

# Multidrug-resistant *Acinetobacter baumannii* strains with NDM-1: Molecular characterization and *in vitro* efficacy of meropenem-based combinations

JINGJING WANG<sup>1\*</sup>, YONGZHONG NING<sup>2\*</sup>, SHU LI<sup>3</sup>, YUN WANG<sup>3</sup>, JINHUA LIANG<sup>3</sup>,  
CHUNMING JIN<sup>3</sup>, HAIRUN YAN<sup>3</sup> and YONGCUN HUANG<sup>4</sup>

<sup>1</sup>Department of Laboratory Medicine, The Second Affiliated Hospital of Henan University of Science and Technology, Luoyang, Henan 471000; <sup>2</sup>Department of Laboratory Medicine, Beijing ChuiYangLiu Hospital Affiliated to Tsinghua University, Beijing 100022; <sup>3</sup>Department of Laboratory Medicine, Hongqi Hospital Affiliated to Mudanjiang Medical University, Mudanjiang, Heilongjiang 157011; <sup>4</sup>Department of Laboratory Medicine, The Second Affiliated Hospital of Mudanjiang Medical College, Mudanjiang, Heilongjiang 157001, P.R. China

Received July 11, 2018; Accepted December 31, 2018

DOI: 10.3892/etm.2019.7927

**Abstract.** *Acinetobacter baumannii* is an important cause of hospital-acquired, multidrug-resistant (MDR) infections occurring worldwide. Anti-microbial combination regimens may be the only feasible treatment option for affected patients. In the present study, the efficacy of the combined therapy of meropenem with colistin, ampicillin-sulbactam, tazobactam and vancomycin against clinical strains of MDR *A. baumannii* was determined. Anti-microbial susceptibility testing was performed and resistance genes were characterized by a multiplex polymerase chain reaction (PCR)-reverse line blot assay. The genetic background of New Delhi metallo- $\beta$ -lactamase 1 (NDM-1) was analysed by primer walking. The presence of NDM-1 was detected using the modified Hodge test and the EDTA-combined disk test. To screen for synergistic drug effects, the fractional inhibitory concentration index was calculated using a checkerboard assay. The results of the PCR as well as the sequence analyses suggested that NDM-1 was located downstream of the ISAbal25 element. In addition, a synergistic effect was determined for meropenem + vancomycin, meropenem + tazobactam and meropenem + ampicillin + sulbactam in two strains each, and in four strains for meropenem + colistin. A total of five

*A. baumannii* strains with resistance to numerous antibiotics and carrying numerous resistance genes were identified. In the strains of *A. baumannii*, the NDM-1 gene was integrated in a transposon structure with a copy of the ISAbal25 insertion sequence. However, the genetic background was not identical among the different species and strains. The genetic variability of NDM-1 may facilitate the rapid dissemination of this gene. In conclusion, meropenem may enhance the efficacy of antibiotics in *A. baumannii* strains with NDM-1-associated MDR.

## Introduction

*Acinetobacter baumannii* is a significant infectious microbial factor in hospitalized patients throughout the world, and the associated mortality and morbidity have been increasing (1). It is an opportunistic bacterial pathogen, with a major involvement in sepsis, pneumonia, urinary tract infection and primary bacteremia. According to the US National Healthcare Safety Network surveillance data for 2009-2010, *A. baumannii* caused 1.8% of all healthcare-associated infections (2). Isolates of the strains of *A. baumannii* with extensive multi-drug resistance (MDR) have raised significant concern. Carbapenem- and colistin-resistant *A. baumannii* strains are considered an emerging, serious public health problem (3). The emergence of carbapenem resistance genes and  $\beta$ -lactamase gene in *A. baumannii* has arisen as a significant public health concern (4). The carbapenem-resistant attribute of *A. baumannii* is principally associated with OXA-type  $\beta$ -lactamases, particularly OXA-23. The development of antibiotic resistance of this species has been associated with the emergence of New Delhi metallo- $\beta$ -lactamase 1 (NDM-1), the potent carbapenem resistance gene (5). Several distinct species of *Enterobacteriaceae* have been reported to harbour NDM-1. Studies have reported on *A. baumannii* with NDM-1 from environmental and clinical isolates in various countries (6,7).

Meropenem is a carbapenem antibiotic with the ability to bind to penicillin-binding proteins and inhibit  $\beta$ -lactamase

**Correspondence to:** Dr Jinhua Liang, Department of Laboratory Medicine, Hongqi Hospital Affiliated to Mudanjiang Medical University, Mudanjiang, Heilongjiang 157011, 5 Tongxiang Road, Aimin, Mudanjiang, Heilongjiang 157011, P.R. China  
E-mail: liangjhhmmmc@163.com

\*Contributed equally

**Key words:** *Acinetobacter baumannii*, New Delhi metallo- $\beta$ -lactamase 1, multidrug-resistant, metallo- $\beta$ -lactamase, antimicrobial effect

with the broadest spectrum of activity among  $\beta$ -lactam antibiotics (8). Vancomycin is a glycopeptide antibiotic that is an inhibitor of bacterial peptidoglycan synthesis (9). Colistin is a cationic polypeptide antibiotic with the ability to increase the permeability of the cell membrane, ultimately leading to bacterial death. Tazobactam and sulbactam only inhibit selected class A enzymes, excluding *Klebsiella pneumoniae* carbapenemase (KPC)-type carbapenemases, generally have a minimal effect on AmpC  $\beta$ -lactamases, and have been reported to exhibit the intrinsic anti-bacterial activity on strains of *Acinetobacter* at concentrations attainable in the human body (5.5–51 and ~40 mg/l, respectively) (10–12).

In another study, the reintroduction of colistin, which is the only remaining active microbial factor with an *in vitro* anti-bacterial effect on MDR Gram-negative bacteria, has been reported for treating carbapenem-resistant strains of *A. baumannii* (13). The colistin resistance of *A. baumannii* strains is well-documented (3), and it is based on the following two mechanisms: Modification of lipid A and lipopolysaccharide loss (14,15). Vancomycin is an inhibitor of bacterial peptidoglycan synthesis, which lacks activity against Gram-negative bacteria due to its large size and hydrophobicity. When colistin was combined with vancomycin, a molecule that should ordinarily have no effect on Gram-negative organisms, due to the relative impermeability of the outer membrane to such a large hydrophobic molecule, a synergistic effect is achieved, and this treatment may become the most common therapy for MDR *A. baumannii* infections in the future (16).

The present study explored the genetic environment of clinical MDR strains of *A. baumannii* carrying the NDM-1 gene, and determined the *in vitro* effects of meropenem in conjunction with colistin, ampicillin-sulbactam, tazobactam as well as vancomycin.

## Materials and methods

**Bacterial strains and growth conditions.** Strains of *A. baumannii* (n=264) were selected from the pool of clinical isolates from the North of China that were obtained between June 2000 and August 2015. The isolates were obtained from urine (n=55), blood (n=73), bronchoalveolar lavage fluid (n=72), abdominal samples (n=18), cerebrospinal fluid (CSF) (n=29), catheter tips (n=8) and stool specimens (n=9) at hospitals including the Second Affiliated Hospital of Henan University of Science and Technology (Luoyang, China), the Beijing ChuiYangLiu Hospital affiliated to Tsinghua University (Beijing, China), Hongqi Hospital of Mudanjiang Medical College (Mudanjiang, China) and the Second Affiliated Hospital of Mudanjiang Medical College (Mudanjiang, China). Standard strains of *A. baumannii* (American Type Culture Collection (ATCC) 19606; ATCC, Manassas, VA, USA) and *Escherichia coli* (ATCC 25922; ATCC) were used in each run as the control. *A. baumannii* strains were identified by the use of a Vitek 2 system (BioMérieux Inc., Marcy-l'Étoile, France). The isolates were stored at -80°C. Mueller-Hinton broth II (MHB II; Difco Laboratories; BD Biosciences, San Jose, CA, USA) was used for all *in vitro* experiments. The strains were grown at 37°C with 5% CO<sub>2</sub>.

**Determination of anti-microbial susceptibility.** Antibiotic susceptibility was measured with the use of the standard disc diffusion process suggested by the Clinical and Laboratory Standards Institute (CLSI) guidelines by broth microdilution and E-test (cat. nos. 537300, 501800, 533500, 501300, 501600, 506710, 513800, 526000, 523600, 525508, 522000, 503500 and 521400; AB Biodisk; BioMérieux Inc.) (17). A total of 14 antibiotics were tested, including ampicillin-sulbactam, trimethoprim-sulfamethoxazole, aminoglycoside antibiotic amikacin, macrolide antibiotic azithromycin,  $\beta$ -lactam antibiotic aztreonam,  $\beta$ -lactamase inhibitor tazobactam, cephalosporin antibiotics ceftazidime and cephalothin, rifampin and tigecycline, carbapenem antibiotic meropenem and colistin, and the glycopeptide antibiotics teicoplanin and vancomycin (Bio-Rad, Laboratories, Inc., Hercules, CA, USA). These antibiotics are of different classes and have different killing mechanisms on the bacteria. The E-test technique was used to determine the minimum inhibitory concentrations (MICs) of meropenem. The results were interpreted according to the CLSI guidelines from 2015 (18).

**Detection of antibiotic resistance genes.** With reference to previous studies, a multiplex polymerase chain reaction (PCR)-reverse line blot assay was employed in the present study for detecting various clinically significant antibiotic resistance genes [NDM-1, VIM, IMP, SHV-5/12-like, VEB, KPC, OXA-10-like, CTX-M, TEM, OXA-23-like, OXA-30-like, DHA, CMY-2-like, armA, rmtC, aac(3)-IIc, aadB, aacC1, aac(6')-Ib-cr, qnrA, qnrB and qnrS] (17,19). The primers are provided in Table I. PCR and genome walking were used to extend uncharacterized flanking regions of the NDM-1 gene (20–23).

**Molecular typing by multilocus sequence typing (MLST).** Strains were identified at the species level using the Vitek 2 system (BioMérieux) and then confirmed by 16S ribosomal DNA sequencing. Housekeeping genes (cpn60, gltA, gdhB, gpi, gyrB, recA and rpoD) were also detected by MLST and then sequenced (24). Tools obtained from the *A. baumannii* MLST database (<http://pubmlst.org/abaumannii/>) were employed to assign the isolates to sequence types (STs).

## Phenotypic detection of NDM-1 production

**Modified Hodge test.** In the present study, the modified Hodge test for *Enterobacteriaceae* was performed according to the CLSI guidelines from 2015 (18). In brief, a 0.5 McFarland standard *E. coli* ATCC 25922 suspension was diluted at 1:10 and then spread onto Mueller-Hinton agar plates. Ertapenem (10  $\mu$ g), imipenem (10  $\mu$ g), and meropenem (10  $\mu$ g) disks were placed. Subsequently, with the use of a sterile wire loop, 3–5 colonies of the isolated strain were inoculated in a straight line out from the rim on the same plate. *K. pneumoniae* ATCC 1705 was used as the Mueller-Hinton Test (MHT)-positive quality control (QC) organism and *K. pneumoniae* ATCC 1706 as the MHT-negative QC organism. The plates were then incubated for 16 to 20 h at a temperature of 37°C in ambient air (25). Those isolates with an intermediate or susceptible zone, i.e. 16–21 mm on disc diffusion, were considered to be positive for carbapenemase production.

Table I. Primers used for the amplification of selected carbapenemase genes.

Gene	Primer name	Sequence	Fragment size (bp)
NDM-1	NDM-1 F	5'-ATGGAATTGCCCAATATTATGCACCCGG-3'	813
	NDM-1 R	5'-TCAGCGCAGCTTGTTCGGCCATG-3'	
VIM-1 and -2	VIM F	GATGGTGTGTTGGTCGCATA	390
	VIM R	CGAATGCGCAGCACCAG	
IMP	MultiIMP F	5'-TTGACACTCCATTTACDG-3'	139
	MultiIMP R	5'-GATYGAGAATTAAGCCACYCT-3'	
SHV-5/12-like	SHV F	5'-GCCTTTATCGGCCCTCACTCAAG-3'	897
	SHV R	5'-TTAGCGTTGCCAGTGCTCGATCA-3'	
VEB	VEB F	CATTTCCCGATGCAAAGCGT	648
	VEB R	CGAAGTTTCTTTGGACTCTG	
KPC	KPC F	5'-TGTCAGTGTATCGCCGTC-3'	1010
	KPC R	5'-CTCAGTGCTCTACAGAAAACC-3'	
OXA-10-like	OXA-10 F	5'-CCACCAAGAAGGTGCCATGA-3'	835
	OXA-10 R	5'-GCGACCTTGAGCGACTTGTT-3'	
CTX-M	CTX-M Gp1 F	5'-TTAGGAARTGTGCCGCTGYA-3'	688
	CTX-M Gp1 R	5'-CGATATCGTTGGTGGTRCCAT-3'	
TEM	TEM F	5'-ATAAAATTCTTGAAGACGAAA-3'	1079
	TEM R	5'-GACAGTTAGCAATGCTTAATCA-3'	
OXA-23-like	OXA-23 F	5'-GATGTGTCATAGTATTCGTCG-3'	1067
	OXA-23 R	5'-TCACAACAATAAAAAGCACTG-3'	
OXA-30-like	OXA-30 F	5'-GGCACCAGATTCAACTTTCAAG-3'	564
	OXA-30 R	5'-GACCCCAAGTTTCTCTGTAAGTG-3'	
DHA	DHA F	5'-AACTTTTACAGGTGTGCTGGGT-3'	405
	DHA R	5'-CCGTACGCATACTGGCTTTGC-3'	
CMY-2-like	CMY-2 F	5'-GCTGAGAGCTCATGATGAAAAAATCG-3'	1146
	CMY-2 R	5'-GGTACGGATCCTTATTGCAGC-3'	
<i>armA</i>	<i>armA</i> F	5'-ATTCTGCCTATCCTAATTGG-3'	315
	<i>armA</i> R	5'-ACCTATACTTTATCGTCGTC-3'	
<i>rmtC</i>	<i>rmtC</i> F	5'-CGAAGAAGTAACAGCCAAAG-3'	711
	<i>rmtC</i> R	5'-ATCCCAACATCTCTCCCACT-3'	
<i>aac(3)-IIc</i>	<i>aac(3)-IIc</i> F	5'-ACGCGGAAGGCAATAACGGA-3'	854
	<i>aac(3)-IIc</i> R	5'-TAACCTGAAGGCTCGCAAGA-3'	
<i>aac(6')-Ib-cr</i>	<i>aac(6')-Ib-cr</i> F	5'-TTGCGATGCTCTATGAGTGGCTA-3'	482
	<i>aac(6')-Ib-cr</i> R	5'-CTCGAATGCCTGGCGTGTGTT-3'	
<i>aadB</i>		5'-AACGCAGGTCACATTGATACA-3'	266
		5'-ACCAAGCAGGTTTCGCAGTC-3'	
<i>aacC1</i>	<i>aacC1</i> F	5'-CACCTACTCCCAACATCAGC-3'	329
	<i>aacC1</i> R	5'-CTTCCCGTATGCCCAACT-3'	
<i>qnrA</i>	<i>qnrA</i> F	5'-ATTTCTCACGCCAGGATTTG-3'	
	<i>qnrA</i> R	5'-GATCGGCAAAGGTTAGGTCA-3'	516
<i>qnrB</i>	<i>qnrB</i> F	5'-ACGATGCCTGGTAGTTGTCC-3'	469
	<i>qnrB</i> R	5'-GATCGTGAAAGCCAGAAAGG-3'	
<i>qnrS</i>	<i>qnrS</i> F	5'-ACGACATTCGTCAACTGCAA-3'	417
	<i>qnrS</i> R	5'-TAAATTGGCACCCCTGTAGGC-3'	

F, forward; R, reverse; NDM-1, New Delhi metallo-β-lactamase 1; *bla*, β-lactamase.

**Combined disk test (CDT) with EDTA.** To detect NDM-1, the EDTA-CDT was performed using disks with meropenem (10 µg) and imipenem (10 µg), utilizing 750 µg EDTA (26-28). The inhibition zone on the disk containing one antibiotic was compared

with that on the disk with the combination of one antibiotic and EDTA. If the increase in the inhibition zone on the combined disk was >7 mm compared with that on the disc with imipenem alone, the strain was considered metallo-beta-lactamase-positive.

Table II. MIC values of NDM-1-producing and colistin-resistant *A. baumannii* strains.

Isolate	Resistance genes	MLST	Sample type	MIC (μg/ml)													
				SAM, ampicillin/sulbactam					SXT, trimethoprim/sulfamethoxazole								
				TZP	CST	ampicillin/sulbactam	TGC	AMK	AZM	ATM	CAZ	MEM	RIF	trimethoprim/sulfamethoxazole	VAN	TEC	CEP
1	<i>blaNDM-1</i> , <i>VIM-16</i> , <i>blaKPC</i> , <i>aadB</i> , <i>blaOXA-23</i>	ST191	Blood	8	8	64/32	8	256	128	64	64	128	4	64/1216	64	128	1024
2	<i>blaNDM-1</i> , <i>blaCTX-M</i> , <i>aacCI</i>	ST191	Blood	128	8	32/16	8	128	64	64	64	32	4	32/1216	32	128	512
3	<i>blaNDM-1</i> , <i>blaCMY-2-like</i> , <i>aadB</i>	ST357	Cerebrospinal fluid	32	4	128/64	4	32	64	128	32	64	8	64/608	32	256	512
4	<i>blaNDM-1</i> , <i>blaOXA-23</i> , <i>blaCTX-M</i> , <i>aac(6')-Ib-cr</i>	ST357	Urine	32	8	64/32	16	32	32	32	128	32	8	32/608	64	512	256
5	<i>blaNDM-1</i> , <i>blaOXA-10</i> , <i>blaCMY-2-like</i> , <i>aac(6')-Ib-cr</i>	ST191	Cerebrospinal fluid	16	8	32/16	32	128	64	32	64	128	4	128/608	128	256	1024

MIC, minimum inhibitory concentration; CST, colistin; SAM, ampicillin-sulbactam; TGC, tigecycline; AMK, amikacin; AZM, azithromycin; ATM, aztreonam; CAZ, ceftazidime; MEM, meropenem; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin; TEC, teicoplanin; CEP, cephalothin; TZP, tazobactam; NDM-1, New Delhi metallo-β-lactamase 1; ST, sequence type.

MIC, minimum inhibitory concentration; CST, colistin; SAM, ampicillin-sulbactam; TGC, tigecycline; AMK, amikacin; AZM, azithromycin; ATM, aztreonam; CAZ, ceftazidime; MEM, meropenem; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin; TEC, teicoplanin; CEP, cephalothin; TZP, tazobactam; NDM-1, New Delhi metallo- $\beta$ -lactamase 1; ST, sequence type.



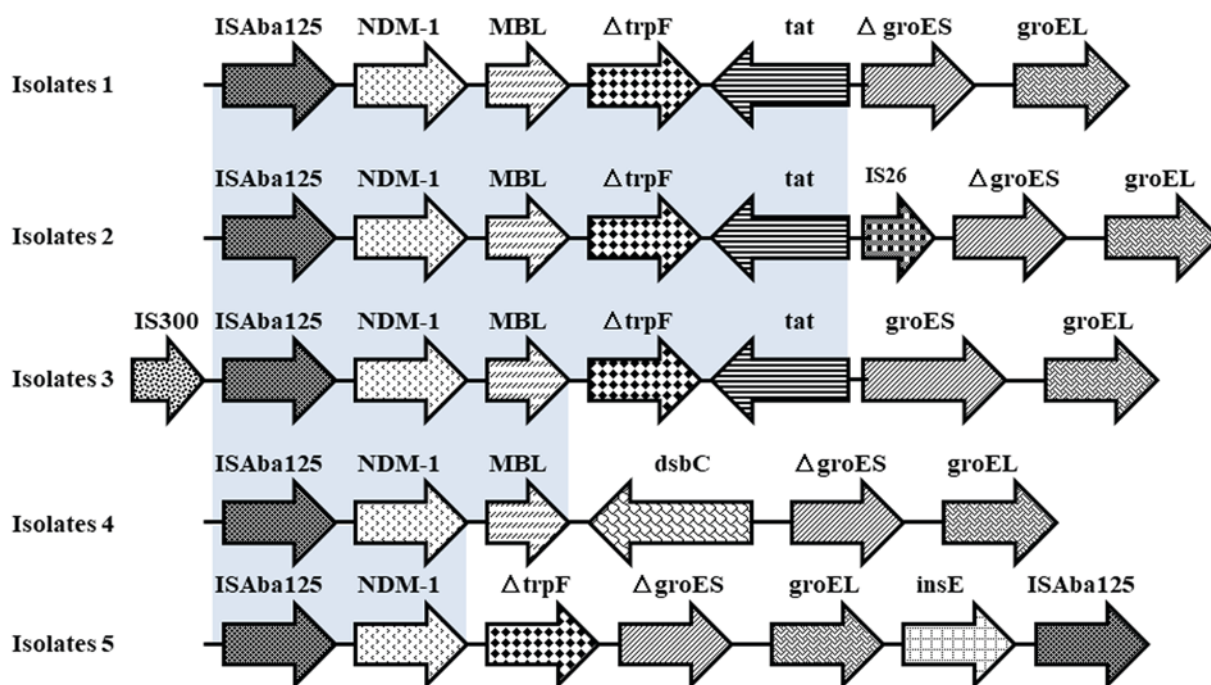


Figure 1. Comparison of the *bla*NDM-1 gene environments identified in the present study. The area highlighted in light blue indicates the homologous regions around the NDM-1 gene in the five strains. The arrows indicate the transcriptional direction of genes. NDM-1, New Delhi metallo-β-lactamase 1.

**Checkerboard assay.** The checkerboard tests aimed to validate the presence of synergism among anti-microbial agents at fixed concentrations. Checkerboard analysis was performed with colistin, vancomycin and β-lactamase inhibitors combined with meropenem. The checkerboard assay was used to determine antibiotic interactions, as previously described (29,30). The anti-microbials or β-lactamase inhibitors were applied in cation-adjusted Mueller-Hinton II broth (MHB II) at the following concentrations: Meropenem, 1-512 mg/l; ampicillin-sulbactam, 1/0.5-512/256 mg/l, tazobactam, 0.25-512 mg/l; and colistin, 0.124-32 mg/l. MHB II was filled into each well of 96-well, round-bottomed microtiter plates. The essential volume of drug solution at a concentration corresponding to the desired final concentration was then added to the wells. The final inoculum concentration of the *A. baumannii* was  $\sim 5 \times 10^5$  CFU/ml in a total volume of 200 μl. The plates were incubated at a temperature of 37°C for 48 h under aerobic conditions.

The turbidity of each well was assumed to represent microbiological growth. To define the interaction between anti-microbials, the fractional inhibitory concentration index (FICI) was employed, which was rated as follows:  $FICI \leq 0.5$ , synergism;  $0.5 < FICI \leq 4$ , indifference;  $FICI > 4$ , antagonism (31).

**Time-kill curve analysis.** *A. baumannii* strain was diluted to  $\sim 5 \times 10^5$  CFU/ml with Mueller-Hinton broth. The drug concentrations of meropenem and colistin were adjusted to the 1xMIC or 0.5xMIC in the time-kill curve analysis. The Erlenmeyer flask was incubated at 37°C under aerobic conditions. The number of bacteria was determined at 0, 2, 4, 6, 8, 12, 24, 30, 36 and 48 h (32).

**Statistical analysis.** Values are expressed as the mean  $\pm$  standard deviation. Statistical analysis was performed using GraphPad

prism version 5.01 (GraphPad Inc., La Jolla, CA, USA). Two-way analysis of variance with Bonferroni's post-hoc tests was used to compare each group of antibiotics to the control group at the same time.

## Results

**In vitro susceptibility and genetic characterization of isolates.** In the present study, five NDM-1-containing *A. baumannii* strains were identified among 264 isolates by PCR screening. Of these isolates, 3 were from blood specimens, one was from a cerebrospinal fluid sample and one was a urine isolate. All of the strains were resistant to the test antibiotics (Table II). By MLST, two strains were identified to be of ST357, while the remaining three strains were of ST191. Different genetic elements can influence the transmission of NDM-1 (Fig. 1). The ISAbal25 element was present upstream of the NDM-1 gene in the five strains and may facilitate the rapid dissemination of the NDM-1 gene. As the NDM-1 *A. baumannii* strains were resistant to numerous antibiotics and carried a number of resistance genes, the characteristics of these strains were next studied.

**Phenotypic detection of NDM-1 production.** The modified Hodge test was employed to detect carbapenemase production in the five isolates. The CDT in conjunction with EDTA revealed that the activity of NDM-1 was inhibited by EDTA.

**Checkerboard assay.** A synergistic interaction was identified for colistin-meropenem in four strains, for meropenem-ampicillin-sulbactam in two strains, for meropenem-vancomycin in two strains, as well as for meropenem-tazobactam in two strains (Table III). No antagonistic activity was detected for any of the combinations.

Table III. Results of the checkerboard synergy test of *A. baumannii* harboring New Delhi metallo- $\beta$ -lactamase 1.

Isolate	Meropenem + vancomycin		Meropenem + ampicillin-sulbactam		Meropenem + tazobactam		Meropenem + colistin	
	FICI	Result	FICI	Result	FICI	Result	FICI	Result
<i>A. baumannii</i> ATCC 19606	0.25	Synergism	0.5	Synergism	0.37	Synergism	0.26	Synergism
1	0.310	Synergism	0.265	Synergism	1.25	Indifference	1.25	Indifference
2	0.750	Indifference	0.532	Indifference	0.281	Synergism	0.281	Synergism
3	0.562	Indifference	1.301	Indifference	0.375	Synergism	0.375	Synergism
4	0.257	Synergism	1.057	Indifference	1.642	Indifference	0.281	Synergism
5	0.255	Indifference	0.124	Synergism	2.000	Indifference	0.140	Synergism

FICI, fractional inhibitory concentration index; ATCC, American Type Culture Collection.

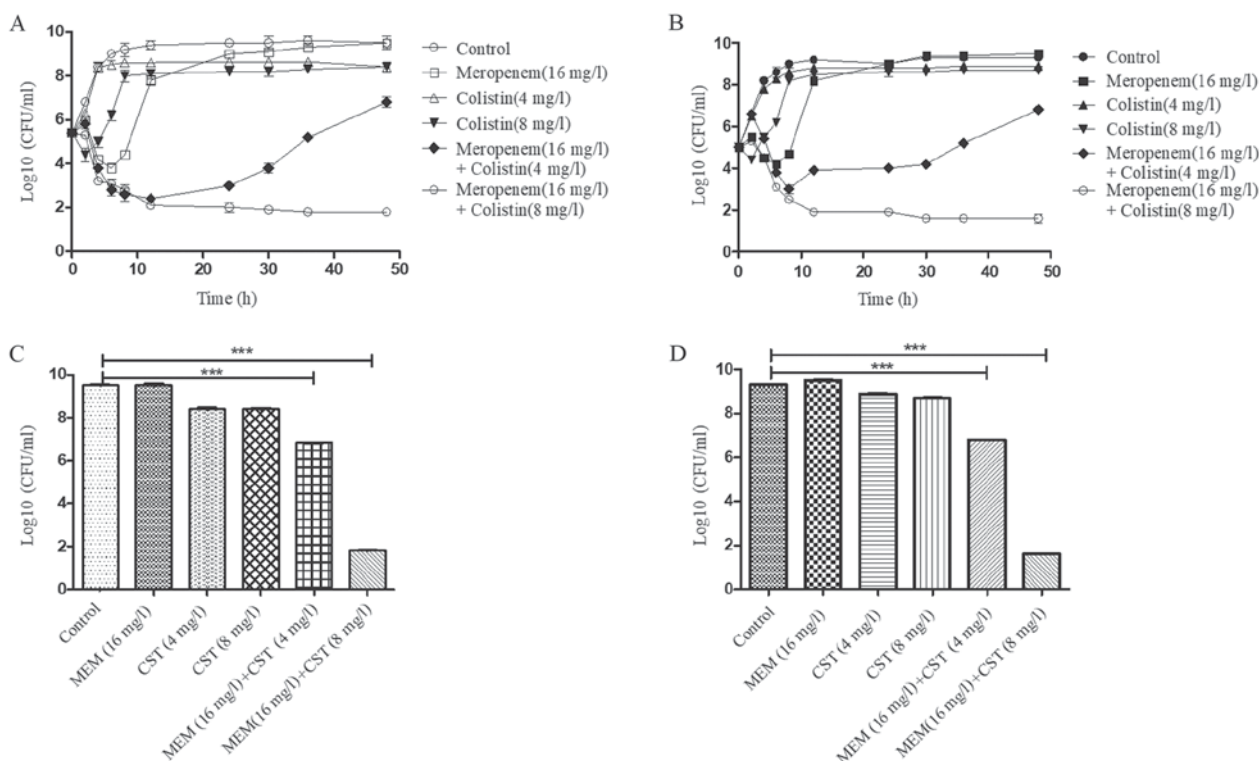


Figure 2. Time-kill curves of two strains of *Acinetobacter baumannii* treated with MEM (16 mg/l), CST [4 mg/l (0.5xMIC) or 8 mg/l (MIC)] or their combination. (A) Time-kill curves of the second strain; (B) Time-kill curves of the fourth strain; (C and D) Number of viable cells in (C) the second strain and (D) the fourth strain with different antibiotic treatments after 48 h of cultivation. \*\*\*P<0.001. MIC, maximum inhibitory concentration; CST, colistin; CFU, colony-forming units; MEM, meropenem.

*Activities of meropenem, colistin and their combination in a time-kill curve analysis.* The second strain and the fourth strain harbored the NDM-1 and CTX-M genes. A synergistic interaction of colistin and meropenem was detected in these two strains, and the strains were therefore selected to study the inhibitory activities of the combination of colistin and meropenem in a time-kill curve analysis. When each strain was incubated with meropenem at a concentration of 0.5xMIC (16 mg/l), the bacterial growth was transiently inhibited but then regrew again (Fig. 2). Colistin at concentrations of 0.5xMIC (4 mg/l) and 1xMIC (8 mg/l) did not inhibit the

growth of the two strains, although the concentration of 1xMIC (8 mg/l) had a transient inhibitory activity lasting for <6 h. The combination of meropenem and colistin at a concentration of 0.5xMIC had an inhibitory effect on the strains that lasted for longer than either antibiotic alone (up to 24 h). However, a sustained synergistic inhibitory activity lasting for >48 h was obtained with meropenem at a concentration of 0.5xMIC combined with colistin at a concentration of 1xMIC. After 48 h of cultivation, the bacterial quantity of the second strain was significantly affected in the presence of meropenem and colistin combined at a concentration of 0.5xMIC, as well

as meropenem at 0.5xMIC combined with colistin at 1xMIC compared with that in the control group ( $P<0.001$  for either; Fig. 2C). Furthermore, for the fourth strain, the number of cells was also significantly affected by the combination of meropenem and colistin at a concentration of 0.5xMIC and by the combination of meropenem at 0.5xMIC and colistin at 1xMIC ( $P<0.001$  for either; Fig. 2D).

## Discussion

*A. baumannii*, a significant nosocomial pathogen, particularly in intensive care units, is a major public health concern. Certain strains of this bacterium are resistant to a range of anti-microbial factors, including  $\beta$ -lactams, aminoglycosides, carbapenems and fluoroquinolones (33). The concern over this organism is mainly attributed to the rising MDR, encompassing colistin and carbapenems. NDM-1 is a  $\beta$ -lactamase belonging to an Ambler class B, and resistance to all  $\beta$ -lactams with the exception of aztreonam is conferred by this organism (34). NDM-1-positive *Acinetobacter spp.*, with *A. baumannii*, *A. pittii* as well as *A. lwoffii* included, have been reported in China (35,36). Studies have also reported NDM-1 in *A. baumannii* isolated in certain other countries from 2010 onwards, and it was hypothesized that NDM-1 associated with the Tn125 transposon originates from the *A. baumannii* strain in a specific area of North Africa prior to the transfer to *Enterobacteriaceae* (37). Tn125 may be the major vehicle for the dissemination of NDM-1 genes in strains of *A. baumannii*. ISAbal25 is present upstream of the NDM-1 gene and is also associated with the horizontal transfer of NDM-1 in *A. baumannii* (38). In the present study, ISAbal25 was located upstream of NDM-1 in the five strains. The genes encoding the GroEL and GroES chaperonin proteins were identified downstream of NDM-1 in the five strains. These genes may be associated with the transfer of NDM-1.

Due to the emerging resistance and insufficient efficacy of the remaining monotherapy options, antibiotic combination therapy is increasingly used (39). However, clinical evidence for specific antibiotic combinations is lacking. Therefore, *in vitro* data derived from checkerboard or time-kill experiments are used to support the choice of treatment (40). Drug combinations have been used to defeat extensively MDR *A. baumannii* isolates, and the efficacy of combinations amongst the drugs has also been demonstrated *in vitro* (41). A previous study has demonstrated the synergy of imipenem and amikacin in a mouse model, but there was no improvement on imipenem monotherapy (42). Combinations of antimicrobial agents including rifampicin have been reported to have synergistic effects on MDR *A. baumannii* isolates. In a clinical study, imipenem + rifampicin combination therapy led to rifampicin resistance in the treatment of infections with carbapenem-resistant *A. baumannii* strains (43). In spite of good efficacy against infections of *A. baumannii*, colistin treatment has been abandoned due to its toxicity, whereas its use is now re-emerging (44). Despite the lack of sufficient evidence for the clinical benefit, the combination of colistin with rifampin has appeared to be one of the most commonly researched combinations *in vitro* (3). Another study demonstrated the effectiveness of sulbactam in the treatment of infections with carbapenem-resistant *A. baumannii* strains (45). The synergistic

effect of sulbactam in conjunction with colistin on colistin-resistant *A. baumannii* strains has been reported (46). Normally, due to the relative impermeability of the outer membrane to such large hydrophobic molecules, vancomycin should exert no effect against Gram-negative organisms. However, colistin combined with vancomycin exhibited synergy (44). Meropenem combined with sulbactam exerted a more potent anti-microbial effect on certain *A. baumannii* strains than meropenem or sulbactam alone (32). In the present study, meropenem in combination with colistin, ampicillin-sulbactam, tazobactam and vancomycin exhibited synergistic effects against most strains harboring the NDM-1 gene in the checkerboard test. Meropenem with colistin was mostly effective against the MDR isolates of *A. baumannii*, which had a synergistic effect of 80% against *A. baumannii*, while other groups only had a synergistic effect of 40%. In the time-kill curve analysis, the combination of meropenem (0.5xMIC) and colistin (1xMIC) had a sustained synergistic bactericidal effect lasting for at least 48 h. Further clinical studies on the combination of the two drugs are required for delineating its clinical significance.

In conclusion, the present study indicated that meropenem plus colistin, ampicillin-sulbactam, tazobactam and vancomycin were effective against certain MDR strains of *A. baumannii* carrying the NDM-1 gene *in vitro*, and the combination therapy should be assessed in future *in vivo* pharmacological and toxicological studies.

## Acknowledgements

Not applicable.

## Funding

No funding was received.

## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Authors' contributions

JHL conceived and designed the experiments and wrote the paper. JJW conceived, designed and performed the experiments. YZN, SL and YW performed the experiments. CMJ, HRY and YCH analyzed the data.

## Ethical approval and consent to participate

The present study was approved by the Ethics Committee of Hongqi Hospital of Mudanjiang Medical College (Mudanjiang, China) and all participants provided informed consent.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.



## References

- Joly-Guillou ML: Clinical impact and pathogenicity of *Acinetobacter*. Clin Microbiol Infect 11: 868-873, 2005.
- Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B and Spellberg B: Clinical and pathophysiological overview of acinetobacter infections: A century of challenges. Clin Microbiol Rev 30: 409-447, 2017.
- Cai Y, Chai D, Wang R, Liang B and Bai N: Colistin resistance of *Acinetobacter baumannii*: Clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother 67: 1607-1615, 2012.
- Novovic K, Mihajlovic S, Vasiljevic Z, Filipic B, Begovic J and Jovicic B: Carbapenem-resistant *Acinetobacter baumannii* from Serbia: Revision of CarO classification. PLoS One 10: e0122793, 2015.
- Boulanger A, Naas T, Fortineau N, Figueiredo S and Nordmann P: NDM-1-producing *Acinetobacter baumannii* from Algeria. Antimicrob Agents Chemother 56: 2214-2215, 2012.
- Revathi G, Siu LK, Lu PL and Huang LY: First report of NDM-1-producing *Acinetobacter baumannii* in East Africa. Int J Infect Dis 17: e1255-e1258, 2013.
- Jones LS, Toleman MA, Weeks JL, Howe RA, Walsh TR and Kumarasamy KK: Plasmid carriage of bla NDM-1 in clinical *Acinetobacter baumannii* isolates from India. Antimicrob Agents Chemother 58: 4211-4213, 2014.
- El-Gamal MI, Ibrahim I, Hisham N, Aladdin R, Mohammed H and Bahaeldin A: Recent updates of carbapenem antibiotics. Eur J Med Chem 131: 185-195, 2017.
- McGuinness WA, Malachowa N and DeLeo FR: Vancomycin resistance in *Staphylococcus aureus*. Yale J Biol Med 90: 269-281, 2017.
- Acar JF, Goldstein FW and Kitzis MD: Susceptibility survey of piperacillin alone and in the presence of tazobactam. J Antimicrob Chemother 31 (Suppl A): S23-S28, 1993.
- Sörgel F and Kinzig M: The chemistry, pharmacokinetics and tissue distribution of piperacillin/tazobactam. J Antimicrob Chemother 31 (Suppl A): S39-S60, 1993.
- Joly-Guillou ML, Decré D, Herrman JL, Bourdelier E and Bergogne-Bérézin E: Bactericidal in-vitro activity of beta-lactams and beta-lactamase inhibitors, alone or associated, against clinical strains of *Acinetobacter baumannii*: Effect of combination with aminoglycosides. J Antimicrob Chemother 36: 619-629, 1995.
- Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR and Paterson DL: Colistin: The re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis 6: 589-601, 2006.
- Arroyo LA, Herrera CM, Fernandez L, Hankins JV, Trent MS and Hancock RE: The pmrCAB operon mediates polymyxin resistance in *Acinetobacter baumannii* ATCC 17978 and clinical isolates through phosphoethanolamine modification of lipid A. Antimicrob Agents Chemother 55: 3743-3751, 2011.
- Moffatt JH, Harper M, Harrison P, Hale JD, Vinogradov E, Seemann T, Henry R, Crane B, St Michael F, Cox AD, *et al*: Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. Antimicrob Agents Chemother 54: 4971-4977, 2010.
- Kimberly CC, Anna DF and Michael JR: A review of novel combinations of colistin and lipopeptide or glycopeptide antibiotics for the treatment of multidrug-resistant *Acinetobacter baumannii*. Infect Dis Ther 3: 69-81, 2014.
- Patel J, Cockerill F, Bradford P, Eliopoulos G, Hindler J, Jenkins S, Lewis J, Limbago B, Miller L, Nicolau D, *et al*: Performance standards for antimicrobial susceptibility testing: Twenty-fifth informational supplement; M100-S25. Wayne, PA: CLSI, 35, 2015.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement (M100-S25). Wayne (PA): The Institute; 2015.
- Shoma S, Kamruzzaman M, Ginn AN, Iredell JR and Partridge SR: Characterization of multidrug-resistant *Klebsiella pneumoniae* from Australia carrying blaNDM-1. Diagn Microbiol Infect Dis 78: 93-97, 2014.
- Rodriguez-Martinez JM, Nordmann P, Fortineau N and Poirel L: VIM-19, a metallo-beta-lactamase with increased carbapenemase activity from *Escherichia coli* and *Klebsiella pneumoniae*. Antimicrob Agents Chemother 54: 471-476, 2010.
- Qin S, Fu Y, Zhang Q, Qi H, Wen JG, Xu H, Xu L, Zeng L, Tian H, Rong L, *et al*: High incidence and endemic spread of NDM-1-positive Enterobacteriaceae in Henan Province, China. Antimicrob Agents Chemother 58: 4275-4282, 2014.
- Guo H and Xiong J: A specific and versatile genome walking technique. Gene 381: 18-23, 2006.
- Li P, Yang C, Xie J, Liu N, Wang H, Zhang L, Wang X, Wang Y, Qiu S and Song H: *Acinetobacter calcoaceticus* from a fatal case of pneumonia harboring bla(NDM-1) on a widely distributed plasmid. BMC Infect Dis 15: 131, 2015.
- Pfeifer Y, Wilharm G, Zander E, Wichelhaus TA, Göttig S, Hunfeld KP, Seifert H, Witte W and Higgins PG: Molecular characterization of blaNDM-1 in an *Acinetobacter baumannii* strain isolated in Germany in 2007. J Antimicrob Chemother 66: 1998-2001, 2011.
- Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H and Rodriguez-Valera F: Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 43: 4382-4390, 2005.
- Memish ZA, Assiri A, Almasri M, Roshdy H, Hathout H, Kaase M, Gatermann SG and Yezli S: Molecular characterization of carbapenemase production among gram-negative bacteria in Saudi Arabia. Microb Drug Resist 21: 307-314, 2015.
- Poirel L, Héritier C, Tolün V and Nordmann P: Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48: 15-22, 2004.
- Chatterjee S, Datta S, Roy S, Ramanan L, Saha A, Viswanathan R, Som T and Basu S: Carbapenem resistance in *Acinetobacter baumannii* and other acinetobacter spp. causing neonatal sepsis: Focus on NDM-1 and its linkage to ISAbal25. Front Microbiol 7: 1126, 2016.
- Aaron SD, Ferris W, Henry DA, Speert DP and Macdonald NE: Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with Burkholderia cepacia. Am J Respir Crit Care Med 161: 1206-1212, 2000.
- Aaron SD, Ferris W, Ramotar K, Vandemheen K, Chan F and Saginur R: Single and combination antibiotic susceptibilities of planktonic, adherent, and biofilm-grown Pseudomonas aeruginosa isolates cultured from sputa of adults with cystic fibrosis. J Clin Microbiol 40: 4172-4179, 2002.
- Percin D, Akyol S and Kalin G: In vitro synergism of combinations of colistin with selected antibiotics against colistin-resistant *Acinetobacter baumannii*. GMS Hyg Infect Control 9: Doc14, 2014.
- Ko WC, Lee HC, Chiang SR, Yan JJ, Wu JJ, Lu CL and Chuang YC: In vitro and in vivo activity of meropenem and sulbactam against a multidrug-resistant *Acinetobacter baumannii* strain. J Antimicrob Chemother 53: 393-395, 2004.
- Abbo A, Navon-Venezia S, Hammer-Muntz O, Krichali T, Siegmán-Igra Y and Carmeli Y: Multidrug-resistant *Acinetobacter baumannii*. Emerg Infect Dis 11: 22-29, 2005.
- Nordmann P, Poirel L, Toleman MA and Walsh TR: Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J Antimicrob Chemother 66: 689-692, 2011.
- Chen Y, Zhou Z, Jiang Y and Yu Y: Emergence of NDM-1-producing *Acinetobacter baumannii* in China. J Antimicrob Chemother 66: 1255-1259, 2011.
- Zhou Z, Guan R, Yang Y, Chen L, Fu J, Deng Q, Xie Y, Huang Y, Wang J, Wang D, *et al*: Identification of New Delhi metallo-beta-lactamase gene (NDM-1) from a clinical isolate of *Acinetobacter junii* in China. Can J Microbiol 58: 112-115, 2012.
- Tran DN, Tran HH, Matsui M, Suzuki M, Suzuki S, Shibayama K, Pham TD, Van Phuong TT, Dang DA, Trinh HS, *et al*: Emergence of New Delhi metallo-beta-lactamase 1 and other carbapenemase-producing *Acinetobacter calcoaceticus*-baumannii complex among patients in hospitals in Ha Noi, Viet Nam. Eur J Clin Microbiol Infect Dis 36: 219-225, 2017.
- Poirel L, Bonnin RA, Boulanger A, Schrenzel J, Kaase M and Nordmann P: Tn125-related acquisition of blaNDM-like genes in *Acinetobacter baumannii*. Antimicrob Agents Chemother 56: 1087-1089, 2012.
- Karaiskos I and Giamarellou H: Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: Current and emerging therapeutic approaches. Expert Opin Pharmacother 15: 1351-1370, 2014.
- Tamma PD, Cosgrove SE and Maragakis LL: Combination therapy for treatment of infections with gram-negative bacteria. Clin Microbiol Rev 25: 450-470, 2012.
- Marques MB, Brookings ES, Moser SA, Sonke PB and Waites KB: Comparative in vitro antimicrobial susceptibilities of nosocomial isolates of *Acinetobacter baumannii* and synergistic activities of nine antimicrobial combinations. Antimicrob Agents Chemother 41: 881-885, 1997.



42. Rodríguez-Hernández MJ, Pachón J, Pichardo C, Cuberos L, Ibáñez-Martínez J, García-Curiel A, Caballero FJ, Moreno I and Jiménez-Mejías ME: Imipenem, doxycycline and amikacin in monotherapy and in combination in *Acinetobacter baumannii* experimental pneumonia. *J Antimicrob Chemother* 45: 493-501, 2000.
43. Wolff M, Joly-Guillou ML, Farinotti R and Carbon C: In vivo efficacies of combinations of beta-lactams, beta-lactamase inhibitors, and rifampin against *Acinetobacter baumannii* in a mouse pneumonia model. *Antimicrob Agents Chemother* 44: 1406-1411, 1999.
44. Gordon NC, Png K and Wareham DW: Potent synergy and sustained bactericidal activity of a vancomycin-colistin combination versus multidrug-resistant strains of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 54: 5316-5322, 2010.
45. Levin AS: Multiresistant *Acinetobacter* infections: A role for sulbactam combinations in overcoming an emerging worldwide problem. *Clin Microbiol Infect* 8: 144-153, 2002.
46. Kempf M, Djouhri-Bouktab L, Brunel JM, Raoult D and Rolain JM: Synergistic activity of sulbactam combined with colistin against colistin-resistant *Acinetobacter baumannii*. *Int J Antimicrob Agents* 39: 180-181, 2012.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.