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

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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-β-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 ISAba125 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 ISAba125 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 β-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 β-lactamases, particularly OXA-23. The development of antibiotic resistance of this species has been associated with the emergence of New Delhi metallo-β-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 β-lactamase.
with the broadest spectrum of activity among β-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 β-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 Vitrek 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% CO₂.

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, β-lactam antibiotic aztreonam, β-lactamase inhibitor tazobactam, cephalosporin antibiotics ceftazidime and cefephalothin, rifampin and tigecycline, carbapenem antibiotic meropenem and colistin, and the glycopeptide antibiotic 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 1. 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 Vitrek 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 µg), imipenem (10 µg), and meropenem (10 µg) 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.
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 I. Primers used for the amplification of selected carbapenemase genes.

| Gene         | Primer name | Sequence                                      | Fragment size (bp) |
|--------------|-------------|-----------------------------------------------|--------------------|
| NDM-1        | NDM-1 F     | 5'-ATGGAATTGCCCAATTTATGACCCCGG-3'             | 813                |
|              | NDM-1 R     | 5'-TCAGCGCGACTTCTGCGGCCATG-3'                 |                    |
| VIM-1 and -2 | VIM F       | GATGTTCTTTGGTGTCGATA                        | 390                |
|              | VIM R       | CGAATGCAGACCCAG                                |                    |
| IMP          | MultiIMP F  | 5'-TGACACTTTACATCGACTCGA-3'                  | 139                |
|              | MultiIMP R  | 5'-GATYGAGAAATTAAGCCACCCCT-3'                |                    |
| SHV-5/12-like| SHV F       | 5'-GCTTTATTAGCCCTCATCAAG-3'                  | 897                |
|              | SHV R       | 5'-TTAGCGTTGCGACTGATCA-3'                    |                    |
| VEB          | VEB F       | CATTGCCGAGCAAGCGT                           | 648                |
|              | VEB R       | CGAAGTTCTTTGGGACCTG                         |                    |
| KPC          | KPC F       | 5'-TGTCACGTATCGCCCTG-3'                      | 1010               |
|              | KPC R       | 5'-CTCAGTGCTCTACAGAAAACC-3'                 |                    |
| OXA-10-like  | OXA-10 F    | 5'-CCACCAAGAAGTGCCATG-3'                     | 835                |
|              | OXA-10 R    | 5'-GCGACCTTGAGCAGACCTTT-3'                  |                    |
| CTX-M        | CTX-M Fp1   | 5'-TTAGGAARTGTGGCGCTGA-3'                    | 638                |
|              | CTX-M Fp1 R | 5'-CGATATCGTGTGGTGTRCCAT-3'                 |                    |
| TEM          | TEM F       | 5'-ATAAAATCTTGAAAGCGAAA-3'                   | 1079               |
|              | TEM R       | 5'-GACAGTTGACGACTGTTAATCA-3'                |                    |
| OXA-23-like  | OXA-23 F    | 5'-GATGTTGCTATGTATCTGC-3'                    | 1067               |
|              | OXA-23 R    | 5'-TCAAAACAACTAAAAGACACTG-3'                 |                    |
| OXA-30-like  | OXA-30 F    | 5'-GGCACCAAGATTTACTTCAAG-3'                  | 564                |
|              | OXA-30 R    | 5'-GACCCCGATTTCTGTAAGTG-3'                   |                    |
| DHA          | DHA F       | 5'-AACCTTTCACAGTGTTGCTGGT-3'                 | 405                |
|              | DHA R       | 5'-CCGTACGCACTGGCTTGGC-3'                    |                    |
| CMY-2-like   | CMY-2 F     | 5'-GCTGAGAGCTCATGATGAAAAATCG-3'              | 1146               |
|              | CMY-2 R     | 5'-GGTACGGAATCTTATTTGACG-3'                 |                    |
| armA         | armA F      | 5'-ATTCTGCTTCTATCTTAAATGG-3'                 | 315                |
|              | armA R      | 5'-ACCTGACTATTTACCTGTC-3'                    |                    |
| rmtC         | rmtC F      | 5'-CGAAAGAATACAGCAGCAAG-3'                   | 711                |
|              | rmtC R      | 5'-ATCCCAAATCCTCCTCCACT-3'                   |                    |
| aac(3)-Iic   | aac(3)-Iic F| 5'-ACGCGGAAGCAATAACCGA-3'                    | 854                |
|              | aac(3)-Iic R| 5'-TAACTGGAAGCCTCAGAAAG-3'                  |                    |
| aac(6')-Ib-cr| aac(6')-Ib-cr F | 5'-TTGCGATGCTCTATGAGTCGCTA-3' | 482 |
|              | aac(6')-Ib-cr R | 5'-CTCGAATGCGCGTTGGATT-3'            |                    |
| aadB         | aadB F      | 5'-AACCGAGTTAACATTGATA-3'                    | 266                |
|              | aadB R      | 5'-ACCAAGGATGTTGCGCAGTC-3'                   |                    |
| aacC1        | aacC1 F     | 5'-CACCTACTTCCCAATACGAGC-3'                  | 329                |
|              | aacC1 R     | 5'-CTTCCGCTATGCGACAC-3'                      |                    |
| qnrA         | qnrA F      | 5'-ATTTCTCAGGCCAGATTT-3'                     | 516                |
|              | qnrA R      | 5'-GATCGGGCAAGGTTAGTTGCA-3'                  |                    |
| qnrB         | qnrB F      | 5'-ACGATCTGTATGGCTG-3'                       | 469                |
|              | qnrB R      | 5'-GATCGTGAAAACGCAGAAAGG-3'                  |                    |
| qnrS         | qnrS F      | 5'-ACGACATTGCACTGCA-3'                       | 437                |
|              | qnrS R      | 5'-TAAATTGGGAACCTGTTAGGC-3'                  |                    |

F, forward; R, reverse; NDM-1, New Delhi metallo-β-lactamase 1; bla, β-lactamase.
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 | TGC | AMK | AZM | ATM | CAZ | MEM | RIF | SXT, trimethoprim/sulfamethoxazole | VAN | TEC | CEP |
| 1        | *blaNDM-1, VIM-16, blaKPC, aadB, blaOXA-23* | ST191 | Blood       | 8          | 8       | 64/32   | 8        | 256 | 128 | 64 | 64 | 128 | 4     | 64/1216   | 64 | 128 | 1024 |
| 2        | *blaNDM-1, blaCTX-M, aacC1*               | ST191 | Blood       | 128        | 8       | 32/16   | 8        | 128 | 64 | 64 | 64 | 32 | 4     | 32/1216   | 32 | 128 | 512  |
| 3        | *blaNDM-1, blaCMY-2-like, aadB*           | ST357 | Cerebrospinal fluid | 32        | 4       | 128/64  | 4        | 32 | 64 | 32 | 64 | 8  | 64/608 | 32 | 256 | 512  |
| 4        | *blaNDM-1, blaOXA-23, blaCTX-M, aac(6')-Ib-cr* | ST357 | Urine       | 32          | 8       | 64/32   | 16       | 32 | 32 | 32 | 128 | 32 | 8     | 32/608    | 64 | 512 | 256  |
| 5        | *blaNDM-1, blaOXA-10, blaCMY-2-like, aac(6')-Ib-cr* | 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.
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 ~5x10^5 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≤0.5, synergism; 0.5<FICI≤4, indifference; FICI>4, antagonism (31).

Time-kill curve analysis. A. baumannii strain was diluted to ~5x10^5 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 ± 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 ISAba125 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-vancocmycin in two strains, as well as for meropenem-tazobactam in two strains (Table III). No antagonistic activity was detected for any of the combinations.
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 at 1xMIC.}

Table III. Results of the checkerboard synergy test of A. baumannii harboring New Delhi metallo-β-lactamase 1.

| Isolate       | Meropenem + vancomycin | Meropenem + ampicillin-sulbactam | Meropenem + tazobactam | Meropenem + colistin |
|---------------|------------------------|----------------------------------|------------------------|----------------------|
| A. baumannii  | 0.25                   | Synergism                        | 0.5                    | Synergism            |
| ATCC 19606    | 1                      | 0.310 Synergism                  | 0.265                  | Synergism            |
| 2             | 0.750 Indifference     | 0.532 Indifference               | 0.281                  | Synergism            |
| 3             | 0.562 Indifference     | 1.301 Indifference               | 0.375                  | Synergism            |
| 4             | 0.257 Synergism        | 1.057 Indifference               | 1.642                  | Indifference         |
| 5             | 0.255 Indifference     | 0.124 Synergism                  | 2.000                  | Indifference         |

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.
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 β-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 β-lactamase belonging to an Ambler class B, and resistance to all β-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. lwoeffii 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. ISAba125 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, ISAba125 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 mono therapy (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.
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