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Antimicrobial resistance among imipenem-non-susceptible *Escherichia coli* and *Klebsiella pneumoniae* isolates, with an emphasis on novel β-lactam/β-lactamase inhibitor combinations and tetracycline derivatives: The Taiwan surveillance of antimicrobial resistance program, 2020–2022

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ABSTRACT

Background: To determine susceptibility of imipenem-non-susceptible *Escherichia coli* (INS-EC) and *Klebsiella pneumoniae* (INS-KP) isolates collected during 2020–2022 through a national surveillance program in Taiwan to novel antibiotics, and to compare the results with those obtained during 2012–2018.

Methods: Minimum inhibitory concentrations were determined by broth microdilution methods. Genes encoding carbapenemases including bla_{KPC}, metallo-β-lactamase (MBL) genes, and bla_{OXA-48} were detected via multiplex PCR. Data retrieved from our 2012–2018 study were used for comparison.

Results: Of 3260 *E. coli* and 1457 *K. pneumoniae* isolates collected during 2020–2022, 0.9 % and 9.5 %, were INS-EC and INS-KP, respectively. Cefepime-zidebactam, ceftazidime-avibactam, imipenem-relebactam, and meropenem-vaborbactam were active against 100 %, 75.9 %, 65.5 %, and 79.3 % of 29 INS-EC isolates respectively; and against 100 %, 90.6 %, 64.5 %, and 67.4 % of 138 INS- KP isolates, respectively. Susceptibility was contingent upon carbapenemase types. Susceptibility rates of cefepime-zidebactam and ceftazidime-avibactam remained constant from 2012 to 2018 through 2020–2022 but those of imipenem-relebactam and meropenem-vaborbactam decreased significantly, which may be partially attributable to the increasing prevalence of bla_{OXA-48}. Eighteen MBL-gene-positive isolates and two bla_{KPC}-positive isolates were resistant to ceftazidime-avibactam, whereas all were susceptible to cefepime-zidebactam. Tigecycline had a higher susceptibility rate than eravacycline and omadacycline for *K. pneumoniae* isolates. Lascufloxacin and delafloxacin were effective against fewer than 10 % of INS isolates. Susceptibilities to novel tetracyclines and fluoroquinolones remained similar from 2012 to 2018 through 2020–2022.

Abbreviations: BL-BLI, β-lactam/β-lactamase inhibitor combination; CZA, ceftazidime-avibactam; IMR, imipenem-relebactam; INS-EC, imipenem-non-sensitive *Escherichia coli*; INS-KP, imipenem-non-sensitive *Klebsiella pneumoniae*; MEV, meropenem-vaborbactam; TSAR, Taiwan Surveillance of Antimicrobial Resistance.

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Conclusions: This study highlights significant resistance patterns of INS-EC and INS-KP isolates in Taiwan. The declining susceptibility rates and the rising prevalence of genetic resistance determinants highlight the importance of ongoing surveillance and antimicrobial stewardship.

Introduction

The increasing prevalence of carbapenem-resistant Enterobacterales (CRE) has become a significant public health challenge, primarily due to the dearth of effective antibiotics and consequent impact on morbidity and mortality rates.[1–4] Invasive CRE infections, which are associated with mortality rates ranging from 20 % to 50 %, predominantly afflict the most vulnerable patient populations.[5] Historically, treatment options for CRE were limited to polymyxins, tigecycline, fosfomycin, and aminoglycosides. However, the clinical utility of these agents is hampered by renal toxicity, variable *in vivo* efficacy, and inadequate drug concentrations at infected sites, highlighting the urgent need for new therapeutic solutions.[6]

Over the past decade, antibiotics that specifically target multidrug-resistant (MDR) Gram-negative bacilli have been approved for clinical use. These include various combinations of a β-lactam and a novel β-lactamase inhibitor (BL-BLI), eravacycline, and cefiderocol.[6,7] Notably, the US Food and Drug Administration (FDA) approved ceftazidime-avibactam (CZA) in 2015, imipenem-relebactam (IMR) in 2017, and meropenem-vaborbactam (MEV) in 2019.[8,9] In Taiwan, CZA became available in 2019, whereas MEV and IMR were not introduced for clinical use until the end of 2023.[10] Clinical data have demonstrated the efficacy of innovative BL-BLIs and other novel agents in treating infections caused by MDR pathogens. However, it is crucial to note that resistance mechanisms that impede the activities of new antibiotics have already emerged. The rapid expansion of resistant strains underscores the necessity of *in vitro* susceptibility testing, surveillance, and the implementation of antimicrobial stewardship strategies.[11]

Our previous study reported the *in vitro* susceptibilities of imipenem-non-susceptible *Escherichia coli* (INS-EC) and *Klebsiella pneumoniae* (INS-KP) to novel antibiotics through a nationwide surveillance project, the Taiwan Surveillance of Antimicrobial Resistance (TSAR), conducted from 2012 to 2018.[12] This follow-up surveillance study investigated the activities of the same novel antibiotics using identical methodology and protocols; recognized changing susceptibility patterns; and disclosed underlying mechanisms.

Methods

Bacterial isolates and surveillance

TSAR is a longitudinal, multicenter surveillance program conducted biennially, focusing on clinical bacterial isolates. Isolates were preserved at low temperatures and subsequently sub-cultured on appropriate agar media to ensure purity prior to further analysis. Species identification in 2020 was achieved through conventional biochemical assays, supplemented by VITEK II (bioMérieux, Marcy l'Étoile, France) as required. Speciation during 2022 was performed using Bruker MALDI-TOF MS systems. TSAR collected approximately 160 isolates of INS-EC and INS-KP with minimum inhibitory concentrations (MICs) $\geq$ 2 mg/L during 2020–2022. The selection process is detailed in Fig. S1 (see supplementary online material).

Antimicrobial susceptibility testing

MICs of novel antimicrobials were determined by broth microdilution conducted in internally prepared 96-well microtiter plates according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[13] The panel of novel antimicrobials included IMR, MEV, CZA, cefepime-zidebactam, lascufloxacin, delafloxacin, eravacycline, and omadacycline. For comparison, MICs of imipenem, meropenem, ceftazidime, cefepime, levofloxacin, minocycline, and tigecycline were also tested simultaneously using an in-house panel, and MICs for other standard antibiotics were tested using commercially available panels (Sensititre®). Sources of antimicrobials included MedChemExpress (USA), MedKoo Biosciences (USA), and Sigma–Aldrich (USA). BL-BLIs were assessed at fixed concentrations with their respective β-lactam partners, and quality control was ensured through the inclusion of reference strains such as *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *K. pneumoniae* ATCC700603 and *K. pneumoniae* ATCC BAA-2814. Susceptibility was interpreted using CLSI breakpoints. The interpretative criteria for cefepime-zidebactam were referenced to the previous study,[14] and those for lascufloxacin were referenced to the CLSI breakpoints for levofloxacin. For agents without available CLSI breakpoints, we adopted FDA (eravacycline, omadacycline, and tigecycline).[15,16] Breakpoints are listed in Table S1 (see online supplementary material).

PCR screening for β-lactamase genes

Multiplex PCR was used to detect genes encoding class A (bla_{KPC}), class B (metallo-β-lactamases, MBLs, including bla_{IMP}, bla_{NDM}, and bla_{VIM}), and class D ($bla_{OXA\text{-}48\text{-}like}$) carbapenemases.[12] INS isolates were also screened for genes encoding extended-spectrum (ESBLs, $bla_{CTX\text{-}M}$ and bla_{SHV}) and AmpC (bla_{CMY} and bla_{DHA}) β-lactamases.

Data analysis

Susceptibility rates of INS-EC and INS-KP isolates to novel antibiotics were computed using Whonet software, with interpretations based on criteria listed in Table S1 (see online supplemental material). Results obtained during the 2012–2018 and 2020–2022 timeframes were compared by using two-tailed chi-square or two-tailed Fisher's exact tests, with a p-value of less than 0.05 considered statistically significant. Statistical evaluations were performed utilizing MedCalc software (MedCalc Software Ltd, Los Angeles, CA, USA).

Ethics statement

Clinical samples for the TSAR program were collected as part of routine patient care, with the surveillance project receiving approval from the Research Ethics Committee of the National Health Research Institutes (EC1050606-E).

Results

Demographic and clinical characteristics

From 2020 to 2022, the TSAR program collected 3260 *E. coli* and 1457 *K. pneumoniae* isolates nationwide. Among these, 29 *E. coli* isolates (0.9 %) and 138 *K. pneumoniae* isolates (9.5 %) were non-susceptible to imipenem (Fig. S1, see online supplementary material). Demographic and clinical characteristics of patients from whom isolates were obtained are detailed in Table S2 (see online supplementary material). These isolates were predominantly collected from adult patients in non-intensive care unit wards of regional hospitals. Specific anatomic sites of specimen collection varied.

Antimicrobial susceptibilities of INS-EC and INS-KP isolates

The antimicrobial susceptibility patterns of INS-EC and INS-KP isolates are displayed in Table 1. These pathogens exhibited substantially decreased susceptibility to a wide range of standard non-carbapenem β-lactam antibiotics. Notably, piperacillin-tazobactam inhibited only 17.2 % of INS-EC and a mere 2.2 % of INS-KP isolates.

The addition of advanced β-lactamase inhibitors significantly improved the activity of β-lactam antibiotics. Specifically, the susceptibility of INS-EC isolates to cefepime, ceftazidime, imipenem, and meropenem was enhanced by 82.8 % (100 % vs. 17.2 %), 72.5 % (75.9 % vs. 3.4 %), 65.5 % (65.5 % vs. 0 %), and 55.2 % (79.3 % vs. 24.1 %), respectively. The increments for INS-KP isolates were 89.9 % (100 % vs. 10.1 %), 89.2 % (90.6 % vs. 1.4 %), 64.5 % (64.5 % vs. 0 %), and 48.6 % (67.4 % vs. 18.8 %), respectively. Furthermore, novel BL-BLIs consistently shifted the MICs of these β-lactam antibiotics to lower values (Table S3, see online supplementary material).

INS-EC and INS-KP isolates also demonstrated comparably low susceptibility rates to both traditional fluoroquinolones, such as ciprofloxacin and levofloxacin, and newer agents in the class, including lascufloxacin and delafloxacin (Table 1). The susceptibility rate of INS-EC isolates to these fluoroquinolones ranged between 3.4 % and 10.3 %, while that of INS-KP isolates was even lower (below 5 %).

Among the tetracyclines, tigecycline was active against over 90 % of isolates. Eravacycline presented a MIC range comparable to that of tigecycline, (Table S3, see online supplementary material). According to FDA criteria, the susceptibility rates of INS-EC isolates to eravacycline, minocycline, omadacycline, and tigecycline were 100 %, 62.1 %, 51.7 %, and 100 %, respectively, whereas those of INS-KP isolates were significantly lower at 38.4 %, 39.1 %, 15.2 %, and 91.3 %, respectively. By using the EUCAST breakpoint of tigecycline against *E. coli* and *Citrobacter koseri* (MIC ≤0.5 mg/L), the susceptibility rate of INS-EC isolates to tigecycline remained at 93.1 % (27/29), but that of INS-KP isolates dropped from 91.3 % (126/138) to 9.4 % (13/138).

Effect of carbapenemase genotypes on susceptibility

The bla_{OXA-48} (n = 6), bla_{NDM} (4), bla_{IMP} (3) and bla_{KPC} (12) genes were detected in 22 of 29 (75.9 %) INS-EC isolates. Several isolates carried two carbapenemase genes; two carried $bla_{OXA-48-like}$ + bla_{KPC}, and one carried $bla_{OXA-48-like}$ + bla_{NDM}. Of 138 INS-KP isolates, 108 (78.3 %) carried carbapenemase genes including bla_{OXA-48} (35), bla_{NDM} (2), bla_{IMP} (1), bla_{VIM} (8) and bla_{KPC} (64). Two INS-KP isolates carried two carbapenemase genes. One exhibited $bla_{OXA-48-like}$ + bla_{KPC}, and the other carried $bla_{OXA-48-like}$ + bla_{NDM}.

None of the INS-KP and INS-EC isolates carrying bla_{KPC} were susceptible to cefepime, ceftazidime, imipenem or meropenem; zidebactam, avibactam, relebactam, and vaborbactam increased their susceptibility rates to 100 %, 97.3 %, 90.4 % and 79.5 %, respectively (Table 2). The novel BL-BLIs were also highly active against isolates without detectable carbapenemases. All isolates carrying only $bla_{OXA-48-like}$ genes were susceptible to CZA and cefepime-zidebactam. The addition of relebactam and vaborbactam to imipenem and meropenem marginally decreased the MICs by 2-4-fold in 8 $bla_{OXA-48-like}$-positive isolates, which increased susceptibility rates by about 20 % for both BL-BLIs. None of the BL-BLIs except cefepime-zidebactam and MEV were active against isolates with MBL genes. However, in contrast to cefepime-zidebactam, the MIC distribution of MEV in these isolates was almost the same as that of meropenem. The susceptibility rate was increased by the change of breakpoint (MEV ≤4/8 mg/L vs meropenem ≤1 mg/L).

Among the 18 MBL-positive isolates, cefepime-zidebactam (100 %, 18/18), tigecycline (94.4 %, 17/18), and eravacycline (66.7 %, 12/18) exhibited susceptibility rates greater than 60 %. The remaining agents showed poor activity against the MBL-positive isolates. In our study, cefepime-zidebactam demonstrated superior susceptibility compared to ceftazidime-avibactam. This difference was primarily due to cefepime-zidebactam's better activity against MBL-positive isolates. As shown in Table 2, all 18 MBL-positive isolates were susceptible to cefepime-zidebactam but resistant to ceftazidime-avibactam. Additionally, two

Table 1
Antimicrobial susceptibility of imipenem-non-susceptible *Escherichia coli* and *Klebsiella pneumoniae* from the Taiwan Surveillance of Antimicrobial Resistance program, 2020–2022.

Antibiotics	*E. coli* (n = 29)						*K. pneumoniae* (n = 138)					
	R	I	S	MIC_{50}	MIC_{90}	MIC Range	R	I	S	MIC_{50}	MIC_{90}	MIC Range
Ampicillin[a]	100	0	0	>16	>16	>16	Intrinsic resistance					
Cefazolin[a]	100	0	0	>32	>32	>32	100	0	0	>32	>32	8 - >32
Cefepime[b]	82.8	0	17.2	>32	>32	≦0.25 - >32	89.9	0	10.1	>32	>32	≦0.25 - >32
Cefepime/zidebactam	0	0	100	≦0.25	1	≦0.25–8	0	0	100	2	4	≦0.25–8
Cefotaxime[a]	96.6	0	3.4	>32	>32	≦1 - >32	98.6	0	1.4	>32	>32	≦1 - >32
Ceftazidime	96.6	0	3.4	>64	>64	0.5 - >64	97.8	0.7	1.4	>64	>64	0.5 - >64
Ceftazidime/avibactam	24.1	0	75.9	1	>64	0.25 - >64	9.4	0	90.6	4	8	0.125 - >64
Cefuroxime[a]	96.6	0	3.4	>16	>16	8 - >16	98.6	0.7	0.7	>16	>16	8 - >16
Ceftolozane/tazobactam	82.8	10.3	6.9	>32	>32	1 - >32	94.2	0.7	5.1	>32	>32	≦0.25 - >32
Imipenem	79.3	20.7	0	4	>16	2 - >16	78.3	21.7	0	>16	>16	2 - >16
Imipenem/relebactam	20.7	13.8	65.5	0.5	16	≦0.12 - >16	25.4	10.1	64.5	1	8	≦0.12 - >16
Meropenem	69	6.9	24.1	8	32	≦0.06 - >32	76.1	5.1	18.8	>32	>32	≦0.06 - >32
Meropenem/vaborbactam	13.8	6.9	79.3	0.25	32	≦0.06 - >32	23.2	9.4	67.4	2	32	≦0.06 - >32
Piperacillin/tazobactam[a]	79.3	3.4	17.2	>64	>64	≦4 - >64	94.9	2.9	2.2	>64	>64	≦4 - >64
Ciprofloxacin[a]	89.7	6.9	3.4	>2	>2	≦0.06 - >2	95.7	1.4	2.9	>2	>2	≦0.06 - >2
Delafloxacin	93.1	0	6.9	32	>32	≦0.06 - >32	97.1	0.7	2.2	>32	>32	≦0.06 - >32
Lascufloxacin	93.1	0	6.9	64	>64	0.06 - >64	95.7	1.4	2.9	>64	>64	0.25 - >64
Levofloxacin	89.7	0	10.3	32	>32	≦0.25 - >32	89.9	5.8	4.3	>32	>32	≦0.25 - >32
Eravacycline	0	0	100	0.5	0.5	0.125–0.5	61.6	0	38.4	1	2	0.125–8
Minocycline	27.6	10.3	62.1	4	64	0.5 - >64	37	23.9	39.1	8	64	1 - >64
Omadacycline	3.4	44.8	51.7	4	8	2–16	48.6	36.2	15.2	8	32	2 - >32
Tigecycline	0	0	100	0.25	0.5	0.125–1	2.9	5.8	91.3	1	2	0.125–8
Amikacin[a]	41.4	3.4	55.2	≦4	>32	≦4 - >32	34.1	2.2	63.8	≦4	>32	≦4 - >32
Gentamicin[a]	69	0	31	>8	>8	≦1 - >8	63	1.4	35.5	>8	>8	≦1 - >8
Aztreonam	93.1	0	6.9	>64	>64	0.125 - >64	94.9	0.7	4.3	>64	>64	0.125 - >64
Colistin[a]	0	0	100	≦0.5	≦0.5	≦0.5–1	18.1	0	81.9	≦0.5	>4	≦0.5 - >4

[a] Minimum inhibitory concentrations (MICs) were determined using Sensititre.
[b] Susceptible dose-dependent (SDD) and susceptible breakpoints (≤8 mg/L) for cefepime were used here.

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

Species with different genotypes and phenotypes	No.	% susceptible								
		FEP[a]	FPZ	CAZ	CZA	IPM	IMR	MEM	MEV	CZT
E. coli and *K. pneumoniae*	167	11.4	100	1.8	88	0	64.7	19.8	69.5	5.4
with carbapenemase genes	130	2.3	100	2.3	84.6	0	57.7	6.2	61.5	2.3
with class B carbapenemase genes	18	0	100	0	0	0	0	5.6	27.8	0
with $bla_{\text{KPC-like}}$ only	73	0	100	0	97.3	0	90.4	0	79.5	0
with $bla_{\text{OXA-48-like}}$ only	36	8.3	100	8.3	100	0	22.2	19.4	41.7	8.3
without carbapenemase genes	37	43.2	100	0	100	0	89.2	67.6	97.3	16.2
with ESBL genes[b] only	2	0	100	0	100	0	100	50	100	0
with AmpC genes only	16	93.8	100	0	100	0	93.8	68.8	93.8	31.3
with ESBL and AmpC genes[b]	17	0	100	0	100	0	88.2	70.6	100	0

Abbreviations: FEP, cefepime; FPZ, cefepime/zidebactam; CAZ, ceftazidime; CZA, ceftazidime/avibactam; IPM; imipenem; IMR, imipenem/relebactam; MEM, meropenem; MEV, meropenem/vaborbactam. CZT, ceftolozane/tazobactam.
[a] Susceptible dose-dependent (SDD) and susceptible breakpoints (≤8 mg/L) for cefepime were used here.
[b] Only prevalent ESBL or AmpC genes were tested

bla_{KPC}-positive isolates that were resistant to ceftazidime-avibactam remained susceptible to cefepime-zidebactam.

Susceptibility to either new or traditional fluoroquinolones and tetracyclines was independent of carbapenemase genotypes and phenotypes. Tigecycline susceptibility exceeded 80 % in all subgroups (Table S4, see online supplementary material).

Changing epidemiology of genotypes and susceptibility: TSAR 2012–2018 versus TSAR 2020–2022.

Carbapenemase genes were more prevalent among INS isolates collected during 2020–2022 compared to 2012–2018 (92/180 [51.1 %] vs 130/167 [77.8 %], $p < 0.001$). The prevalence of carbapenemase-producing INS-EC and INS-KP isolates increased from 29.4 % and 53.4 % to 75.9 % and 78.3 %, respectively (Fig. 1). The bla_{KPC} remained the most prevalent carbapenemase gene, but the prevalence of $bla_{\text{OXA-48}}$-positive isolates expanded (21/180, 11.7 % vs 41/167, 24.6 %, $p = 0.002$). Fig. 1 illustrates the proportion of carbapenemase-producing, KPC-positive, MBL-positive, and OXA-48-positive isolates among INS-EC and INS-KP isolates in this study. In *E. coli*, an emergence of KPC-positive isolates was observed. Additionally, an increase in MBL-positive and OXA-48-positive isolates was observed, although the numbers were too small to reach statistical significance. In *K. pneumoniae*, the increase in carbapenemase-producing isolates from 2020 to 2022 was primarily driven by a higher proportion of OXA-48-positive isolates (19/163, 11.7 % vs 35/138, 25.4 %, p = 0.002) compared to the period from 2012 to 2018. Concurrently, the susceptibility rates of IMR and MEV decreased significantly between the 2012–2018 and 2020–2022 timeframes ($p = 0.002$ and <0.001, Fig. 2) while those of CZA and cefepime-zidebactam were similar during the two periods. The susceptibility rates of cefepime, meropenem, piperacillin-tazobactam, amikacin, and omadacycline decreased from 2012 to 2018 to 2020–2022. Conversely, minocycline and eravacycline exhibited better susceptibility rates in the present study than before (Fig. 2).

Discussion

This study evaluated the activities of novel and traditional antibiotics against INS-EC and INS-KP isolates collected in Taiwan from 2020 to 2022. The combination of novel β-lactamase inhibitors with β-lactam antibiotics significantly enhanced their activity against INS Enterobacterales, with variations influenced by β-lactamase genotypes and phenotypes. However, IMR and MEV susceptibility rates decreased between the 2012–2018 and 2020–2022 timeframes, consistent with an increasing prevalence of isolates harboring $bla_{\text{OXA-48}}$. Novel tetracyclines and fluoroquinolones were not more active than traditional agents against these INS bacteria.

The susceptibility of CRE isolates to novel BL-BLI combinations varies according to the types of carbapenemase genes. A study conducted in 74 American medical centers disclosed that the prevalence of MEV-susceptible isolates declined from 91.7 % in 2019 to 76.5 % in 2021. This decline coincided with an increased prevalence of class B (MBL) and OXA-48-type carbapenemases.[17] Following the introduction

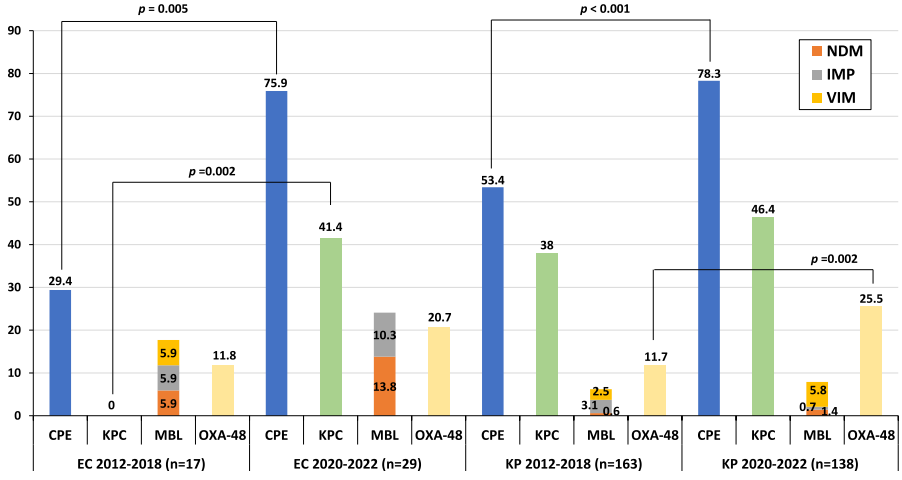

Fig. 1. Proportion of β-Lactamase genes for imipenem-non-susceptible (INS) *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) between two study periods, 2012–2018 versus 2020–2022, from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) Program. CPE: carbapenemase producing Enterobacterales. MBL: metallo-β-lactamase

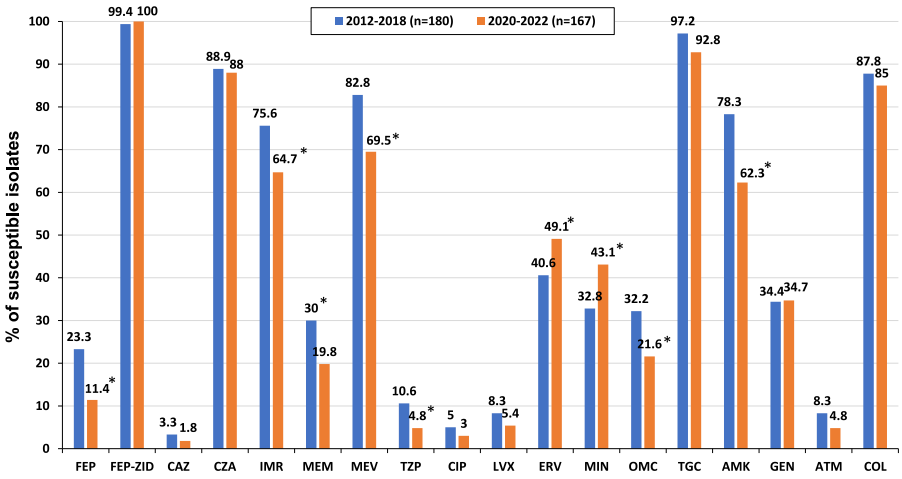

Fig. 2. Comparison of *in vitro* susceptibility to antibiotics among imipenem-non-susceptible *Escherichia coli* and *Klebsiella pneumoniae* isolates between two study periods, 2012–2018 (n = 180) versus 2020–2022 (n = 167), from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) Program. *Significant difference between two study periods. FEP: cefepime; FEP-ZID: cefepime-zidebactam; CAZ: ceftazidime; CZA: ceftazidime-avibactam; IMR: imipenem-relebactam; MEM: meropenem; MVB: meropenem-vaborbactam; TZP: piperacillin-tazobactam; CIP: ciprofloxacin; DLX: delafloxacin; LSFX: lascufloxacin; LVX: levofloxacin; ERV: eravacycline; MIN: minocycline; OMC: omadacycline; TGC: tigecycline; AMK: amikacin; GEN: gentamicin; ATM: aztreonam; COL: colistin.

of CZA to Taiwan during 2019, CZA resistance emerged rapidly due to the increased prevalence of CRE carrying various MBLs.[18] The decreased MEV and IMR susceptibility rates observed in our study might be partially explained by the increasing prevalence of the OXA-48-like carbapenemase in Taiwan from 21.8 % during 2012–2018 to 31.5 % during 2020–2022. In addition, we also found an MEV susceptibility rate of bla_{KPC}-positive isolates of only 74.0 %, which was significantly lower than the CZA (95.9 %) and IMR (91.8 %) susceptibilities observed in present study. A recent Italian study identified MEV resistance in 8 % (n = 5/62) of KPC-producing *K. pneumoniae* isolates. Genomic analysis linked the resistance mechanism to truncated OmpK35 and the insertion of glycine and aspartic acid in OmpK36 at positions 134–135.[19] Vaborbactam crosses the outer membrane of Enterobacterales using both OmpK35 and OmpK36; consequently, MEV resistance in KPC-producing Enterobacterales is due primarily to the loss of these porins.[20,21] A recent molecular characterization of 17 CR K. *pneumoniae* isolates at Taipei Medical University Hospital in Taiwan revealed that most (13/17, 76 %) isolates contained amino acid substitutions or frameshift mutations in outer membrane proteins OmpK35 and OmpK36.[22] Therefore, local epidemiologic data would be important to guide the application of MEV in clinical practice in Taiwan. The associated resistance mechanisms warrant further investigation.

The potent activity of the phase three clinical candidate cefepime-zidebactam against Gram-negative pathogens that harbor diverse antibiotic-resistant mechanisms is conferred by its β-lactam enhancer mechanism and binding to multiple penicillin binding proteins (PBPs).[23–25] Although zidebactam does not inhibit MBL enzymes, it exerts direct antibacterial activity through its high-affinity binding to PBP2. The combination of cefepime (targeting PBP3) and zidebactam (targeting PBP2) significantly improves *in vitro* and *in vivo* activity compared with cefepime alone.[26] In the present study, cefepime-zidebactam displayed the most favorable susceptibility rates of all tested β-lactams and BL-BLIs, demonstrating activity against 100 % (18/18) of INS-EC and INS-KP isolates with MBLs. Recent studies have linked the emergence of KPC variants to resistance to ceftazidime-avibactam.[27–29] In this study, two KPC-positive isolates resistant to ceftazidime-avibactam remained susceptible to cefepime-zidebactam. Thus, cefepime-zidebactam may offer a potential salvage therapy for KPC-positive strains resistant to ceftazidime-avibactam.

Although eravacycline is not yet licensed in Taiwan, its non-susceptibility rate among INS-KP isolates was unsatisfactory (38.4 %). A recent study by Huang et al. also revealed an eravacycline susceptibility rate of only 47.1 % among meropenem-non-susceptible Enterobacterales in Taiwan.[30] In contrast, susceptibility rates as high as 84 % were reported in Taiwan between 2017 and 2020.[31] The differences in susceptibility rates between eravacycline and tigecycline in the previous study and ours may be explained by the breakpoints used.[32] No CLSI criteria for new tetracycline derivatives have been determined. Most studies adopt FDA criteria, which consider Enterobacterales with MICs of ≤2 mg/L, ≤0.5 mg/L, and ≤4 mg/L as susceptible to tigecycline, eravacycline, and omadacycline, respectively. However, the EUCAST criteria for tigecycline and eravacycline susceptibility in *E. coli* are stricter, with MICs of ≤0.5 mg/L.[15] Using these stringent criteria, the susceptibility rates of *K. pneumoniae* to tigecycline would decrease from 91.3 % (126/138) to 9.4 % (13/138) during 2020–2022 and from 96.9 % (158/163) to 28.8 % (47/163) during 2012–2018. A right shift of the MIC distribution of INS-KP isolates against tigecycline was noted over the two study periods.

Several limitations of our study must be noted. First, the inclusion of novel antibiotic candidates was restricted due to the regulatory challenges of accessing investigational drugs. Second, the roles of efflux pumps or porins were not investigated in our study. Some isolates were carbapenemase-negative, suggesting that porin alterations and/or efflux pump overexpression may have contributed to resistance in these strains. In Taiwan, porin loss in combination with AmpC or ESBLs has been reported as the primary mechanism of carbapenem resistance in *K. pneumoniae* and *E. coli*.[33,34] Third, our PCR assays targeted a limited range of genes, focusing on prevalent ESBLs (bla_{CTX-M}-type but not bla_{TEM}). Whole-genome sequencing would have provided a more complete analysis of resistance mechanisms.

Conclusion

Data collected through the TSAR program from 2020 to 2022 revealed that INS-EC and INS-KP isolates exhibited low susceptibility rates to commonly used agents, while advanced BL-BLI antibiotics remained highly active. Compared with the 2012–2018 timeframe, susceptibility to IMR and MVR decreased significantly, congruent with the increased prevalence of bla_{OXA-48}-positive isolates. These findings highlight the impact of dynamics of carbapenemase genes on the activities of novel BL-BLIs and the importance of phenotypic and genotypic

surveillance.

CRediT authorship contribution statement

Yu-Lin Lee: Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Chun-Eng Liu:** Writing – review & editing, Conceptualization. **Wei-Yao Wang:** Writing – review & editing, Formal analysis, Data curation. **Mei-Chen Tan:** Methodology, Investigation. **Pei-Jing Chen:** Methodology, Investigation. **Yih-Ru Shiau:** Methodology, Investigation. **Hui-Ying Wang:** Methodology, Investigation. **Jui-Fen Lai:** Methodology, Investigation. **I-Wen Huang:** Methodology, Investigation. **Ya-Sung Yang:** Supervision, Funding acquisition. **Shu-Chen Kuo:** Supervision, Funding acquisition, Data curation, Conceptualization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2025.01.006.

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