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Original Article

Distribution of β-lactamases and emergence of carbapenemases co-occurring *Enterobacterales* isolates with high-level antibiotic resistance identified from patients with intra-abdominal infection in the Asia–Pacific region, 2015–2018

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Abstract *Purpose:* In this study, we aimed to assess the geographic distribution and molecular characteristics of β-lactamases among *Enterobacterales* isolates causing intra-abdominal infections (IAIs) from 2015 to 2018 in the Asia–Pacific region.
Method: Isolates were investigated for extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases using multiplex PCR assays and full-gene DNA sequencing.
Result: A total of 832 *Enterobacterales* isolates from 8 different countries with β-lactamase genes were analysed. Plasmid-mediated ESBLs and AmpC β-lactamases were encoded in 598 (71.9 %) and 314 (37.7 %) isolates, respectively. In 710 (85.3 %) carbapenemase-negative isolates, positivity for both AmpC β-lactamases and ESBLs was identified in 51 (8.5 %) *Escherichia coli* and 24 (3.4 %) *Klebsiella pneumoniae* isolates. The most prevalent countries were Taiwan and Vietnam, and the co-occurrence of CMY/CTX-M in *E. coli* and DHA-1/ESBLs in *K. pneumoniae* was predominant. All isolates showed high susceptibility to colistin, but susceptibility to

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carbapenems varied among different resistance mechanism combinations. Among 122 (14.7 %) isolates encoding carbapenemase, NDM (n = 67, including 64.2 % NDM-1) was the most common, followed by the OXA-48-type (n = 49), KPC (n = 24) and IMP (n = 4). The most prevalent country was Thailand (n = 44), followed by Vietnam (n = 35) and the Philippines (n = 21). Twenty-two isolates were found to encode multiple carbapenemases, 16 of which were collected from Thailand and harbored NDM-1, OXA-232 and CTX-M-15. Despite high susceptibility to amikacin, susceptibility to colistin was only 56 %.

Conclusion: The emergence of carbapenem-non-susceptible AmpC/ESBL co-occurring *Enterobacterales* and colistin non-susceptible carbapenemases co-occurring *K. pneumoniae* highlights potential therapeutic challenges in the Asia—Pacific region.

Introduction

Increasing antimicrobial resistance is a great challenge to public health worldwide.[1–3] Resistant pathogens are associated with increased mortality, prolonged hospitalization and high medical costs.[4,5] The burden of multidrug-resistant (MDR) organisms has increased in Asia. Among all carbapenem-resistant *Klebsiella pneumoniae* infections, the mortality rate was 44.8 % (95 % CI 37.8—51.9 %) in Asia.[6] In Singapore, one retrospective cohort study demonstrated 29.8 % 30-day mortality rate after ESBL-producing *Escherichia coli* or *K. pneumoniae* bacteremia, even under carbapenem treatment.[7] The molecular epidemiology of regional antibiotic resistance affects choices of empirical antibiotics and policies regarding infection control.

Of the various antimicrobial resistance mechanisms, β-lactamase is one of the most important hydrolyzing enzymes. This enzyme is found in both gram-positive and gram-negative bacteria. Due to the presence of plasmids, some *Enterobacterales* are able to transfer circular DNA molecules horizontally between bacteria. This mechanism contributes to the harboring and transmission of multiple drug resistance genes simultaneously, limiting the effectiveness of antibiotics. Regular resistance surveillance is essential to guide effective therapies and public health policies.

The Study for Monitoring Antimicrobial Resistance Trends (SMART) was initiated in 2002 and continuously monitors the antimicrobial resistance of clinical aerobic and facultative gram-negative bacterial isolates from intra-abdominal infections (IAIs). In a previous study, emergence of CTX-M-15-, CMY-2-, and NDM-1-producing *Enterobacterales* isolates was reported.[8] A high resistance burden in Vietnam and the Philippines was noted in subsequent surveillance reports.[9] In this study, we aimed to investigate the current distribution and molecular characteristics of extended-spectrum β-lactamases and carbapenemases in the Asia—Pacific region.

Material and methods

Bacterial isolates

Forty-two medical centers in 8 Asia—Pacific countries/regions participated in SMART from 2015 to 2018, including the Hong Kong Special Administrative Region of China (n = 4), Malaysia (n = 4), the Philippines (n = 4), Singapore (n = 2), South Korea (n = 7), Taiwan (n = 9), Thailand (n = 5), and Vietnam (n = 7). Nonduplicate isolates of aerobic and facultative gram-negative bacilli were collected from patients with a diagnosis of IAI. The clinical specimens were obtained from intra-abdominal abscess drainage fluid, gastrointestinal tissues, paracentesis or peritoneal fluid aspiration, and deep wound cultures obtained intraoperatively. Isolates collected within 48 h of hospital admission were presumptively classified as community-associated (CA) IAIs, and those collected more than 48 h after admission were classified as hospital-associated (HA) IAIs.

Antimicrobial susceptibility testing

All isolates were sent to a central laboratory (International Health Management. Associates, Inc., Schaumburg, IL). Prior to antimicrobial susceptibility testing, isolates were confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Billerica, MA). Susceptibility was determined by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method. Minimum inhibitory concentration (MIC) breakpoints for defining susceptibility to antimicrobial agents were tested following the CLSI M100-S30 standards, except for colistin. For colistin, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint of MIC ≤2 was considered susceptible.

Isolates of *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* with ceftriaxone and/or ceftazidime MICs of ≥2 μg/ml were confirmed to be ESBL producers using the CLSI confirmatory disc diffusion method for phenotypic confirmation (i.e., cefotaxime and ceftazidime tested alone and in combination with 4 μg/ml of clavulanic acid). These isolates were further characterized molecularly. All *Enterobacterales* that were non-susceptible to ertapenem (MIC ≥1 μg/ml), ceftolozane/tazobactam (MIC ≥4 μg/ml) or imipenem (MIC ≥2 μg/ml, *E. coli*, *K. pneumoniae* and *K. oxytoca*) were also screened for genes encoding carbapenemases.

Detection of β-lactamase genes

Isolates were tested for the presence of genes encoding β-lactamases using published multiplex PCR assay methods, followed by full-gene DNA sequencing as described previously.[10] The screened genes included ESBLs (CTX-M, TEM, SHV, VEB, PER and GES), acquired AmpC β-lactamases (ACC, ACT, CMY, DHA, FOX, MIR, MOX), serine carbapenemases (KPC, GES and OXA-48-like) and metallo-β-lactamases (IMP, VIM, NDM, GIM and SPM).

Ethical approval

The study was approved by the Institutional Review Boards and Ethical Committees of Chi Mei Medical Center (10908-E02).

Results

Isolates harboring β-lactamase genes

A total of 8383 isolates were collected from 2015 to 2018. *Enterobacterales* (n = 7144) accounted for 85.2 % of the total isolates. In total, 965 isolates were subjected to molecular testing. Isolates that tested negative for ESBLs, AmpC β-lactamases, and carbapenemases and those harboring only original spectrum β-lactamase were excluded. Finally, 832 isolates were analysed, including *E. coli* (n = 429), *K. pneumoniae* (n = 260), *Enterobacter cloacae* (n = 75), *Citrobacter freundii* (n = 20), *Enterobacter asburiae* (n = 14), *P. mirabilis* (n = 9), *K. oxytoca* (n = 9), *Klebsiella aerogenes* (n = 6), *Klebsiella variicola* (n = 2), *Citrobacter koseri* (n = 2), *Enterobacter kobei* (n = 4), *Morganella morganii* (n = 1), and *Serratia marcescens* (n = 1). Table 1 shows the distribution of ESBLs, AmpC β-lactamases and carbapenemases among 832 isolates from the Asia–Pacific region. In isolates harboring ESBL genes, *E. coli* (58.2 %) accounted for the majority, followed by *K. pneumoniae* (35.1 %) and *E. cloacae* (3.0 %). The most frequent AmpC β-lactamase-producing bacteria were *E. coli* (42.3 %), followed by *E. cloacae* (22.0 %) and *K. pneumoniae* (21.0 %). Among 122 isolates positive for carbapenemase, the most commonly identified bacteria were *K. pneumoniae* (67.2 %), followed by *E. coli* (21.3 %) and *E. cloacae* (7.4 %). Regarding the origin of the isolates, most of them (63.4 %) were recovered from patients with nosocomial infections.

ESBLs and AmpC β-lactamases

In Table 2, the geographic distributions of the major ESBLs and AmpC β-lactamases among the *Enterobacterales* isolates are displayed. CTX-M-type ESBLs were dominant in most countries. In *E. coli* isolates, the CTX-M-1 group (CTX-M-15 and CTX-M-55) and the CTX-M-9 group (CTX-M-14 and CTX-M-27) were the most common. CTX-M-15 was dominant in the Philippines and Thailand. In Taiwan and Vietnam, CTX-M-55 and CTX-M-27 were the most prevalent, respectively. In *K. pneumoniae* isolates, the pattern was similar, with CTX-M-15 predominant in most countries except Taiwan. In Taiwan, CTX-M-14 was predominant in the circulating isolates. Some *E. cloacae* isolates in the CTX-M-1 group were identified in Malaysia, the Philippines, Taiwan, Thailand and Vietnam. Among the AmpC β-lactamases, CMY-2 was the dominant variant in *E. coli*. The DHA-1 variant was more common in *K. pneumoniae* isolates. ACT/MIR were the dominant variants among *E. cloacae* isolates.

During our analysis, we observed co-occurrence of plasmid-mediated AmpC β-lactamase and ESBLs in carbapenemase-negative *E. coli* and *K. pneumoniae*. *E. coli* with this genotype was more prevalent in Taiwan and Vietnam, with some sporadic isolates in other countries. No β-lactamase co-occurring *E. coli* isolates were found in Hong Kong. The most common combination among *E. coli* isolates was the CMY/CTX-M type followed by the DHA-1/CTX-M type. Among *K. pneumoniae* isolates, those from Taiwan represented the majority of co-occurring isolates, with various AmpC and ESBL combinations. No co-occurring *K. pneumoniae* isolates were found in Hong Kong, Thailand or Vietnam. The detailed data are summarized in Table 3.

In Table 4, the antimicrobial susceptibility rates in isolates with combinations of AmpC β-lactamases and ESBLs are displayed. Among *E. coli* encoding either CMY/CTX-M or DHA-1/CTX-M, excellent inhibition by amikacin (98 %) and colistin (100 %) was observed. Meropenem and imipenem remained active against the CMY/CTX-M type, with a susceptibility rate greater than 90 %; however, the susceptibility rate decreased to approximately 70 % in *E. coli* harboring DHA-1/CTX-M. Among the *K. pneumoniae* isolates, colistin still has high susceptibility (95.8 %) to *K. pneumoniae* isolates. However, susceptibility to amikacin decreased (69.2 %) among isolates positive for both DHA-1/CTX-M or even combinations (25 %) of three different β-lactamases (DHA-1, SHV and CTX-M). Susceptibility to carbapenems varied among different combinations. The overall susceptibility rates were 75 % and 83.3 % for imipenem and meropenem, respectively.

Carbapenemases

In Table 5, the distribution of carbapenemases in our study is described. A total of 122 isolates were carbapenemase positive. *K. pneumoniae* (67.2 %) was the most common pathogen, followed by *E. coli* (21.3 %) and *E. cloacae* (7.4 %). Most of the isolates were isolated from Thailand (n = 44), Vietnam (n = 35), the Philippines (n = 21) and Taiwan (n = 14). KPC-2-positive isolates mainly comprised *K. pneumoniae*, especially in Taiwan and Vietnam. OXA-48-like producers were also predominantly *K. pneumoniae*. Among 22 OXA-232-positive isolates, 20 (91 %) were isolated from Thailand. Twelve isolates from Vietnam were positive for OXA-48-like carbapenemases. Among the isolates encoding NDM carbapenemases, most were collected in Thailand (n = 31) and were predominantly NDM-1-encoding *K. pneumoniae* (n = 30). In Malaysia, the Philippines, Singapore and Vietnam, sporadic NDM-positive isolates were collected (NDM-1, NDM-4, NDM-5, NDM-7). IMP-positive isolates were found in only the Philippines and Taiwan in *E. cloacae*, *Citrobacter* spp. and *Klebsiella* spp. other than *K. pneumoniae*.

Table 1 Distribution and origin of ESBLs, AmpC β-lactamases and carbapenemases among 832 isolates from intra-abdominal infections patients from the Asia—Pacific region.

	Isolates	ESBL	AmpC	Carbapenemase
Total numbers[a]	832	598	314	122
CA (%)	281 (37.6)	197 (37.6)	112 (37.0)	35 (37.2)
Escherichia coli	429 (51.6)	348 (58.2)	133 (42.3)	26 (21.3)
CA (%)	179 (47.0)	136 (44.6)	65 (52)	11 (47.8)
Klebsiella pneumoniae	260 (31.3)	210 (35.1)	66 (21.0)	82 (67.2)
CA (%)	63 (27.8)	50 (27.9)	18 (27.3)	16 (28.1)
Enterobacter cloacae	75 (9.0)	18 (3.0)	69 (22.0)	9 (7.4)
CA (%)	21 (29.2)	7 (38.9)	17 (25.8)	5 (55.6)
Citrobacter spp.[b]	22 (2.6)	1 (0.2)	22 (7.0)	1 (0.8)
CA (%)	4 (18.2)	0 (0)	4 (18.2)	0 (0)
Enterobacter spp.[c]	18 (2.2)	1 (0.2)	18 (5.7)	0 (0)
CA (%)	6 (33.3)	0 (0)	6 (33.3)	0 (0)
Klebsiella spp.[d]	17 (2.0)	12 (2.0)	3 (1.0)	4 (3.3)
CA (%)	6 (35.3)	3 (25)	1 (33.3)	3 (75.0)
Others[e]	11 (1.3)	8 (1.3)	3 (1.0)	0 (0)
CA (%)	2 (18.2)	1 (12.5)	1 (33.3)	0 (0)

[a] One isolate might harbor more than one resistance allele encoding more than one β-lactamase gene. Some data on community-associated infections are missing, so the isolate numbers between CA and total are inconsistent.
[b] The isolates of *Citrobacter* spp. comprised 22 isolates of *Citrobacter freundii* and *Citrobacter koseri*.
[c] The isolates of *Enterobacter* spp. comprised 18 isolates of *Enterobacter asburiae* and *Enterobacter kobei*.
[d] The isolates of *Klebsiella* spp. comprised 17 isolates of *Klebsiella aerogenes*, *Klebsiella oxytoca* and *Klebsiella variicola*.
[e] Other isolates included 11 isolates of *Proteus mirabilis*, *Morganella morganii* and *Serratia marcescens*.
Abbreviation: CA, community associated; ESBL, extended-spectrum β-lactamase.

In Table 6, the molecular characteristics and origins of carbapenemase co-occurring *Enterobacterales* collected from 2015 to 2018 are shown. Of a total of 22 isolates, 16 isolates (72.7 %) positive for both NDM-1/OXA-232 were obtained from Thailand. These 16 isolates also harbored CTX-M-15 simultaneously. The number of carbapenemase co-occurring *Enterobacterales* has increased since 2016. In Vietnam, 2 isolates harboring both NDM-4/OXA-181 were also found in 2016 and 2018. These two isolates were susceptible to colistin but not amikacin. In Table 7, the antimicrobial susceptibility of carbapenemase co-occurring *K. pneumoniae* isolates in Thailand is described. Carbapenemase co-occurring *K. pneumoniae* isolates have a high resistance rate (100 %) to nearly all tested antimicrobial agents except colistin and amikacin. Although the susceptibility rate was 100 % for amikacin, the MIC_{50} and MIC_{90} were 8 mg/L and 16 mg/L, respectively. The non-susceptibility rate to colistin reached 44 %.

Discussion

In our analysis, we summarized the current molecular epidemiology of β-lactamases among patients with IAI in the Asia—Pacific region. CTX-M-15 was still the most prevalent genotype. However, the emergence of AmpC/ESBL and carbapenemase co-occurring *Enterobacterales* is alarming. In previous studies, India showed a high prevalence of AmpC/ESBL co-occurring *Enterobacterales*, ranging from 58.4 % to 84.6 %.[11,12] AmpC and ESBL genes harbored in transmissible plasmids with specific plasmid replicon types are a possible explanation for the rapid spread of co-occurring bacteria.

The most commonly reported plasmid-encoded AmpC enzyme was the CMY-2 variant, especially in *E. coli*. Our analysis demonstrated the same distribution pattern in the Asia—Pacific region. *E. coli* isolates harboring both CMY/CTX-M are common in Vietnam and Taiwan. According to the SMART 2009—2011 in Vietnam, only 5.7 % (6/106) *E. coli* isolates harbored both CMY and CTX-M.[13] A subsequent study between 2011 and 2013 revealed a 6.2 % prevalence of AmpC-encoding gram-negative bacilli in patients with bloodstream infections.[14] In addition, 47 % of the AmpC producers harbored an ESBL gene. In Taiwan, in a longitudinal nationwide surveillance study conducted from 2002 to 2012, *E. coli* harboring both AmpC and ESBL accounted for 1.5 % of the isolates.[15] Cefotaxime non-susceptible rate increased from 8.9 % to 19.6 % during study period. Antimicrobial non-susceptibility rate was higher in co-producers than in non-co-producers. In co-producers, all isolates (100 %) were non-susceptible to cefoxitin, ceftazidime, and 76.9 % isolates were non-susceptible to cefepime. Among isolates encoding solely ESBL, non-susceptibility rate was 29.5 % for cefoxitin and 35.3 % for ceftazidime. In isolates encoding AmpC, non-susceptibility rate to cefepime was only 0.8 %.

In Taiwan, the DHA-1 variant among *K. pneumoniae* was more prevalent than in other countries; therefore, we found more isolates with co-occurrence of DHA-1 and other ESBLs in Taiwan than in other countries. Between 2013 and 2016, DHA-1/SHV co-occurring hypervirulent K1 *K. pneumoniae* strains were identified in 8 out of 18 drug-resistant isolates from one medical center in Taiwan.[16] These isolates all showed the MDR phenotype. Along with the results of our data, possible clonal expansion of isolates encoding more than 2 β-lactamases may need further investigation. Similar dissemination has also been

Table 2 Geographic distribution of major ESBLs and AmpC β-lactamases among *Enterobacterales* causing intra-abdominal infections in different countries.

N = 817	Total	Hong Kong	Korea	Malaysia	Philippines	Singapore	Taiwan	Thailand	Vietnam
E. coli	426	4	22	15	33	19	107	41	185
ESBL	348	4	21	13	18	18	64	38	172
CTX-M-1 group[a]	192								
CTX-M-15	123	2	5	6	14	4	12	16	64
CTX-M-55	68	1	4	2		4	24	10	23
Others	3		1		1			1	
CTX-M-8 group	1		1						
CTX-M-9 group[b]	161								
CTX-M-14	39		5		2	3	11	5	13
CTX-M-27	99		3	1	2	3	13	5	72
Others	25	1	3	4		4	4		9
AmpC[c]	133	0	2	7	16	2	59	5	42
CMY-2	80		2	5	4	2	51	5	11
CMY-42	17			1	1				15
Others	13			1	1		2		9
DHA-1	24				10		7		7
K. pneumoniae	251	2	26	17	27	5	80	55	39
ESBL	210	2	24	17	22	5	51	55	34
CTX-M-1 group[d]	152								
CTX-M-15	140		20	16	19	5	13	48	19
Others	14	2		1	3		1	2	5
CTX-M-9 group	35								
CTX-M-14	25		2		1	1	17		4
Others	10		1				3		6
SHV-5	10						9	1	
SHV-12	17		6		1		8		2
Others	20						11	6	3
AmpC[e]	66	0	6	1	6	1	47	0	5
CMY-2	3						3		
DHA-1	64		6	1	6	1	45		5
E. cloacae	74	3	6	3	9	2	42	6	3
ESBL	18	0	0	1	4	0	11	1	1
CTX-M-3	2				1		1		
CTX-M-15	8			1	3		2	1	1
CTX-M-9	1			1					
SHV-5	1						1		
SHV-12	9						9		1
AmpC[f]	69	3	6	2	6	2	41	6	3
CMY-2	2						2		
DHA-1	3						1	1	1
ACT	56	2	5	2	5	1	35	4	2
MIR	9	1	1		1	1	4	1	

Abbreviation: ESBL, extended-spectrum β-lactamase.

[a] Two isolates in Vietnam harbored both CTX-M-15 and CTX-M-55 alleles.

[b] One isolate in Vietnam harbored both CTX-M-14 and CTX-M-27 alleles, and another isolate in Vietnam harbored both CTX-M-14 and CTX-M-24 alleles.

[c] One isolate in Taiwan harbored both CMY-2 and DHA-1 alleles.

[d] Two isolates in the Philippines harbored both CTX-M-15 and CTX-M-3 alleles.

[e] One isolate in Taiwan harbored both CMY-2 and DHA-1 alleles.

[f] One isolate in Taiwan harbored both CMY-2 and ACT alleles.

reported in Europe. Sporadic cases were found in the Czech Republic and Spain.[17,18] From 2009 to 2013, countrywide spread of the DHA-1 and CTM-X-15 types co-producing the KP053/ST11 clone was noted in Hungary.[19]

There are no CLSI criteria for phenotypic detection of AmpC β-lactamases. The production of ESBLs could be masked by the presence of AmpC, which makes identification of isolates with different resistance mechanisms difficult. Combining piperacillin-tazobactam with cefepime or incorporating inhibitors of the AmpC enzyme (such as boronic acid) may be a feasible method to detect AmpC/ESBL co-occurring isolates in clinical laboratories.[20] In bacteria with AmpC and ESBL co-occurrence, the activity of cephamycin and clavulanic acid is decreased. However, our data also showed decreased susceptibility to other classes of antibiotics, such as aminoglycoside or fluroquinolone. This finding indicated that the plasmid genes encoding ESBLs might carry resistance genes for other antimicrobial agents. Hence, therapeutic options will be limited. Carbapenems might remain the main treatment for severe or life-threatening infections.[21] Some isolates showed a carbapenem-resistant phenotype without carbapenemase, and the mechanism may be largely attributed to reduced outer membrane permeability and mutations in the porin genes.[22,23] In our analysis, the phenotypic profiles of the isolates varied depending on the genetic variant type or whether there were several β-lactamase genes in a single isolate.

Among the carbapenemases, KPC, OXA-48 and NDM cause great concern. *K. pneumoniae* is a major source of transmission. Most of the carbapenemases were carried by *K. pneumoniae* in our study. Endemic or sporadic spread of carbapenem-resistant *K. pneumoniae* has been reported worldwide.[24–26] However, the prevalent genotype of carbapenemase-producing *K. pneumoniae* varies geographically.[27] In a previous estimation, the prevalence of carbapenem-resistant *Enterobacterales* in Vietnam and the Philippines was approximately 5–10 %. The estimated prevalence in Thailand was approximately 1–5%.[28] However, our data showed that the numbers of carbapenemase-encoding isolates were highest in Thailand. NDM-1 and OXA-232 co-occurring isolates were identified in this surveillance study. Although most of the isolates (87.5 %) were restricted to HA IAIs, the isolated number gradually increased from 2015 to 2018. This suggests the potential threat of community transmission and regional spread.

Emergence of NDM- and OXA-48-type carbapenemase co-occurring *Enterobacterales* has been reported in the Middle East and Thailand.[29–31] The co-occurrence of NDM-1/OXA-232 was mainly found in *K. pneumoniae*, with isolated cases in different countries.[32–36] A sporadic case of *E. coli* harboring both NDM-1/OXA-232 was reported.[37] In the absence of selective pressure, plasmids generally impose a fitness cost to host bacteria. However, one *K. pneumoniae* strain from Korea revealed that plasmids harboring both NDM-1 and OXA-232 increased the fitness and virulence of bacterial hosts in animal models.[38] Although the mechanism needs further exploration, this finding supports the emergence of isolates harboring several resistance genes in our analysis. Continuous surveillance is crucial for regional transmission monitoring and containment of dissemination.

Rapid identification of the presence of carbapenemases is important for infection control. OXA-48-type isolates are characterized by susceptibility to broad spectrum cephalosporins but resistance to carbapenems. Nevertheless, in OXA-48-producing *K. pneumoniae*, concomitant outer membrane porin loss and coproduction of other β-lactamases were common,[25] which elevated the MIC levels for cephalosporins and caused universal resistance. It is a great challenge to identify co-occurring carbapenemases. Several commonly used detection methods, such as the modified Hodge test or hydrolytic assay (Carba NP test), cannot differentiate OXA-48-types or NDM alone.[39,40] Molecular-based techniques and newly developed multiplexed lateral flow assays might be useful tools for discriminating different carbapenemase-encoding genes.

Colistin resistance in endemic carbapenem-resistant *Enterobacterales* is a rapidly evolving problem. This combination was verified in our analysis. In our analysis, 7 out of 16 *K. pneumoniae* isolates harboring both NDM-1 and OXA-232 in Thailand showed non-susceptibility to colistin. An increase in the amikacin MIC was also observed. All isolates were non-susceptible to carbapenems or fluoroquinolones.

Table 3 Co-occurrence of plasmid-mediated AmpC beta-lactamase/ESBLs among carbapenemase-negative *Escherichia coli* and *Klebsiella pneumoniae* isolated from intra-abdominal infection patients in different countries.

N = 75	Hong Kong	Korea	Malaysia	Philippines	Singapore	Taiwan	Thailand	Vietnam	Total
E. coli	0	1	3	1	1	16	1	28	51
CMY + CTX-M		1	3	1	1	11	1	22	40
CMY-2 + TEM-12						1			1
CMY-8B + VEB-1								1	1
CMY-148 + CTX-M-14 + CTX-M-15								1	1
DHA-1 + CTX-M						4		3	7
DHA-1 + VEB-1 + CTX-M-15								1	1
K. pneumoniae	0	4	1	1	1	17	0	0	24
CMY-2 + SHV-5						1			1
DHA-1 + CTX-M		3	1	1	1	7			13
DHA-1 + SHV						6			6
DHA-1 + SHV + CTX-M		1				3			4

Abbreviations: ESBL, extended-spectrum β-lactamase.

Table 4 In vitro antimicrobial susceptibility rates of *Klebsiella pneumoniae* and *Escherichia coli* with combinations of AmpC β-lactamase and ESBLs among isolates from intra-abdominal infection patients.

	Isolates	AMK	AZT	FEP	CAZ	CRO	CST	EPM	IPM	MEM	CIP	LVX	C/T	PTZ
E. coli	51	98.0	0	19.6	2.0	0	100	70.6	90.2	95.0	2.0	17.6	33.3	45.1
CMY + CTX-M	41	100	0	12.2	0	0	100	68.3	92.7	100	0	17.1	25.8	36.6
DHA-1 + CTX-M	7	100	0	42.9	14.3	0	100	71.4	71.4	66.7	14.3	14.3	66.7	71.4
K. pneumoniae	24	70.8	4.2	25.0	8.3	0	95.8	54.2	75.0	83.3	4.2	20.8	16.7	20.8
DHA-1 + CTX-M	13	69.2	7.7	23.1	15.4	0	92.3	69.2	84.6	88.9	7.7	23.1	22.2	30.8
DHA-1 + SHV	6	100	0	33.3	0	0	100	33.3	50.0	80.0	0	33.3	20.0	16.7
DHA-1 + SHV + CTX-M	4	25.0	0	25.0	0	0	100	50.0	75.0	66.7	0	0	0	0

Abbreviations: AMK, amikacin; AZT, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CST, colistin; C/T, ceftolozane-tazobactam; EPM, ertapenem; FEP, cefepime; LVX, levofloxacin; IPM, imipenem; PTZ, piperacillin–tazobactam.
Minimum inhibitory concentration breakpoints for defining susceptibility to antimicrobial agents were tested following the CLSI M100-S30 standards, except for CST. The EUCAST breakpoint with minimum inhibitory concentration ≤2 was considered susceptible for CST.

Table 5 Geographic distribution of various carbapenemases among *Enterobacterales* causing intra-abdominal infections in different countries in the Asia–Pacific region.

	Hong Kong	Korea	Malaysia	Philippines	Singapore	Taiwan	Thailand	Vietnam	Total
Total[a]	0	1	6	21	1	14	44	35	122
Escherichia coli	0	0	3	3	0	0	8	12	26
KPC-2				1				4	5
OXA-48				2				3	5
OXA-181			2				4		6
OXA-232			1				3		4
NDM-1							1		1
NDM-4								1	1
NDM-5			1					3	4
NDM-7				1				1	2
Klebsiella pneumoniae	0	1	2	9	1	11	36	22	82
KPC-2		1		2		8		7	18
KPC-17						1			1
OXA-48						2	3	6	11
OXA-181							2	3	5
OXA-232			1				17		18
NDM-1			2	3			29	4	38
NDM-4								5	5
NDM-7				4	1				5
NDM (not typing)							1		1
Enterobacter cloacae	0	0	1	6	0	1	0	1	9
NDM-1			1	1				1	3
NDM-7				4					4
IMP-4				1					1
IMP-8						1			1
***Klebsiella* spp.**	0	0	0	3	0	1	0	0	4
NDM-1				1					1
NDM-7				2					2
IMP-8						1			1
Citrobacter freundii	0	0	0	0	0	1	0	0	1
IMP-8						1			1

[a] One isolate might harbor more than one resistance allele encoding more than two carbapenemases. The detailed data are provided in Table 6.

A high rate of colistin resistance (31.4 %) among *K. pneumoniae* with co-occurring OXA-48-type and NDM carbapenemases has also emerged in Dubai, United Arab Emirates.[29] However, no mcr genes were detected in this study. The mechanism of colistin resistance acquired in the clinical isolates was beyond the scope of our analysis. However, monitoring the antibiotic susceptibility trend of colistin in carbapenemase-endemic areas is warranted.

Table 6 Molecular characteristics and origins of carbapenemase co-occurring *Enterobacterales* causing intra-abdominal infections in the Asia—Pacific region.

	Bacterial isolates	Country	Year	Age	CA or HA	ICU	Carbapenemases	Other β-lactamases
1	*K. pneumoniae*	Malaysia	2015	56	CA	No	NDM-1; OXA-232	CTX-M-15
2	*E. coli*	Malaysia	2018	76	HA	No	NDM-5; OXA-181	CTX-M-15; CMY-2
3	*E. coli*	Philippines	2018	38	HA	Yes	NDM-7; KPC-2	
4	*K. pneumoniae*	Thailand	2016	81	—	No	NDM-1; OXA-232	CTX-M-15; SHV-5
5	*K. pneumoniae*	Thailand	2016	78	—	No	NDM-1; OXA-232	CTX-M-15
6	*K. pneumoniae*	Thailand	2017	42	—	Yes	NDM-1; OXA-232	CTX-M-15
7	*K. pneumoniae*	Thailand	2017	83	—	No	NDM-1; OXA-232	CTX-M-15
8	*K. pneumoniae*	Thailand	2017	70	HA	Yes	NDM-1; OXA-232	CTX-M-15
9	*K. pneumoniae*	Thailand	2017	31	HA	No	NDM-1; OXA-232	CTX-M-15
10	*K. pneumoniae*	Thailand	2017	39	HA	No	NDM-1; OXA-232	CTX-M-15
11	*K. pneumoniae*	Thailand	2018	49	CA	No	NDM-1; OXA-232	CTX-M-15
12	*K. pneumoniae*	Thailand	2018	57	HA	No	NDM-1; OXA-232	CTX-M-15
13	*K. pneumoniae*	Thailand	2018	82	HA	Yes	NDM-1; OXA-232	CTX-M-15
14	*K. pneumoniae*	Thailand	2018	78	HA	No	NDM-1; OXA-232	CTX-M-15
15	*K. pneumoniae*	Thailand	2018	25	HA	Yes	NDM-1; OXA-232	CTX-M-15
16	*K. pneumoniae*	Thailand	2018	71	HA	No	NDM-1; OXA-232	CTX-M-15
17	*K. pneumoniae*	Thailand	2018	62	CA	No	NDM-1; OXA-232	CTX-M-15
18	*K. pneumoniae*	Thailand	2018	48	HA	No	NDM-1; OXA-232	CTX-M-15
19	*K. pneumoniae*	Thailand	2018	52	—	No	NDM-1; OXA-232	CTX-M-15
20	*K. pneumoniae*	Vietnam	2016	32	HA	No	NDM-4; KPC-2	
21	*K. pneumoniae*	Vietnam	2018	83	HA	No	NDM-4; OXA-181	CTX-M-15
22	*K. pneumoniae*	Vietnam	2018	97	HA	No	NDM-4; OXA-181	CTX-M-15

Abbreviation: CA, community associated; HA, hospital associated.

Table 7 Antimicrobial susceptibility of carbapenemase co-occurring *K. pneumoniae* isolated from patients with intra-abdominal infections during 2015—2018 in Thailand.

Antimicrobial agents	MIC (mg/L)			Susceptible rate (%)		
	Range	MIC_{50}	MIC_{90}	S (%)	I (%)	R (%)
Amikacin	<4—16	8	16	100	0	0
Aztreonam	>16	>16	>16	0	0	100
Cefepime	>16	>16	>16	0	0	100
Ceftazidime	>16	>16	>16	0	0	100
Ceftriaxone	>4	>4	>4	0	0	100
Colistin	<1 − >4	2	>4	56	NA	44
Ertapenem	>2	>2	>2	0	0	100
Imipenem	>4	>4	>4	0	0	100
Meropenem	>4	>4	>4	0	0	100
Ciprofloxacin	>2	>2	>2	0	0	100
Levofloxacin	>2	>2	>2	0	0	100
Ceftolozane/Tazobactam	>8/4	>8/4	>8/4	0	0	100
Piperacillin/Tazobactam	>64/4	>64/4	>64/4	0	0	100

Abbreviations: MIC, minimum inhibitory concentration; NA, not applicable; S, susceptible; I, intermediate; R, resistant.

Among patients with IAI in the Asia—Pacific region, AmpC/ESBL co-occurring *Enterobacterales* was common in Taiwan and Vietnam. However, carbapenemase co-occurring *Enterobacterales* has also emerged, especially in Thailand. The isolates of *K. pneumoniae* harboring NDM-1 and OXA-232 increased from 2015 to 2018. Community-associated, colistin non-susceptible isolates warrant continuous monitoring. Monotherapy with colistin or carbapenem alone may not overcome all resistance mechanisms in endemic areas, especially in isolates with different combinations of resistance mechanisms. Understanding the regional epidemiology of resistance could drive effective combination therapies or the new development of antibiotics with novel mechanisms.

Declaration of competing interest

All authors have no conflicts of interest to disclose.

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