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Nationwide surveillance of antimicrobial resistance among clinically important Gram-negative bacteria, with an emphasis on carbapenems and colistin: Results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) in 2018

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ABSTRACT

Multicentre surveillance of antimicrobial susceptibility of clinically important Gram-negative bacteria (GNB) from 16 Taiwanese hospitals was performed. *Escherichia coli* ($n = 398$), *Klebsiella pneumoniae* ($n = 346$), *Pseudomonas aeruginosa* ($n = 252$) and *Acinetobacter baumannii* complex (ABC) ($n = 188$) bloodstream isolates, non-typhoidal *Salmonella* ($n = 230$) and *Shigella flexneri* ($n = 18$) from various sources were collected. Antimicrobial MICs were determined using broth microdilution. Genes encoding *K. pneumoniae* carbapenemases (KPCs), New Delhi metallo-β-lactamases (NDMs), Verona integron-encoded metallo-β-lactamase (VIM), OXA-48-like carbapenemase (OXA-48) as well as *mcr-1–5* genes were detected by molecular methods. Rates of carbapenem non-susceptibility were 2.8%, 9.0%, 0.4%, 0%, 10.3% and 48.8% for *E. coli, K. pneumoniae, Salmonella, Shigella, P. aeruginosa* and ABC, respectively. For carbapenemases, one (0.3%) *E. coli* harboured $bla_{\text{NDM-1}}$. Fifteen (4.3%), two (0.6%) and two (0.6%) *K. pneumoniae* contained bla_{KPC}, $bla_{\text{OXA-48}}$ and bla_{VIM}, respectively. Two (0.5%) *E. coli* and fourteen (4.0%) *K. pneumoniae* were non-wild-type according to the colistin MIC. Among Enterobacteriaceae with a colistin MIC $\geq$ 2 mg/L, *mcr-1* was detected in one *E. coli*, two *K. pneumoniae* and three *Salmonella* spp. All three *mcr-1*-positive *Salmonella* isolates were collected from community-acquired infections; none of the six *mcr-1*-positive Enterobacteriaceae were carbapenem-resistant. Carbapenem resistance has increased among clinically important GNB, especially among hospital-acquired infections. bla_{KPC}, especially the $bla_{\text{KPC-2}}$ variant, was detected in approximately one-half of the carbapenem-resistant *K. pneumoniae* isolates in this study. Although resistance rates to colistin remained low among Enterobacteriaceae, the finding of *mcr-1* from different species raises concern of potential dissemination.

1. Introduction

Widespread antimicrobial resistance is one of the greatest public-health threats and is associated with increased medical burden [1–3]. It has been estimated that annual deaths due to antimicrobial resistance will reach 10 million a year by 2050 [4]. The continuously increasing antimicrobial resistance has drawn the attention of the World Health Organization (WHO), which has declared a list of antibiotic-resistant 'priority pathogens' including a collection of 12 families of bacteria [5,6]. The top priority pathogens are all Gram-negative bacteria (GNB), including carbapenem-resistant *Acinetobacter baumannii* complex, carbapenem-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant or extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, that urgently require research and development of new antibiotics.

Antimicrobial resistance is also a serious problem in Taiwan. The percentage of carbapenem-resistant *A. baumannii* complex increased from <20% in 2001–2003 to approximately 63–71.2% in 2010–2012 according to data from the Taiwan Nosocomial Infections Surveillance (TNIS) System [7–9]. A significant trend of increasing carbapenem-resistant *P. aeruginosa* prevalence was also observed in data from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) programme [10,11]. ESBL-producing Enterobacteriaceae have become prevalent in recent decades [12,13]. Moreover, carbapenem-resistant Enterobacteriaceae have emerged since the arrival of *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase 1 (NDM-1) enzymes in 2010 [14,15].

The Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) programme has monitored trends in the antimicrobial susceptibility of clinically important pathogens collected from hospitals all over Taiwan since 2002. The continuing increase in carbapenem resistance has raised major concerns regarding clinically important GNB, whether in community-associated or healthcare-associated infections. Because of limited therapeutic options, colistin, a polymyxin antibiotic discovered more than 50 years ago, is being increasingly used as a last-resort antibiotic to treat multidrug-resistant GNB [16–18]. However, the mobilised colistin resistance gene (*mcr-1*), which makes horizontal transfer of colistin resistance possible among different strains of GNB, was recently discovered in plasmids of *Escherichia coli* [19–21]. Therefore, the nationwide SMART study in 2018 was conducted to survey the susceptibility of clinically important GNB by investigating the type and prevalence of antimicrobial resistance genes associated with carbapenem and colistin resistance.

2. Materials and methods

2.1. Settings and bacterial isolates

The SMART programme was conducted by the Taiwan Centers for Disease Control. This nationwide resistance surveillance programme involved 16 hospitals (11 medical centres and 5 regional hospitals) located in different parts of Taiwan (8 from Northern, 4 from Central, 3 from Southern and 1 from Eastern Taiwan) (Fig. 1). Clinically important GNB were collected, including bloodstream *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* complex (two and one non-duplicate isolates were required from each medical centre and regional hospital, respectively, each month). Meanwhile, non-duplicate non-typhoidal *Salmonella* species and *Shigella* spp. were collected irrespective of the source of the isolates.

Community-acquired isolates were defined as pathogens collected <48 h after admission to hospital with signs or symptoms of infection upon admission. Hospital-acquired isolates were defined as those collected >48 h after admission from patients who initially lacked signs of infection. In addition, basic patient characteristics were collected, including age, sex, hospital setting and outcome, using a standardised case record form. The study was approved by the Research Ethics Committees or Institutional Review Boards of the 16 participating hospitals. Informed consent was waived because the in vitro antimicrobial susceptibility surveillance presented no more than minimal risk to participating subjects.

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of the isolates for 17 antimicrobial agents, including colistin, were determined using a commercial VITEK®2 antimicrobial susceptibility system (AST-NB card; bioMérieux, Marcy-l'Étoile, France) as described previously [6]. Colistin MICs were also determined by the reference broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [22]. The results were illustrated as resistance categories according to the MIC breakpoints recommended by the CLSI in 2018 [22]. For colistin, the epidemiological cut-off value was used to separate microbial populations into

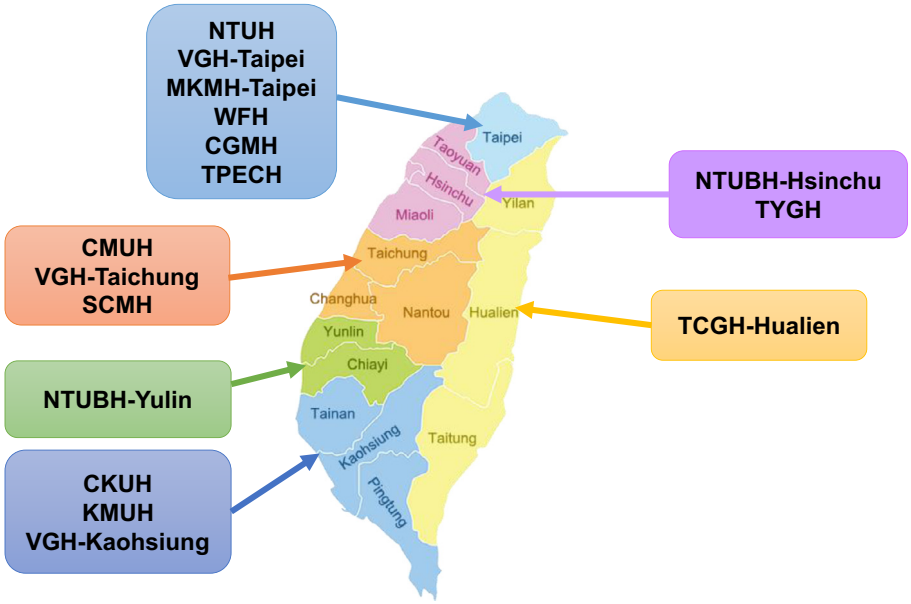

Fig. 1. Distribution of 16 participating hospitals in Taiwan in 2018. CGMH, Chang Gung Memorial Hospital; CKUH, National Cheng Kung University Hospital; CMUH, China Medical University Hospital; KMUH, Kaohsiung Medical University Hospital; MKMH, MacKey Memorial Hospital; NTUH, National Taiwan University Hospital; NTUHB, National Taiwan University Hospital Branch; TCGH, Buddhist Tzu Chi General Hospital; TPECH, Taipei City Hospital; TYGH, Taoyuan General Hospital; SCMH, Show Chwan Memorial Hospital; VGH, Veterans General Hospital; and WFH, Wan Fang Hospital.

those with and without phenotypically detectable resistance [non-wild-type (NWT) or wild-type (WT), respectively], and an MIC $\leq$ 2 mg/L was considered WT among Enterobacteriaceae.

2.3. Detection of key genes for carbapenem and colistin resistance

Enterobacteriaceae, *P. aeruginosa* and *A. baumannii* complex isolates that were non-susceptible to any carbapenem in vitro were tested for carbapenemase-encoding genes, including bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM} and $bla_{OXA\text{-}48\text{-}like}$, using an Xpert® Carba-R assay (Cepheid, Sunnyvale, CA). Enterobacteriaceae with a colistin MIC $\geq$ 2 mg/L and *P. aeruginosa* and *A. baumannii* complex with colistin resistance (MICs > 2 mg/L) were subjected to PCR detection of plasmid-mediated transferable resistance determinants. The *mcr* genes (*mcr-1, mcr-2, mcr-3, mcr-4* and *mcr-5*) were screened by PCR and were sequenced as previously described [6].

2.4. Molecular typing of bla_{KPC}-positive Klebsiella pneumoniae

The sequence type (ST) of *K. pneumoniae* isolates positive for bla_{KPC} was determined by multilocus sequence typing (MLST) according to a previously described protocol [23]. The MLST protocol was performed according to the *K. pneumoniae* MLST website (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). MLST results were typed according to the international *K. pneumoniae* MLST database created in 2005 at the Institut Pasteur (Paris, France).

Pulsed-field gel electrophoresis (PFGE) with the restriction enzyme *Xba*I was further performed on isolates with the same MLST for analysis of relatedness as suggested by the manufacturer [24]. Banding patterns with >80% similarity were considered to belong to the same pulsotype.

2.5. Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics v.20.0 (IBM Corp., Armonk, NY). Categorical variables were compared using the χ^2 test or Fisher's exact test, whereas non-categorical variables were compared using the Mann–Whitney *U*-test. All tests were two-tailed, and a *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. Antimicrobial susceptibility profiles

During the study period, 1184 bloodstream isolates were collected, including *E. coli* (n = 398), *K. pneumoniae* (n = 346), *P. aeruginosa* (n = 252) and *A. baumannii* complex (n = 188). In addition, *Salmonella* spp. (n = 230) and *Shigella flexneri* (n = 18) were also collected from any sources. Clinically important GNB were collected monthly for monitoring the dynamics of resistance trends to avoid involving clusters of strains in short-term outbreaks. The MIC range and percentage of antimicrobial susceptibility are shown in Table 1. The rates of ESBL phenotype were 17.3% and 16.5% among bloodstream isolates of *E. coli* and *K. pneumoniae*, respectively. Hospital-acquired isolates had a higher prevalence of ESBL phenotype in *E. coli* (29.5% vs. 15.1%; $P < 0.05$) and *K. pneumoniae* (29.9% vs. 10.4%; $P < 0.05$).

In general, hospital-acquired isolates had higher non-susceptibility rates than community-acquired isolates. Regarding *E. coli* and *K. pneumoniae*, amikacin and tigecycline showed >90% susceptibility rates in either hospital-acquired or community-acquired isolates. The different susceptibility between community-acquired and hospital-acquired isolates was more obvious among *K. pneumoniae* than *E. coli*. Among *K. pneumoniae*, not only cefotaxime and cefepime but also piperacillin/tazobactam (PIP/TAZ), ertapenem and levofloxacin showed significantly lower susceptibility rates among hospital-acquired isolates (Fig. 2). It should be noted that ertapenem was highly effective against *E. coli*, with susceptibility rates of 99.1% and 96.7% in community-acquired isolates and hospital-acquired isolates, respectively; however, the susceptibility rate decreased from 97.1% in community-acquired isolates to 77.6% in hospital-acquired isolates among *K. pneumoniae*.

Salmonella spp. and *S. flexneri* showed better carbapenem susceptibility, with only one *Salmonella* sp. isolate showing intermediate resistance to ertapenem. For other commonly used antibiotics for *Salmonella* and *Shigella* spp., most antibiotics, including

Table 1
In vitro susceptibility to tested antimicrobial agents of bloodstream isolates of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex, as well as non-typhoidal *Salmonella* and *Shigella flexneri* from various body sites collected from patients at 16 participating hospitals in Taiwan, 2018.

Bacterial species (no. of isolates)/antimicrobial agent	MIC (mg/L)			No. (%) of isolates with indicated susceptibility		
	Range	MIC_{50}	MIC_{90}	S	I	R
Escherichia coli (398)						
Ampicillin/sulbactam	≤2 to ≥32	≥32	≥32	145 (36.4)	46 (11.6)	207 (52.0)
Cefazolin[a]	≤4 to ≥64	≤4	≥64	269 (67.6)	NA	129 (32.4)
Cefmetazole	≤1 to ≥64	≤1	8	367 (92.2)	15 (3.8)	16 (4.0)
Cefotaxime	≤1 to ≥64	≤1	≥64	297 (74.6)	1 (0.3)	100 (25.1)
Ceftazidime	≤1 to ≥64	≤1	16	337 (84.7)	1 (0.3)	60 (15.1)
Cefepime	≤1 to ≥64	≤1	2	359 (90.2)	16 (4.0)	23 (5.8)
Piperacillin/tazobactam	≤4 to ≥128	≤4	64	354 (88.9)	18 (4.5)	26 (6.5)
Ertapenem	≤0.5 to ≥8	≤0.5	≤0.5	390 (98.0)	0 (0)	8 (2.0)
Imipenem	≤0.25 to ≥16	≤0.25	≤0.25	387 (97.2)	9 (2.3)	2 (0.5)
Meropenem	≤0.25 to ≥16	≤0.25	≤0.25	392 (98.5)	0 (0)	6 (1.5)
Ciprofloxacin	≤0.25 to ≥4	≤0.25	≥4	273 (68.6)	1 (0.3)	124 (31.2)
Levofloxacin	≤0.12 to ≥8	0.5	≥8	273 (68.6)	5 (1.3)	120 (30.2)
Gentamicin	≤1 to ≥16	≤1	≥16	305 (76.6)	4 (1.0)	89 (22.4)
Amikacin	≤2–32	≤2	4	397 (99.7)	1 (0.3)	0 (0)
Trimethoprim/sulfamethoxazole	≤20 to ≥320	≤20	≥320	229 (57.5)	–[b]	169 (42.5)
Tigecycline[c]	≤0.5–4	≤0.5	≤0.5	NA	NA	NA
Colistin[d]	0.25–4	1	1	WT: 396 (99.5)		NWT: 2 (0.5)
Klebsiella pneumoniae (346)						
Ampicillin/sulbactam	≤2 to ≥32	8	≥32	247 (71.4)	9 (2.6)	90 (26.0)
Cefazolin[a]	≤4 to ≥64	≤4	≥64	264 (76.3)	NA	82 (23.7)
Cefmetazole	≤1 to ≥64	≤1	≥64	292 (84.4)	8 (2.3)	46 (13.3)
Cefotaxime	≤1 to ≥64	≤1	≥64	276 (79.8)	11 (3.2)	59 (17.1)
Ceftazidime	≤1 to ≥64	≤1	≥64	280 (80.9)	3 (0.9)	63 (18.2)
Cefepime	≤1 to ≥64	≤1	8	307 (88.7)	7 (2.0)	32 (9.2)
Piperacillin/tazobactam	≤4 to ≥128	≤4	≥128	285 (82.4)	13 (3.8)	48 (13.9)
Ertapenem	≤0.5 to ≥8	≤0.5	≤0.5	315 (91.0)	6 (1.7)	25 (7.2)
Imipenem	≤0.25 to ≥16	≤0.25	1	324 (93.6)	4 (1.2)	18 (5.2)
Meropenem	≤0.25 to ≥16	≤0.25	≤0.25	327 (94.5)	1 (0.3)	18 (5.2)
Ciprofloxacin	≤0.25 to ≥4	≤0.25	≥4	291 (84.1)	3 (0.9)	52 (15.0)
Levofloxacin	≤0.12 to ≥8	≤0.12	≥8	291 (84.1)	8 (2.3)	47 (13.6)
Gentamicin	≤1 to ≥16	≤1	≥16	282 (81.5)	13 (3.8)	51 (14.7)
Amikacin	≤2 to ≥64	≤2	≤2	337 (97.4)	0 (0)	9 (2.6)
Trimethoprim/sulfamethoxazole	≤1 to ≥16	≤1	≥16	272 (78.6)	–	74 (21.4)
Tigecycline[c]	≤0.5 to ≥8	≤0.5	2	NA	NA	NA
Colistin[d]	0.5 to >32	1	1	WT: 332 (96.0)		NWT: 14 (4.0)
Salmonella spp. (230)						
Ampicillin/sulbactam	≤2 to ≥32	≤2	≥32	129 (56.1)	3 (1.3)	98 (42.6)
Cefotaxime	≤1 to ≥64	≤1	4	197 (85.7)	0 (0)	33 (14.3)
Ceftazidime	≤1 to ≥64	≤1	16	197 (85.7)	0 (0)	33 (14.3)
Cefepime	≤1 to ≥64	≤1	≤1	225 (97.8)	0 (0)	5 (2.2)
Piperacillin/tazobactam	≤4 to ≥128	≤4	≤4	217 (94.3)	9 (3.9)	4 (1.7)
Ertapenem	≤0.5–1	≤0.5	≤0.5	229 (99.6)	1 (0.4)	0 (0)
Imipenem	≤0.25–1	≤0.25	≤0.25	230 (100)	0 (0)	0 (0)
Meropenem	≤0.25	≤0.25	≤0.25	230 (100)	0 (0)	0 (0)
Ciprofloxacin	≤0.25 to ≥4	≤0.25	0.5	206 (89.6)	7 (3.0)	17 (7.4)
Levofloxacin	≤0.12–4	≤0.12	1	181 (78.7)	42 (18.3)	7 (3.0)
Trimethoprim/sulfamethoxazole	≤1 to ≥16	≤1	≥16	164 (71.3)	–	66 (28.7)
Tigecycline[c]	≤0.5–4	≤0.5	1	NA	NA	NA
Colistin[c]	0.5 to >32	1	8	NA	NA	NA
Shigella flexneri (18)						
Ampicillin/sulbactam	≤2 to ≥32	≤2	≥32	11 (61.1)	0 (0)	7 (38.9)
Cefotaxime	≤1–32	≤1	≤1	17 (94.4)	0 (0)	1 (5.6)
Ceftazidime	≤1 to ≥64	≤1	≤1	17 (94.4)	0 (0)	1 (5.6)
Cefepime	≤1	≤1	≤1	18 (100)	0 (0)	0 (0)
Piperacillin/tazobactam	≤4	≤4	≤4	18 (100)	0 (0)	0 (0)
Ertapenem	≤0.5	≤0.5	≤0.5	18 (100)	0 (0)	0 (0)
Imipenem	≤0.25	≤0.25	≤0.25	18 (100)	0 (0)	0 (0)
Meropenem	≤0.25	≤0.25	≤0.25	18 (100)	0 (0)	0 (0)
Ciprofloxacin	≤0.25 to ≥4	≥4	≥4	7 (38.9)	0 (0)	11 (61.1)
Levofloxacin	≤0.12–4	4	4	6 (33.3)	1 (5.6)	11 (61.1)
Trimethoprim/sulfamethoxazole	≤1 to ≥16	≤1	≥16	12 (66.7)	–	6 (33.3)
Tigecycline[c]	≤0.5	≤0.5	≤0.5	NA	NA	NA
Colistin[c]	0.5–1	0.5	1	NA	NA	NA
Pseudomonas aeruginosa (252)						
Ceftazidime	≤1 to ≥64	4	32	209 (82.9)	15 (6.0)	28 (11.1)
Cefepime	≤1 to ≥64	2	16	223 (88.5)	11 (4.4)	18 (7.1)
Piperacillin/tazobactam	≤4 to ≥128	8	≥128	189 (75.0)	29 (11.5)	34 (13.5)
Imipenem	≤0.25 to ≥16	2	2	227 (90.1)	0 (0)	25 (9.9)
Meropenem	≤0.25 to ≥16	≤0.25	4	226 (89.7)	11 (4.4)	15 (6.0)
Ciprofloxacin	≤0.25 to ≥4	≤0.25	2	224 (88.9)	5 (2.0)	23 (9.1)
Levofloxacin	≤0.12 to ≥8	0.5	4	217 (86.1)	10 (4.0)	25 (9.9)
Gentamicin	≤1 to ≥16	≤1	4	235 (93.3)	3 (1.2)	14 (5.6)

(*continued on next page*)

Table 1 (*continued*)

Bacterial species (no. of isolates)/antimicrobial agent	MIC (mg/L)			No. (%) of isolates with indicated susceptibility		
	Range	MIC_{50}	MIC_{90}	S	I	R
Amikacin	≤2 to ≥64	≤2	4	247 (98.0)	1 (0.4)	4 (1.6)
Colistin	0.5–8	2	2	230 (91.3)	–	22 (8.7)
Acinetobacter baumannii complex (188)						
Ampicillin/sulbactam	≤2 to ≥32	≤2	≥32	120 (63.8)	8 (4.3)	60 (31.9)
Ceftazidime	≤1 to ≥64	8	≥64	96 (51.1)	21 (11.2)	71 (37.8)
Cefepime	≤1 to ≥64	8	≥64	107 (56.9)	6 (3.2)	75 (39.9)
Piperacillin/tazobactam	≤4 to ≥128	16	≥128	98 (52.1)	2 (1.1)	88 (46.8)
Imipenem	≤0.25 to ≥16	≤0.25	≥16	111 (59.0)	0 (0)	77 (41.0)
Meropenem	≤0.25 to ≥16	≤0.25	≥16	106 (56.4)	5 (2.7)	77 (41.0)
Ciprofloxacin	≤0.25 to ≥4	≤0.25	≥4	104 (55.3)	1 (0.5)	83 (44.1)
Levofloxacin	≤0.12 to ≥8	0.25	≥8	106 (56.4)	24 (12.8)	58 (30.9)
Gentamicin	≤1 to ≥16	≤1	≥16	110 (58.5)	8 (4.3)	70 (37.2)
Amikacin	≤2 to ≥64	≤2	≥64	156 (83.0)	3 (1.6)	29 (15.4)
Trimethoprim/sulfamethoxazole	≤1 to ≥16	≤1	≥16	103 (54.8)	–	85 (45.2)
Tigecycline[c]	≤0.5 to ≥8	≤0.5	2	NA	NA	NA
Colistin	0.5 to >32	1	2	172 (91.5)	–	16 (8.5)

MIC, minimum inhibitory concentration; $MIC_{50/90}$, MIC for 50% and 90% of the isolates, respectively; S, susceptible; I, intermediate; R, resistant; NA, not available.

[a] Isolates with MIC ≤ 4 mg/L among *E. coli* and *K. pneumoniae* were recorded in the susceptible column because the lower limit of MIC detection was 4 mg/L in this study. The intermediate category column of cefazolin among *E. coli* and *K. pneumoniae* was marked as not available (NA).

[b] No MIC for the intermediate category.

[c] Susceptibility categories not available (NA) as there are no current CLSI interpretive criteria for these bacteria.

[d] The epidemiological cut-off value was used for the colistin MIC among isolated *E. coli* and *K. pneumoniae*. An MIC ≤ 2 mg/L was considered wild-type (WT) and an MIC ≥ 4 mg/L was considered non-wild-type (NWT).

third- and fourth-generation cephalosporins, PIP/TAZ and carbapenems, maintained good susceptibility levels. However, there were increased levels of non-susceptibility to trimethoprim/sulfamethoxazole and quinolones. The susceptibility rate to levofloxacin was only 78.7% in *Salmonella* spp. isolates. *Shigella flexneri* showed even greater resistance to ciprofloxacin and levofloxacin, with only 38.9% and 33.3% susceptibility, respectively.

In this study, *P. aeruginosa* showed good susceptibility to most antipseudomonal antibiotics, and only PIP/TAZ had a susceptibility rate of <80%. Susceptibility to PIP/TAZ and imipenem was significantly lower among isolates from hospital-acquired isolates, but other antipseudomonal agents showed similar susceptibility rates (Fig. 2).

In contrast, *A. baumannii* complex showed more extensive antibiotic resistance, especially for isolates from hospital-acquired infections, for which the highest susceptibility rate was <60% for most agents. Amikacin and colistin were two agents with preserved antimicrobial activity against *A. baumannii* complex regardless of whether the isolates were from community-acquired or hospital-acquired infections (Fig. 2). For tigecycline, the percentage of isolates with an MIC ≥ 4 mg/L was low (6.9%).

3.2. Detection of the main carbapenemase genes among carbapenem-non-susceptible isolates, and genotyping of bla_{KPC}-positive Klebsiella pneumoniae

For all carbapenem-non-susceptible isolates, including *E. coli* ($n = 11$), *K. pneumoniae* ($n = 31$), *Salmonella* sp. ($n = 1$), *P. aeruginosa* ($n = 26$) and *A. baumannii* complex ($n = 82$), genes associated with carbapenem resistance were studied and the results are shown in Table 2. No carbapenemase-related genes were detected among the *Salmonella* sp., *P. aeruginosa* and *A. baumannii* complex isolates. One *E. coli* isolate was positive for bla_{NDM-1}. Most carbapenemase-related genes were detected in *K. pneumoniae*, including 15 bla_{KPC} (11 bla_{KPC-2}, 2 bla_{KPC-17} and 2 non-typeable bla_{KPC}), 2 bla_{OXA-48} and 2 bla_{VIM}. The positive identification rate of carbapenemases in carbapenem-non-susceptible *K. pneumoniae* was 61.3% (19/31). Most bla_{KPC}-positive *K. pneumoniae* isolates (14/15; 93.3%) belonged to ST11 according to MLST genotyping. The 14 *K. pneumoniae* ST11 were further analysed by PFGE for clonality and the results showed four clusters from four hospitals (Fig. 3). Three clusters contained isolates from one hospital, indicating intrahospital transmission. The remaining cluster, which might be associated with interhospital transmission, contained one isolate from Northern Taiwan and another from Central Taiwan. Susceptibility to some key antibiotics for isolates harbouring target resistance genes is shown in Table 3. Colistin, tigecycline and amikacin are commonly used agents for multidrug-resistant Enterobacteriaceae. None of the isolates in this study with a carbapenemase gene were simultaneously resistant to colistin, tigecycline and amikacin (Table 3). Both bla_{OXA-48}-positive *K. pneumoniae* isolates were isolated from a single hospital in Central Taiwan and had relatively low MICs for imipenem (2 mg/L) and meropenem (1 mg/L) but a high MIC for ertapenem (≥8 mg/L).

3.3. Detection of mcr-1 in Enterobacteriaceae with a non-wild-type phenotype to colistin

The *mcr-1* gene encoding plasmid-mediated resistance to colistin was investigated in all Enterobacteriaceae with a colistin MIC ≥ 2 mg/L, including *E. coli* ($n = 2$), *K. pneumoniae* ($n = 22$), *Salmonella* spp. ($n = 82$), colistin-resistant *P. aeruginosa* ($n = 22$) and colistin-resistant *A. baumannii* complex ($n = 16$). Six isolates were positive for *mcr-1*, including one *E. coli*, two *K. pneumoniae* and three *Salmonella* spp. (Table 3). None of the colistin-resistant *P. aeruginosa* and *A. baumannii* complex strains were positive for *mcr-1*. The complete antimicrobial susceptibility of the six *mcr-1*-positive isolates is listed in Table 3. None of the six *mcr-1*-positive isolates showed advanced antimicrobial resistance, e.g. *mcr-1*-positive isolates were fully susceptible to all tested carbapenems, including ertapenem, imipenem and meropenem. All GNB with specific antimicrobial resistance genes, including bla_{KPC}, bla_{NDM-1}, bla_{OXA-48}, bla_{VIM} and *mcr-1*, identified by the SMART programme in 2018 are illustrated in Fig. 4 with reference to the results in 2017 in Taiwan [6].

4. Discussion

For the entire year of national surveillance, the status of antimicrobial resistance and respective antimicrobial resistance genes among clinically important GNB in Taiwan was analysed. The data

Table 2
Distribution of carbapenemase-related genes, including bla_{KPC}, bla_{OXA-48}, bla_{NDM-1} and bla_{VIM}, among carbapenem-non-susceptible isolates, and of the plasmid-mediated transferable resistance determinant *mcr-1* among Enterobacteriaceae with a colistin MIC ≥ 2 mg/L and colistin-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex isolates.

	Prevalence [*n* (%)]				
	E. coli (*n* = 398)	*K. pneumoniae* (*n* = 346)	*Salmonella* spp. (*n* = 230)	*P. aeruginosa* (*n* = 252)	*A. baumannii* complex (*n* = 188)
Carbapenem-non-susceptible	11 (2.8)	31 (9.0)	1 (0.4)	26 (10.3)	82 (43.6)
bla_{KPC}	0	15 (48.4)	0	0	0
bla_{OXA-48}	0	2 (6.5)	0	0	0
bla_{NDM-1}	1 (9.1)	0	0	0	0
bla_{VIM}	0	2 (6.5)	0	0	0
High colistin MIC[a]	2 (0.5)	22 (6.4)	82 (35.7)	22 (8.7)	16 (8.5)
mcr-1	1 (50.0)	2 (9.1)	3 (3.7)	0	0

MIC, minimum inhibitory concentration.
[a] An MIC ≥ 2 mg/L among Enterobacteriaceae and an MIC ≥ 4 mg/L among *P. aeruginosa* and *A. baumannii* complex isolates.

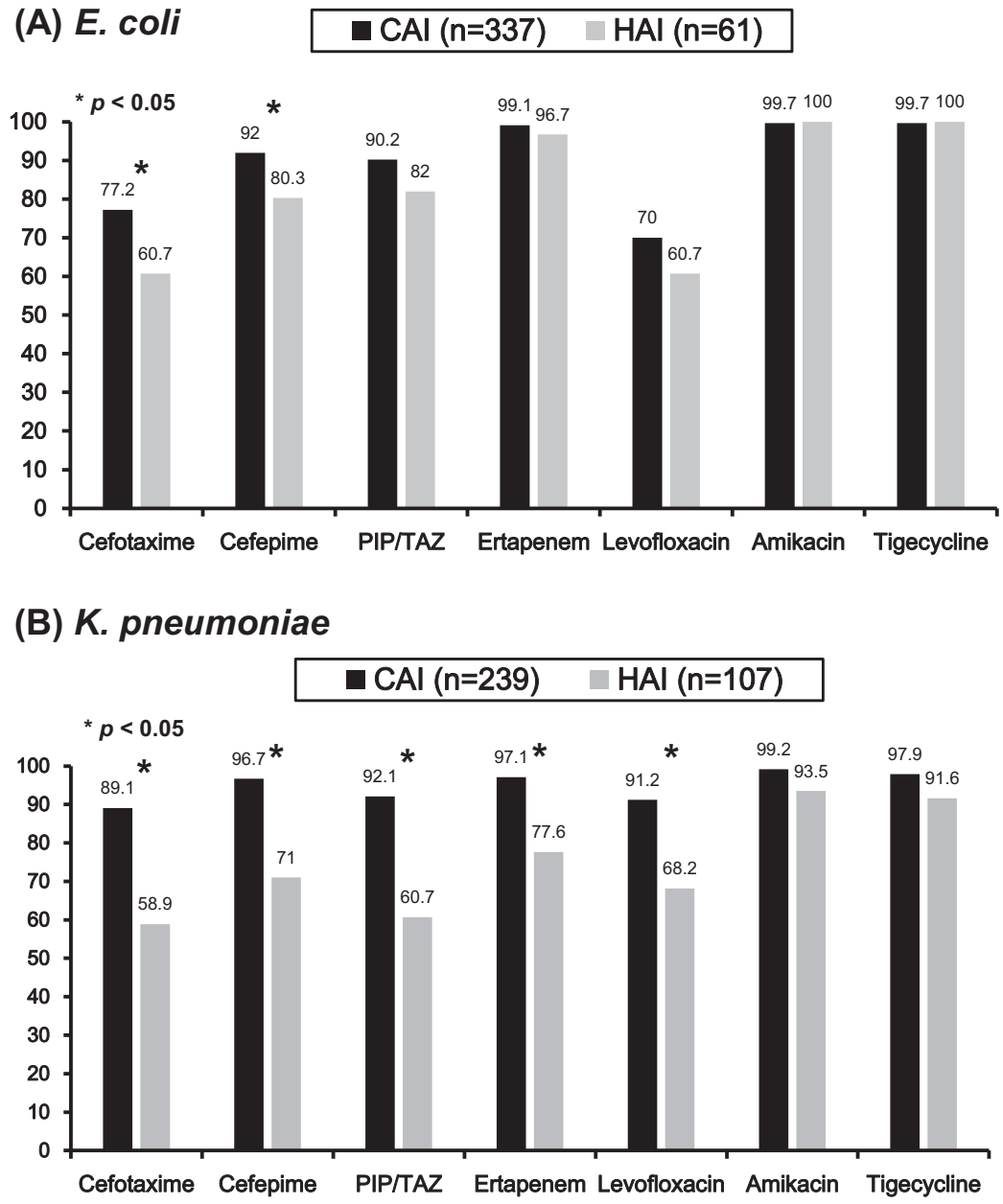

Fig. 2. Comparison of the in vitro susceptibility to seven selected antibiotics among hospital-acquired and community-acquired bacteraemia isolates caused by (A) *Escherichia coli* (*n* = 398), (B) *Klebsiella pneumoniae* (*n* = 346), (C) *Pseudomonas aeruginosa* (*n* = 252) and (D) *Acinetobacter baumannii* complex (*n* = 188) collected from 16 major teaching hospitals across Taiwan in 2018. CAI, community-acquired infection; HAI, hospital-acquired infection; PIP/TAZ, piperacillin/tazobactam; colistin-BMD, colistin MIC determined by broth microdilution.

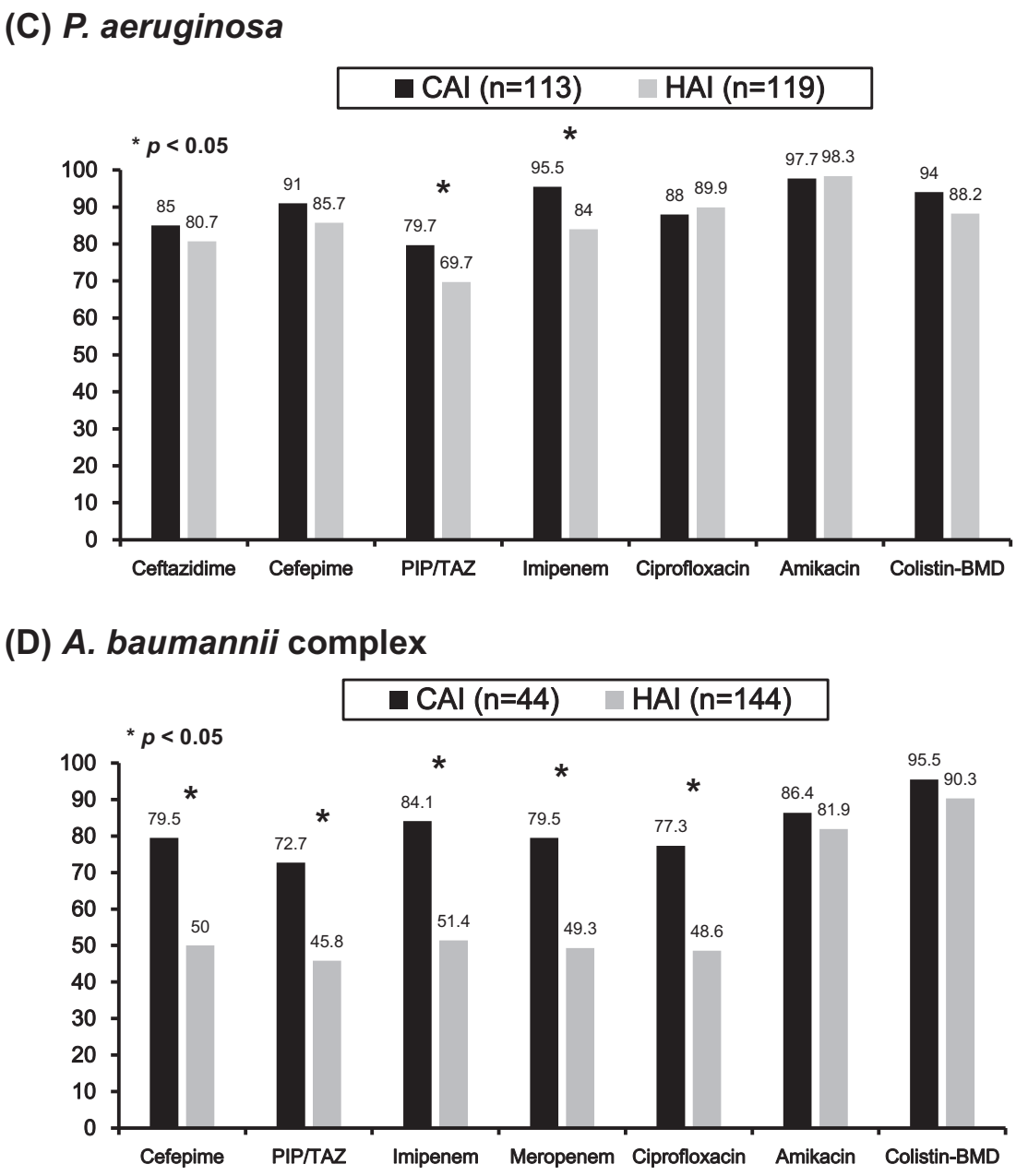

Fig. 2. Continued

provide important antimicrobial resistance epidemiology information and fill the gap of continuous monitoring. Furthermore, most isolates in this surveillance programme were collected from bloodstream sources that represent true clinical infections rather than other sources that might be due to contamination or colonisation. Carbapenems maintained good efficacy against Enterobacteriaceae, with non-susceptibility rates of 2.8%, 9.0%, 0.4% and 0% among isolates of *E. coli*, *K. pneumoniae*, *Salmonella* spp. and *S. flexneri*, respectively. As expected, the non-susceptibility rate to ertapenem was significantly higher in hospital-acquired isolates than in community-acquired bacteraemic *K. pneumoniae* (22.4% vs. 2.9%; $P < 0.05$). Similarly, carbapenemase-associated genes were more frequently detected in *K. pneumoniae* isolates than in *E. coli* isolates. Only one isolate of *E. coli* harboured the bla_{NDM-1} gene, whereas 61.3% (19/31) of carbapenem-non-susceptible *K. pneumoniae* isolates harboured antimicrobial resistance genes, including bla_{KPC}, bla_{OXA-48} and bla_{VIM}. Six isolates showed positive results for *mcr-1* gene detection. However, all six isolates, including three *Salmonella* spp., two *K. pneumoniae* and one *E. coli*, showed no advanced multidrug resistance, and the three *Salmonella* spp. isolates were collected from community-acquired infections.

Carbapenem resistance mediated by carbapenemases among Enterobacteriaceae is a growing public-health threat, especially in Asia in recent decades [25]. bla_{KPC} is predominant among carbapenemases in East Asia and represents a major epidemic circulating clone. In China, bla_{KPC} was first reported in 2004 and then disseminated to South Korea and Singapore in the following years [26,27]. Taiwan was free from bla_{KPC} until the first arrival of bla_{KPC-2}-positive *K. pneumoniae* in 2010 [14]. However, once bla_{KPC} was introduced into Taiwan, it spread rapidly. In an early study conducted in Northern Taiwan in 2011, bla_{KPC-2} was detected in 16 (7.3%) of 219 ertapenem-non-susceptible *K. pneumoniae* isolates. All bla_{KPC-2}-positive *K. pneumoniae* isolates belonged to the same pulsotype by PFGE analysis, indicating intrahospital and

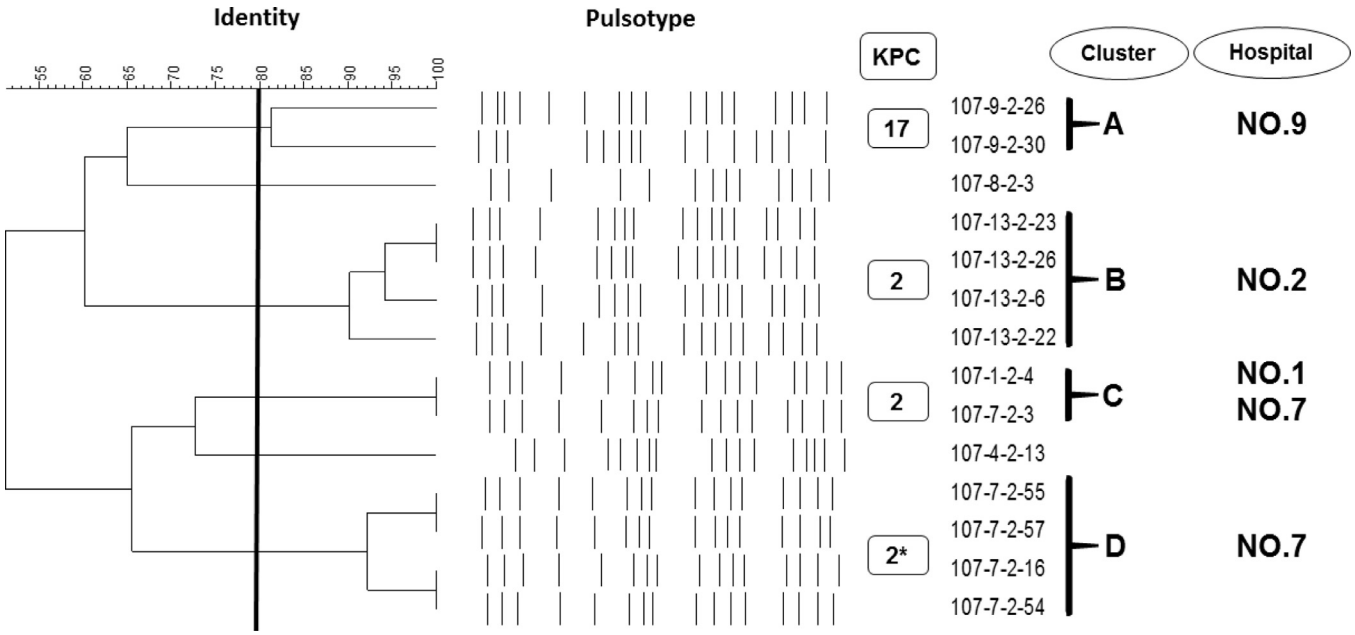

Fig. 3. Pulsed-field gel electrophoresis (PFGE) patterns of 14 *Klebsiella pneumoniae* ST11 isolates, including 11 bla_{KPC-2}-positive isolates, 2 bla_{KPC-17}-positive isolates and 1 non-typeable bla_{KPC}-positive isolate, from six hospitals in Taiwan in 2018. Four clusters (>80% identity) were identified, including three clusters related to intrahospital transmission and one cluster associated with interhospital transmission. ST, sequence type.

Table 3
Minimum inhibitory concentrations (MICs) of some key antibiotics against isolates harbouring antimicrobial resistance genes (ARGs), including *Escherichia coli* ($n=2$), *Klebsiella pneumoniae* ($n=21$) and *Salmonella* spp. ($n=3$) in Taiwan, 2018.

ARG type/species	ARG	MIC (mg/L)											
		COL	TGC	AMK	CMZ	CAZ	FEP	SXT	PIP/TAZ	CIP	ETP	IPM	MEM
Carbapenemase gene-positive organisms													
E. coli	bla_{NDM-1}	1	≤0.5	4	32	≥64	8	≥320	≥128	≥4	4	≥16	≥16
K. pneumoniae	bla_{KPC-2}	1	2	≤2	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-2}	16	4	≤2	≥64	≥64	≥64	≥320	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-2}	1	2	≥64	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-2}	0.5	≥8	≥64	≥64	≥64	≥64	≥320	≥128	≥4	≥8	8	≥16
K. pneumoniae	bla_{KPC-2}	>32	2	≥64	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	$bla_{KPC-non-typeable}$	1	1	≥64	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-2}	1	1	≥64	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-2}	1	2	≥64	≥64	≥64	≥64	≥320	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-17}	32	2	≤2	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-17}	1	2	≤2	≥64	≥64	≥64	≥320	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-2}	1	2	≤2	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-2}	1	1	≤2	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-2}	1	2	≤2	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	$bla_{KPC-non-typeable}$	32	2	≤2	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{KPC-2}	1	1	≤2	≥64	≥64	≥64	≤20	≥128	≥4	≥8	≥16	≥16
K. pneumoniae	bla_{OXA-48}	32	2	≤2	≥64	≥64	≥64	80	≥128	≥4	≥8	2	1
K. pneumoniae	bla_{OXA-48}	>32	2	≥64	≥64	16	≥64	≥320	≥128	≥4	≥8	2	1
K. pneumoniae	bla_{VIM}	2	≤0.5	≤2	≥64	≥64	≥64	≥320	≥128	1	≥8	8	≥16
K. pneumoniae	bla_{VIM}	16	≥8	≥64	≥64	≥64	≥64	≥320	≥128	≥4	≥8	8	≥16
mcr-1-positive organisms													
E. coli	*mcr-1*	4	≤0.5	≤2	≤1	4	32	≥320	≤4	≥4	≤0.5	≤0.25	≤0.25
K. pneumoniae	*mcr-1*	8	2	≤2	≥64	2	≤1	≥320	32	≤0.25	≤0.5	0.5	≤0.25
K. pneumoniae	*mcr-1*	2	1	≤2	≤1	≤1	≤1	≥320	≤4	≤0.25	≤0.5	≤0.25	≤0.25
Salmonella sp.	*mcr-1*	8	4	≤2	≤1	≥64	≥64	≥320	8	2	≤0.5	≤0.25	≤0.25
Salmonella sp.	*mcr-1*	2	1	≤2	≤1	≤1	≤1	≥320	≤4	1	≤0.5	≤0.25	≤0.25
Salmonella sp.	*mcr-1*	8	1	≤2	≤1	≤1	≤1	≤20	≤4	0.5	≤0.5	≤0.25	≤0.25

COL, colistin; TGC, tigecycline; AMK, amikacin; CMZ, cefmetazole; CAZ, ceftazidime; FEP, cefepime; SXT, trimethoprim/sulfamethoxazole; PIP/TAZ, piperacillin/tazobactam; CIP, ciprofloxacin; ETP, ertapenem; IPM, imipenem; MEM, meropenem.

interhospital clonal dissemination [23]. In a later national surveillance in 2013, the bla_{KPC} prevalence increased to 16.6% (41/247) among carbapenem-non-susceptible *K. pneumoniae* isolates. All isolates were bla_{KPC-2} variants with the same MLST genotype (ST11) and belonged to a major pulsotype according to PFGE analysis [24]. In the current study, all bla_{KPC} genes were found in *K. pneumoniae* isolates, and the prevalence increased to 48.4% (15/31) among carbapenem-non-susceptible *K. pneumoniae*, with the major variant being bla_{KPC-2} (73.3%; 11/15). One molecular study of a plasmid carrying bla_{KPC-2} from *K. pneumoniae* showed that a lack of one of the origins of replication in the bla_{KPC-2}-carrying plasmid restricted bacterial conjugation and confined the transmission between different clones and bacterial species [28]. In contrast to a previous study, PFGE analysis in the current study showed more

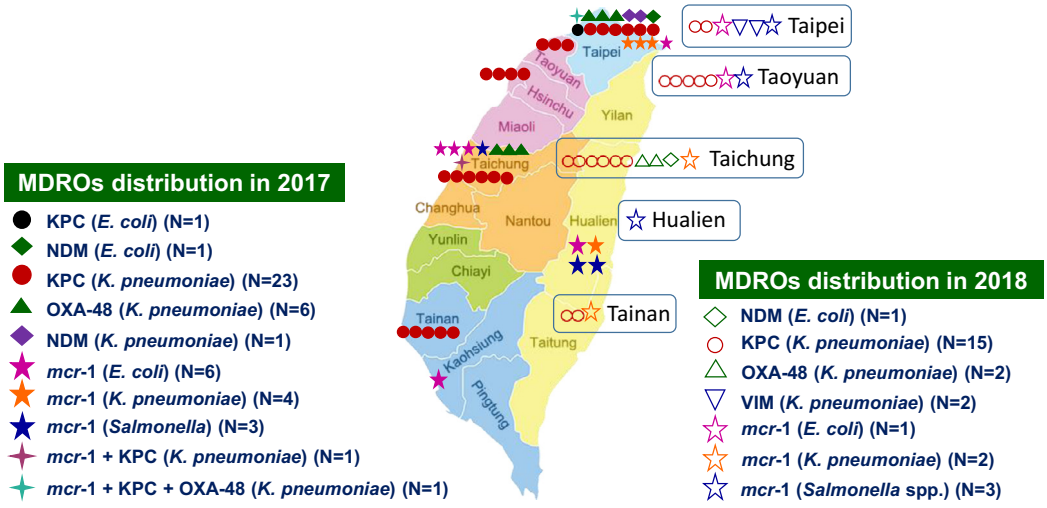

Fig. 4. Geographic distribution of *Escherichia coli, Klebsiella pneumoniae* and *Salmonella* spp. isolates harbouring specific antimicrobial resistance genes, including bla_{KPC}, bla_{NDM-1}, bla_{OXA-48}, bla_{VIM} and *mcr-1*, identified by the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) programme in 2017 and 2018. MDRO, multidrug-resistant organism; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase-48 carbapenem-hydrolysing class D β-lactamase; *mcr-1*, mobilised colistin resistance-1; VIM, Verona integron-encoded metallo-β-lactamase.

heterogeneity. Although four clusters were identified, no major pulsotype was found in this study to indicate the evolution of bla_{KPC}-positive *K. pneumoniae* since 2010. In addition to bla_{KPC-2}, two isolates were found to harbour bla_{KPC-17}, and the result was identical to a previous investigation in which bla_{KPC-17} emerged as the second most common bla_{KPC} in Taiwan [29]. In contrast to bla_{KPC}, bla_{NDM-1} is seldom found in Taiwan. In the current study, only one *E. coli* strain carried bla_{NDM-1}, and a previous national survey also showed that bla_{NDM-1} was carried by only 1 *Klebsiella oxytoca* among 1135 screened carbapenem-resistant Enterobacteriaceae [24]. In addition, most patients with bla_{NDM-1}-positive Enterobacteriaceae carriage had a recent history of travel to highly endemic areas such as India or China [15,30,31].

bla_{OXA-48}-positive *K. pneumoniae* isolates were from a single hospital in Central Taiwan. bla_{OXA-48}-producing *K. pneumoniae* appeared in Taiwan in late 2013 [32]. In addition, an investigation of an outbreak of carbapenem-resistant *K. pneumoniae* in a single hospital in Central Taiwan also showed a high prevalence of bla_{OXA-48} carriage among carbapenem-resistant *K. pneumoniae* from March 2014 to June 2015 [33]. In contrast to bla_{KPC-2}, carbapenem-resistant *K. pneumoniae* with bla_{OXA-48} carriage generally belonged to multiple pulsotypes according to PFGE analysis, implying the high transmissibility of the bla_{OXA-48}-carrying plasmid, as shown in recent reports [34,35]. Most reported bla_{OXA-48}-positive carbapenem-resistant *K. pneumoniae* cases occurred in Central Taiwan, and clinical physicians and infection control specialists should be warned of a possible regional outbreak.

In this study, the *mcr-1* gene encoding plasmid-mediated colistin resistance was detected in six Enterobacteriaceae isolates, but not among *P. aeruginosa* or *A. baumannii* complex isolates. The prevalence of the *mcr-1* gene remained low in clinical isolates, with rates of 0.3% (1/398), 0.6% (2/346) and 1.3% (3/230) of *E. coli, K. pneumoniae* and *Salmonella* spp., respectively. The results are similar to a recent study on *E. coli* in which *mcr-1* was detected in 0.3% (14/4589) of clinical *E. coli* isolates [36]. However, the prevalence of *mcr-1* among *E. coli* isolated from retail meat increased from 1.1% in 2012 to 8.7% in 2018 [37]. In addition, *mcr-1* was also found among *Salmonella* isolates from humans (0.2%; 10/5178) and diseased food-producing animals (0.7%; 9/1208) [37]. Both *E. coli* and *Salmonella* spp. studied revealed that *mcr-1* could be detected in isolates with diverse genetic backgrounds carrying different plasmid types. Dissemination of *mcr-1* between different bacterial species and between human and food-producing animals is a major concern in Taiwan. However, it was fortunate that none of the six *mcr-1*-positive Enterobacteriaceae were carbapenem-resistant and therefore remained susceptible to multiple classes of antibiotics. The complex relationship between bacterial resistance and virulence factors warrants further investigation [38].

In Taiwan, imipenem-resistant *A. baumannii* complex increased from 3.4% in 2002 to 58.7% in 2010 according to data from the TSAR programme, which is conducted biennially [39]. Although the imipenem resistance rate of *A. baumannii* in the current study (41.0%) was lower than in a previous report, this result should be interpreted with caution because all of the isolates were from bloodstream sources. *Acinetobacter baumannii* isolates from blood had a better susceptibility profile than isolates from sputum, urine and pus [39]. The situation of multidrug resistance in *A. baumannii* complex is still critical. In contrast, the rate of imipenem resistance among *P. aeruginosa* was 9.9%, which is similar to the TSAR data (10.2%) from 2000–2010 [10]. In the current study, bla_{KPC} was not found in *P. aeruginosa* or *A. baumannii* complex isolates. Amber class B (metallo-β-lactamase) and D (OXA-type) enzymes are often associated with carbapenem resistance among *P. aeruginosa* and *A. baumannii*; nevertheless, there were also no bla_{NDM-1} or bla_{OXA-48} genes detected in the two species in the present study [39,40]. Colistin resistance rates of *P. aeruginosa* and *A. baumannii* complex were 8.7% and 8.5%, respectively.

There are some limitations to this surveillance study. First, clinically important GNB, except *Salmonella* spp. and *S. flexneri,* were collected from bloodstream sources. Therefore, the susceptibility results are not applicable to isolates of various origins that might have different rates of resistance. Second, it is worth performing further molecular investigations on the bla_{KPC-17}-carrying plasmid to evaluate the transmissibility compared with the bla_{KPC-2}-carrying plasmid. Third, it was noted that the PFGE genotypes became more heterogeneous among bla_{KPC-2}-positive *K. pneumoniae* than previous studies, and further study is warranted to elucidate the evolution of *K. pneumoniae* isolates and transmission routes.

In conclusion, carbapenem resistance increased among clinically important GNB, especially among hospital-acquired infections. bla_{KPC} genes, especially the bla_{KPC-2} variant, were detected in approximately one-half of the carbapenem-resistant *K. pneumoniae*

isolates, with intrahospital and interhospital transmission around Taiwan. However, colistin still had low resistance rates among Enterobacteriaceae. Identification of *mcr-1* in different species raises concern regarding the potential dissemination of this gene. Continuous and close monitoring is required.

Declaration of Competing Interest

None declared.

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Ethical approval

This study was approved by the Research Ethics Committees or Institutional Review Boards of the 16 participating hospitals. The requirement for informed consent from each patient was waived.

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