Supplemental Material

Rapid Detection of New Delhi Metallo-β-Lactamase Gene and Variants coding Carbapenemase with Different Activity by a PCR-based *in vitro* protein expression method

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Supplementary Materials and Methods

Clonal relationship of 15 *A. baumannii* isolates

To test the cloneship between the 15 *A. baumannii* isolates, a multilocus sequence typing (MLST) method was used to determine the sequence types (STs) the *A. baumannii* isolates as described. The seven housekeeping genes (*gltA*, *gyrB*, *gdhB*, *RecA*, *cpn60*, *gpi*, *rpoD*) were sequenced by BGI Tech Inc. (Wuhan, China) and analyzed using the *A. baumannii* MLST database (http://pubmlst.org/abaumannii/). As shown in supplementary Table S2, 8 different STs and three new STs (42, 65, 69 ABA) were found in the 15 *A. baumannii* isolates. The results were also analyzed by

eBURST (http://eburst.mlst.net/default.asp). Using group defined by 6 or more loci matches, eBURST results showed that there were 2 groups and 3 singletons in the 8 STs. Compared with all the STs in the database, the ST208, ST368 and ST368 belong to group 1, in which the predicted founder was ST118 isolated from Thailand.

Preparation of DNA templates of clinical isolates by heat lysis.

Briefly, 1.4 mL bacteria culture (with McFarland between 3 to 4) were centrifuged first. Then the precipitates were re-suspended in 0.1 mL sterile milliQ water, and finally inactivated at 100 °C for 10 min.

Long fragment real-time PCR (LF-qPCR) assay.

The whole $bla_{\text{NDM-1}}$ fragments (822 bp) were amplified by LF-qPCR with primers F-rt and R-rt designed in house and shown in Table S2. The LF-qPCR was performed on a StepOnePlus™ qPCR System (Applied Biosystems Inc., USA) under the following conditions: 2 min at 98 °C and 35 cycles of 10 s at 98 °C and 75 s at 68 °C. The LF-qPCR mixture contained 10 μL MightyAmp™ (SYBR® Plus, Takara Biotechnology Co. Ltd, China), 0.2 μL primer F-rt (20 μmol/L), 0.2 μL primer R-rt (20 μmol/L), 4 μL DNA template and 5.6 μL sterile milliQ water. The threshold cycle (C_T) values were calculated by the StepOne™ Software v2.2.2.

The analytical sensitivity of the LF-qPCR for $bla_{\text{NDM-1}}$ contained plasmids were determined using the plasmid solution extracted from *A. baumanii* 65. The qPCR sensitivity for bacteria suspensions was also determined by spiking different amounts

of *A. baumanii* 65 into water. To quantify the viable bacteria in each dilution step, a nutrient agar plate was inoculated with 100 μL of a suspension and incubated overnight at 37 °C. The number of colonies that grew was counted on the following day. All experiments were repeated three times to determine the sensitivity and the linearity of LF-qPCR.

PCR amplification for *in vitro* expression (ePCR).

The ePCR is performed to add T7 promoter and 5' untranslated region (5' UTR) for *in vitro* expression using the primers F-1-UTR and R-1 shown in Table S2. The ePCR amplifications were conducted with an initial 30 s denaturation step at 98 °C, 37 cycles of 98 °C for 30 s, 70 °C for 1 min, and 72 °C for 30 s, and a final 10 min elongation on a thermal cycler (Biometra, Germany). Each reaction tube contained 0.6 U of Phusion®High-Fidelity DNA Polymerase (NEB, UK), 10 μL 5×GC buffer, 200 μmol/L of each dNTP, 20 μmol/L of each primers, 4 μL Dimethyl-sulfoxide and 3 μL DNA template. The amplified products were analyzed by electrophoresis using 1.5% agarose gel with ethidium bromide.

***In vitro* synthesis of NDM-1**

The amplified $bla_{\text{NDM-1}}$ DNA in the ePCR were concentrated by adding 3M CH_3COONa (pH 5.2) according to a volume ratio 1:10 and 4 μL DNAmate (Takara Biotechnology Co. Ltd, Dalian, China) first, and then by the ethanol precipitation. The concentration and the quality of the concentrated DNAs were evaluated by a

Nanodrop 2000 spectrophotometer (Thermo, USA). The *in vitro* synthesis of NDM-1 was carried out at 24 °C for 20 h using RTS^{TM} 100 Wheat Germ CECF Kit (5 Prime Inc., USA) following the procedures suggested by the manufacturer. An equal amount of the Wheat Germ lysate without adding the ePCR amplicon was also incubated simultaneously as the negative control.

Antibiotic degradation assay

After *in vitro* synthesis of NDM-1, 3 μL of the lysates were mixed with 297 μL of 50 mmol/L HEPES buffer (containing 100 μmol/L imipenem and 1 μmol/L $ZnSO_4$, pH 7.5) at 30 °C, respectively. Measurements of NDM-1 activities were performed on a Synergy H1 Hybrid Reader (BioTek, USA) by observing the decrease in absorption at 300 nm. The inhibition assays were performed by pre-incubating the lysates with EDTA for 2 min at the 25 °C before addition of the imipenem contained HEPES buffer, as described previously. All experiments were repeated three times

Tables

TABLE S1 Information of clinical samples.

Sample no.	Strain	Isolated from	Isolated time
Negative control	*E. coli* 53	urine	2009/5/3
Positive control	*A. baumannii* 65	sputum	2010/7/20
1	*E. coli* 23	urine	2008/3/5
2	*E. coli* 59	ascites	2009/8/1
3	*E. coli* 75	urine	2008/12/12
4	*K. pneumoniae* 17	sputum	2011/11/17
5	*K. pneumoniae* 19	sputum	2009/2/24
6	*K. pneumoniae* 76	-	2007
7	*E. cloacae* 60	sputum	2009/1/12
8	*E. cloacae* 63	sputum	2009/3/4
9	*K. ozaenae* 16	urine	2008/12/12
10	*A. baumannii* 26	sputum	2009/10/22
11	*A. baumannii* 30	sputum	2009/2/2
12	*A. baumannii* 31	sputum	2011/11/17
13	*A. baumannii* 33	sputum	2009/4/29
14	*A. baumannii* 39	sputum	2009/7/29
15	*A. baumannii* 40	trachea	2010/3/9
16	*A. baumannii* 41	sputum	2010/3/1
17	*A. baumannii* 42	trachea	2010/6/3
18	*A. baumannii* 59	sputum	2009/12/1
19	*A. baumannii* 62	sputum	2010/1/20
20	*A. baumannii* 63	trachea	2010/1/20
21	*A. baumannii* 66	sputum	2010/9/8
22	*A. baumannii* 69	ascites	2010/6/14
23	*A. baumannii* 70	sputum	2008/8/2

86 **TABLE S2** Results of MLST.

Strain no.	Allele no.							ST
	gtlA	*gyrB*	*gdhB*	*recA*	*cpn60*	*gpi*	*rpoD*	
26	21	15	3	2	35	111	4	254
30	21	15	3	2	35	111	4	254
31	21	15	3	2	35	111	4	254
33	21	15	3	2	35	111	4	254
39	21	15	3	2	35	111	4	254
40	21	15	3	2	35	111	4	254
41	21	15	3	2	35	111	4	254
42	21	15	3	49	35	111	4	new
59	1	3	3	2	2	97	3	208
62	1	3	3	2	2	97	3	208
63	34	81	3	2	2	16	3	381
65	9	3	34	2	39	125	20	new
66	1	3	3	2	2	140	3	368
69	1	15	3	7	39	111	22	new
70	1	15	2	28	1	107	32	229

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TABLE S3 Primer sequences used for amplification of the *bla*$_{NDM-1}$ gene and construction of *bla*$_{NDM-1}$ variants.

	Primers	Sequence (5'- 3')
LF-qPCR	F-rt	AAACTTGATGGAATTGCCCA
	R-rt	GCTCAGCGCAGCTTGTC
ePCR	F-rt-UTR[a]	TAATACGACTCACTATAGG**ATACTCCCCCACAACAGCTTA CAATACTCCCCCACACAGCTTACAAATACTCCCCC**AAA CTTGATGGAATTGCCCA
	F-1-UTR[a]	TAATACGACTCACTATAGG**ATACTCCCCCACAACAGCTTA CAATACTCCCCCACACAGCTTACAAATACTCCCCC**ATGG AATTGCCCAATATTATGCA
	R-1	TCAGCGCAGCTTGTCGGCCATGC
PCR[b]	F-38	GGCGGAATGGCTCATCACGA
	R-344	CGCAACACAGCCTGACTTTC
	Pre-A	CACCTCATGTTTGAATTCGCC
	Pre-B	CTCTGTCACATCGAAATCGC
Variants	F-K211E	CTGTCCTCGATCAGGCAGCCAC
	R-K211E	CTGCCTGATCGAGGACAGCAAG
	F-P28A	CTGAGCGGGTGCATGGCCGGTGAAATCCGCC
	R-P28A	GGCGGATTTCACCGGCCATGCACCCGCTCAG
	F-D95N	TCGATACCGCCTGGACCAATGACCAGACCGCCCAGA
	R-D95N	TCTGGGCGGTCTGGTCATTGGTCCAGGCGGTATCGA
	F-V88L	GCGGCCGCGTGCTGTTGGTCGATACCGCCTG
	R-V88L	CAGGCGGTATCGACCAACAGCACGCGGCCGC
	F-A233V	ACTACGCCGCGTCAGTGCGCGCGTTTGGTGC
	R-A233V	GCACCAAACGCGCGCACTGACGCGGCGTAGT
	F-M154L	GGGCTGGTTGCGGCGCAA
	R-M154L	CCGCAACCAGCCCCTCTT

Abbreviations: 5' UTR, 5' untranslated regulatory regions that could enhance protein expression in the wheat germ system.

[a] The 5' UTR sequence is shown in boldface type.

[b] PCR here means the conventional PCR to test *bla*$_{NDM-1}$ gene.

Figure legends

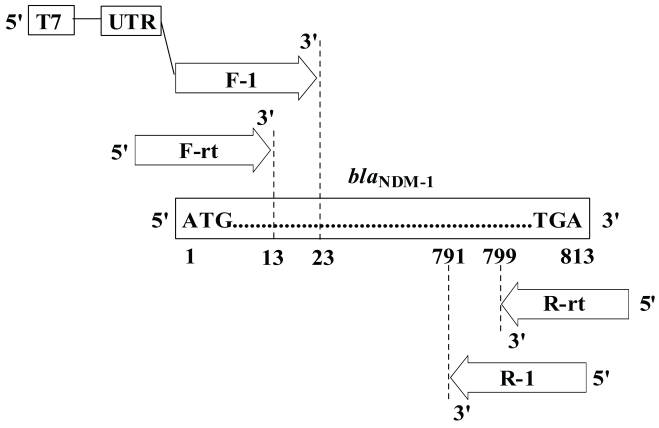

FIG S1 Positions of the PCR primers used. The frame was the open reading frame (ORF) of *bla*$_{\text{NDM-1}}$.

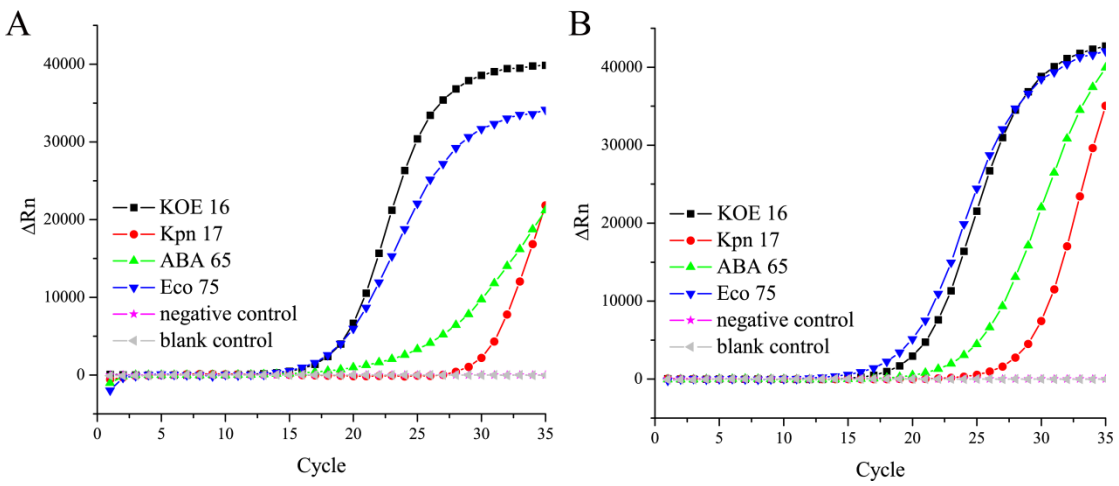

FIG S2 Assay of the clinical samples. (A) Typical amplification curves of *bla*$_{\text{NDM-1}}$ LF-qPCR assay using the plasmid templates extracted from four positive samples. (B) Typical amplification curves of *bla*$_{\text{NDM-1}}$ LF-qPCR assay using the templates prepared by boiling the four positive samples.