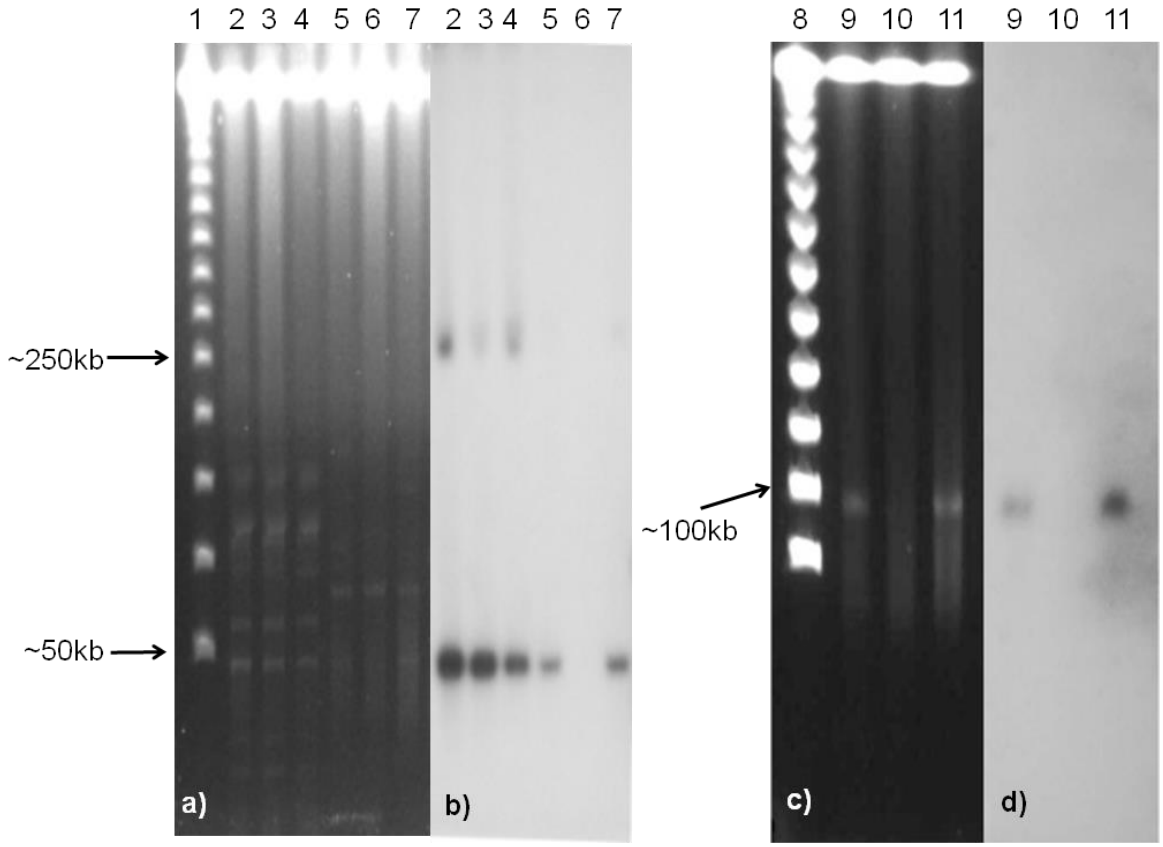
Figure S3 – Gene maps of complete sequence of pNDM-BJ01 and related plasmids.

Colour codes and abbreviations as in main text Figure 2. The putative plasmid replicase, *repB,* in pWCA157-71 is lime green. Dark blue and red lines mark the boundaries between *A. ursingii* NIPH 706 contigs. Percentages above genes represent degree of amino-acid sequence identity of translated protein sequences in pNDM-40-1, based on MUSCLE alignments. Percentages are not shown for pNDM-AB as all amino-acid sequences are 99-100% similar.

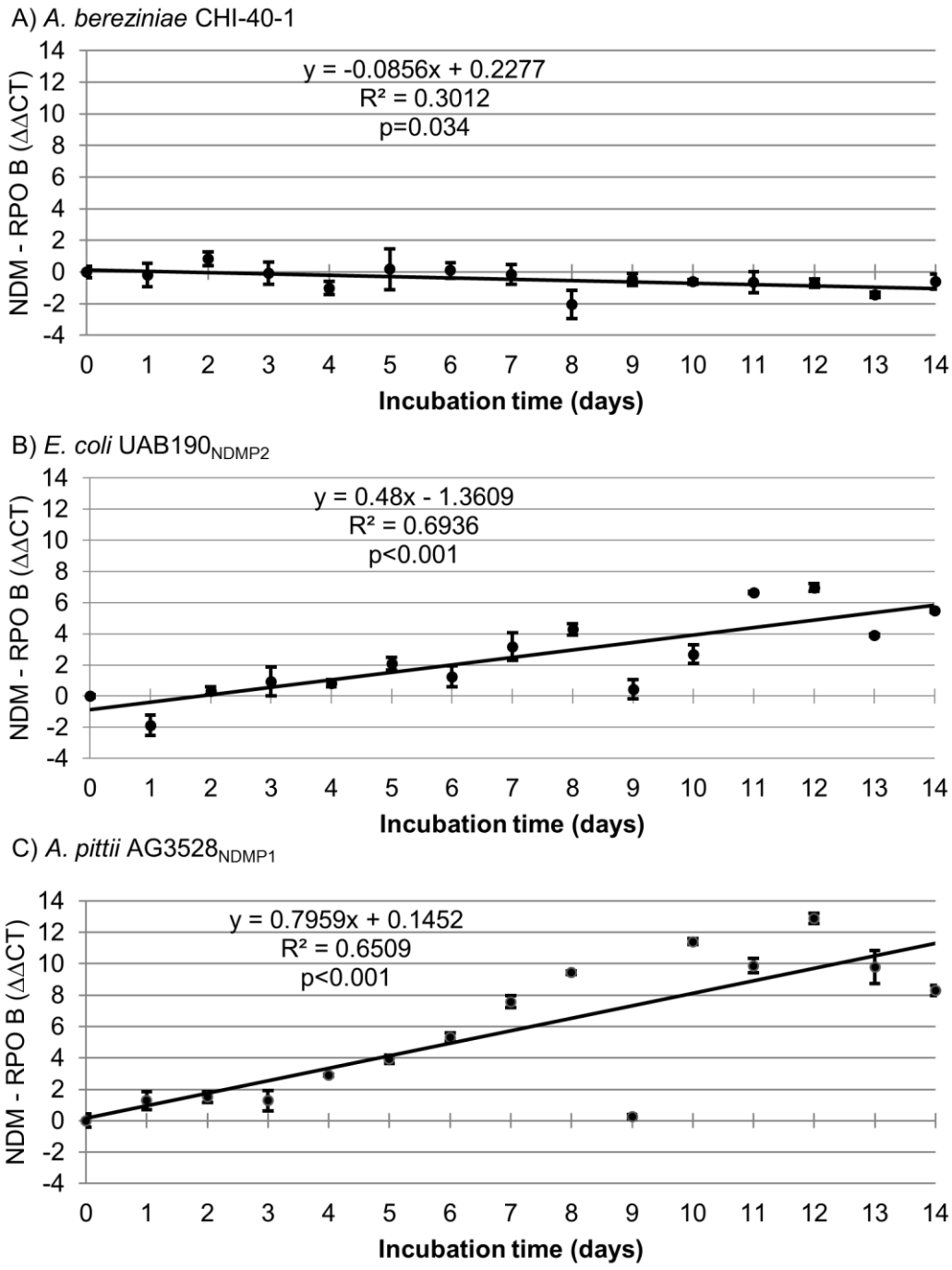


Supplementary Material Figure S4 – Pulsed field gels of S1 digested genomic DNA from passaged isolates and in gel hybridisation with *bla*$_{NDM-1}$ gene probe. a) Pulsed field gel of CHI-40-1 and AG3528$_{NDMP1}$ at start of passage (D0) and after 14 day passage without antibiotics (D14N) and with meropenem (D14M); b) in gel hybridisation of a); c) Pulsed field gel of UAB190$_{NDMP2}$ at D0, D14N and D14M; d) in gel hybridisation of b).

1 - λ concatamer (~50-1000kb); 2 – CHI-40-1 D0; 3 – CHI-40-1 D14N; 4 – CHI-40-1 D14M; 5 - AG3528$_{NDMP1}$ D0; 6 - AG3528$_{NDMP1}$ D14N; 7 - AG3528$_{NDMP1}$ D14M; 8 – λ; 9 – UAB190$_{NDMP2}$ D0; 10 – UAB190$_{NDMP2}$ D14N; 11 – UAB190$_{DMP2}$ D14M



Supplementary Material Figure S5 – Estimated quantity of *bla*$_{NDM-1}$ gene present relative to *rpoB* gene over the course of the passage experiment with meropenem selection versus no antibiotic selection by ΔΔCT method. Results are shown for a) the *bla*$_{NDM-1}$ positive donor strain CHI-40-1 and transconjugants b) UAB190$_{NDMP2}$ and c) AG3528$_{NDMP1}$. Note that a positive slope indicates a fall in quantity of *bla*$_{NDM-1}$ gene detected relative to reference in the absence of antibiotic selection. Results based on means of three replicate real time PCRs, error bars show 2 standard deviations.



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