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In vitro activity of imipenem/relebactam, meropenem/vaborbactam, ceftazidime/avibactam, cefepime/zidebactam and other novel antibiotics against imipenem-non-susceptible Gram-negative bacilli from Taiwan

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Objectives: To investigate the susceptibility of imipenem-non-susceptible *Escherichia coli* (INS-EC), *Klebsiella pneumoniae* (INS-KP), *Acinetobacter baumannii* (INS-AB) and *Pseudomonas aeruginosa* (INS-PA) to novel antibiotics.

Methods: MICs were determined using the broth microdilution method. Carbapenemase and ESBL phenotypic testing and PCR for genes encoding ESBLs, AmpCs and carbapenemases were performed.

Results: Zidebactam, avibactam and relebactam increased the respective susceptibility rates to cefepime, ceftazidime and imipenem of 17 INS-EC by 58.8%, 58.8% and 70.6%, of 163 INS-KP by 77.9%, 88.3% and 76.1% and of 81 INS-PA by 45.7%, 38.3% and 85.2%, respectively. Vaborbactam increased the meropenem susceptibility of INS-EC by 41.2% and of INS-KP by 54%. Combinations of β-lactams and novel β-lactamase inhibitors or β-lactam enhancers (BLI-BLE) were inactive against 136 INS-AB. In 58 INS-EC and INS-KP with exclusively $bla_{\text{KPC-like}}$ genes, zidebactam, avibactam, relebactam and vaborbactam increased the susceptibility of the partner β-lactams by 100%, 96.6%, 84.5% and 75.9%, respectively. In the presence of avibactam, ceftazidime was active in an additional 85% of 20 INS-EC and INS-KP with exclusively $bla_{\text{OXA-48-like}}$ genes while with zidebactam, cefepime was active in an additional 75%. INS-EC and INS-KP with MBL genes were susceptible only to cefepime/zidebactam. The β-lactam/BLI-BLE combinations were active against INS-EC and INS-KP without detectable carbapenemases. For INS-EC, INS-KP and INS-AB, tigecycline was more active than omadacycline and eravacycline but eravacycline had a lower MIC distribution. Lascufloxacin and delafloxacin were active in <35% of these INS isolates.

Conclusions: β-Lactam/BLI-BLE combinations were active in a higher proportion of INS-EC, INS-KP and INS-PA. The susceptibility of novel fluoroquinolones and tetracyclines was not superior to that of old ones.

Introduction

Antimicrobial resistance (AMR) is a major public health problem worldwide.[1] In addition to worse patient outcome, AMR is associated with higher healthcare costs and productivity losses. In the EU, an estimated 33 110 deaths and 874 541 disability-adjusted life-years were attributable to AMR in 2015.[2] Carbapenems have been successfully used to treat antibiotic-resistant Gram-negative pathogens but resistance emerges quickly. Among carbapenem-resistant Gram-negative pathogens, carbapenem-resistant Enterobacterales, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are of greater clinical importance due to their high prevalence, concomitant resistance to other antibiotics and negative impact on patient outcome. They are therefore on the priority list of bacteria for which new antibiotics are urgently needed.[1]

The assessment of the pipeline in 2019 by WHO revealed that 8 new antibiotics and combination agents gained market authorization between July 2017 and September 2019, with

another 32 antibiotics targeting WHO priority pathogens in the Phase 1–3 development stage.[3] Some of these recently developed antibiotics have shown good *in vitro* activity against drug-resistant pathogens.[4–8] However, the demographic characteristics of patients from whom isolates were collected differed in each study, i.e. infection sites, healthcare settings or geographical area. These factors are associated with *in vitro* activity of antibiotics. For example, ceftolozane/tazobactam was less active against Gram-negative pathogens isolated in Vietnam than against those from other Asia-Pacific regions.[8] In addition, the various inclusion criteria, such as the definition of carbapenem resistance or types of carbapenemase gene, also affect the susceptibility results. Avibactam has better inhibitory activity against OXA-48 compared with relebactam and vaborbactam but neither inhibit MBLs.[4,5] In contrast, the activities of eravacycline and plazomicin are not affected by the types of carbapenemases *per se*.[7] The interpretation of the susceptibility results of recently developed antibiotics from different studies is therefore difficult. This multicentre study concomitantly determined susceptibility to eight recently developed antibiotics of imipenem-non-susceptible pathogens from a nationwide surveillance programme in Taiwan and their activities were compared with commonly used antibiotics. Susceptibility was further analysed according to phenotypic and molecular characteristics.

Materials and methods

Ethics

The Taiwan Surveillance of Antimicrobial Resistance (TSAR) bacterial isolates were recovered from clinical samples taken as part of standard care and the TSAR project was approved by the Research Ethics Committee of the National Health Research Institutes (EC1010602-E, EC1030406-E and EC1050606-E).

Bacterial isolates

TSAR is a biennial longitudinal multicentre surveillance programme on clinical isolates.[9] Isolates were stored frozen and subcultured onto appropriate agar plates for purity check before subsequent testing. Speciation was confirmed using conventional biochemical tests and API 20E, 32GN or VITEK II (bioMérieux, Marcy l'Étoile, France) as needed and *A. baumannii* was additionally identified using multiplex PCR.[10] In this study, around 400 imipenem-non-susceptible isolates were selected, including *Escherichia coli* and *Klebsiella pneumoniae* with imipenem MIC $\geq$ 2 mg/L from 2012–18, and *A. baumannii* and *P. aeruginosa* with MIC $\geq$ 4 mg/L from 2018. The selection algorithm is shown in Figure S1, available as Supplementary data at *JAC* Online.

Antimicrobial susceptibility testing

The MICs of recently developed antibiotics were determined by broth microdilution using 96-well microtitre plates prepared in-house, following CLSI-recommended protocols.[11] Recently developed antibiotics that we had access to included imipenem/relebactam, meropenem/vaborbactam, ceftazidime/avibactam, cefepime/zidebactam, lascufloxacin, delafloxacin, eravacycline and omadacycline. Relebactam, vaborbactam, avibactam, eravacycline and omadacycline were obtained from MedChemExpress (USA), zidebactam and lascufloxacin were from MedKoo Biosciences (USA) and delafloxacin was from Sigma–Aldrich (USA). Relebactam, vaborbactam and avibactam were tested at fixed concentrations of 4, 8 and 4 mg/L, respectively, in combination with doubling dilutions of their partner β-lactams. Zidebactam was tested in combination with cefepime at a 1:1 ratio. The following quality control strains were included: *E. coli* ATCC 25922 (eravacycline, lascufloxacin and omadacycline) and *P. aeruginosa* ATCC 27853 (imipenem/relebactam, meropenem/vaborbactam, ceftazidime/avibactam, cefepime/zidebactam and delafloxacin).

MICs of cefepime, ceftazidime, ciprofloxacin, levofloxacin, imipenem, meropenem, minocycline and tigecycline that were meant to be compared with the aforementioned antibiotics were also determined using 96-well microtitre plates prepared in-house. MICs of other commonly used antibiotics were determined using custom-designed NHRIGN8, NHRIGN9 or NHRIGN10 panels prepared by Sensititre (Trek Diagnostics, West Sussex, UK) for *E. coli* and *K. pneumoniae* and standard GNX3F panels for *A. baumannii* and *P. aeruginosa*. *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains.

PCR screening for β-lactamase genes

Multiplex PCRs[12–14] were performed on imipenem-non-susceptible isolates to detect the genes encoding class A (bla_{NMC}, bla_{SME}, bla_{IMI}, bla_{KPC} and bla_{GES}), class B (bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SPM}, bla_{SIM-1} and bla_{NDM}) and class D ($bla_{OXA-48-like}$, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-58-like}$ and $bla_{OXA-51-like}$) β-lactamases with carbapenemase activity (Figure S1). *E. coli* and *K. pneumoniae* isolates that were negative for carbapenemase genes were subjected to PCR targeting genes encoding ESBLs (bla_{SHV} as well as $bla_{CTX-M-group-1}$, $_{-2}$, $_{-8}$, $_{-9}$ and $_{-25}$) and AmpCs (bla_{MOX}, bla_{CIT}, bla_{DHA}, bla_{ACC}, bla_{MIR} and bla_{FOX}).[15–18] The bla_{SHV} amplicon was further digested by restriction endonuclease NheI to identify the presence of Gly238Ser in ESBLs.[16]

Phenotypic testing

The modified carbapenem inactivation method (mCIM) and the EDTA-modified carbapenem inactivation method (eCIM) were performed on *E. coli* and *K. pneumoniae* to detect the activity of serine- and metal-dependent carbapenemases. Isolates without carbapenemase production were further subjected to ESBL phenotypic testing (Figure S1). The experimental procedures were in accordance with CLSI recommendations.[11]

Data analysis

Susceptibilities were calculated using Whonet software (Stelling and O'Brien).[9] The interpretation of susceptibility was in accordance with 2019 CLSI criteria,[19] and US FDA criteria if CLSI breakpoints were not available. The breakpoints of novel antibiotics used in this study are listed in Table S1. For comparison with cefepime/zidebactam (susceptible $\leq$8/8 mg/L), the susceptible dose-dependent (SDD) and susceptible breakpoints ($\leq$8 mg/L) for cefepime were used in this study. Due to the lack of tigecycline MIC breakpoints for *Acinetobacter* spp., the US FDA breakpoints for Enterobacterales were used ($\leq$2 mg/L susceptible; >4 mg/L resistant). The breakpoints of colistin followed 2019 EUCAST recommendations ($\leq$2 mg/L susceptible; >2 mg/L resistant).

Results

Clinical characteristics

Among the 6806 *E. coli* and 3021 *K. pneumoniae* isolates from 2012–2018, and the 191 *A. baumannii* and 557 *P. aeruginosa* isolates from 2018, the numbers of isolates that were non-susceptible to imipenem were 17 (0.2%), 163 (5.4%), 136 (71.2%) and 81(14.5%), respectively (Figure S1). The clinical characteristics of patients from whom these isolates were recovered are listed in Table S2. These isolates were mostly from adults, in non-ICU wards

and in regional hospitals but the specimen sites and hospital geographical regions varied.

Antimicrobial susceptibility of imipenem-non-susceptible E. coli and K. pneumoniae

Table 1 shows the antimicrobial susceptibility of imipenem-non-susceptible *E. coli* and *K. pneumoniae*. Both bacteria were highly resistant to commonly used non-carbapenem β-lactam antibiotics. Even piperacillin/tazobactam, which exerted the highest inhibitory effect, inhibited only 17.6% and 9.8% of imipenem-non-susceptible *E. coli* and *K. pneumoniae*, respectively. The addition of novel β-lactamase inhibitors or β-lactam enhancers (BLI-BLE) greatly increased the susceptibility of β-lactam antibiotics. Zidebactam, avibactam, relebactam and vaborbactam increased the susceptibility to cefepime, ceftazidime, imipenem and meropenem of *E. coli* by 58.8% (100% versus 41.2%), 58.8% (70.6% versus 11.8%), 70.6% (70.6% versus 0%) and 41.2% (94.1% versus 52.9%) and of *K. pneumoniae* by 77.9% (99.4% versus 21.5%), 88.3% (90.8% versus 2.5%), 76.1% (76.1% versus 0%) and 54.0% (81.6% versus 27.6%), respectively. Concurrently, there was a consistent left shift of MIC distribution for these β-lactam antibiotics in the presence of the novel BLI-BLEs (Table S3).

Imipenem-non-susceptible *E. coli* and *K. pneumoniae* had similar high rates of non-susceptibility to ciprofloxacin and levofloxacin and the newer fluoroquinolones lascufloxacin and delafloxacin (Table 1). Susceptibility to these fluoroquinolones ranged from 17.6% to 23.5% in *E. coli* and was even lower (<10%) in *K. pneumoniae*. Tigecycline was the most active tetracycline tested (>95% susceptibility) but the MIC range of eravacycline was similar to or lower than that of tigecycline (Table S3 and Figure S2). The rates of susceptibility to minocycline, eravacycline and

Table 1. Antimicrobial susceptibility of imipenem-non-susceptible *E. coli* and *K. pneumoniae* from the TSAR programme, 2012–18

	E. coli ($n = 17$)						*K. pneumoniae* ($n = 163$)					
Antibiotic	R (%)	I (%)	S (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	R (%)	I (%)	S (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)
Ampicillin	100	0	0	>16	>16	>16	100	0	0	>16	>16	>16
Cefazolin	100	0	0	>16	>16	>16	99.4	0	0.6	>16	>16	4 to >16
Cefepime[a]	58.8	0	41.2	16	>32	≤0.25 to >32	78.5	0	21.5	>32	>32	≤0.25 to >32
Cefepime/ zidebactam	0	0	100	1	4	≤0.25–4	0.6	0	99.4	1	4	≤0.25 to >32
Cefotaxime	94.1	0	5.9	>32	>32	≤1 to >32	96.3	1.2	2.5	>32	>32	≤1 to >32
Cefoxitin	94.1	5.9	0	>16	>16	16 to >16	98.2	0	1.8	>16	>16	≤4 to >16
Ceftazidime	88.2	0	11.8	>32	>32	≤0.25 to >32	96.3	1.2	2.5	>32	>32	≤0.25 to >32
Ceftazidime/ avibactam	29.4	0	70.6	2	>32	≤0.25 to >32	9.2	0	90.8	2	8	≤0.25 to >32
Cefuroxime	100	0	0	>16	>16	>16	97.5	0	2.5	>16	>16	8 to >16
Imipenem	47.1	52.9	0	2	>16	2 to >16	71.2	28.8	0	8	>16	2 to >16
Imipenem/ relebactam	29.4	0	70.6	1	8	≤0.125 to >16	16	8	76.1	0.5	4	≤0.125–16
Meropenem	35.3	11.8	52.9	1	>32	0.125 to >32	67.5	4.9	27.6	8	>32	≤0.06 to >32
Meropenem/ vaborbactam	5.9	0	94.1	0.5	4	≤0.06 to >32	6.7	11.7	81.6	2	8	≤0.06 to >32
Piperacillin/ tazobactam	70.6	11.8	17.6	>64	>64	≤4 to >64	87.1	3.1	9.8	>64	>64	≤4 to >64
Ciprofloxacin	82.4	0	17.6	>16	>16	≤0.03 to >16	91.4	4.9	3.7	>16	>16	≤0.03 to >16
Delafloxacin	76.5	0	23.5	8	>32	≤0.06 to >32	97.5	0	2.5	>32	>32	≤0.06 to >32
Lascufloxacin	76.5	0	23.5	32	>64	0.06 to >64	96.3	1.8	1.8	>64	>64	0.125 to >64
Levofloxacin	82.4	0	17.6	32	>32	≤0.25 to >32	89	3.7	7.4	>32	>32	≤0.25 to >32
Eravacycline	23.5	0	76.5	0.5	1	0.125–2	63.2	0	36.8	1	2	0.25 to >8
Minocycline	23.5	11.8	64.7	4	16	1–32	27.6	42.9	29.4	8	32	1 to >64
Omadacycline	11.8	17.6	70.6	4	16	1–32	27.6	44.2	28.2	8	16	2 to >32
Tigecycline	0	0	100	0.5	2	0.25–2	0.6	2.5	96.9	1	2	0.25–8
Amikacin	5.9	0	94.1	≤4	16	≤4 to >32	22.7	0.6	76.7	≤4	>32	≤4 to >32
Gentamicin	41.2	0	58.8	2	>8	≤1 to >8	66.9	1.2	31.9	>8	>8	≤1 to >8
Aztreonam	88.2	5.9	5.9	>16	>16	≤1 to >16	86.5	4.9	8.6	>16	>16	≤1 to >16
Colistin	5.9	0	94.1	≤0.5	≤0.5	≤0.5 to >2	12.9	0	87.1	≤0.5	>2	≤0.5 to >2

R, resistant; I intermediate; S, susceptible.
[a]SDD and susceptible breakpoints (≤8 mg/L) for cefepime were used here.

omadacycline were 64.7%, 76.5% and 70.6% in *E. coli* but were only 29.4%, 36.8% and 28.2%, respectively, in *K. pneumoniae*.

Effect of carbapenemase genotypes and phenotypes on susceptibility

The $bla_{\text{OXA-48-like}}$ ($n = 2$), $bla_{\text{VIM-like}}$ (1), $bla_{\text{IMP-like}}$ (1) and $bla_{\text{NDM-like}}$ (1) genes were found in 5 of the 17 *E. coli* (29.4%). Among 87 of the 163 *K. pneumoniae* harbouring carbapenemase genes (53.4%), $bla_{\text{KPC-like}}$ ($n = 62$) was the most commonly identified gene, followed by $bla_{\text{OXA-48-like}}$ (19), $bla_{\text{IMP-like}}$ (5), $bla_{\text{VIM-like}}$ (5) and $bla_{\text{NDM-like}}$ (1). The low prevalence of carbapenemases in imipenem-non-susceptible *E. coli* and *K. pneumoniae* in Taiwan has been reported.[20,21] Among the 88 *E. coli* and *K. pneumoniae* isolates without any detectable carbapenemase gene, 85 had *ampC* and/or bla_{ESBL}.

None of the 58 *K. pneumoniae* and *E. coli* isolates with $bla_{\text{KPC-like}}$ genes was susceptible to cefepime, ceftazidime, imipenem or meropenem but zidebactam, avibactam, relebactam and vaborbactam restored their susceptibility to 100%, 96.6%, 84.5% and 75.9%, respectively (Table 2). Avibactam and zidebactam increased the susceptibility of isolates with $bla_{\text{OXA-48-like}}$ genes to ceftazidime and cefepime by 85% and 75%, respectively. Intriguingly, the addition of relebactam increased imipenem susceptibility in six isolates with $bla_{\text{OXA-48-like}}$ genes, whose MICs were marginally decreased by 2–4-fold. The β-lactam/novel BLI-BLE combinations were highly active *in vitro* against isolates without detectable carbapenemases regardless of the presence of *ampC* and/or bla_{ESBL}.

The types of carbapenemases identified by phenotypic testing were in accordance with the PCR except seven *K. pneumoniae* isolates that were falsely categorized as class B carbapenemase producers by the phenotypic testing. They were excluded from the class B carbapenemase producers because WGS showed only the presence of $bla_{\text{KPC-like}}$ genes and they were susceptible to β-lactam/novel BLI-BLE combinations (data not shown). After

Table 2. Susceptibility of imipenem-non-susceptible bacteria with different genotypes and phenotypes to β-lactams with and without novel BLI-BLEs

		Susceptible (%)							
Species with different genotypes and phenotypes	*N*	FEP[a]	FPZ	CAZ	CZA	IPM	IMR	MEM	MEV
E. coli and *K. pneumoniae*	180	23.3	99.4	3.3	88.9	0	75.6	30	82.8
genotype									
with carbapenemase gene	92	5.4	100	2.2	82.6	0	59.8	7.6	70.7
with class B carbapenemase gene	13	0	100	0	0	0	0	7.7	53.8
with $bla_{\text{KPC-like}}$ only	58	0	100	0	96.6	0	84.5	0	75.9
with $bla_{\text{OXA-48-like}}$ only	20	25	100	10	95	0	30	30	70
without carbapenemase gene	88	42	98.9	4.5	95.5	0	92	53.4	95.5
with ESBL gene[a] only	1	0	100	100	100	0	100	100	100
with AmpC gene only	41	85.4	97.6	2.4	95.1	0	95.1	63.4	95.1
with ESBL and AmpC gene[b]	43	0	100	0	95.3	0	90.7	41.9	95.3
phenotype[c]									
class B carbapenemase producer[d]	11	0	100	0	0	0	0	9.1	45.5
non-MBL producer	82	7.3	100	2.4	93.9	0	68.3	8.5	74.4
non-carbapenemase producer	87	41.4	98.9	4.6	95.4	0	92	52.9	95.4
ESBL producer	22	4.5	100	4.5	100	0	100	72.7	100
A. baumannii	136	1.5	8.1	2.2	1.5	0	0.7	0	0
genotype									
with class D carbapenemase gene	135	1.5	8.1	2.2	1.5	0	0	0	0
$bla_{\text{OXA-23-like}}$	117	0	6.8	1.7	0.9	0	0	0	0
$bla_{\text{OXA-24-like}}$	22	4.5	13.6	0	4.5	0	0	0	0
P. aeruginosa	81	45.7	91.4	43.2	81.5	0	85.2	28.4	42.0
genotype									
with class B carbapenemase gene	4	0	75	0	0	0	0	0	0
without carbapenemase gene	77	48.1	92.2	45.5	85.7	0	89.6	29.9	44.2

FEP, cefepime; FPZ, cefepime/zidebactam; CAZ, ceftazidime; CZA, ceftazidime/avibactam; IPM; imipenem; IMR, imipenem/relebactam; MEM, meropenem; MEV, meropenem/vaborbactam.

[a]SDD and susceptible breakpoints (≤8 mg/L) for cefepime were used here.

[b]Only prevalent ESBL genes were tested.

[c]Isolates with positive mCIM and eCIM were defined as class B carbapenemase (MBL) producers; those with positive mCIM but negative eCIM were non-MBL carbapenemase producers; those with negative mCIM were non-carbapenemase producers.

[d]Seven of the 18 isolates positive for both eCIM and mCIM were only positive for $bla_{\text{KPC-like}}$ by PCR, which was further confirmed by WGS. Therefore, these seven isolates were categorized as non-MBL carbapenemase producers.

Table 3. Antimicrobial susceptibility of imipenem-non-susceptible *A. baumannii* and *P. aeruginosa* from the TSAR programme, 2018

	A. baumannii (*n* = 136)						*P. aeruginosa* (*n* = 81)					
Antibiotics	R (%)	I (%)	S (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	R (%)	I (%)	S (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)
Ampicillin/sulbactam	86.8	11.8	1.5	64	>64	≤4 to >64	Intrinsic resistance					
Cefepime	97.1	1.5	1.5	>32	>32	8 to >32	28.4	25.9	45.7	16	32	1 to >32
Cefepime/zidebactam	38.2	53.7	8.1	16	32	8 to >32	0	8.6	91.4	4	8	0.5–16
Cefotaxime	96.3	3.7	0	>32	>32	16 to >32	Intrinsic resistance					
Ceftazidime	97.1	0.7	2.2	>32	>32	4 to >32	44.4	12.3	43.2	16	>32	1 to >32
Ceftazidime/avibactam	98.5	0	1.5	>32	>32	8 to >32	18.5	0	81.5	4	32	1 to >32
Imipenem	99.3	0.7	0	>16	>16	4 to >16	70.4	29.6	0	16	>16	4 to >16
Imipenem/relebactam	99.3	0	0.7	>16	>16	1 to >16	7.4	7.4	85.2	1	4	0.25–16
Meropenem	99.3	0.7	0	>32	>32	4 to >32	61.7	9.9	28.4	8	32	0.125 to >32
Meropenem/vaborbactam	98.5	1.5	0	>32	>32	8 to >32	35.8	22.2	42	8	32	0.25 to >32
Piperacillin/tazobactam	98.5	1.5	0	>64	>64	32 to >64	29.6	19.8	50.6	16	>64	≤8 to >64
Ciprofloxacin	99.3	0	0.7	>16	>16	1 to >16	56.8	7.4	35.8	2	>16	0.06 to >16
Delafloxacin	98.5	0	1.5	4	16	0.25 to >32	56.8	12.3	30.9	2	32	0.125 to >32
Lascufloxacin	79.4	18.4	2.2	8	16	1 to >64	97.5	2.5	0	64	>64	2 to >64
Levofloxacin	93.4	5.9	0.7	16	>32	≤0.25 to >32	65.4	6.2	28.4	4	>32	≤0.25 to >32
Eravacycline	80.9	0	19.1	1	2	0.125–8	Intrinsic resistance					
Minocycline	17.6	41.9	40.4	8	16	≤0.5–16	Intrinsic resistance					
Omadacycline	8.1	55.9	36	8	8	1–32	Intrinsic resistance					
Tigecycline	6.6	22.8	70.6	2	4	0.25–16	Intrinsic resistance					
Amikacin	88.2	0.7	11	>32	>32	≤4 to >32	2.5	2.5	95	≤4	8	≤4 to >32
Gentamicin	90.4	2.9	6.6	>8	>8	≤1 to >8	19.8	1.2	79	2	>8	≤1 to >8
Tobramycin	89	2.2	8.8	>8	>8	≤1 to >8	19.8	0	80.2	≤1	>8	≤1 to >8
Aztreonam	Intrinsic resistance						33	13.6	53.1	8	>16	≤2 to >16
Colistin	0.7	0	99.3	≤0.25	0.5	≤0.25 to >4	3.7	0	96.3	1	2	≤0.25 to >4

R, resistant; I intermediate; S, susceptible.

re-categorizing these seven isolates into the non-MBL-producer group, the effect of phenotypes on susceptibility to the β-lactam/novel BLI-BLE combinations was also similar to the observed effect of genotypes (Table 2). Susceptibility to fluoroquinolones and tetracyclines, either new or old agents, was not contingent on the genotypes and phenotypes of carbapenemase (Table S4) and susceptibility to tigecycline and colistin remained at >80% in all subgroups.

Antimicrobial susceptibility in imipenem-non-susceptible A. baumannii

The 136 imipenem-non-susceptible *A. baumannii* isolates were highly resistant to all β-lactams, with or without BLI-BLE, as well as all fluoroquinolones tested, with susceptibility rates of less than 10% (Table 3). Among tetracyclines, tigecycline was the most active, followed by minocycline, omadacycline and eravacycline (susceptibility rates of 70.6%, 40.4%, 36% and 19.1%, respectively). However, the MIC distribution, MIC_{50} and MIC_{90} were the lowest for eravacycline, followed by tigecycline (Table 3 and Table S3).

The $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, IS*Aba1*-$bla_{OXA\text{-}51\text{-}like}$ and $bla_{OXA\text{-}58\text{-}like}$ genes were found in 117, 22, 11 and 1 isolates, respectively. In isolates with $bla_{OXA\text{-}23\text{-}like}$ or $bla_{OXA\text{-}24\text{-}like}$ genes, susceptibility to the β-lactam/novel BLI-BLE combinations, as well as newly developed fluoroquinolones and tetracyclines, remained similar to the overall trend (Table 2 and Table S4).

Antimicrobial susceptibility in imipenem-non-susceptible P. aeruginosa

The susceptibility rates to many commonly used broad-spectrum β-lactams, i.e. aztreonam, cefepime, ceftazidime and piperacillin/tazobactam, of imipenem-non-susceptible *P. aeruginosa* were around 40%–60%; only a few isolates (15 of 81; 18.5%) were resistant concomitantly to these commonly used broad-spectrum β-lactams. The addition of a novel BLI-BLE increased the

susceptibility by 45.7%, 38.3% and 85.2% for cefepime, ceftazidime and imipenem, respectively, with a corresponding left shift in MICs (Table 3 and Table S3). In contrast, the addition of vaborbactam only marginally increased the meropenem susceptibility from 28.4% to 42%, without an MIC shift. Similar rates of susceptibility were observed to the new fluroquinolone delafloxacin and old fluoroquinolones (<40%) but no isolate was susceptible to lascufloxacin.

Most imipenem-non-susceptible *P. aeruginosa* isolates (77 of 81) lacked carbapenemase genes and were susceptible to β-lactam/novel BLI-BLE combinations, except meropenem/vaborbactam. Three of the four *P. aeruginosa* with $bla_{\text{VIM-like}}$ genes were susceptible to cefepime/zidebactam (Table 2), with an MIC decrease of 2–4-fold. Overall, susceptibility rates for amikacin and colistin were the highest.

Discussion

This study compared the *in vitro* activity of recently developed antibiotics with commonly used ones against four WHO priority bacteria. The addition of novel BLI-BLEs to β-lactams greatly increased their effect on imipenem-non-susceptible *E. coli* and *K. pneumoniae*; the degree of change depended on β-lactamase phenotypes and genotypes. Imipenem-non-susceptible *A. baumannii* were highly resistant to the β-lactam/novel BLI-BLEs tested. Cefepime/zidebactam, ceftazidime/avibactam and imipenem/relebactam had >80% inhibitory effect on imipenem-non-susceptible *P. aeruginosa*. Omadacycline, delafloxacin and lascufloxacin were not superior to commonly used antibiotics against imipenem-non-susceptible bacteria.

The activity of β-lactams in combination with novel BLI-BLEs varied depending on the resistance mechanisms in *E. coli* and *K. pneumoniae*. Our results were generally in accordance with previous studies.[4,5] All new BLI-BLEs increased the susceptibility of partner β-lactams in isolates with $bla_{\text{KPC-like}}$, bla_{ESBL} and/or *ampC* genes. Ceftazidime/avibactam was additionally active against isolates with $bla_{\text{OXA-48-like}}$ genes. However, meropenem/vaborbactam, which lacks activity against class B or D carbapenemase,[22] appeared to inhibit isolates with these genes in our study (Table 2). The difference in breakpoints used for meropenem/vaborbactam ($S \leq 4$ mg/L) and meropenem ($S \leq 1$ mg/L) was the main factor responsible for the increased susceptibility since the MIC distributions of meropenem and meropenem/vaborbactam were similar in our isolates with class B or D carbapenemase genes (data not shown). A similar situation was also observed for *P. aeruginosa* (Table S1).

Cefepime/zidebactam was reported to be active against various pathogens harbouring class A, B or D carbapenemase genes.[23–25] The effect was also observed in our *E. coli* and *K. pneumoniae* as well as *P. aeruginosa* with different resistance mechanisms. It is postulated that zidebactam promotes cefepime killing by targeting different PBPs but not inhibition of MBL.[23–25] Other studies further showed its modest effect on *A. baumannii*; one study reported that the addition of zidebactam increased the proportion of isolates with cefepime MICs of ≤8 mg/L from 3.8% to 25.6%.[6] However, the addition of zidebactam did not significantly lower the cefepime MICs to ≤8 mg/L in our *A. baumannii* isolates (Table S3).

Sfeir *et al.*[26] demonstrated that eCIM had a sensitivity of 100% but a specificity of 90%–100%, due to two *K. pneumoniae* isolates with $bla_{\text{OXA-232}}$ that tested positive initially but negative upon repeat testing. Our study showed that seven *K. pneumoniae* isolates with $bla_{\text{KPC-like}}$ genes repeatedly tested positive on eCIM under the same conditions.[26] Using PCR as a standard, the sensitivity and specificity of eCIM were 100% (11/11) and 91.5% (75/82), respectively, which are similar to those shown by Sfeir *et al.* The eCIM had high accuracy but a small chance of false positivity is still possible.

Omadacycline, delafloxacin and lascufloxacin are indicated against Gram-positive pathogens and community-acquired infections since their activity against healthcare-associated Gram-negative pathogens was not expected to be superior to commonly used comparators, i.e. levofloxacin or tigecycline, as shown by previous studies and ours.[27–29] Comparison of eravacycline and tigecycline activity was difficult due to different breakpoints set by the US FDA (≤0.5 and ≤2 mg/L, respectively), which resulted in lower susceptibility to eravacycline despite having lower MICs compared with tigecycline MICs seen in our isolates (Table S3 and Figure S2) and in previous studies.[30] It is further complicated by the different breakpoints set by the US FDA and EUCAST. An agreement on breakpoints via harmonization of various regulatory agencies would facilitate future comparative studies.

There were limitations of our study. First, not all recently developed antibiotics were included due to the difficulty of access to drugs under development. Second, some isolates were carbapenemase negative so porin alteration and/or efflux pump overexpression likely played a role in these isolates.[21] In Taiwan, porin loss in combination with the presence of AmpC or ESBL has been reported to be the main mechanism of carbapenem resistance in *K. pneumoniae* and *E. coli*.[31] However, the roles of efflux pumps or porins were not determined in our study. Third, our PCR only targeted a limited number of genes, i.e. those encoding prevalent ESBLs ($bla_{\text{CTX-M-type}}$ but not bla_{TEM}).[21,32,33] Resistance mechanisms identified by WGS would be more comprehensive. Fourth, our study did not select for certain resistance mechanisms, i.e. class B carbapenemases; therefore, the number of isolates with a specific resistance mechanism was limited. However, our results for these isolates were still in concordance with previously published studies.[4–8]

In conclusion, β-lactam plus novel BLI-BLE combinations inhibited most imipenem-non-susceptible *E. coli*, *K. pneumoniae* and *P. aeruginosa* but none was active against imipenem-non-susceptible *A. baumannii*. Cefepime/zidebactam was the most active, even against isolates with an MBL. New fluoroquinolones and tetracyclines were not superior to old ones but eravacycline had lower MICs compared with tigecycline.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 to S4 and Figures S1 and S2 are available as Supplementary data at *JAC* Online.

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