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Original Article

In vitro activity of imipenem/relebactam, meropenem/vaborbactam and comparators against *Enterobacterales* from patients with intra-abdominal infections: Results of the study for Monitoring Antimicrobial Resistance Trends (SMART) in Taiwan, 2020

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Cross resistance

Abstract *Background:* Multi-drug resistant *Enterobacterales* is a growing health threat. Imipenem/relebactam and meropenem/vaborbactam, are not clinically used in Taiwan and the susceptibility is lack from routine laboratory tests.
Methods: Broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints criteria. Isolates that were not susceptible to imipenem (MIC $\geq$ 2 mg/L), imipenem/relebactam (MIC $\geq$ 2 mg/L), or ceftolozane-tazobactam (MIC $\geq$ 4 mg/L) were selected for further molecular testing for genes encoding extended-spectrum beta-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases by multiplex PCR assays.
Results: A total of 290 *Enterobacterales* isolates from 9 participating hospitals were collected in 2020. *Escherichia coli* (n = 135, 46.6%) and *Klebsiella pneumoniae* (n = 88, 30.3%) were two

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leading pathogens of all *Enterobacterales* isolates. The antimicrobial agents with susceptibility rates more than 90% included amikacin (99.3%, 288/290), ertapenem (90.0%, 261/290), meropenem (97.2%, 282/290), imipenem/relebactam (94.8%, 275/290) and meropenem/vaborbactam (99.3%, 288/290). *K. pneumoniae* isolates were less susceptible to ertapenem, imipenem, meropenem, piperacillin-tazobactam and ceftozolane/tazobactam than *E. coli* (all $p < 0.05$). ESBL, AmpC, and carbapenemase were detected in 40.5% (17/42), 45.2% (19/42) and 11.9% (5/42) among tested isolates, respectively. The 5 carbapenemase genes included 4 bla_{KPC} and 1 bla_{IMP}. The imipenem-non-susceptible isolates (n = 30) had higher susceptibility rates to meropenem/vaborbactam (93.3%, 28/30) than imipenem/relebactam (50%, 12/30) ($p < 0.05$).
Conclusions: Imipenem/relebactam and meropenem/vaborbactam had excellent efficacy against *Enterobacterales* isolates. Meropenem/vaborbactam allowed better salvage therapy for carbapenem-resistant *Enterobacterales* infections.

Introduction

Complicated intra-abdominal infections (cIAIs) are common clinical diseases but often associated with certain morbidity and mortality particularly in higher risk patients.[1] Both adequate infection source control and appropriate antimicrobial therapy are cornerstones in the management of IAIs.[2,3] The cIAIs often caused by microbial contamination from the gastrointestinal tract and the infecting organisms depend on the source of contamination. Among all etiology, *Enterobacterales* are the predominant pathogens isolated from patients with cIAIs.[4,5] With extensive use of antibiotics, the emergence and dissemination of antibiotic resistance are growing threats to public health, especially for β-lactams that are the most widely prescribed antibiotics in treating *Enterobacterales* related infections.[6] β-Lactamase production has been reported globally and is responsible for the increased resistance to β-lactams in *Enterobacterales* isolates.[3,7,8] Extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases (AmpC), and carbapenemases are the three major classes of β-lactamases commonly produced by *Enterobacterales*.[9–13]

Recently, novel beta-lactamase inhibitors have been developed to combat multidrug-resistant *Enterobacterales*.[12,14,15] Relebactam, a bicyclic diazabicyclooctane, and vaborbactam, a cyclic boronic acid are two new beta-lactamase inhibitors with activity against Ambler class A β-lactamase including ESBLs and *Klebsiella pneumoniae* carbapenemases (KPCs), and class C β-lactamases (AmpC).[14–17] In combination with ordinary carbapenems, imipenem/relebactam and meropenem/vaborbactam were licensed by U.S. Food & Drug Administration (FDA) in 2019 and 2017, respectively.[16,17] However, these two agents have not been introduced to Taiwan. As a result, susceptibility to them has not been routinely tested in clinical laboratories and the epidemiological data are lacking.

The Study for Monitoring Antimicrobial Resistance Trends (SMART) is a surveillance program to investigate the *in vitro* antimicrobial susceptibility of clinical important Gram-negative bacilli causing IAIs.[18] The purpose of this study was to evaluate the *in vitro* activity of two novel carbapenem-β-lactamase inhibitor combinations and other comparators among *Enterobacterales* isolates from patients with IAIs in Taiwan.

Material and methods

Bacterial isolates and identification

Bacterial identification was verified by using MALDI-TOF spectrometry in the central laboratory. Community-acquired infections were defined as isolates collected <48 h after hospitalization with symptoms and signs of infection upon admission. On the other hand, hospital-acquired isolates were defined as those collected ≧48 h after hospitalization from patients who initially did not have symptoms or signs of infection. The Institutional Review Board or Research Ethics Committees of each participating hospitals have approved the SMART program and informed consent was waived due to no more than minimal risk to the participating patients.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined at IHMA (Schaumburg, IL) using the CLSI broth microdilution method with frozen panels prepared at IHMA. MIC interpretive criteria followed the Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S31, 2021).[20] Minimum inhibitory concentrations (MICs) were determined for tested agents including amikacin, aztreonam, cefepime, ceftazidime, ceftriaxone, ceftolozane/tazobactam, colistin, ertapenem, imipenem, imipenem/relebactam, levofloxacin, meropenem, meropenem/vaborbactam and piperacillin/tazobactam. Isolates except *Morganellaceae* and *Serratia* spp. were selected for further molecular test of genes encoding common β-lactamases including ESBLs, AmpCs β-lactamases and carbapenemases if the isolates showed non-susceptible to imipenem (MIC ≥ 2 mg/L), imipenem/relebactam (MIC ≥ 2 mg/L) and/or ceftolozane/tazobactam (MIC ≥ 4 mg/L). *Morganellaceae* (*Proteus* spp., *Morganella* spp., and *Providencia* spp.) were excluded for molecular gene detection because these isolates are often intrinsically non-susceptible to imipenem mostly due to weak affinity to penicillin binding proteins (PBPs) or porin loss but not carbapenemase production.[13] On the other hand, *Serratia* spp. isolates were also not characterized because genes encoding acquired β-lactamases were rarely detected in

isolates that met above criteria. A previous report stated that carbapenemase-producing *Serratia marcescens* accounted for only 45 isolates amongst 7800 carbapenem-resistant isolates.[19]

Detection of β-lactamase genes

An overnight single colony grown on a blood agar plate (Thermo Fisher Scientific, Waltham, MA, USA) at 35 °C was collected for genomic DNA extraction. Whole genomic DNA was extracted using the QIAamp DNA minikit and a QIAcube instrument (Qiagen, Valencia, CA). A multiplex PCR for the rapid detection of presence of ESBL (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{VEB}, bla_{PER}, and bla_{GES}) and AmpC β-lactamases (bla_{CMY}, bla_{DHA}, bla_{FOX}, bla_{MOX}, bla_{ACC}, bla_{MIR}, and bla_{ACT}) genes and carbapenemase (bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, and bla_{OXA}) genes among selected *Enterobacterales* isolates as previously described.[11]

Statistical analysis

Statistical analyses were performed using the MedCalc software (MedCalc Software Ltd, Los Angeles, CA, USA). Categorical variables were compared using the Chi-square or Fisher's exact tests. All tests were two-tailed, and a p value of <0.05, was considered as significant.

Results

Characteristics of *Enterobacterales* isolates

A total of 290 *Enterobacterales* isolates from participating hospitals were collected and tested in 2020 including *Escherichia coli* (n = 135, 46.6%), *K. pneumoniae* (n = 88, 30.3%), *Enterobacter cloacae* (n = 16, 5.5%), *Enterobacter bugandensis* (n = 10, 3.4%), *Proteus mirabilis* (n = 9, 3.1%), *Klebsiella aerogenes* (n = 9, 3.1%), *Klebsiella oxytoca* (n = 8, 2.8%), *S. marcescens* (n = 6, 2.1%), *Citrobacter koseri* (n = 5, 1.7%) and *Morganella morganii* (n = 4, 1.4%). With regard to the infection sources, gall bladders (n = 143, 49.3%) and peritoneal fluids (n = 95, 32.8%) were two leading sites where *Enterobacterales* isolated from. The ranking of species in community-acquired IAIs (isolates obtained within 48 h) was similar to that in nosocomial IAIs (isolates obtained ≥ 48 h), otherwise, isolates from appendix were more found in community-acquired IAIs (13.8% vs. 1.0%, $p < 0.05$). The number of *Enterobacterales* isolates fulfilled the criteria to undergo molecular resistance analysis was also found more among isolates from nosocomial IAIs than that from community-acquired IAIs (18.4% (36/196) vs. 6.4% (6/94), $p < 0.001$). Genes encoding for ESBL and carbapenemase were not found among isolates from community-acquired IAIs but high proportion among isolates from nosocomial IAIs (Table 1).

Antimicrobial susceptibility

For all 290 *Enterobacterales* isolates, the antimicrobial agents with susceptibility rates more than 90% included amikacin (99.3%, 288/290), ertapenem (90.0%, 261/290), meropenem (97.2%, 282/290), imipenem/relebactam (94.8%, 275/290) and meropenem/vaborbactam (99.3%, 288/290). A total of 32 (11.0%, 32/290) of *Enterobacterales* isolates were resistant to colistin (MIC of colistin ≧ 4 mg/L). "On the contrast, other agents, including aztreonam, ceftazidime, ceftriaxone, and levofloxacin all had susceptibility rates less than 70%. Isolates collected 48 h or more after admission had higher resistant rates than those collected less than 48 h, especially to aztreonam, cephalosporins (cefepime, ceftazidime, and ceftriaxone), piperacillin/tazobactam, and ceftolozane/tazobactam (Fig. 1)".

For two novel β-lactam/β-lactamase inhibitor combinations, 9 isolates (1 *E. coli*, 2 *K. pneumoniae*, 2 *M. morganii*, and 4 *P. mirabilis*) had intermediate results and 6 isolates (1 *E. coli*, 1 *E. cloacae*, 1 *M. morganii*, and 3 *P. mirabilis*) had resistant results to imipenem/relebactam. Besides, only two isolates (1 *E. coli* and 1 *K. pneumoniae*) showed intermediate results to meropenem/vaborbactam.

A total of 30 *Enterobacterales* isolates were found to have intermediate or resistant MIC results to imipenem (MIC ≥2 mg/L) including 11 *K. pneumoniae*, seven *P. mirabilis*, four *M. morganii*, three *E. coli*, two *E. cloacae*, two *K. aerogenes*, and one *E. bugandensis* isolates. For 14 isolates with intermediate MIC results to imipenem (2 mg/L) were susceptible to imipenem/relebactam (57.1%, 8/14), meropenem (100%, 14/14), and meropenem/vaborbactam (100%, 14/14), respectively. Besides, 16 *Enterobacterales* isolates with resistance to imipenem (MIC ≥ 4 mg/L) were susceptible to meropenem (56.3%, 9/16), imipenem/relebactam (43.8%, 7/16), and meropenem/vaborbactam (87.5%, 14/16), respectively. The range of the MIC and antimicrobial susceptibility rates of *E. coli* and *K. pneumoniae* were presented in Table 2. In general, the susceptibility rates of *E. coli* were similar to *K. pneumoniae*. However, *K. pneumoniae* isolates were less susceptible to ertapenem (78.4% [69/88] vs 97.0% [131/135], $p < 0.001$), imipenem (87.5% [77/88] vs 97.8% [132/135], $p = 0.003$), meropenem (92.0% [81/88] vs 99.3% [131/135], $p = 0.007$), piperacillin/tazobactam (78.4% [69/88] vs 97.0% [131/135], $p < 0.001$) and ceftozolane/tazobactam (78.4% [69/88] vs 94.1% [127/135], $p < 0.001$) than *E. coli*. In addition, the difference in susceptible rates to tested antimicrobial agents of *E. coli* and *K. pneumoniae* isolates collected <48 h or ≧ 48 h after hospitalization were illustrated on Fig. 2. The difference in susceptible rates was more obvious among isolates collected ≧48 h.

Detection of beta-lactamase-encoding genes in *Enterobacterales*

A total of 42 *Enterobacterales* isolates fulfilled the criteria for further molecular resistance mechanism survey including 1 *C. koseri*, 2 *E. bugandensis*, 5 *E. cloacae*, 10 *E. coli*, 2 *K. aerogenes* and 22 *K. pneumoniae* isolates. ESBL, AmpC β-lactamase and carbapenemase were detected in 40.5% (17/42), 45.2% (19/42) and 11.9% (5/42) of all tested isolates, respectively. The distribution of β-lactamase among different species of *Enterobacterales* was illustrated in Fig. 3. The detected ESBLs included bla_{SHV} (9/42) and bla_{CTX-M} (10/42), AmpC β-lactamases included bla_{CMY} (4/

Table 1 Distribution of bacterial species, sample sources and resistance mechanism among *Enterobacterales* causing intra-abdominal infections in patients from whom isolates were obtained within 48 h (<48 h) or after 48 h (≥48 h) of hospitalization.

Character	No. (%) of isolates		
	<48 h (n = 94)	≥48 h (n = 196)	*p* value
Species			0.493
Escherichia coli	48 (51.1)	87 (44.4)	
Klebsiella pneumoniae	23 (24.5)	65 (33.2)	
Enterobacter cloacae	3 (3.2)	13 (6.6)	
Enterobacter bugandensis	3 (3.2)	7 (3.6)	
Klebsiella aerogenes	4 (4.3)	5 (2.6)	
Serratia marcescens	1 (1.1)	5 (2.6)	
Klebsiella oxytoca	4 (4.3)	4 (2)	
Proteus mirabilis	5 (5.3)	4 (2)	
Citrobacter koseri	2 (2.1)	3 (1.5)	
Morganella morganii	1 (1.1)	3 (1.5)	
Sources of isolates			<0.001
Gall Bladder	42 (44.7)	101 (51.5)	
Peritoneal fluid	26 (27.7)	69 (35.2)	
Abscess	7 (7.4)	9 (4.6)	
Liver	5 (5.3)	7 (3.6)	
Colon	0 (0)	3 (1.5)	
Stomach	0 (0)	3 (1.5)	
Appendix	13 (13.8)	2 (1)	
Pancreas	0 (0)	2 (1)	
Small intestine	1 (1.1)	0 (0)	
Resistance mechanisms			
Isolates fulfilled criteria of resistance mechanism survey	6 (6.4)	36 (18.4)	<0.001
ESBL	0 (0)[a]	17 (47.2)[a]	<0.001
AmpC β-lactamases	4 (66.7)[a]	15 (41.7)[a]	0.384
Carbapenemase	0 (0)[a]	5 (13.9)[a]	<0.001

[a] The percentage indicated number of isolates with detected ESBL, AmpC or carbaepenemase genes among isolates fulfilled criteria for resistance mechanism survey. ESBL, extended-spectrum β-lactamases.

42), bla_{DHA} (11/42), bla_{MIR} (1/42), and bla_{ACT} (3/42) and carbapenemases included bla_{KPC} (4/42), and bla_{IMP} (1/42). Some isolates had more than one β-lactamase gene detected including one *K. pneumoniae* isolate had bla_{SHV}, bla_{DHA}, and bla_{KPC}, another *K. pneumoniae* isolate had bla_{CTX-M} and bla_{KPC}, the other *K. pneumoniae* isolate had bla_{SHV} and bla_{KPC} and one *E. cloacae* isolate had bla_{SHV} and bla_{IMP}.

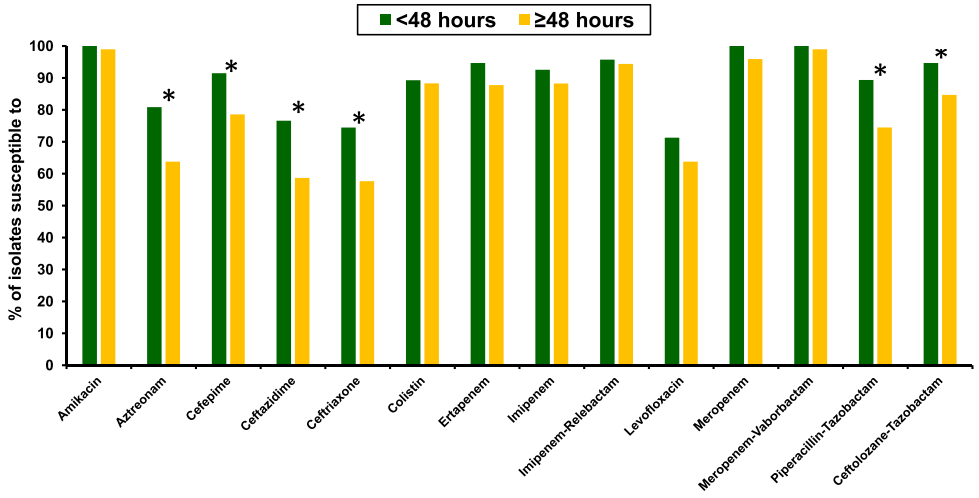

Figure 1. In vitro susceptibility rates to antimicrobial agents of *Enterobacterales* collected from patients with intra-abdominal infections in the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) in 2020 who were hospitalized at <48 or ≥48 h.

Table 2 Minimum inhibitory concentrations (MIC_{50}, MIC_{90}, MIC range [mg/L]) and antimicrobial susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* in the SMART program in 2020.

Organism/agent	MIC (mg/L)			% of isolates with indicated susceptibility category		
	MIC_{50}	MIC_{90}	Range	Susceptible	Intermediate	Resistant
***E. coli* (n = 135)**						
Amikacin	8	8	8–32	99.3	0.7	0
Aztreonam	1	8	1–8	65.2	34.8	0
Cefepime	1	16	1–16	77.8.	6.7	15.6
Ceftazidime	1	4	1–4	62.2	5.9	31.9
Ceftriaxone	1	4	1–4	57.8	0	42.2
Ceftolozane-tazobactam	0.5	1	0.06–16	94.1	1.5	4.4
Colistin	1	2	1–4	0	99.3	0.7
Ertapenem	0.12	0.12	0.12–2	97.0	1.5	1.5
Imipenem	0.25	0.5	0.12–8	97.8	1.5	0.7
Imipenem-relebactam	0.12	0.25	0.12–8	98.5	0.7	0.7
Levofloxacin	0.5	4	0.5–4	60.0	3.7	36.3
Meropenem	0.12	0.12	0.12–16	99.3	0	0.7
Meropenem-vabobactam	0.06	0.06	0.06–8	99.3	0.7	0
Piperacillin-tazobactam	4	16	4–64	89.6	2.2	8.1
***K. pneumoniae* (n = 88)**						
Amikacin	8	8	8–32	98.9	1.1	0
Aztreonam	1	8	1–8	72.7	27.3	0
Cefepime	1	16	1–16	81.8	2.3	15.9
Ceftazidime	1	16	1–16	63.6	5.7	30.7
Ceftriaxone	1	4	1–4	65.9	6.8	27.3
Ceftolozane-tazobactam	0.5	8	0.06–16	78.4	2.3	19.3
Colistin	1	2	1–4	0	98.9	1.1
Ertapenem	0.12	2	0.12–2	78.4	8.0	13.6
Imipenem	0.25	2	0.12–16	87.5	3.4	9.1
Imipenem-relebactam	0.12	0.5	0.12–2	97.7	2.3	0
Levofloxacin	0.5	4	0.5–4	60.2	14.8	25.0
Meropenem	0.12	0.25	0.12–16	92.0	1.1	6.8
Meropenem-vaborbactam	0.06	0.25	0.06–8	98.9	1.1	0
Piperacillin-tazobactam	4	64	4–64	69.3	6.8	23.9

S, susceptible; I, intermediate; R, resistant.

For 30 *Enterobacterales* isolates showed non-susceptible to imipenem (MIC $\geq$ 2 mg/L), the detailed MICs to imipenem/relebactam, meropenem, and meropenem/vaborbactam were listed on Table 3. For 14 *Enterobacterales* isolates with imipenem MIC equal to 2 mg/L, the susceptible rates to imipenem/relebactam, meropenem, and meropenem/vaborbactam were 57.1% (8/14), 100% (14/14) and 100% (14/14), respectively. On the other hand, the susceptible rates to imipenem/relebactam, meropenem, and meropenem/vaborbactam among 16 *Enterobacterales* isolates resistant to imipenem were 43.8% (7/16), 56.3% (9/16) and 87.5% (14/16), respectively. Only two strains were non-susceptible to all carbapenems with or without β-lactamases inhibitors including one *E. coli* and one *K. pneumoniae*. However, the *E. coli* harbored only ESBL genes (bla_{SHV} and $bla_{CTX\text{-}M}$), and the *K. pneumoniae* harbored bla_{SHV} and bla_{KPC}.

Discussion

Multi-drug resistant *Enterobacterales* is an emerging threat to public health and novel antimicrobial therapy is urgently needed. In our study, we had evaluated the *in vitro* susceptibility of *Enterobacterales* isolates to a variety of antimicrobial agents in a nationwide surveillance program, SMART study, in 2020 including two novel β-lactamases inhibitor combinations. Both imipenem/relebactam and meropenem/vaborbactam posed excellent activity to *Enterobacterales* in our study and the susceptible rates were 94.8% (275/290) and 99.3% (288/290), respectively. Even for imipenem-non-susceptible *Enterobacterales*, imipenem/relebactam and meropenem/vaborbactam still preserved 50% (15/30) and 93.3% (28/30) activity that could play roles in salvage treatment.

In IAIs, *E. coli* and *K. pneumoniae* were still the two leading *Enterobacterales* pathogens. Comparing to previous data from the SMART from 2006 to 2010 and 2018, we noted increasing resistant rates among *Enterobacterales* to multiple antimicrobial agents such as 3rd, 4th generation cephalosporins, fluoroquinolones and ertapenem in 2020.[4,8] The resistant rate to imipenem among *Enterobacterales* isolates was 5.5% (16/290) in our study which was higher than a previous study that only 0.6% *Enterobacterales* isolates were resistant to imipenem from 19 countries in Asia

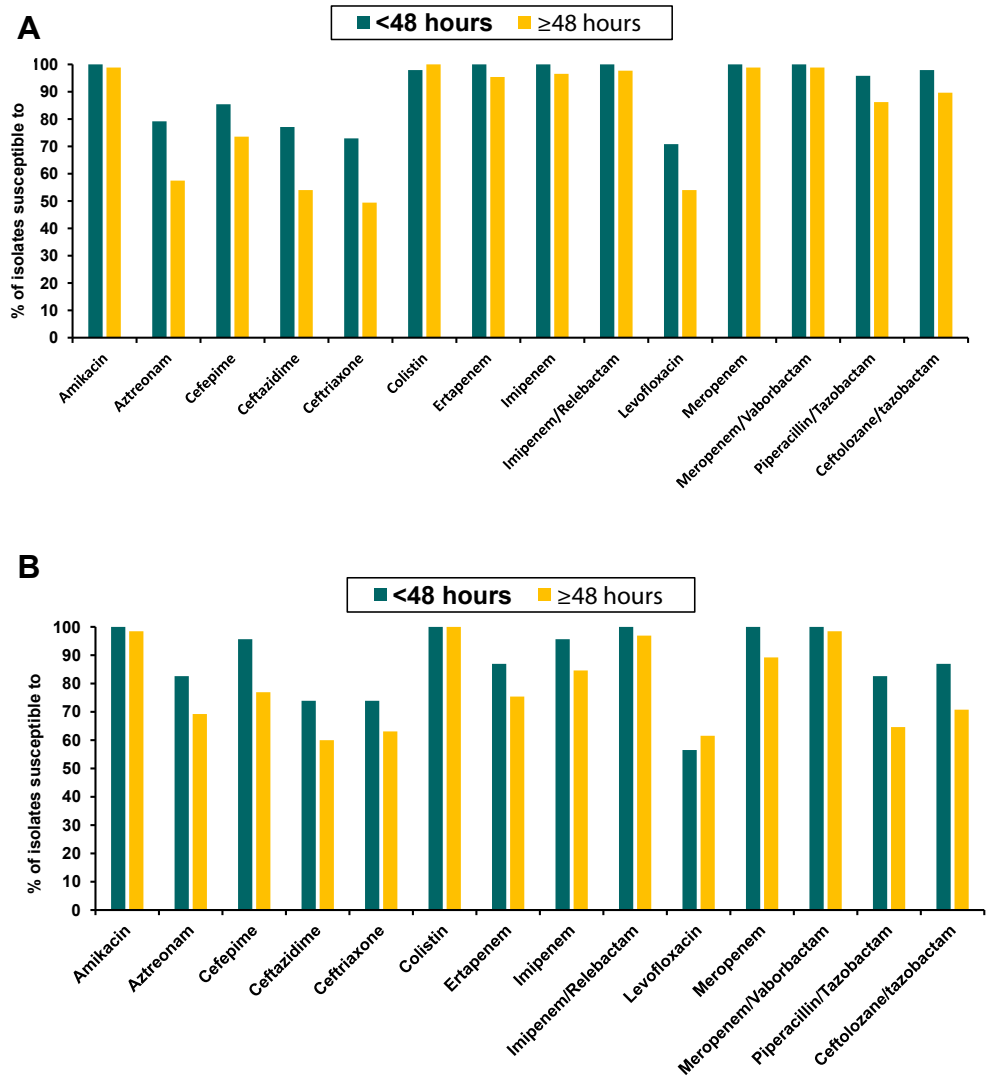

Figure 2. *In vitro* susceptibility rates to (A) *Escherichia coli* (n = 135) and (B) *Klebsiella pneumoniae* (n = 88) collected from patients with intra-abdominal infections in the SMART in 2020 who were hospitalized for <48 or ≥48 h.

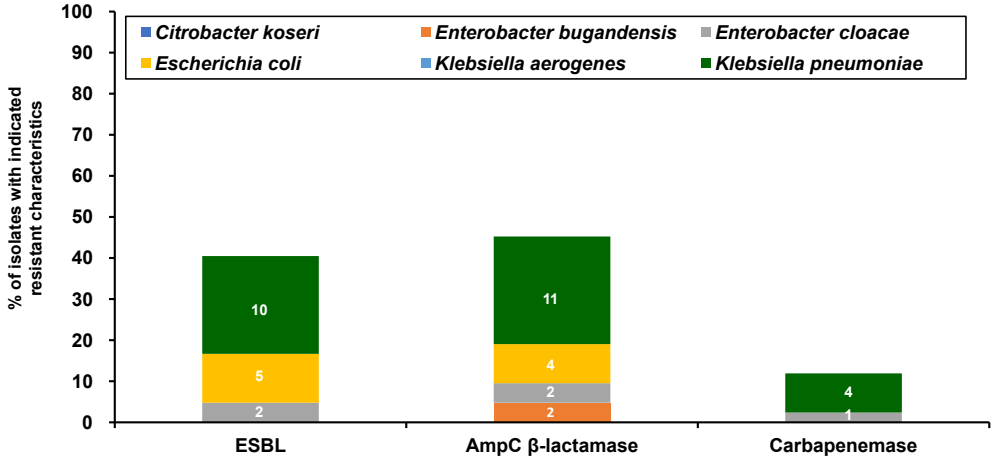

Figure 3. Distribution of extended-spectrum β-lactamases (ESBL), AmpC β-lactamase and carbapenemse gene among 42 isolates with imipenem minimum inhibitory concentration (MIC) ≥ 2 mg/L including 10 *Escherichia coli*, 22 *Klebsiella pneumoniae*, 5 *Enterobacter cloacae*, 2 *Klebsiella aerognenes*, 2 *Enterobacter bugandensis* and 1 *Citrobacter koseri*.

Table 3 List of minimum inhibitory concentration (MIC) of carbapenems and β-lactam combination agents against *Enterobacterales* with imipenem MIC ≥2 mg/L and associated resistance mechanisms collected from patients with intra-abdominal infections in the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) in 2020.

Species	MIC (mg/L)				Resistance mechanisms		
	Imipenem	Imipenem/ relebactam	Meropenem	Meropenem/ vaborbactam	ESBL	AmpC	carbapenemase
Enterobacter bugandensis	2.00	0.25	0.12	0.06	—	MIR-TYPE	—
Enterobacter cloacae	2.00	0.50	0.12	0.06	—	—	—
Enterobacter cloacae	4.00	4.00	1.00	1.00	SHV	—	IMP-8
Escherichia coli	2.00	2.00	0.12	0.06	TEM	—	—
Escherichia coli	2.00	1.00	0.12	0.06	—	—	—
Escherichia coli	8.00	8.00	16.00	8.00	SHV, CTX-M-9	—	—
Klebsiella aerogenes	2.00	0.25	0.12	0.06	—	—	—
Klebsiella aerogenes	2.00	0.25	0.12	0.06	—	—	—
Klebsiella pneumoniae	2.00	1.00	0.12	0.06	—	—	—
Klebsiella pneumoniae	2.00	0.50	0.12	0.06	—	—	—
Klebsiella pneumoniae	2.00	2.00	0.12	0.06	CTX-M-1	CMY-2	
Klebsiella pneumoniae	16.00	2.00	16.00	**8.00**	CTX-M-9	—	KPC-2
Klebsiella pneumoniae	16.00	0.25	16.00	1.00	SHV	—	KPC-17
Klebsiella pneumoniae	4.00	0.25	2.00	0.25	—	DHA	—
Klebsiella pneumoniae	4.00	0.25	1.00	0.25	—	DHA	—
Klebsiella pneumoniae	4.00	0.25	0.25	0.06	—	DHA	—
Klebsiella pneumoniae	16.00	0.12	16.00	0.25	—	—	KPC-type
Klebsiella pneumoniae	8.00	0.25	4.00	0.50	SHV	DHA	—
Klebsiella pneumoniae	16.00	0.25	16.00	2.00	SHV	DHA	KPC-2
Morganella morganii	2.00	1.00	0.12	0.06	ND	ND	ND
Morganella morganii	4.00	2.00	0.12	0.06	ND	ND	ND
Morganella morganii	8.00	2.00	0.12	0.06	ND	ND	ND
Morganella morganii	4.00	4.00	0.12	0.12	ND	ND	ND
Proteus mirabilis	2.00	2.00	0.12	0.06	ND	ND	ND
Proteus mirabilis	2.00	2.00	0.12	0.06	ND	ND	ND
Proteus mirabilis	2.00	2.00	0.12	0.06	ND	ND	ND
Proteus mirabilis	2.00	2.00	0.12	0.06	ND	ND	ND
Proteus mirabilis	4.00	4.00	0.12	0.06	ND	ND	ND
Proteus mirabilis	4.00	4.00	0.12	0.06	ND	ND	ND
Proteus mirabilis	4.00	4.00	0.12	0.06	ND	ND	ND

ND, not done; ESBL, extended-spectrum β-lactamases; OSBL, older-spectrum β-lactamases.

during 2002—2012.[21] Although some bias came from study design or sample sources, a significant increasing trend of carbapenem resistant *Enterobacterales* was observed with time.[22,23] Among carbapenem-resistant *Enterobacterales* (CRE), carbapenemase-producing *Enterobacterales* (CPE) comprised 31.3% (5/16) including 4 bla_{KPC} and 1 bla_{IMP} genes detected in present study. The proportion of CPE to CRE was similar to previous surveillance in Taiwan with rates which steadily increased from 24.1% in 2012 to 46.5% in 2017.[22,24] All 4 bla_{KPC} were detected in *K. pneumoniae* isolates and the only one bla_{IMP} was found in an *E. cloacae* isolate. *K. pneumoniae* still played the most important role in CPE.[25] Lai et al. had reviewed the carbapenemase genes in *K. pneumoniae* over 20 years from 1998 to 2019 in Taiwan. Since first case of KPC-2-producing *K. pneumoniae* reported in a businessman returned from China to Taiwan in 2010, the endemicity of carbapenemase evolved from IMP, NDM-1 to predominant KPC in recent decades in Taiwan.[25]

In general, isolates in our study had lower MICs to meropenem than imipenem. The susceptible rates to meropenem (MIC ≦ 1 mg/L) were 100% (14/14) and 56.3% (9/16) among *Enterobacterales* isolates with imipenem MIC equal to 2 mg/L and ≧4 mg/L, respectively. Although laboratory factors had been previously reported to cause pseudo- or false resistance to imipenem resulting from degradation of imipenem in an automated susceptibility testing system,[26—29] meropenem is slightly more active than imipenem against Gram-negative organisms in some studies.[30] A review article conducted by Zhanel et al. revealed that meropenem is 2-fold—16-fold more active than imipenem against Gram-negative organisms and even 4-fold—16-fold more active against *Enterobacterales* than imipenem.[30—32] In clinical situations, meropenem had similar efficacy to imipenem such as clinical and bacteriologic improvement, but associated with fewer drug-related adverse reactions.[33—35]

Two novel β-lactam/β-lactamase inhibitor combinations were found to have excellent *in vitro* efficacy for *Enterobacterales* in our study. Meropenem/vabobactam had slightly higher susceptibility than imipenem/relebactam (99.3% vs. 94.8%) because several *M. morganii* and *P. mirabilis* showed discordant results to the two β-lactam/β-

lactamase inhibitor combinations. *Morganellaceae*, particularly *P. mirabilis* have intrinsic decreased susceptibility to imipenem due to weak affinity of penicillin binding proteins or porin loss which less impact on meropenem and ertapenem.[36] "In addition, the MIC breakpoints for imipenem/relebactam and meropenem/vaborbactam were $\leq$1/4 mg/L and $\leq$4/8 mg/L despite the MIC breakpoints for imipenem and meropenem were both $\leq$1 mg/L, respectively, according to the CLSI guidelines. Although a recent review by Zhanel et al. described the MIC fold reduction was similar by adding relebactam and vaborbactam to imipenem and meropenem, respectively,[37] meropenem/vaborbactam was found to have slightly better susceptible rates than imipenem/relebactam in our study due to the higher MIC breakpoints. Our result was similar to several recent studies that the two combinations revealed good efficacy in treating *Enterobacterales* either carbapenem resistant or not.[37–39] Only one *E. coli* was found to have resistance to imipenem/relebactam and intermediate susceptibility to meropenem/vaborbactam. We only detected ESBL including SHV and CTX-M-9 in the *E. coli* but neither AmpC β-lactamase nor carbapenemase. Mechanism other than β-lactamase production was considered for its increased MICs. Recently, impaired permeability due to porin mutations such as OmpK35 and OmpK36, and increases in efflux pump production with or without overexpression of β-lactamase were reported to be associated with resistance to imipenem/relebactam and meropenem-vabobactam.[40] The detailed molecular study is warranted. Besides, an IMP-8 positive *E. cloacae* was found to be resistant to imipenem and susceptible to meropenem in our study. The isolate was also resistant to imipenem/relebactam and susceptible to meropenem/vaborbactam. Our finding was similar to a study conducted by Wang et al. that investigated resistance mechanism for CRE in Taiwan between 2010 and 2012. The susceptible rates for the IMP-8-positive CRE, mostly *E. cloacae*, to ertapenem, imipenem, and meropenem were 4%, 37.8%, and 86.5%, respectively.[24]

Our study has several limitations. First, it was a cross-sectional study the involved only data in 2020, the trend of resistance change was unable to be demonstrated. To monitor the dynamics of resistance change would be important if those novel β-lactam/β-lactamase inhibitor combinations are integrated to clinical use in near future. Second, the resistance mechanisms other than the presence of β-lactamase genes such as porin deficiency or overexpression of pumping efflux were not performed. It would be interesting if more resistant isolates to novel antimicrobial agents could be collected for further molecular research.

In conclusion, the results of this study show that the two β-lactam combination agents, imipenem/relebactam and meropenem/vaborbactam, had excellent efficacy to *Enterobacterales* in Taiwan. It is important to monitor the resistance trend through continuous surveillance especially the time after the two agents are put into clinical practice.

Ethics statement

This study was approved by the Institutional Review Board of National Taiwan University Hospital (Taipei, Taiwan) [NTUH 9561709108]. The Institutional Review Board or Research Ethics Committees of each participating hospitals have approved the SMART program and informed consent was waived due to no more than minimal risk to the participating patients.

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Declaration of competing interest

All authors declare no conflicts of interest.

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