

Molecular characteristics of carbapenem-resistant Enterobacterales harboring multiple carbapenemase genes in Asian countries

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ABSTRACT

Background: Carbapenem-resistant Enterobacterales (CRE) have emerged as a major global public health concern, particularly when resistance is mediated by carbapenemase production. Infections caused by carbapenemase-producing CRE are associated with limited therapeutic options and poor clinical outcomes. While most previous studies have focused on isolates producing a single carbapenemase, the phenotypic and molecular characteristics of CRE harboring multiple carbapenemase genes remain poorly understood. We investigated the phenotypic and molecular characteristics of carbapenem-resistant *Escherichia coli* (CREC) and *Klebsiella pneumoniae* (CRKP) harboring multiple carbapenemase genes in healthcare-associated infections (HAIs) among high-risk populations in five Asian countries.

Methods: CREC and CRKP clinical isolates collected from Asian countries between 2021 and 2023 as part of a multicenter surveillance study of the Asian Network for Surveillance of Resistant Pathogen (ANSORP). Antimicrobial susceptibility test was performed by the broth microdilution method according to CLSI guidelines. Carbapenemase genes (*bla*NDM, *bla*KPC, *bla*OXA-48-like, *bla*VIM, and *bla*IMP) were detected by multiplex PCR and Multilocus sequence typing (MLST) was performed for molecular epidemiological analysis.

Results: Among 27 CREC isolates, six (22.2%) harbored multiple carbapenemase genes, most frequently the NDM-5/OXA-181 combination (n=5) and belonged to diverse sequence types including the high-risk clone ST410 (n=2). Among 163 CRKP isolates, 29 (17.8%) carried multiple carbapenemase genes, predominantly combinations of NDM and OXA-48-like enzymes; one isolate harbored three distinct carbapenemase genes. High-risk clones such as ST11, ST16, and ST147 were identified and identical sequence types exhibited distinct carbapenemase gene combinations according to country. All 35 isolates with multiple carbapenemase genes showed extensively drug-resistant phenotypes, with susceptibility retained in only 17% of isolates to imipenem/relebactam and ceftazidime/avibactam.

Conclusion: CRE harboring multiple carbapenemase genes were frequently associated with high-risk clones and demonstrated marked heterogeneity in carbapenemase gene combinations across countries, even within identical sequence types. These isolates exhibited extensively drug-resistant phenotypes with very limited remaining therapeutic options. Our findings highlight the complexity of resistance mechanisms in CRE and underscore the importance of integrated molecular surveillance to inform treatment and containment strategies.

INTRODUCTION

- Carbapenem-resistant Enterobacterales (CRE) are a major cause of antimicrobial resistance-associated mortality worldwide, with high mortality rates driven by delayed detection and limited treatment options (Sung-woon Kang., 2024).
- Continuous surveillance and multinational epidemiological monitoring of CRE are essential to understand the global spread of antimicrobial resistance and to support effective infection prevention and control strategies (Sunish Shah., 2026).
- In this study, we investigated the molecular characteristics of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* harboring multiple carbapenemase genes across five Asian countries.**

MATERIALS AND METHODS

- Bacterial isolates**
27 CREC and 163 CRKP isolates causing HAIs collected from high-risk patients in 5 Asian countries as a part of prospective study.
- Detection of carbapenemase genes by PCR**
Identification of carbapenemase genes (*bla*KPC, *bla*NDM, *bla*IMP, *bla*VIM, and *bla*OXA-48-like) by PCR.
- Antimicrobial susceptibility testing (CLSI 2025)**
13 antimicrobial agents including ceftolozane/tazobactam, imipenem/relebactam, ceftazidime/avibactam, meropenem, imipenem, ertapenem, cefepime, ceftriaxone, ceftazidime, piperacillin/tazobactam, amikacin, gentamicin, and ciprofloxacin.
- Molecular typing by MLST**
Multilocus sequencing typing (MLST) was performed for *E. coli* and *K. pneumoniae* isolates using species-specific schemes (Achtman scheme for *E. coli* and Pasteur scheme for *K. pneumoniae*).

RESULTS

Figure 1. Distribution of CRE isolates across five Asian countries

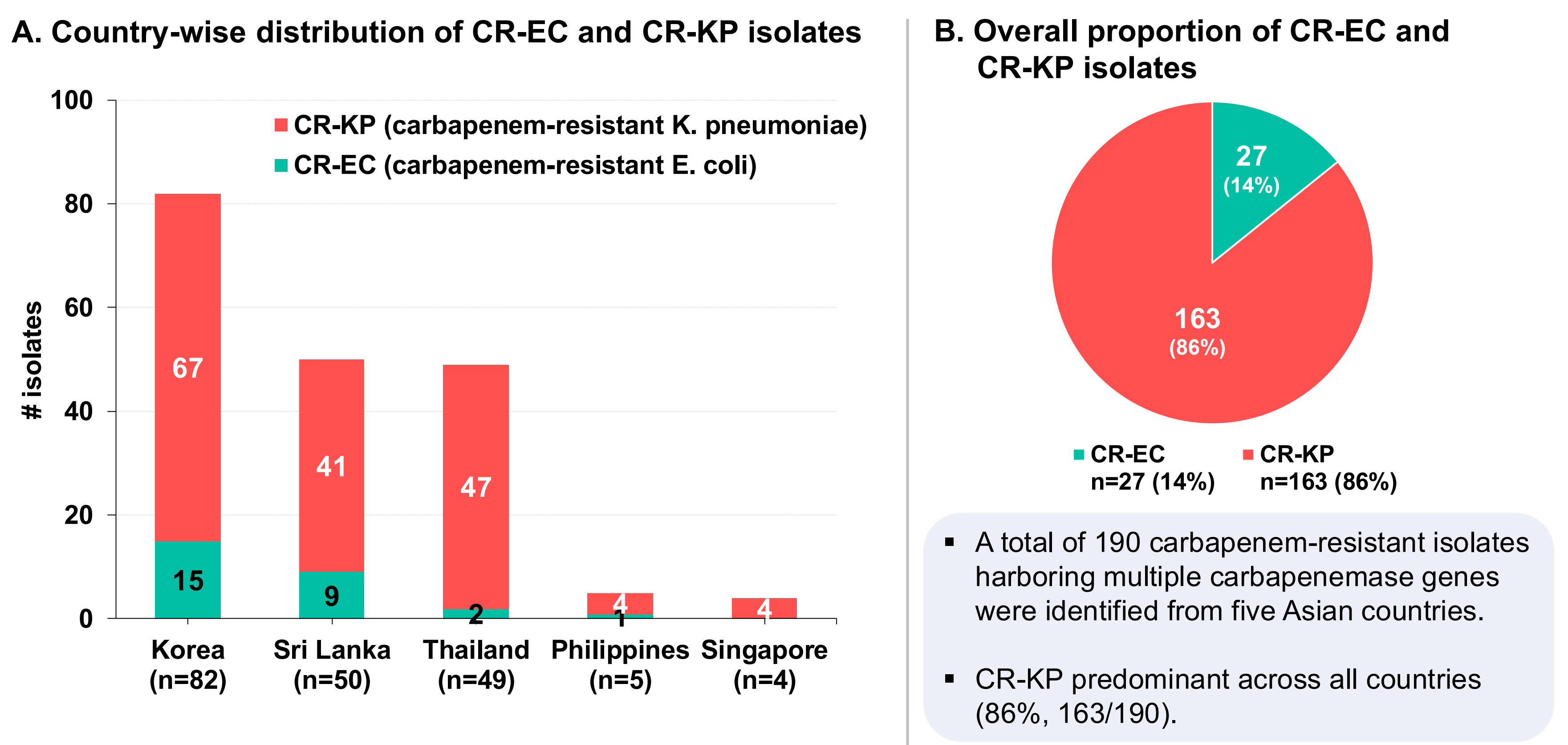


Figure 2. Carbapenemase Gene Profiles among CRE isolates (N=190)

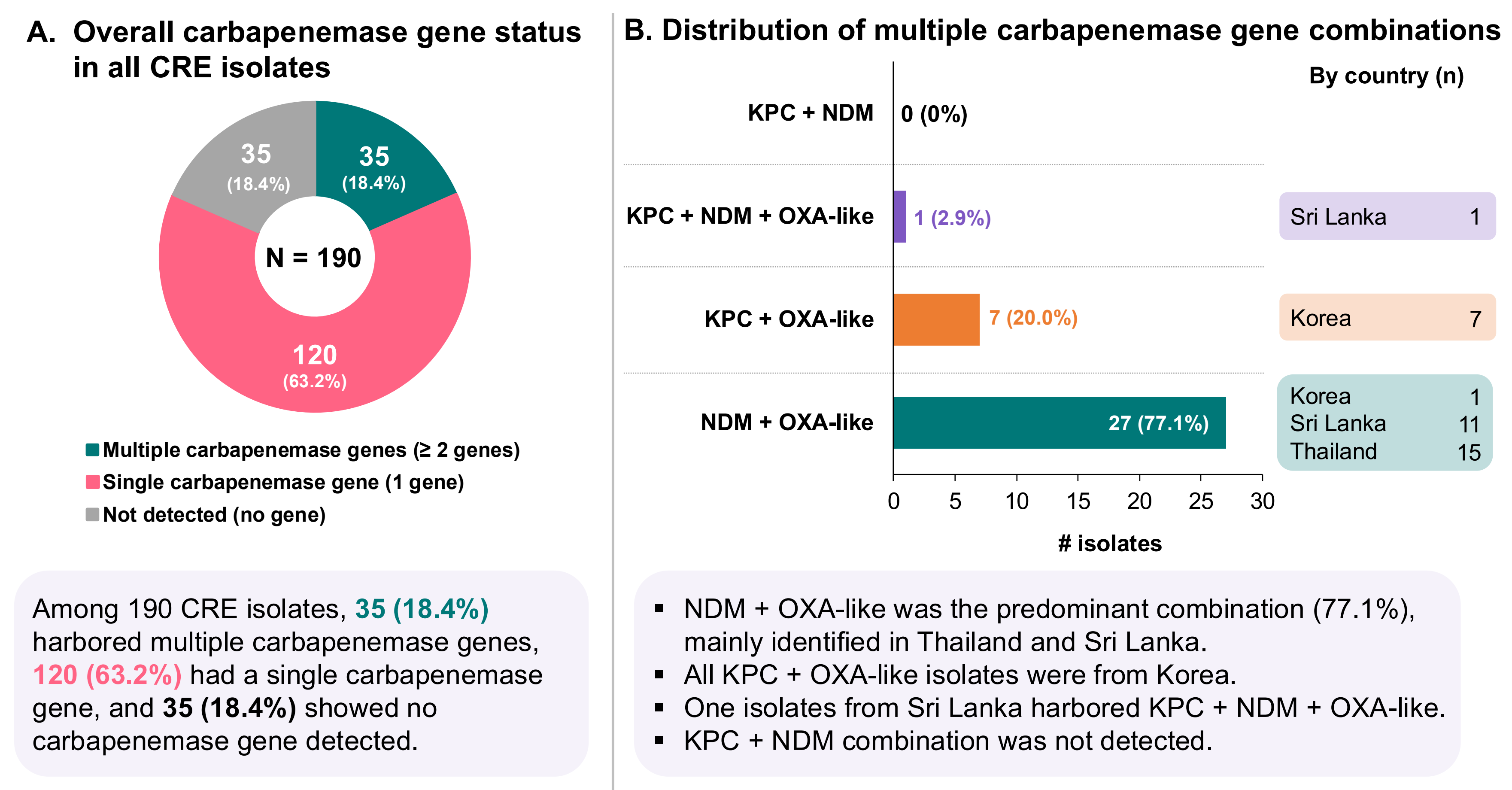


Figure 3. Antimicrobial resistance profiles of CR-EC and CR-KP

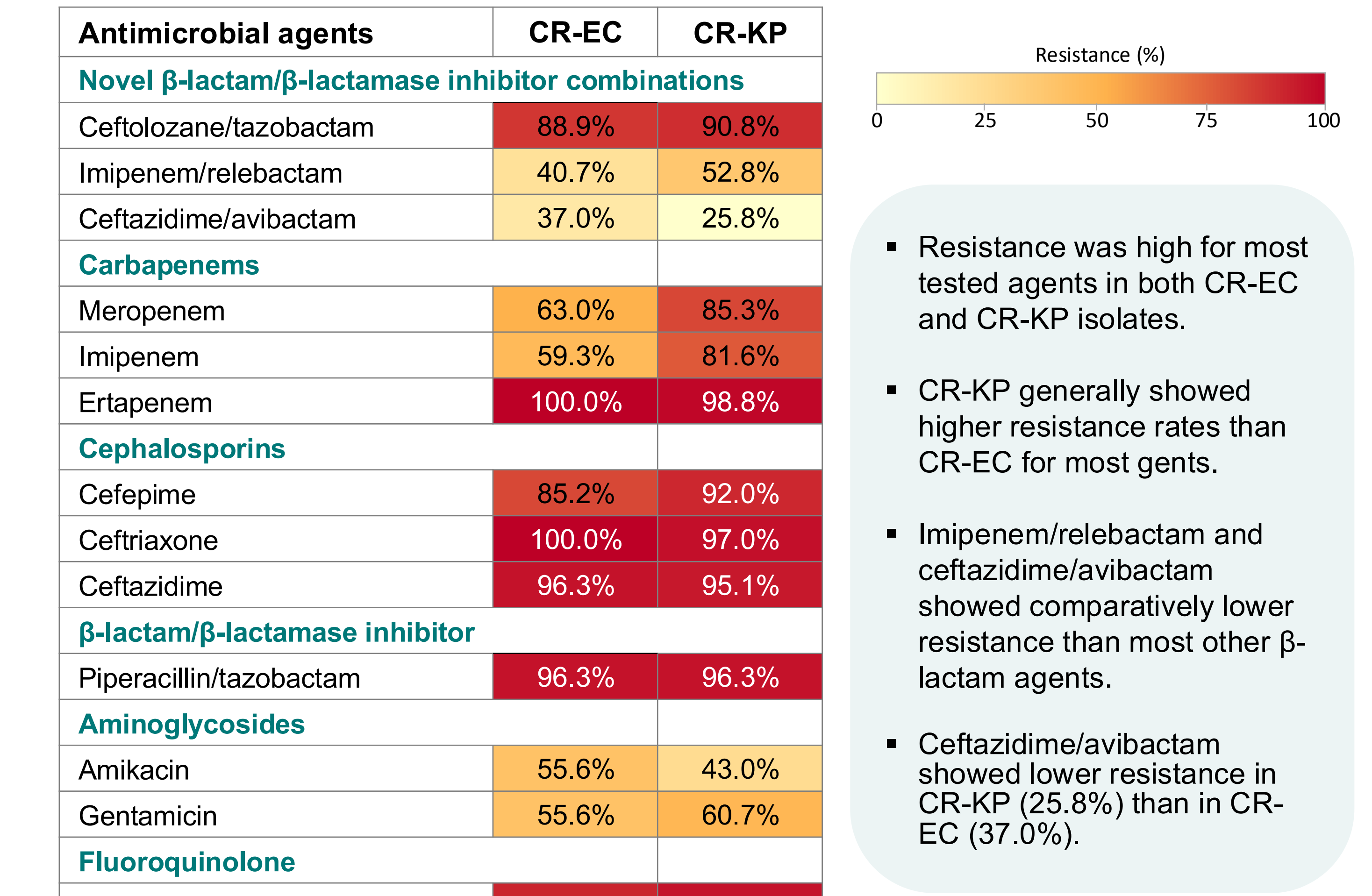
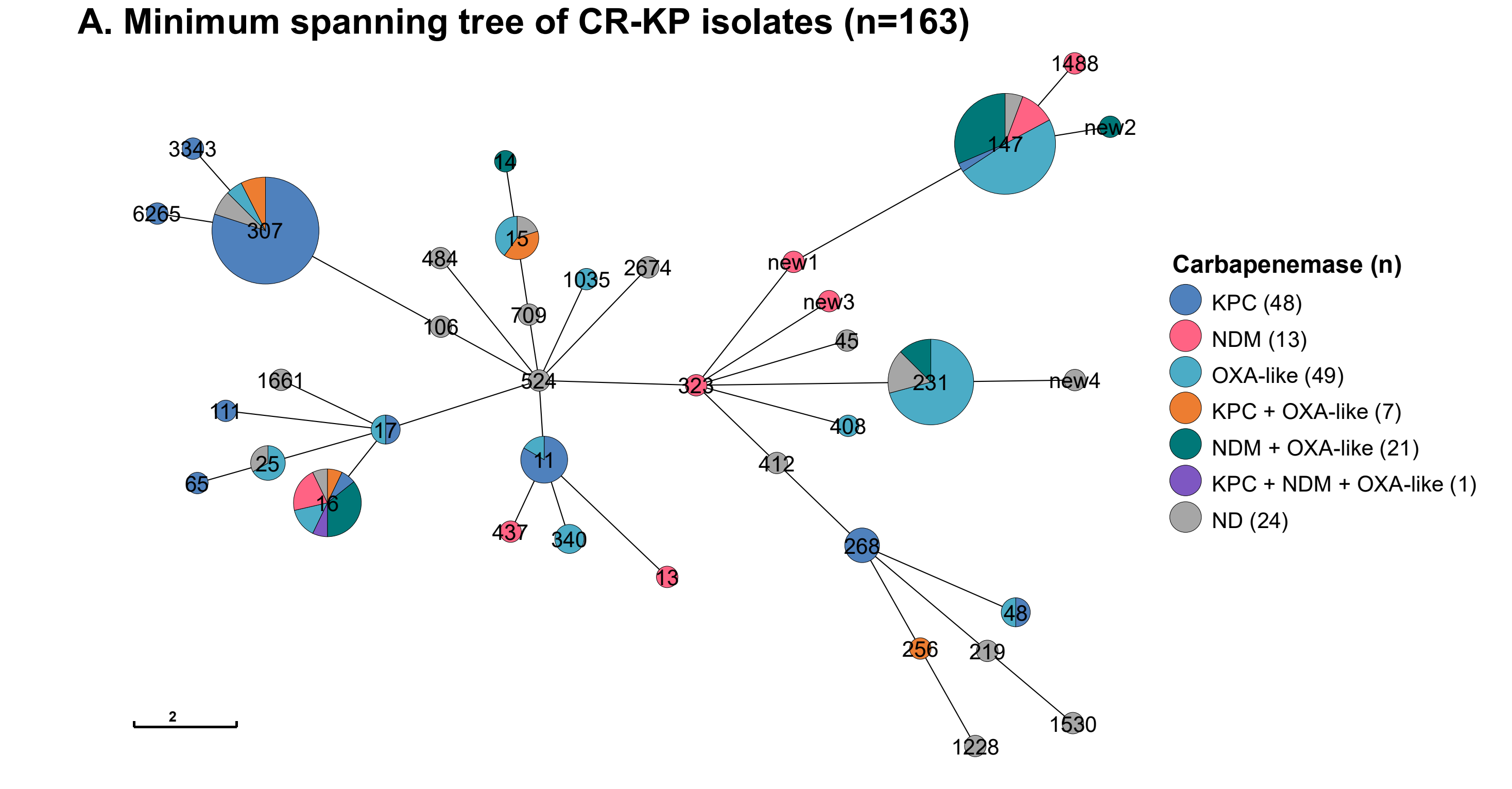


Figure 4. MLST and carbapenemase gene profiles CRE isolates



B. MLST and carbapenemase gene profiles of CR-EC isolates (n=27)

MLST	# isolates	Country (n)	Carbapenemase profile (# isolates)					ND
			KPC	NDM	OXA-like	KPC+ OXA-like	NDM+ OXA-like	
ST131	6	Korea (6)	3	0	0	0	0	3
ST410	6	Korea (2)	0	1	1	0	3	1
		Sri Lanka (2)						
		Philippines (1)						
ST405	4	Thailand (1)	0	1	0	0	1	2
		Korea (1)						
ST361	2	Sri Lanka (2)	0	1	0	0	1	0
ST2659	2	Sri Lanka (2)	0	0	1	0	1	0
ST450	1	Korea (1)	0	0	0	0	0	1
ST602	1	Thailand (1)	0	0	1	0	0	0
ST1994	1	Korea (1)	0	0	0	0	0	1
ST3180	1	Korea (1)	0	0	0	0	0	1
ST8019	1	Korea (1)	0	0	0	0	0	1
ST10539	1	Korea (1)	0	0	0	0	0	1
ST11672	1	Korea (1)	0	0	1	0	0	0
Total	27		3	3	4	0	6	11

CONCLUSIONS

- CR-KP was the predominant CRE species across the five Asian countries, with substantial geographic variation in isolates distribution.
- Carbapenemase gene profiles were heterogeneous, with most isolates carrying a single carbapenemase gene, while 18.4% harbored multiple carbapenemase genes.
- CR-KP isolates showed high antimicrobial resistance and diverse MLST-based clonal distribution, highlighting the need for continuous molecular surveillance of CRE in Asia.

REFERENCES

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- Shah, Sunish. *et al.* The changing epidemiology and long-term outcomes of patients with CRE infections. *Antimicrobial Stewardship & Healthcare Epidemiology.* 2026;6(1):e110.