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A longitudinal seven-year multicenter study on the molecular epidemiology of carbapenemase-producing *Enterobacterales* in Taiwan: The burden of metallo-β-lactamases

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ABSTRACT

Background: With the introduction of novel antimicrobial agents, the threat posed by multidrug-resistant organisms has rapidly emerged. Among these, metallo-β-lactamase-producing carbapenem-resistant Enterobacterales (MBL-CRE) represent a significant challenge, severely limiting therapeutic options for clinicians. Our aim is to investigate the current burden and molecular epidemiology of carbapenemase-producing Enterobacterales (CPE) in Taiwan, with a particular focus on MBL producers.

Methods: We conducted a multicenter, seven-year longitudinal study from 2018 to 2024, collecting carbapenem-resistant Enterobacterales isolates, and screening them for carbapenemase genes.

Results: A total of 1743 CPE isolates were included in this study. The most common species were Klebsiella pneumoniae (58.5 %), Escherichia coli (21.2 %), and Enterobacter cloacae complex (7.8 %), primarily recovered from urine and respiratory specimens. Among these isolates, 67.3 % harbored non-MBL genes and 32.7 % harbored MBL genes, with both types increasing significantly over time (both $p < 0.001$). In addition, a notable emergence of multiple-carbapenemase-producing Enterobacterales (M-CPE) was observed among various species, particularly K. pneumoniae, which exhibited the greatest diversity of carbapenemase gene combinations while E. coli co-harboring $bla_{OXA-181}$ and bla_{NDM-5} accounted for the largest proportion of M-CPE isolates. Overall, the proportion of MBL producers among total CPE isolates increased from 22.3 % (95 % CI, 15.2–30.4 %) in 2018–40 % (95 % CI, 34.9–45 %) in 2024 with a statistical significance ($p = 0.02$). Among the six most frequently identified CPE species, K. pneumoniae and Citrobacter koseri were largely linked to non-MBL genes, while E. coli, Enterobacter cloacae complex, Citrobacter freundii, and Serratia marcescens were almost exclusively associated with MBL genes.

Conclusion: The rising prevalence of MBL-CPE underscores the urgent need for strengthened infection control measures, ongoing genomic surveillance, and robust antimicrobial stewardship programs to prevent further emergence and transmission.

Introduction

Following the introduction of penicillin by Alexander Fleming in the early 20th century, numerous antibiotics with diverse mechanisms of action were developed. Nonetheless, this advancement was soon followed by a persistent and escalating challenge posed by multidrug-resistant organisms (MDROs)—a consequence closely tied to the ongoing overuse of antibiotics [1,2]. Carbapenem-resistant *Enterobacterales* (CRE) have been identified by the World Health Organization in 2025 as one of the critical priority MDROs requiring urgent attention, due to their limited treatment options and high associated mortality rates [2]. CRE can be broadly classified

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into two major categories: carbapenemase-producing *Enterobacterales* (CPE) and non-CPE. CPE can be further subdivided based on the Ambler classification of β-lactamases into serine β-lactamases (Ambler classes A, C, and D) and metallo-β-lactamases (MBL; Ambler class B) [3].

In recent years, the development of novel antimicrobial agents has expanded treatment options against CREs. The introduction of new β-lactam/β-lactamase inhibitor combinations, including ceftazidime-avibactam, imipenem-cilastatin-relebactam, and meropenem-vaborbactam, has significantly improved therapeutic strategies. However, these agents remain ineffective against MBL-producing pathogens, which represent a growing clinical threat [4]. Cefiderocol, a siderophore cephalosporin, has demonstrated efficacy against MBL producers; nevertheless, emerging resistance mechanisms—particularly in multiple-carbapenemase-producing *Enterobacterales* (M-CPE)—have raised concerns regarding its susceptibility [5–7]. Therefore, understanding the current burden and molecular epidemiology of CPE, especially MBL producers, is essential. To address this, we conducted a seven-year longitudinal multicenter study investigating the molecular characteristics of CPE isolated in Taiwan.

Methods

Bacterial isolates and surveillance rationale

From 2018–2024, we prospectively collected clinical isolates from patients in three hospitals in Taiwan (Fig. 1). Clinical specimens were processed at each hospital and further identified by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS; Bruker Daltonik, Bremen, Germany). The antibiotic susceptibility of all available isolates was determined by the routine disk diffusion method and interpreted according to Clinical & Laboratory Standards Institute guidelines [8]. In this study, only CRE isolates that were non-susceptible to at least two of the following carbapenems—ertapenem, meropenem, imipenem, or doripenem—were included.

Isolates that met these criteria were then transferred to the central laboratory for carbapenemase screening. Our institution has included common carbapenemase resistance genes in the routine surveillance protocol, including bla_{KPC}, $bla_{OXA-48-like}$, bla_{NDM}, bla_{IMP}, and bla_{VIM}. After excluding non-CPE isolates, clinical CPE isolates were considered duplicates if they were obtained from the same individual, belonged to the same species, and harboured identical carbapenemase resistance genes, even if from different specimens. For the duplicates, isolates collected at later time points were excluded; when isolates were obtained concurrently, those derived from blood were given preference. This study was approved by the Institutional Review Board of Chi Mei Medical Center (Approval No. 11410–015).

Molecular identification of carbapenemase resistance genes

Both genomic and plasmid DNA were extracted using the Geneaid Genomic DNA Mini Kit (Geneaid Biotech, New Taipei City, Taiwan) and the Qiagen Plasmid Midi Kit (Qiagen, Hilden, Germany). The presence of carbapenemase genes, including bla_{NDM}, bla_{KPC}, $bla_{OXA-48-like}$, bla_{IMP}, and bla_{VIM}, was investigated by conventional polymerase chain reaction (PCR) using gene-specific primers, as previously described [9]. PCR amplicons corresponding to β-lactamase genes were purified with a PCR clean-up kit (Roche Diagnostics GmbH, Penzberg, Germany) and subsequently sequenced using an ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

We assessed the temporal trend of CPE isolate counts across 2018–2024 using a linear regression model implemented in R version 4.5.1. and a $p < 0.05$ is statistically significant. The yearly proportion of MBL-producing CRE was calculated, and 95 % confidence intervals (CIs) were estimated using the Clopper-Pearson exact method and displayed as error bars.

Results

Species and specimen distribution of different CPE

A total of 1743 CPE isolates were included in this study. Fig. 2 illustrates the distribution of different *Enterobacterales* species as well as the sources of the clinical specimens from which they were isolated.

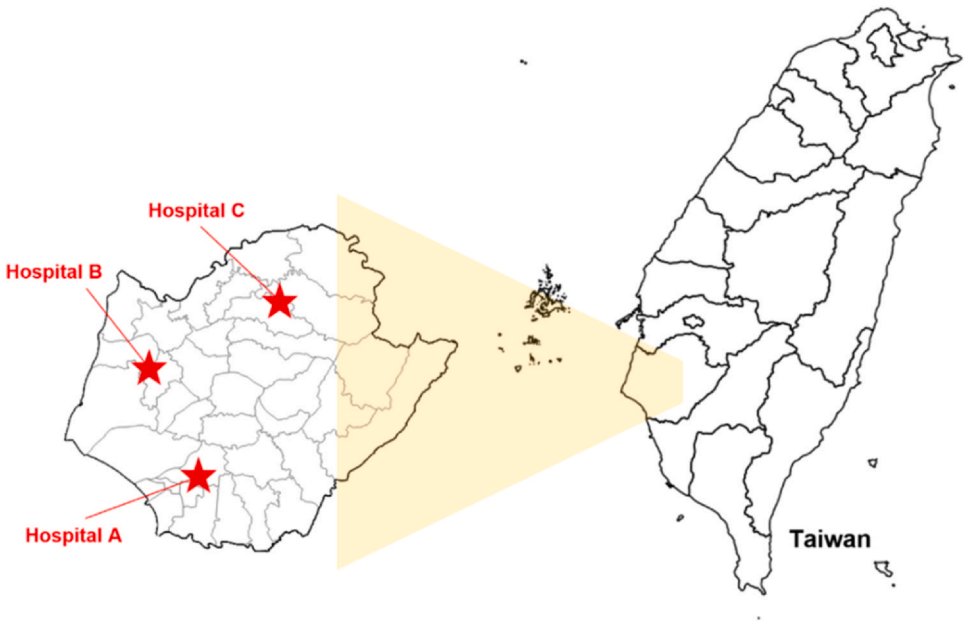

Fig. 1. The geographic information of three referral hospitals in Taiwan.

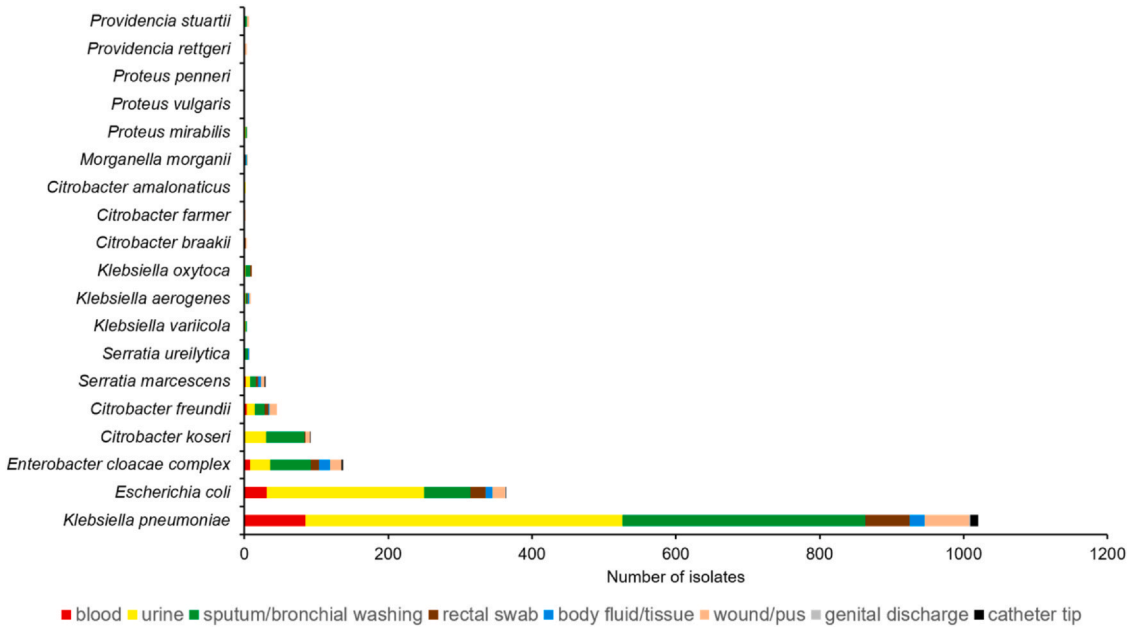

Fig. 2. Specimen distribution of different carbapenemase-producing *Enterobacterales* (CPE) from 2018 to 2024. The dominant species among CPE was *Klebsiella pneumoniae* (n = 1020, 58.5 %), followed by *Escherichia coli* (n = 370, 21.2 %), and *Enterobacter cloacae* complex (ECC) (n = 136, 7.8 %). The remaining CPE isolates accounted for only a small proportion, comprising just 12.5 % of the 1743 isolates. In addition, most of the isolates were obtained from urine (n = 744, 42.7 %) and sputum/bronchial washing (n = 549, 31.5 %), making them the predominant specimen sources.

Annual trend of non-MBL-CPE and MBL-CPE

The annual distribution of different carbapenem-resistance genes in CPE is shown in Fig. 3. Overall, isolates harbouring non-MBL genes remained predominant (n = 1173, 67.3 %), while the remaining CPE isolates (n = 570, 32.7 %) carried MBL genes. Both exhibited significant increasing trend from 2018 to 2024 ($p < 0.001$).

Distribution of non-MBL-CPE

Among the group with bla_{KPC} genes, bla_{KPC-17} and bla_{KPC-2} accounted for the majority (n = 757, 99.6 %). In addition, a small number of isolates with bla_{KPC} variants, including bla_{KPC-22}, bla_{KPC-37}, and bla_{KPC-84}, were identified during this period (n = 3, 0.4 %), all of which were found in *K. pneumoniae* (Table 1).

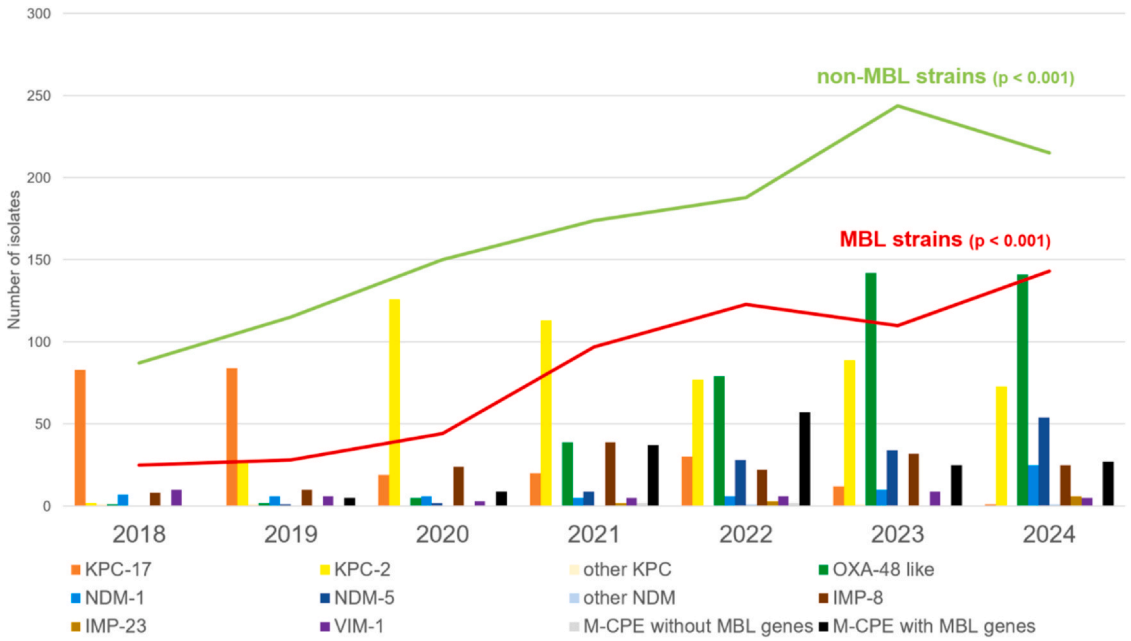

Fig. 3. Annual trends of different carbapenem-resistant genes in carbapenem-producing *Enterobacterales* (CPE) from 2018 to 2024. Both metallo-β-lactamases (MBL)-producing *Enterobacterales* and non-MBL-producing *Enterobacterales* exhibited significant increasing trend from 2018 to 2024 ($p < 0.001$). Among the group with bla_{KPC} genes, bla_{KPC-17} and bla_{KPC-2} accounted for the majority, with the former predominant before 2020, while the latter emerged after 2020 and gradually became the dominant bla_{KPC} strains in Taiwan. As for the group carrying $bla_{OXA-48-like}$ genes, a noticeable rise in their prevalence was observed in 2021, and they gradually emerged as the dominant CPE isolates after 2022. Moreover, a pronounced increase in MBL-producing strains was noted beginning in 2021, particularly among bla_{IMP-8}–carrying isolates. Other *Enterobacterales* harboring additional types of MBL genes, notably bla_{NDM-1} and bla_{NDM-5}, gradually emerged in subsequent years and continued to rise. Alongside the progressive increase in isolates carrying single MBL genes, multiple-carbapenemase-producing *Enterobacterales* (M-CPE) with MBL genes also emerged from 2021 and even became the most prevalent MBL-producing group by 2022.

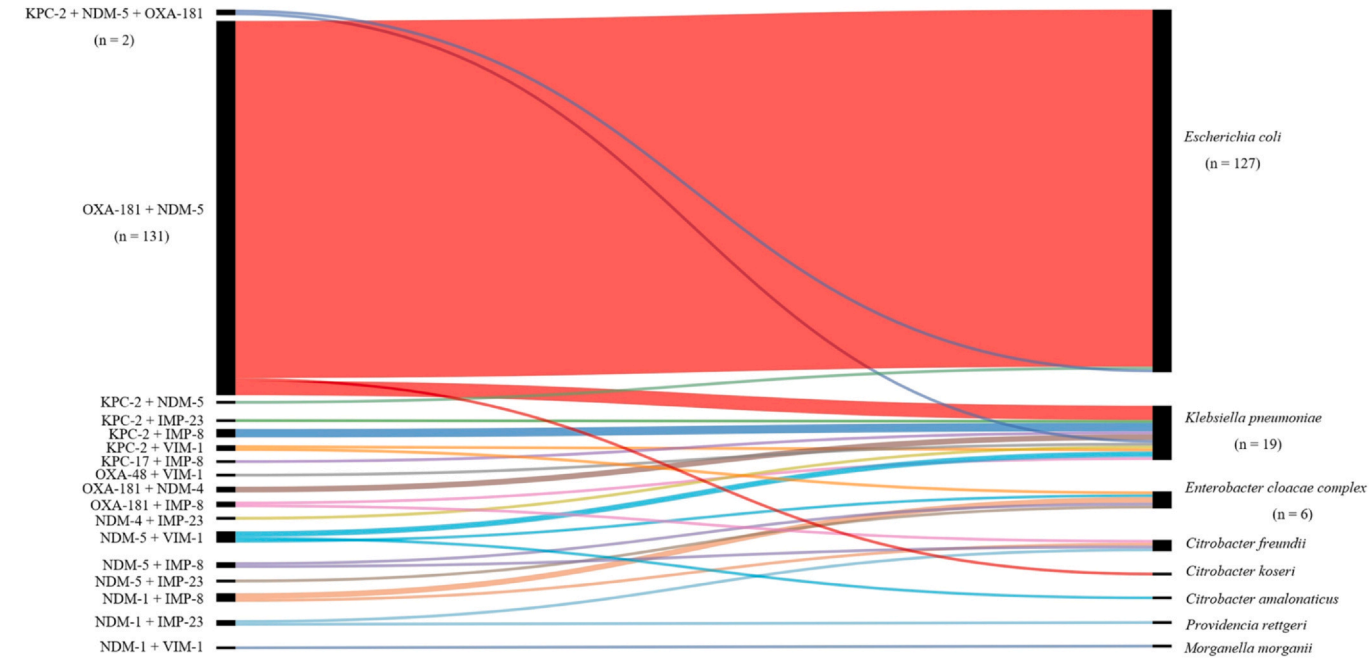

Fig. 4. Sankey diagram illustrating the distribution of multiple carbapenemase-producing *Enterobacterales* (M-CPE). The first column represents the various combinations of carbapenemase genes, each comprising at least one metallo-β-lactamase (MBL) gene, while the second column indicates the corresponding bacterial species of the M-CPE isolates. A total of 17 distinct genotypes were identified among eight bacterial species of M-CPE. The same resistance gene combination could be expressed by different bacterial species, whereas distinct gene combinations were also identified within the same species. Moreover, 81.9 % (131/160) carried both $bla_{\text{OXA-181}}$ and $bla_{\text{NDM-5}}$, with *E. coli* notably representing 95 % (125/131) of total. Among the M-CPE isolates, *E. coli* also accounted for the largest proportion of total (n = 127, 79.4 %) while *K. pneumonia* exhibited the greatest diversity of carbapenemase gene combinations. Notably, one *K. pneumoniae* and one *E. coli* even harboured three different types of carbapenemase genes–$bla_{\text{KPC-2}}$, $bla_{\text{NDM-5}}$, and $bla_{\text{OXA-181}}$. For more detailed information on the M-CPE isolates, the number of strains corresponding to each genotype across the eight bacterial species is summarized in Table S1.

Four variants of $bla_{\text{OXA-48-like}}$ were identified during this period, including $bla_{\text{OXA-48}}$, $bla_{\text{OXA-181}}$, $bla_{\text{OXA-232}}$, and $bla_{\text{OXA-484}}$, with $bla_{\text{OXA-181}}$ accounting for nearly 96 % of these isolates (Table 1). In addition, four isolates were found to harbour two different non-MBL genes concurrently, referred to as M-CPE without MBL genes in our study, all of which were identified in *K. pneumoniae* (Table 1). It is also evident that *K. pneumoniae* (n = 957, 81.6 %), *E. coli* (n = 97, 8.3 %), and *C. koseri* (n = 88, 7.5 %) together account for nearly all 1173 isolates with non-MBL genes (Table 1).

Table 1
Distribution of CRE strains with non-MBL genes from 2018 - 2024 in three hospitals.

Bacteria	Non-MBL										Total of isolates
	$bla_{\text{KPC-2}}$	$bla_{\text{KPC-17}}$	$bla_{\text{KPC-22}}$	$bla_{\text{KPC-37}}$	$bla_{\text{KPC-84}}$	$bla_{\text{OXA-48}}$	$bla_{\text{OXA-181}}$	$bla_{\text{OXA-232}}$	$bla_{\text{OXA-484}}$	M-CPE without MBL	
Klebsiella pneumoniae	448	244	1	1	1	6	251	1	0	4	957
Escherichia coli	46	2	0	0	0	1	48	0	0	0	97
Enterobacter cloacae complex	0	0	0	0	0	0	1	0	0	0	1
Citrobacter freundii	0	0	0	0	0	0	1	0	0	0	1
Citrobacter koseri	0	0	0	0	0	2	85	0	1	0	88
Citrobacter amalonaticus	0	0	0	0	0	0	0	0	0	0	0
Citrobacter farmer	0	0	0	0	0	0	0	0	0	0	0
Citrobacter braakii	0	0	0	0	0	0	1	0	0	0	1
Serratia marcescens	5	2	0	0	0	2	2	0	0	0	11
Serratia ureilytica	0	0	0	0	0	0	1	0	0	0	1
Klebsiella aerogenes	1	0	0	0	0	1	1	0	0	0	3
Klebsiella oxytoxa	4	0	0	0	0	0	0	0	0	0	4
Klebsiella variicola	0	0	0	0	0	0	0	0	0	0	0
Proteus mirabilis	2	0	0	0	0	0	0	0	0	0	2
Proteus penneri	0	0	0	0	0	0	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0	0	0	0	0	0
Providencia stuartii	2	0	0	0	0	0	2	0	0	0	4
Providencia rettgeri	0	0	0	0	0	0	0	0	0	0	0
Morganella morganii	0	1	0	0	0	0	2	0	0	0	3
Total of isolates	508	249	1	1	1	12	395	1	1	4	1173

Abbreviations: CRE, carbapenem-resistant *Enterobacterales*; MBL, metallo-β-lactamases; M-CPE, multiple-carbapenemase-producing *Enterobacterales*; KPC, *Klebsiella pneumoniae* carbapenemase; OXA, Oxacillinase
Data are presented as different non-MBL carbapenemase genes (which may consist of one or multiple gene combinations) corresponding to the number of carbapenemase-producing *Enterobacterales* isolates recovered during the study period.

Table 2
Distribution of CRE strains with MBL genes from 2018 - 2024 in three hospitals.

Bacteria	MBL								Total of isolates
	bla_{NDM-1}	bla_{NDM-4}	bla_{NDM-5}	bla_{NDM-7}	bla_{IMP-8}	bla_{IMP-23}	bla_{VIM-1}	M-CPE with MBL	
Klebsiella pneumoniae	11	0	3	0	15	4	11	19	63
Escherichia coli	14	0	120	1	6	1	4	127	273
Enterobacter cloacae complex	28	0	4	0	79	5	13	6	135
Citrobacter freundii	1	0	1	0	27	1	9	4	43
Citrobacter koseri	0	0	0	0	1	0	0	1	2
Citrobacter amalonaticus	0	0	0	0	0	0	1	1	2
Citrobacter farmer	1	0	0	0	1	0	0	0	2
Citrobacter braakii	1	0	0	0	1	0	0	0	2
Serratia marcescens	0	0	0	0	18	0	0	0	18
Serratia ureilytica	0	0	0	0	6	0	0	0	6
Klebsiella aerogenes	0	1	0	0	4	0	0	0	5
Klebsiella oxytoxa	0	0	0	0	0	0	5	0	5
Klebsiella variicola	0	0	0	0	2	0	1	0	3
Proteus mirabilis	2	0	0	0	0	0	0	0	2
Proteus penneri	1	0	0	0	0	0	0	0	1
Proteus vulgaris	1	0	0	0	0	0	0	0	1
Providencia stuartii	2	0	0	0	0	0	0	0	2
Providencia rettgeri	2	0	0	0	0	0	0	1	3
Morganella morganii	1	0	0	0	0	0	0	1	2
Total of isolates	65	1	128	1	160	11	44	160	570

Abbreviations: CRE, carbapenem-resistant *Enterobacterales*; MBL, metallo-β-lactamases; M-CPE, multiple-carbapenemase-producing *Enterobacterales*; NDM, New Delhi metallo-β-lactamase; IMP, Imipenemase metallo-β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase
Data are presented as different MBL carbapenemase genes (which may consist of one or multiple gene combinations) corresponding to the number of carbapenemase-producing *Enterobacterales* isolates recovered during the study period.

Distribution of MBL-CPE

Among these isolates, a variety of MBL genes have been identified in different *Enterobacterales*, including bla_{NDM-1}, bla_{NDM-4}, bla_{NDM-5}, bla_{NDM-7}, bla_{IMP-8}, bla_{IMP-23}, bla_{VIM-1}, and M-CPE with at least one of MBL genes (Table 2). The genotypes among 570 isolates with MBL genes primarily involve *E. coli* (n = 273, 47.9 %), ECC (n = 135, 23.7 %), *K. pneumoniae* (n = 63, 11.1 %), *C. freundii* (n = 43, 7.5 %), and *S. marcescens* (n = 18, 3.2 %) (Table 2). Among 160 M-CPE with MBL genes, the majority of isolates were *E. coli* (n = 127, 79.4 %) and *K. pneumoniae* (n = 19, 11.9 %) (Table 2).

Distribution of M-CPE

Proportion of strains with MBL genes

Although the total number of non-MBL-CPE isolates exceeded that of MBL-CPE each year (Fig. 3), the proportion of MBL producers among total CPE isolates increased year by year as shown in Fig. 5.

Annual number of strains carrying MBL genes and non-MBL genes

In Fig. 6A–F, we presented the annual numbers of isolates carrying MBL genes and non-MBL genes from 2018 to 2024, specifically for *K. pneumoniae*, *E. coli*, ECC, *C. freundii*, *C. koseri*, and *S. marcescens*, which represent the top six most frequently identified CPE species. Notably, *K. pneumoniae* and *C. koseri* isolates were predominantly associated with non-MBL genes, whereas *E. coli*, ECC, *C. freundii*, and *S. marcescens* were almost exclusively associated with MBL genes.

Discussion

This seven-year, multicenter study provides a comprehensive overview of the molecular epidemiology of CPE in Taiwan. Our findings reveal a diverse array of CPE species and resistance genes circulating within these hospitals. Notably, while non-MBL producers continue to be the predominant CPE, there has been a significant increase in the number of isolates harbouring MBL genes. Not only has the proportion of MBL-producing isolates among all CPE markedly increased (from 22.3 % in 2018–40 % in 2024), but the emergence of M-CPE has also posed significant challenges in both clinical practice and public health. This shift reflects the dynamic and evolving nature of carbapenem resistance in the clinical setting.

Among the bla_{KPC} variants, bla_{KPC-2} has emerged as the dominant pandemic genotype and was the most prevalent carbapenemase within *K. pneumoniae* isolates in Taiwan, according to a nationwide surveillance conducted from 2011 to 2013. Multilocus sequence typing revealed ST11 as the predominant clone, indicating its critical role in the local dissemination of bla_{KPC}-producing strains [10,11]. However, a regional outbreak of bla_{KPC-17}-producing *K. pneumoniae* was documented in a hospital in southern Taiwan, with all isolates sharing an identical pulsotype and belonging to ST11, suggesting a clonal origin [12]. In our surveillance from 2018 to 2019, bla_{KPC-17}-producing CRE emerged as the predominant genotype (Fig. 3), likely linked to this earlier outbreak. Notably, nearly all bla_{KPC-17}-producing CRE isolates were *K. pneumoniae* (Table 1). Conjugation experiments by Yu et al. demonstrated that bla_{KPC-17} was not transferrable to the recipient strain *E. coli* J53, indicating limited horizontal gene transfer (HGT) potential [12]. This may account for the limited interspecies dissemination of bla_{KPC-17}. Furthermore, the poor transferability of bla_{KPC-17} may also explain its subsequent replacement by the more transmissible bla_{KPC-2} after 2020 [13].

Unlike other carbapenemases, bla_{OXA-48} lacks intrinsic activity against extended-spectrum cephalosporins. However, the concomitant loss of outer membrane porins and co-production of other β-lactamases, such as ESBLs and AmpC enzymes, can markedly confer resistance to nearly all β-lactam antibiotics [14]. A multicenter study in Taiwan has revealed both nosocomial and community dissemination of bla_{OXA-48}-producing *Enterobacterales* [14]. Notably, bla_{OXA-48}-producing *K. pneumoniae* isolates, like those producing bla_{KPC}, were assigned to ST11, which represents the predominant clone in Taiwan [14]. Furthermore, plasmid analysis indicated that the bla_{OXA-48}-producing *K. pneumoniae* isolates carried IncA/C-type plasmids, rather than the globally dominant IncL/M-type [14]. In our study, a notable emergence of $bla_{OXA-48-like}$ was observed after 2021 (Fig. 3), with the majority of isolates carrying $bla_{OXA-181}$ (Table 1). $bla_{OXA-181}$, a variant of bla_{OXA-48} first identified in India in 2007, originates from *Shewanella xiamenensis* and has demonstrated considerable potential for global dissemination, as evidenced by reports from multiple countries [15,16]. Different from bla_{OXA48}, the IncX3-type plasmids, which appear to serve as the

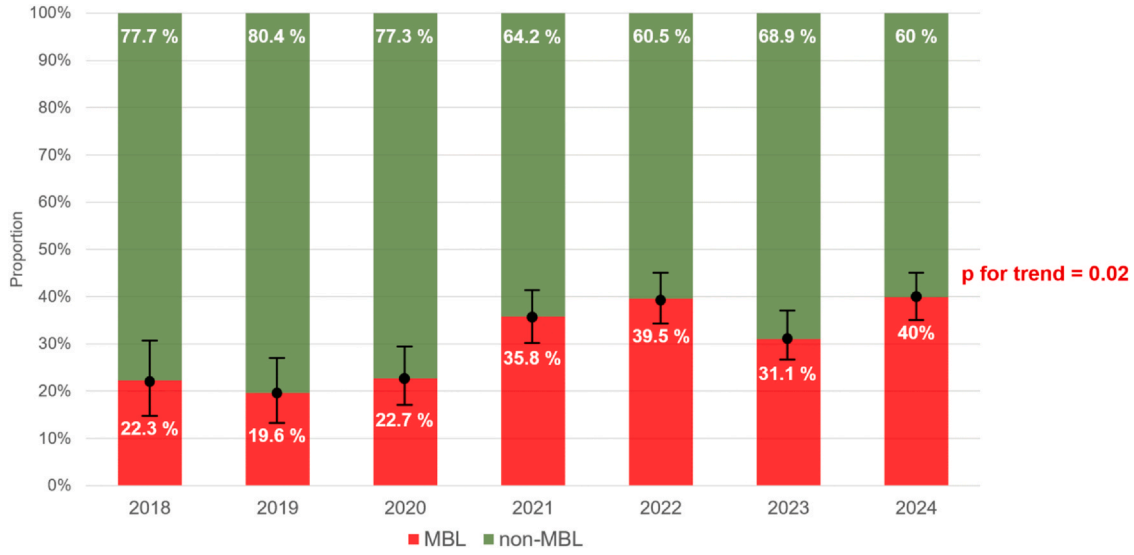

Fig. 5. Proportion of strains with MBL and non-MBL genes from 2018 to 2024. There is a mark increase for the proportion of MBL producers among total CPE isolates from 22.7 % (95 % CI, 17.0–28.9 %) in 2020–35.8 % in 2021 (95 % CI, 30.3–41.7 %). This proportion then declined sharply in 2023 but soon rebounded in 2024, finally reaching a seven-year peak. Overall, this proportion showed a statistically significant upward trend, increasing from 22.3 % (95 % CI, 15.2–30.4 %) in 2018–40 % (95 % CI, 34.9–45 %) in 2024 (p for trend = 0.02).

primary vehicle for the spread of *bla*$_{OXA-181}$, are likely the product of complex genetic rearrangements involving direct truncations, insertions, and recombination of multiple mobile genetic elements, highlighting their high transposition activity. Furthermore, the presence of homologous *bla*$_{OXA-181}$-associated transposons across diverse bacterial species supports the potential for HGT [15]. Notably, the ability of *bla*$_{OXA-181}$ to cross species boundaries raises concern for both spillover into humans and spillback into animal reservoirs [17]. In addition to the commonly identified *bla*$_{OXA-181}$-producing *K. pneumoniae* and *E. coli*, our study revealed nine additional *Enterobacterales* species harbouring *bla*$_{OXA-181}$, most notably *C. koseri*, with 85 non-duplicate isolates (Table 1). The clonality of these isolates remains undetermined and warrants further investigation to elucidate whether this represents a clonal outbreak event, which, has not been previously reported. Moreover, it is essential to assess whether the presence of *bla*$_{OXA-181}$ across diverse bacterial species is mediated by a homologous plasmid backbone, indicating potential interspecies plasmid dissemination. Urgent molecular surveillance is needed to characterize these clinical isolates and to monitor the spread of *bla*$_{OXA-48-like}$ variants among CRE.

The emergence of M-CPE represents a concerning and increasingly recognized phenomenon. The first report of M-CPE appeared in Greece in 2009 [18]. Since then, their prevalence has risen globally, with reports spanning Asia, Europe, North America, South America, and Africa [19]. In addition to the commonly observed dual-carbapenemase producers, strains encoding three or even up to six different carbapenemases have been documented [19]. Among the various plasmid combinations, IncFII and ColKP3 are the most frequently identified [19]. These plasmids often carry non-overlapping resistance genes and distinct virulence factors, thereby broadening the antimicrobial resistance spectrum and enhancing the pathogenic potential of the host strains [19]. Moreover, the aforementioned IncX3-type plasmid has the potential to serve as a common vehicle for both bla$_{OXA-181}$ and bla$_{NDM-1}$ in *E. coli* ST410, an internationally recognized high-risk clone associated with the co-harbouring of multiple carbapenemases, thereby facilitating the dissemination of M-CPE [20]. In our study, a notable emergence of M-CPE was observed after 2021, predominantly involving E. coli co-harbouring bla$_{OXA-181}$ and bla$_{NDM-5}$ (Fig. 4). Our research team, Tsai et al., previously demonstrated that E. coli ST8346, which carries both bla$_{OXA-181}$ and bla$_{NDM-5}$, has emerged independently from E. coli ST410. Pulsed-Field Gel Electrophoresis (PFGE) revealed high clonality among the isolates, suggestive of a potential outbreak event. In addition, a combination of S1-nuclease–PFGE-based plasmid profiling and southern blot hybridization demonstrated that bla$_{OXA-181}$ and bla$_{NDM-5}$ were located on IncX3- and IncF-type plasmids, respectively [9]. It is crucial to determine whether other bla$_{OXA-181}$ and bla$_{NDM-5}$ co-producing M-CPE isolates share plasmids similar to those identified in E. coli ST8346, which may suggest HGT across different bacterial species. In addition, investigating the plasmid origin of other M-CPE strains and whether the carbapenemase genes are located on separate or a single plasmid would provide important insights into the potential for large-scale dissemination. In our study, a paradoxical phenomenon was observed: most M-CPE isolates carried at least one MBL gene, while only four isolates without any MBL genes. Further investigations are warranted to explore underlying mechanisms, including plasmid incompatibility, conjugation potential, fitness cost, and plasmid host range.

After the initial discovery of *bla*$_{IMP-1}$ in Japan, *bla*$_{IMP}$-producing bacteria have been detected globally and there are 88 *bla*$_{IMP}$ variants reported currently [16,21]. The distribution of *bla*$_{IMP}$ variants varies geographically, with a significant concentration in Asia, where *bla*$_{IMP-1}$, *bla*$_{IMP-4}$, *bla*$_{IMP-6}$, *bla*$_{IMP-8}$, and *bla*$_{IMP-14}$ have been reported. Europe also exhibits considerable diversity, with multiple variants—including *bla*$_{IMP-7}$, *bla*$_{IMP-11}$, *bla*$_{IMP-13}$, and *bla*$_{IMP-29}$—identified in various countries. Meanwhile, *bla*$_{IMP-18}$, *bla*$_{IMP-27}$, *bla*$_{IMP-56}$, and *bla*$_{IMP-83}$ have been detected in the Americas [21]. Mostly, *bla*$_{IMP}$ genes are embedded within class 1 integrons located on broad-host-range plasmids. These mobile genetic elements play a crucial role in the intercontinental and interspecies dissemination of *bla*$_{IMP}$. For instance, *bla*$_{IMP-8}$-producing *Enterobacter cloacae* ST78 from Taiwan has been identified as a high-risk global clone associated with the integron In73 [22]. As early as 2010–2012, surveillance data from Taiwan demonstrated that endemic clonal outbreaks of *bla*$_{IMP}$-producing CRE had already emerged, with *bla*$_{IMP-8}$-producing ECC being the predominant strains [23]. Recent studies in Taiwan have not only demonstrated an increasing trend in the prevalence of *bla*$_{IMP-8}$-producing CRE, but also disclosed that these isolates are distributed across multiple PFGE clusters and are frequently associated with IncF, IncY, or IncA/C plasmid types—broad-host-range plasmids known for their conjugative transferability [24]. Conjugation assays further confirmed that *bla*$_{IMP-8}$ can be horizontally transferred

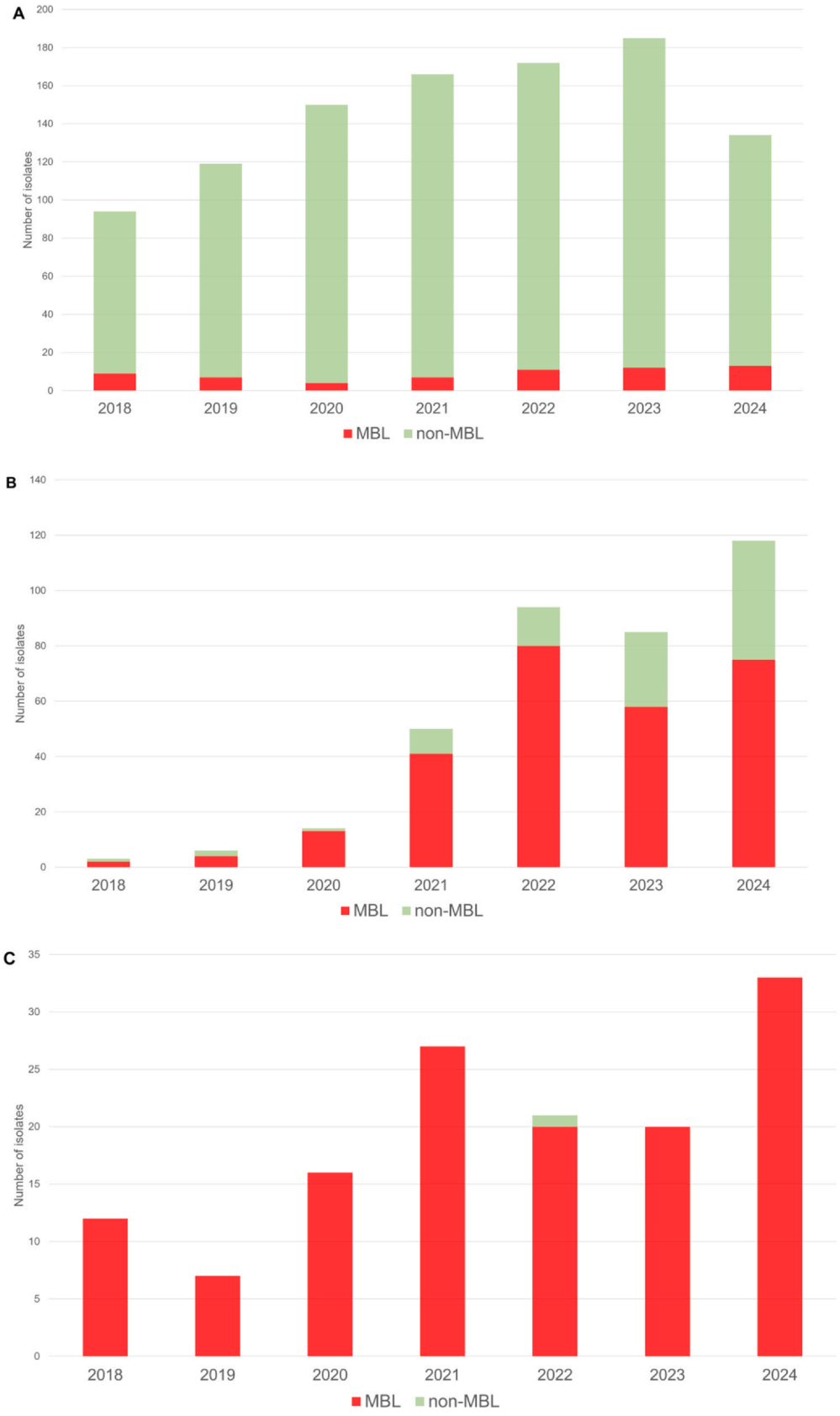

Fig. 6. Annual number of strains carrying MBL genes and non-MBL genes from 2018 to 2024. (A) *Klebsiella pneumoniae.* (B) *Escherichia coli.* (C) *Enterobacter cloacae* complex. (D) *Citrobacter freundii.* (E) *Citrobacter koseri.* (F) *Serratia marcescens.*

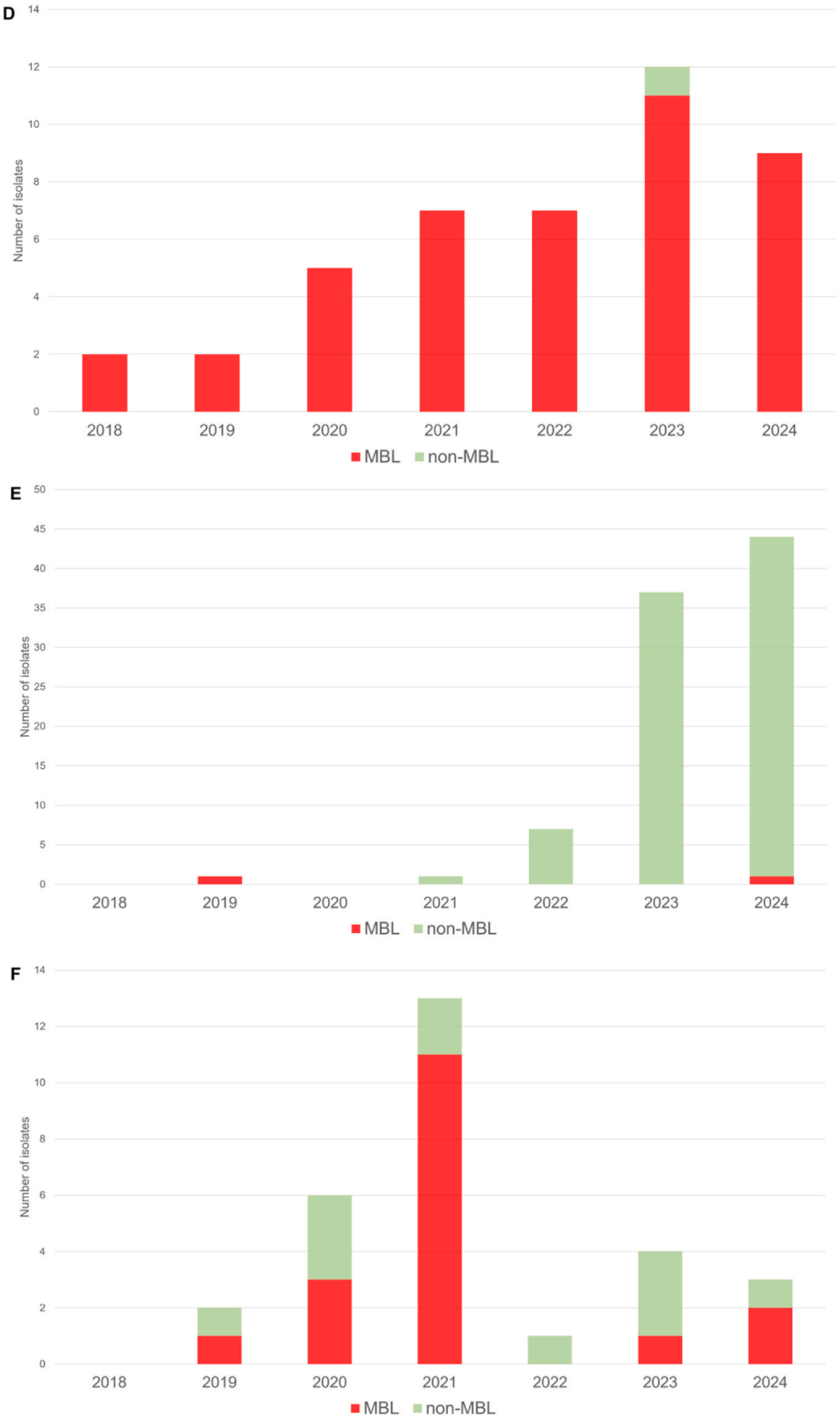

Fig. 6. (*continued*)

among different bacterial species [24]. In our study, bla_{IMP-8}-producing CRE represented one of the most common MBL-producing groups, predominantly involving ECC isolates (Table 2)—consistent with findings from other regions in Taiwan. Additionally, most MBL-producing *C. freundii* and *Serratia* spp. also harboured bla_{IMP-8}. Whether this distribution reflects HGT remains a critical question. Notably, several isolates carrying bla_{IMP-23}, a rarely reported variant, were also identified. Further plasmid characterization and whole-genome sequencing (WGS) are warranted to clarify the potential origin and evolutionary dynamics.

There are at least 24 bla_{NDM} variants, some of which exhibit enhanced carbapenemase activity, posing a significant clinical challenge. Notably, they are frequently carried on broad-host-range plasmids such as IncX3, IncFII, and IncA/C, facilitating interspecies transmission [25]. Furthermore, bla_{NDM-1} has been detected in diverse aquatic environments, including rivers, lakes, groundwater, tap water, hospital sewage, livestock wastewater, industrial effluents, and wastewater treatment plants, highlighting the transmission route from human activities to the environment and the associated public health risks [26]. In our study, the widespread distribution of bla_{NDM-1} across diverse bacterial species reflects the remarkable transmissibility of this carbapenemase gene. Whereas, bla_{NDM-5}-producing isolates were predominantly *E. coli*. It is essential to determine whether these *E. coli* strains, along with the few other bla_{NDM-5}-producing CPE, share the same plasmid type as *E. coli* ST8346, underscoring the potential for HGT (Table 2). Notably, three M-CPE isolates were found to carry the bla_{NDM-4} variant, with one of which even co-harbouring bla_{IMP-23}. This raises the question of whether such an evolutionary event was driven by a specific genetic mechanism or represents the emergence of a novel recombinant plasmid. WGS could offer deeper insights into the underlying genetic architecture and evolutionary dynamics.

To date, 11 bla_{VIM} variants have been described, with bla_{VIM-1} remaining the most prevalent [16,27]. Unlike bla_{IMP}, all bla_{VIM} variants are embedded within class 1 integrons, and their geographic distribution is predominantly concentrated in Europe [27]. A recent study by Huang et al. on the *Klebsiella oxytoca* complex revealed that, although bla_{VIM}-producing isolates exhibited lower levels of resistance compared to bla_{NDM}-producing isolates, they were associated with greater virulence and invasive potential [28]. In our study, only bla_{VIM-1} was detected among various CPE isolates, including M-CPE. Further WGS, antibiotic susceptibility profiling, and integration with patient-specific clinical data from corresponding specimens will provide deeper insights into genotype–phenotype correlations.

Interestingly, a decline in the proportion of MBL-CPE was observed in 2023 (Fig. 5). This shift may be attributed to the reduced prevalence of isolates harbouring MBL, accompanied by a simultaneous rise in those carrying non-MBL. According to Tsai et al., an outbreak of *E. coli* ST8346 co-harbouring $bla_{OXA-181}$ and bla_{NDM-5} occurred between 2021 and 2022, contributing to a marked rise in MBL-CPE [9]. It is plausible that infection control interventions implemented thereafter contributed to the decline in MBL-CPE isolates in 2023. However, a notable surge in $bla_{OXA-181}$-producing isolates during the same year led to a substantial increase in non-MBL-CPE, thereby explaining the dip in the MBL-CPE proportion observed in 2023. Between 2020 and 2022, following Taiwan's entry into the COVID-19 pandemic era, the emergence of CRE may be attributed to immunosuppression caused by SARS-CoV-2 infection, the concept of syndemic microbiology, inappropriate antibiotic use, and the unequal redistribution of infection control resources [3,29–31]. The most significant increase among CPE isolates was seen in those carrying $bla_{OXA-181}$ in our study, which spread across multiple species. Unlike the clonal *E. coli* ST8346 outbreak, the dissemination of $bla_{OXA-181}$ was more diverse. In 2023, lifted restrictions and increased human activity likely accelerated the spread, leading to a broader hospital and community outbreak of $bla_{OXA-181}$-producing isolates [30]. In 2024, the total number of MBL-producing isolates continued to increase again, resulting in a rise of MBL-CPE proportion, which even reached its highest level over the seven-year surveillance period.

Infections caused by CPE are often associated with poorer clinical outcomes compared to non-CPE, particularly in cases involving MBL-CPE and M-CPE, due to limited therapeutic options [4,19,32]. A study conducted in Taiwan reported a steady increase in the proportion of CPE among all CRE isolates, rising from 16.7 % in 2011–61 % in 2020, underscoring the growing clinical importance of CPE [33]. In our study, most CP-*K. pneumoniae* and CP-*C. koseri* isolates were classified as non-MBL-CPE, whereas the other four major CPE species—*E. coli*, ECC, *C. freundii*, and *S. marcescens*—were predominantly MBL-CPE (Fig. 6). This suggests that the use of agents lacking activity against MBL may result in treatment failure when managing infections caused by these organisms. Further investigation is warranted to delineate their complete antibiogram, particularly the distribution of minimum inhibitory concentration (MIC) and susceptibility profiles of novel antibiotics.

As shown in Fig. 2, most CPE isolates were recovered from respiratory specimens and urine samples, which are also common sources for the transmission of MDROs. In an era marked by the continuous rise of MBL-producing strains, strict infection control measures are crucial. In addition, robust antibiotic stewardship programs is essential to minimize unnecessary antimicrobial use, reduce selection pressure, and curb the increasing prevalence of MDROs.

To the best of our knowledge, this seven-year, multicenter, longitudinal study currently stands as the largest in terms of sample size, characterizing the burden of MBL-CPE in Taiwan. It provides valuable insights into the epidemiology and genetic diversity of these organisms. Compared with previous regional surveillance studies in Taiwan [11,23,24,33], our investigation included the largest number of CPE isolates analyzed to date and encompassed the most diverse range of bacterial species. In addition, we provided the most up-to-date epidemiological data through 2024, particularly revealing the current burden of MBL-producing CPE, which now accounts for nearly half of all CPE isolates. Furthermore, we identified multiple distinct bacterial species harboring different combinations of carbapenemase genes, indicating the recent emergence of M-CPE.

However, the study has some limitations. First, this was an epidemiological surveillance limited in Taiwan, and our inclusion criteria may have underestimated the true burden of MBL-CPE. This is partly due to the existence of other untested MBL genes and the absence of PFGE or WGS analyses to determine the clonality of excluded CPE isolates [3]. Second, due to cost constraints, only isolates non-susceptible to at least two carbapenems were referred to the central laboratory for carbapenemase testing. Some bla_{IMP}-producing strains, which exhibit weaker hydrolytic activity, may be resistant only to ertapenem [21,23]. As a result, our criteria may have further underestimated the prevalence of MBL producers. Third, isolates harbouring bla_{KPC} variants may confer resistance to ceftazidime-avibactam while reducing carbapenem MICs due to the D179Y mutation. This phenomenon may be associated with the usage of ceftazidime-avibactam [34]. In our study, we included strains only with high carbapenem MICs, which may probably lead to an underestimation of the true prevalence of bla_{KPC} variants. Lastly, ceftazidime–avibactam was gradually introduced into the three hospitals between 2020 and 2021. Further data regarding its defined daily dose per 1000 inhabitants per day in the subsequent years are warranted to elucidate its potential association with the concurrent emergence of MBL-CPE.

Conclusions

This seven-year multicenter study provides the most comprehensive investigation to date of the molecular epidemiology of CPE in Taiwan. Our findings reveal a substantial rise in MBL-producing CPE, increasing from 22.3 % in 2018–40 % in 2024, accompanied by the emergence of M-CPE. The diverse array of CPE isolates identified in this study likely reflects the coexistence of both globally disseminated and locally adapted resistance mechanisms. Moreover, the expansion of carbapenemase genes across various bacterial species suggests potential plasmid-mediated dissemination. These findings underscore the urgent need to strengthen nationwide genomic surveillance, with WGS being essential to determine whether the observed increases result from clonal expansion or extensive horizontal gene transfer. Reinforcing infection control measures, promoting the development of novel antibiotics, and implementing robust antimicrobial stewardship programs are paramount to mitigating further emergence and transmission. In parallel, continuous monitoring of high-risk clones remains essential to inform therapeutic strategies and strengthen containment efforts against these evolving multidrug-resistant pathogens.

Author contributions

All authors contributed equally to this work.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2025.103052.

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