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Original Article

In Vitro Analysis of Activities of 16 Antimicrobial Agents against Gram-Negative Bacteria from Six Teaching Hospitals in China

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SUMMARY: To evaluate the in vitro antimicrobial activities of biapenem, arbekacin, and cefminox against different gram-negative bacterial isolates in China, a total of 100 non-duplicated *Escherichia coli*, 100 *Acinetobacter baumannii*, 100 *Pseudomonas aeruginosa*, and 99 *Klebsiella pneumoniae* isolates were collected from 6 teaching hospitals in China in 2012. The minimal inhibitory concentrations (MICs) of biapenem, arbekacin, cefminox and 13 other antibiotics were determined by the broth microdilution method. The carbapenems (biapenem, meropenem, and imipenem) exhibited high antimicrobial activity against *E. coli* (98%) and *K. pneumoniae* (≥95%), followed by colistin and amikacin. The MIC_{50} and MIC_{90} of biapenem against *E. coli* were ≤0.06 mg/L and 0.25 mg/L, respectively. For *K. pneumoniae*, the MIC_{50} and MIC_{90} of biapenem were 0.25 mg/L and 1.0 mg/L, respectively. The MIC_{50} and MIC_{90} of cefminox against *E. coli* were 1.0 mg/L and 4.0 mg/L, respectively. The resistance rates of *A. baumannii* to most of the antibiotics were more than 50%, except for colistin. Amikacin was the most active antibiotic against *P. aeruginosa* (97%), followed by colistin (93%). The MIC_{50} and MIC_{90} of arbekacin against *P. aeruginosa* were 2.0 mg/L and 8.0 mg/L, respectively. In conclusion, carbapenems, colistin, amikacin, and arbekacin exhibited high antimicrobial activities against gram-negative bacteria, except *A. baumannii*.

INTRODUCTION

At present, multidrug-resistant (MDR) gram-negative bacteria pose a great challenge to clinicians with regard to the choice of appropriate antimicrobial therapy, especially the initial empirical treatment. MDR strains have been associated with higher mortality, prolonged hospital stays, and increased medical costs as compared to susceptible strains (1).

Carbapenems are often the last choice of drugs for therapy of infectious caused by MDR strains. Biapenem is a parenteral carbapenem antibacterial agent that was launched in Japan in 2001 and has a broad spectrum of activity against gram-negative, gram-positive, and anaerobic bacteria (2). Aminoglycoside antibiotics exhibit in vitro activity against a wide variety of clinically important gram-negative bacilli, as well as *Staphylococcus aureus* (*S. aureus*) and some streptococci. Arbekacin is an aminoglycoside licensed for use as an anti-methicillin-resistant *S. aureus* (MRSA) drug in Japan. It binds to the bacterial 30S ribosomal subunit, thereby inhibiting protein synthesis (3). Cefminox is a semisynthetic cephamycin possessing a broad spectrum of antibacterial activity against gram-positive, gram-negative, and anaerobic bacteria, except *Enterococcus faecalis* and *Pseudomonas aeruginosa* (*P. aeruginosa*) (4).

Although several studies have described the antibacterial activity of biapenem, arbekacin, and cefminox against gram-negative bacteria in Asia (4–7), none have evaluated the in vitro antimicrobial activities of these antibiotics in China. In this study, 100 *Escherichia coli* (*E. coli*), 100 *Acinetobacter baumannii* (*A. baumannii*), 100 *P. aeruginosa*, and 99 *Klebsiella pneumonia* (*K. pneumonia*) isolates were collected from 6 hospitals in China in 2012. The minimum inhibitory concentrations (MICs) of biapenem, arbekacin, cefminox and 13 other antibiotics were determined by the broth-microdilution method, to evaluate their in vitro antibacterial activity against gram-negative bacteria that commonly cause nosocomial infections.

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MATERIALS AND METHODS

Bacterial isolates: A total of 100 *E. coli*, *A. baumannii*, and *P. aeruginosa*, and 99 *K. pneumoniae* non-duplicated isolates (Table 1) were collected from 6 teaching hospitals in China in 2012. The isolates were identified at a local laboratory, and their identify was re-confirmed at the central laboratory (Clinical Microbiology, Peking University People's Hospital) using colony morphology, routine biochemical tests, a 42°C growth test (for *A. baumannii*), and/or VITEK System identification cards (bioMerieux, Hazelwood, MO, USA), as required. All isolates were stored at −80°C until MIC testing.

Antimicrobial susceptibility testing: The MICs for each antibiotic against the aforementioned bacteria were determined by the broth-microdilution method using designed MIC panels manufactured by the Eiken Chemical Co., Ltd. (Tokyo, Japan), according to Clinical and Laboratory Standards Institute (CLSI) guidelines (8). The antimicrobials tested included biapenem,

Table 1. Origins of isolates used in the present study

Hospital[1)]	Year of isolation	No. of isolate				City
		E. coli	*K. pneumoniae*	*A. baumannii*	*P. aeruginosa*	
PKUPH	2012	40	40	40	41	Beijing
BJH	2012	30	16	6	15	Beijing
CYH	2012	30	26	29	23	Beijing
PUMCH	2012	0	9	10	12	Beijing
TJH	2012	0	8	4	4	Tianjin
SYH	2012	0	0	11	5	Shenyang

1): PKUPH, Peking University People's Hospital; BJH, Beijing Hospital; CYH, Beijing Chao-Yang Hospital; PUMCH, Peking Union Medical College Hospital; TJH, Tianjin Medical University General Hospital; SYH, The First Hospital of China Medical University.

imipenem, meropenem, cefminox, piperacillin/tazobactam, cefepime, ceftazidime, cefotaxime, ceftriaxone, aztreonam, cefotaxime/cefotaxime-clavulanate, ceftazidime/ceftazidime-clavulanate, arbekacin, amikacin, gentamicin, levofloxacin, fosfomycin, and colistin. Testing procedures were validated by determining the MICs for reference strains, as recommended by CLSI standards. *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used in each set of tests. The MICs results were interpreted according to the CLSI breakpoints (9).

The CLSI extended-spectrum β-lactamases (ESBLs)-screening criteria (MIC $\geq$2 mg/L for ceftazidime, cefotaxime, ceftriaxone, and aztreonam) were applied to *E. coli* and *K. pneumoniae*. Two pairs of drug combinations, cefotaxime/cefotaxime-clavulanate and ceftazidime/ceftazidime-clavulanate, were used to confirm the production of ESBLs for suspicious isolates. Isolates were considered ESBL positive if the MIC of the antimicrobial drug with clavulanate was $\geq$3 two-fold lower that of the drug alone. *E. coli* ATCC 25922 and *K. pneumonia* ATCC 700603 were used as controls in the ESBL test.

Data analysis: For each isolate, every center recorded detailed information, including specimen type, collection date, and location type, and filled out standardized tables. All this information, as well as the MIC data, were analyzed using WHONET5.6 (WHO, Geneva) software at the central laboratory.

RESULTS

Specimen types: Of the 100 non-duplicated *E. coli* isolates, 24% were recovered from blood, 17% from respiratory tract specimens, and 59% from urine. Of the 99 *K. pneumoniae* isolates, 38% were recovered from blood, 31% from respiratory tract specimens, and 31% from urine. Of the 100 *A. baumannii* isolates, 33% were recovered from blood, 61% from respiratory tract specimens, and 6% from urine. Of the 100 *P. aeruginosa* isolates, 40% were recovered from blood, 35% from respiratory tract specimens, and 25% from urine.

***E. coli*:** Antimicrobial susceptibility data for *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* are presented in Table 2. Meropenem and imipenem were the most active agents against *E. coli* isolates (98%), followed by colistin (96%). ESBL phenotypes accounted for 55% of all *E. coli* isolates. For *E. coli*, the MIC_{50} and MIC_{90} were as follows: biapenem, $\leq$0.06 mg/L and 0.25 mg/L; meropenem, $\leq$0.06 mg/L for both; and imipenem, 0.125 mg/L and 0.25 mg/L, respectively. The MIC_{50} of imipenem against *E. coli* was 2-fold higher than that of biapenem and meropenem. The MIC_{90}s of biapenem and imipenem against *E. coli* strains were 4-fold higher than that of meropenem. The MIC_{50} and MIC_{90} of cefminox against *E. coli* were 1.0 mg/L and 4.0 mg/L, respectively. The MIC_{50} of arbekacin against *E. coli* was 4-fold lower than that of amikacin, and 32-fold lower than that of gentamicin. The MIC_{90} of arbekacin against *E. coli* was $\geq$8 fold lower than that of gentamicin. For the 55 ESBL-producing *E. coli* strains, meropenem, imipenem, and colistin were the most active agents (96.4%), followed by amikacin (83.6%) and piperacillin/tazobactam (76.4%). The susceptibility rates of the remaining antibiotics were less than 40%.

For *E. coli*, 98% of isolates were inhibited by biapenem or meropenem MICs of 1.0 mg/L, and 98% of isolates were inhibited by an imipenem MIC of 0.5 mg/L. Ninety percent of *E. coli* isolates were inhibited by amikacin or arbekacin MICs of 16.0 mg/L; further, 89% of the isolates were inhibited by an arbekacin MIC of 8.0 mg/L, and 82% of the isolates were inhibited by an arbekacin MIC of 4.0 mg/L. However, only 34% of *E. coli* isolates were inhibited by a gentamicin MIC of 4.0 mg/L. Ninety-three percent and 96% of *E. coli* isolates were inhibited by cefminox MICs of 8.0 and 16.0 mg/L, respectively. Only 13% of the *E. coli* isolates were inhibited by a levofloxacin MIC of 2.0 mg/L, which is the susceptible breakpoint.

***K. pneumonia*:** For the 99 *K. pneumoniae* strains, meropenem and imipenem were the most active agents against the isolates ($\geq$95%), followed by colistin (95%). The ESBL phenotypes accounted for 45% of *K. pneumoniae*. For *K. pneumoniae*, the MIC_{50} and MIC_{90} for biapenem and imipenem were 0.25 mg/L and 1.0 mg/L, respectively, while those for meropenem were $\leq$0.06 mg/L and 0.125 mg/L, respectively. The MIC_{50} of meropenem against *K. pneumoniae* was 4-fold lower than that of biapenem and imipenem. The MIC_{90} of meropenem against *K. pneumoniae* was 8-fold lower than that of biapenem and imipenem. The MIC_{50} and MIC_{90} of cefminox against *K. pneumoniae* were 1.0 mg/L and 128.0 mg/L, respectively. The MIC_{50} of arbekacin against *K. pneumoniae* was 2-fold lower than that of amikacin and 2-fold higher than that of gentamicin. The MIC_{90} of arbekacin against *K. pneumoniae* was lower than that of amikacin and gentamicin. For the 45 ESBL-producing *K. pneumoniae* strains, meropenem and colistin were the most active agents (93%), followed by imipenem (91%), amikacin (84%), and piperacil-

Table 2. The overall in vitro susceptibility to biapenem, arbekacin, cefminox, and the other thirteen antibiotics of clinical gram-negative isolates in China in 2012

Antimicrobial agent	Organism															
	E. coli (n = 100) ESBL 55%				*K. pneumoniae* (n = 99) ESBL 45%				*A. baumannii* (n = 100)				*P. aerginosa* (n = 100)			
	R%[1]	S%[3]	MIC_{50} (mg/L)	MIC_{90} (mg/L)	R%	S%	MIC_{50} (mg/L)	MIC_{90} (mg/L)	R%	S%	MIC_{50} (mg/L)	MIC_{90} (mg/L)	R%	S%	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Biapenem	—[2]	—	≤0.06	0.25	—	—	0.25	1	—	—	16	64	—	—	0.5	16
Imipenem	1	98	0.125	0.25	4	95	0.25	1	52	47	16	64	29	65	1	16
Meropenem	1	98	≤0.06	≤0.06	4	96	≤0.06	0.125	53	45	16	64	21	69	1	16
Cefminox	—	—	1	4	—	—	1	128	—	—	128	>128	—	—	>128	>128
Piperacillin/ Tazobactam	10	86	4/4	64/4	23	74	4/4	>128/4	60	33	>128/4	>128/4	21	67	8/4	>128/4
Cefepime	46	47	16	>128	21	75	1	128	57	38	32	>128	23	62	8	64
Ceftazidime	33	57	2	64	33	61	2	>64	56	38	32	>64	31	63	4	>64
Cefotaxime	70	30	32	>128	56	44	8	>128	58	15	>128	>128	—	—	64	>128
Ceftriaxone	68	31	128	>128	55	44	8	>128	59	8	>128	>128	—	—	128	>128
Aztreonam	50	41	8	128	36	62	2	128	—	—	64	>128	29	57	8	128
Arbekacin	—	—	1	16	—	—	1	128	—	—	>128	>128	—	—	2	8
Amikacin	8	90	4	16	15	84	2	>128	54	46	>128	>128	2	97	4	8
Gentamicin	64	34	32	>128	41	57	0.5	>128	56	44	>128	>128	13	85	2	32
Levofloxacin	86	13	16	64	35	62	1	64	55	43	8	16	28	58	2	32
Colistin[4]	—	96	0.25	0.5	—	95	0.25	0.5	0	100	0.5	0.5	5	93	1	2
Fosfomycin[5]	—	—	2	>256	—	—	32	>256	—	—	128	>256	—	—	64	>256

[1]: Percentage of resistance defined by Clinical and Laboratory Standards Institute guidelines (CLSI).
[2]: No breakpoints in CLSI guidelines.
[3]: Percentage of susceptibility defined by CLSI guidelines.
[4]: Based on the European Committee on Antimicrobial Susceptivity Testing (EUCAST) breakpoint.
[5]: The breakpoint standard of fosfomycin in CLSI is only used for urinary tract isolates.

lin/tazobactam (62%). The susceptibility rates of the remaining antibiotics were less than 50%.

For *K. pneumoniae*, 94% and 95% of the isolates were inhibited by imipenem or biapenem MICs of 1.0 mg/L, and 95% of isolates were inhibited by a meropenem MIC of 0.5 mg/L. Eighty-three percent of *K. pneumoniae* isolates were inhibited by an amikacin MIC of 8.0 mg/L or an arbekacin MIC of 4.0 mg/L. However, only 56% of *K. pneumoniae* isolates were inhibited by a gentamicin MIC of 4.0 mg/L. Seventy percent and 75% of *K. pneumoniae* isolates were inhibited by cefminox MICs of 2.0 and 16.0 mg/L, respectively. Only 44% of *K. pneumoniae* isolates were inhibited by ceftriaxone or cefotaxime MICs of 1.0 mg/L, which is the susceptible breakpoint.

***A. baumannii*:** Of the 100 *A. baumannii* strains, the susceptibility rates of *A. baumannii* to the all antibiotics, except colistin, were lower than 50%. The MIC_{50} and MIC_{90} of colistin for *A. baumannii* were 0.5 mg/L. For *A. baumannii*, 91% and 100% of isolates were inhibited by colistin MICs of 0.5 mg/L and 1.0 mg/L, respectively. Forty-five percent of *A. baumannii* isolates were inhibited by biapenem or meropenem MICs of 4.0 mg/L, and 47% of isolates were inhibited by an imipenem MIC of 4.0 mg/L. Forty-five percent of *A. baumannii* isolates were inhibited by an amikacin MIC of 4.0 mg/L or an arbekacin MIC of 2.0 mg/L, and 44% of isolates were inhibited by a gentamicin MIC of 4.0 mg/L. Forty-three percent of *A. baumannii* isolates were inhibited by a levofloxacin MIC of 2.0 mg/L, which is the susceptible breakpoint. Thirty-eight percent of *A. baumannii* isolates were inhibited by a ceftazidime or cefepime MIC of 8.0 mg/L. Thirty-three percent of *A. baumannii* isolates were inhibited by a piperacillin/tazobactam MIC of 16.0 mg/L, which is the susceptible breakpoint.

***P. aeruginosa*:** For the 100 *P. aeruginosa* strains, amikacin was the most active agent (≥95%). The second most-active agent against *P. aeruginosa* was colistin (93%). For *P. aeruginosa*, the MIC_{50}s of arbekacin and gentamicin were 2-fold lower than that of amikacin. Further, the MIC_{90}s of arbekacin and amikacin for *P. aeruginosa* was 4-fold lower than that of gentamicin. The MIC_{50} of biapenem for *P. aeruginosa* was 2-fold lower than that of imipenem and meropenem. The MIC_{90} for biapenem, imipenem, and meropenem was 16.0 mg/L.

For *P. aeruginosa*, 94%, 90%, and 87% of isolates were inhibited by arbekacin, amikacin, and gentamicin MICs of 8.0 mg/L, respectively. Further, 95%, 97%, and 89% of isolates were inhibited by arbekacin, amikacin, and gentamicin MICs of 16.0 mg/L, and 75%, 69%, and 65% of *P. aeruginosa* strains were inhibited by biapenem, meropenem, and imipenem MICs of 2.0 mg/L.

DISCUSSION

In this study, carbapenems (biapenem, meropenem, and imipenem) exhibited high antimicrobial activity against most of the *E. coli* and *K. pneumonia* isolates, followed by colistin, arbekacin, and amikacin. Since half of the *E. coli* and *K. pneumonia* isolates were ESBL producers, ceftriaxone and cefotaxime showed high resistance rates. Except for the carbapenems, colistin, amikacin, and piperacillin/tazobactam, the resistance

rates of ESBL-producing *E. coli* and *K. pneumonia* isolates to most of the tested antibiotics were high.

Biapenem has a broad spectrum of in vitro antibacterial activity against gram-negative (including β-lactamase-producing strains and *P. aeruginosa*), gram-positive, and anaerobic bacteria (10,11). A previous study demonstrated that biapenem is as effective and well tolerated as imipenem/cilastatin in the treatment of intermediate and severe bacterial infections (11). A small number of carbapenem-resistant *E. coli* isolates have emerged in China (12,13). Our previous study showed that two resistance mechanisms involved in carbapenem-resistant enterobacteriaceae isolates included the production of carbapenemases and the loss or decreased expression of outer membrane proteins, in combination with the production of AmpC or ESBL enzymes (13). Livermore and Mushtaq (14) reported that biapenem (RPX2003) in combination with the boronate β-lactamase inhibitor RPX7009 (Carbavance) could overcome most of the resistance caused by *K. pneumoniae* carbapenemases and other class A carbapenemases. Class B and D carbapenemases were not inhibited, but conferred less consistent resistance to biapenem than to other carbapenems (14).

Arbekacin and amikacin were the second most-active agents against *E. coli* isolates. Arbekacin, which is a semi-synthetic aminoglycoside antibiotic, is active against both gram-positive and gram-negative bacteria and is not affected by the aminoglycoside-modifying enzymes produced by MRSA. It lyses *E. coli* by causing membrane damage and inhibits translation by binding to bacterial ribosomal subunits (15). A previous study showed that the MIC_{90}s of arbekacin for *E. coli* and *Citrobacter freundii* were 1.0 g/mL and 16.0 g/mL, respectively, which were 2- to 4 fold and 8- to 16-fold lower than those of amikacin and gentamicin, respectively (5). Therefore, most *E. coli* isolates were susceptible to arbekacin.

Cefminox was shown to exhibit high activity against ESBL-producing *E. coli* (16), which is consistent with our current results. Cefminox has broad-spectrum antimicrobial activity and is therefore, it suitable for prophylaxis in surgical procedures, where a mixed infection may occur (17).

Recently, pan-resistant *A. baumannii*, which are resistant to most antibiotics, except for colistin and tigecycline, has become one of the most important causes of nosocomial infections worldwide. Our current study showed that the resistance rates of *A. baumannii* to all antibiotics were more than 50%, except for colistin. Our previous study showed that *A. baumannii* has exhibited decreased susceptibilities to meropenem and imipenem (18). The susceptibility of *A. baumannii* to meropenem reduced significantly, from 94.6% in 2003 to 60.7% in 2008, while its the susceptibility to imipenem reduced from 92.5% to 62.1%. The resistance rates of *A. baumannii* to meropenem and imipenem in this study were 53% and 52%, respectively. For pan-resistant *A. baumannii*, the use of new antibiotics should be explored, and at the same time, measure for strict prevention and control of nosocomial infections should be initiated to avoid outbreaks in hospitals.

P. aeruginosa is one of the most important pathogens causing hospital-acquired pneumonia, urinary tract infections, surgical site infections, and bacteremia. We found that the resistance rates of *P. aeruginosa* to most antibiotics were less than 30%. Our previous study showed that the susceptivility rate of *P. aeruginosa* to meropenem was relatively stable, increasing from 76.0% in 2003 to 86.2% in 2008, while its susceptibility to imipenem increased from 70.5% to 74.8% (18). However, in the current study, the susceptibility rates of *P. aeruginosa* to meropenem and imipenem were 69% and 65%, respectively. The MIC_{50} of biapenem for *P. aeruginosa* (0.5 mg/L) was lower than that of meropenem and imipenem (1.0 mg/L). Biapenem shows a good post-antibiotic effect, similar to imipenem, and has a high bactericidal activity against biofilm-forming *Pseudomonas* strains (19) and several efflux-system mutants (20). Although the in vitro activity of biapenem against *P. aeruginosa* was similar to that of imipenem in most investigations, two studies have shown that biapenem was more active than imipenem (7,21). Another study indicated that a novel metallo-β-lactamase (MBL) inhibitor (ME1071) could potentiate the activity of ceftazidime and carbapenems (especially biapenem) against MBL-producing strains of *P. aeruginosa* (22). Arbekacin and amikacin were the most active agents against *P. aeruginosa* isolates. The MIC_{50} of arbekacin for *P. aeruginosa* (2.0 mg/L) was lower than that of amikacin (4.0 mg/L). Moreover, arbekacin has demonstrated efficacy against clinical isolates of *P. aeruginosa* (6). A previous study indicated that aztreonam + arbekacin was the most promising combination regimen against MDR *P. aeruginosa* strains (23). Therefore, arbekacin can be considered a potential candidate for the future treatment of diseases caused by *P. aeruginosa*.

In summary, carbapenems (biapenem, meropenem, and imipenem) exhibited high antimicrobial activities against gram-negative bacteria, except for *A. baumannii*. Cefminox exhibited high activity against ESBL-producing *E. coli*. Isolates. Arbekacin and amikacin were the second most active agents against enterobacteriaceae isolates. Most antibiotics showed antimicrobial activity against *P. aeruginosa*, and amikacin was the most active agent. Pandrug-resistant *A. baumannii* has become one of the most important causes of nosocomial infections in China, and strict prevention and control measurements are required for such infections.

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Conflict of interest None to declare.

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