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CLINICAL TRIAL REPORT

Susceptibility rates of clinically important bacteria collected from intensive care units against colistin, carbapenems, and other comparative agents: results from Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART)

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Objectives: This study aimed to determine the in vitro susceptibility of commonly encountered Gram-negative bacilli (GNB) recovered from patients admitted to intensive care units (ICUs) in Taiwan against colistin, carbapenems, and other comparative agents.

Methods: In total, 758 nonduplicate GNB isolates were obtained from clinical specimens of ICU patients at seven medical centers in 2016. Minimum inhibitory concentrations (MICs) were determined using the Vitek 2 susceptibility system. The reference broth-microdilution method was performed to determine MICs of colistin. Five main carbapenemase genes among carbapenem-non-susceptible GNB and *mcr-1–mcr5* genes among colistin non-wild-type or -resistant isolates were determined.

Results: After exclusion 38 *Proteus mirabilis* and 13 *Morganella morganii* spp. among 361 Enterobacteriaceae isolates, 34 (9.4%) isolates were carbapenem-insusceptible, 91.1% (n=31) were colistin wild type, and three and one *Klebsiella pneumoniae* isolates carried bla_{KPC} and bla_{OXA48}-like, respectively. Carbapenem-insusceptible isolates were found in 23.4% (30 of 128) and 63.0% (87 of 138) of isolates of the *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex, respectively. *mcr-1* was detected in two (1.8%) *Enterobacter cloacae* isolates. Very major errors between two methods of susceptibility to colistin were found in 1.5% of *K. pneumoniae*, 27.5% of *E. cloacae*, 4.7% of *P. aeruginosa*, and 10.1% of *A. baumannii* complex isolates.

Conclusion: In this study, 8.7% of Enterobacteriaceae isolates from ICUs were not susceptible to carbapenem, and bla_{KPC} and bla_{OXA48}-like were found among three and one carbapenem-insusceptible *K. pneumoniae* isolates, respectively. Colistin MICs determined by Vitek 2 were not reliable, especially for *E. cloacae* and *A. baumannii* complex isolates.

Keywords: colistin, carbapenems, susceptibility, carbapenemase, *mcr-1*, intensive care units, SMART, *P. aeruginosa, A. baumannii*

Introduction

Intensive care units (ICUs) cater to saving the lives of critically ill patients, and their use is rapidly growing worldwide.[1,2] However, the ICU is also a common place for acquiring nosocomial infections, due to the increasing number of immunocompromised patients and the frequent use of catheters, such as endotracheal tubes, central venous catheters, and Foley catheters.[3,4] Moreover, the increasing number of multidrug-resistant organisms (MDROs) that cause health-care-acquired infections in the ICU complicates this

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condition further.[5–7] In 2007, the World Health Organization highlighted in particular the threat of MDR Gram-negative bacteria (GNB), including carbapenem-resistant *Acinetobacter baumannii* complex, *Pseudomonas aeruginosa*, and Enterobacteriaceae as critical priority pathogens. There was no exception for Taiwan.[8–10] In addition to carbapenem, MDR GNB can also develop resistance to colistin, which is one of the limited antibiotic choices for MDRO infections.[11] Several resistance mechanisms, including extended-spectrum β-lactamases, such as the *ampC* gene, carbapenemase genes, and *mcr* genes, are reported to be responsible for carbapenem and colistin resistance.[11,12] To overcome this life-threatening condition, active infection-control programs including infection surveillance and implementation of prevention guidelines should be a priority.

Therapeutic options for MDROs are limited, and carbapenems and colistin are the last drugs of choice. However, the threat of colistin and carbapenem resistance has become another serious concern globally.[13–15] There is an urgent need to address these conditions of MDROs in ICUs. The Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART), launched in 2000, is designed to monitor longitudinally the in vitro susceptibility profiles of clinical pathogens to promising antibiotic agents, particularly pathogens isolated from ICUs over time throughout Taiwan.[5,6,16–19]

This study aimed to determine the in vitro susceptibilities of commonly encountered GNB, including Enterobacteriaceae, and nonfermentative GNB (NFGNB) isolated from patients admitted to ICUs at different locations in Taiwan against colistin, carbapenems, and other comparative agents. It also investigated the prevalence of carbapenemase genes and *mcr* genes among carbapenem-insusceptible and colistin non-wild-type (NWT) isolates, respectively.

Methods

Bacterial isolates

We analyzed 758 nonduplicate isolates of GNB collected from various specimens of patients admitted to ICUs at seven medical centers from January to December 2016. One participating hospital submitted only 57 clinical isolates, whereas the other six hospitals submitted more than 100 each. These clinical isolates included *A. baumannii* complex (n=138), *Klebsiella pneumoniae* (n=137), *P. aeruginosa* (n=128), *Escherichia coli* (n=121), *Stenotrophomonas maltophilia* (n=61), *Enterobacter cloacae* (n=51), *Serratia marcescens* (n=42), *Proteus mirabilis* (n=38), *Burkholderia cepacia* (n=19), *Morganella morganii* (n=13), and *Citrobacter freundii* (n=10) (Table 1). Sputum/endotracheal aspirates were the most common source of isolates (n=495, 65.3%), followed by blood (n=93, 12.3%), urine (n=90, 11.9%), pus/abscess (n=42, 5.5%), and ascites (n=13, 1.7%) (Table 1). All the isolates were stored at –70°C in trypticase soy broth (BD, Franklin Lakes, NJ, USA) supplemented with 15% glycerol prior to testing. The isolates were then transported to National Taiwan University Hospital, Taipei for further identification using the Phoenix PMIC/ID-30 identification system (BD). The institutional review board of National Taiwan University Hospital (201512064RSB) approved this study and waived the requirement for written informed consent.

Antimicrobial-susceptibility testing

Minimum inhibitory concentrations (MICs) of 17 antimicrobial agents to the isolates, including colistin, were determined using the commercial Vitek 2 antimicrobial-susceptibility system (AST-NB card; BioMérieux, Marcy l'Etoile, France). The MICs of colistin were also determined using the reference broth microdilution (BMD) method recommended by the Clinical and Laboratory Standards Institute (CLSI).[20] Ampicillin–sulbactam testing was performed with a 2:1 ratio and piperacillin–tazobactam testing with a fixed concentration (4 mg/L) of tazobactam. Interpretations of all MIC results were in accordance with the CLSI guidelines.[20] *E. coli* ATCC 25922 and *P. aeruginosa* (ATCC 27853) were used as quality-control strains for each run of the MIC tests.

In addition to *Proteus* spp. and *Morganella morganii*, which have intrinsically elevated MICs to imipenem, carbapenem-insusceptible isolates were defined as clinical isolates exhibiting insusceptibility to any of the carbapenems, including ertapenem, imipenem, and meropenem. As per the CLSI, the *E. coli*, *K. pneumoniae*, and *E. cloacae* isolates are known as wild type (WT; MICs ≤2 mg/L) and non-WT (NWT; MICs ≥4 mg/L) based on their susceptibility to colistin. For *P. aeruginosa* and *A. baumannii* complex isolates, MICs of ≤2 and ≥4 mg/L for colistin are identified as susceptible and resistant, respectively.[17] For the six other isolates tested in this study — *P. mirabilis*, *M. morganii*, *C. freundii*, *S. marcescens*, *B. cepacia*, and *S. maltophilia* — there were no CLSI MIC-interpretation criteria for defining susceptibility.[20]

To examine intertest agreement between the two methods for determining susceptibility to colistin, essential and categorical agreement and very major error (VME) were evaluated. Essential agreement between BMD and Vitek 2 susceptibility testing was measured as the difference between MICs of ±1 $\log_2$ dilution or less using BMD as a reference standard. Categorical agreement between the two

Table 1 Clinical isolates obtained from patients admitted to the intensive care units of seven main teaching hospitals in Taiwan in 2016

Sources	Isolates											Isolates (total 758), n (%)
	E. coli (n=121)	*K. pneumoniae* (n=137)	*P. aeruginosa* (n=128)	*A. baumannii* complex (n=138)	*C. freundii* (n=10)	*E. cloacae* (n=51)	*S. marcescens* (n=42)	*P. mirabilis* (n=38)	*M. morganii* (n=13)	*B. cepacia* (n=19)	*S. maltophilia* (n=61)	
Hospital (location within Taiwan)												
NTUH (N)	20	20	20	20	5	10	10	10	4	10	10	139 (18.3)
TMWFH (N)	1	18	8	20	2	1	1	0	1	1	4	57 (7.5)
VGH Taichung (M)	20	20	20	20	0	10	5	1	0	1	9	106 (14.0)
CMMC (S)	20	20	20	20	2	9	10	10	4	0	10	125 (16.5)
NCKUH (S)	20	20	20	20	1	10	4	3	3	0	10	111 (14.6)
KMUH (S)	20	20	20	18	0	3	4	9	0	5	9	108 (14.2)
VGH Kaohsiung (S)	20	19	20	20	0	8	8	5	1	2	9	112 (14.8)
Clinical sources												
Sputum/endotracheal aspirates	40	93	99	115	5	34	29	17	3	9	51	495 (65.3)
Blood	17	16	13	9	2	5	8	4	1	10	8	93 (12.3)
Urine	44	19	7	6	1	3	2	6	1	—	1	90 (11.9)
Pus/wound	7	3	3	5	2	8	3	7	4	—	—	42 (5.5)
Ascites	7	—	1	1	—	—	—	1	2	—	1	13 (1.7)
Abscess fluids	2	2	1	1	—	—	—	1	—	—	—	7 (0.9)
Bile	2	1	—	—	—	1	—	—	—	—	—	4 (0.5)
Cerebrospinal fluid	—	1	—	—	—	—	—	—	—	—	—	1 (0.1)
Others[a]	2	2	4	1	—	—	—	2	2	—	—	13 (1.7)

Note: [a]Vaginal discharges (n=7), intravenous catheters (n=3), and tissue biopsy (n=3).

Abbreviations: CMMC, Chi Mei Medical Center; KMUH, Kaohsiung Medical University Hospital; M, middle; N, Northern; NCKUH, National Cheng Kung University Hospital; NTUH, National Taiwan University Hospital; S, Southern; TMWFH, Taipei Municipal Wan-Fang Hospital; VGH, Veterans General Hospital; *E. coli; Escherichia coli; K. pneumoniae; Klebsiella pneumoniae; P. aeruginosa; Pseudomonas aeruginosa; A. baumannii; Acinetobacter baumannii; C. freundii; Citrobacter freundii; E. cloacae; Enterobacter cloacae; S. marcescens, Serratia marcescens; P. mirabilis, Proteus mirabilis; M. morganii; Morganella morganii; B. cepacia, Burkholderia cepacia; S. maltophilia; Stenotrophomonas maltophilia.*

susceptibility-testing methods was measured as the percentage of isolates that had concordant test results when determining susceptible or WT and resistant or NWT to colistin. A VME for the Vitek 2 was defined as discrepancy in MICs between the methods when a colistin-resistant or NWT isolate determined using the reference BMD method was interpreted as a colistin-susceptible or WT isolate by the Vitek 2.

Determination of carbapenemase-encoding genes among carbapenem-insusceptible Enterobacteriaceae

The Xpert Carba-R assay (Cepheid, Sunnyvale, CA, USA) was used to detect carbapenemase-encoding alleles, including bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and bla_{OXA48}-like, among the carbapenem-insusceptible Enterobacteriaceae.[15,16]

Determination of *mcr-1–mcr5* genes

PCR amplification of whole-cell DNA of isolates showing colistin MICs >2 mg/L was performed using previously described primers for *mcr-1*, *mcr2*, *mcr3*, *mcr4*, and *mcr5*, and PCR products were sequenced.[21]

Statistical analysis

We compared rates of WT susceptibility to colistin among selected isolates from patients admitted to ICUs of seven main teaching hospitals in Taiwan in this study and those from data reported in 2007.[6] Analyses were performed using Excel 2013 (Microsoft, Redmond, WA, USA), and $P<0.05$ was considered statistically significant.

Results

Antimicrobial susceptibility

Table 2 shows the antibiotic susceptibility of 758 clinical isolates. Amikacin showed the highest in vitro activity against both Enterobacteriaceae and *P. aeruginosa*, and resistance rates were <8%. Among Enterobacteriaceae isolates, all three carbapenems — ertapenem, imipenem, and meropenem — exhibited good activity, with a resistance rate <10%, except *E. cloacae* (ertapenem-resistance rate:17.6%) and *C. freundii* (ertapenem-, imipenem-, and meropenem-resistance rates 10%, 20%, and 20%, respectively). Among the 128 *P. aeruginosa* isolates, imipenem- and meropenem-resistance rates were 20.3% and 19.5%, respectively. Among the 138 *A. baumannii* complex isolates, imipenem- and meropenem-resistance rates were 62.3% and 61.6%, respectively. Among the 61 *S. maltophilia* isolates, resistance rates of levofloxacin and trimethoprim–sulfamethoxazole were 16.4% and 29.5%, respectively. For *B. cepacia*, the susceptibility rate of both ceftazidime and meropenem was 100%.

We further analyzed the antimicrobial-susceptibility pattern of Enterobacteriaceae isolated from different sources (Figure 1). Among the pathogens isolated from sputum/endotracheal aspirates, carbapenems had the best activity, with susceptibility >85%, and other commonly used antibiotics — ceftazidime, cefepime, piperacillin–tazobactam, ciprofloxacin, and levofloxacin — exhibited good activity, with susceptibility >70%. Similar patterns were noted for isolates from urinary and blood specimens. However, for the isolates from abscess/pus and intra-abdominal sources, imipenem-resistance rates were 21.9% (n=7) and 33.3% (n=5), respectively, which were much higher than the other two carbapenems, ertapenem and meropenem, which exhibited <10% resistance.

Carbapenem-resistant Enterobacteriaceae, P. aeruginosa, A. baumannii complex, and carbapenemases

After excluding 38 *P. mirabilis* and 13 *M. morganii* isolates among the remaining 361 Enterobacteriaceae isolates, 34 (9.4%) were classified as carbapenem-insusceptible, including *K. pneumoniae* (n=18), *E. cloacae* (n=11), *E. coli* (n=3), and *C. freundii* (n=2). The prevalence of carbapenem-insusceptible Enterobacteriaceae among each Enterobacteriaceae species was highest for *E. cloacae* (21.6%), followed by *C. freundii* (20.0%), *K. pneumoniae* (13.1%), and *E. coli* (2.5%). Only amikacin showed good in vitro activity, with a susceptibility rate of 82.4% (Figure 2A). The susceptibility rates of imipenem and meropenem against 28 ertapenem-insusceptible Enterobacteriaceae were 46.4% (n=13) and 57.1% (n=16), respectively. Susceptibility rates of ertapenem and meropenem against 21 imipenem-insusceptible Enterobacteriaceae were 28.5% (n=6) and 42.9% (n=9), respectively. Susceptibility rates of ertapenem and imipenem against 15 meropenem-insusceptible Enterobacteriaceae were only 6.7% (n=1) and 13.3% (n=2), respectively. In total, 30 (23.4%) and 87 (63.0%) carbapenem-insusceptible *P. aeruginosa* and *A. baumannii* complex isolates, respectively, were identified, and their susceptibility patterns are shown in Figure 2B. For carbapenem-insusceptible *A. baumannii* complex isolates, all antibiotics tested showed poor in vitro activity. For carbapenem-insusceptible *P. aeruginosa* isolates, gentamicin exhibited good in vitro activity. In addition, 91.1% (n=31) of carbapenem-insusceptible Enterobacteriaceae exhibited WT susceptibility to colistin. All carbapenem-insusceptible Enterobacteriaceae isolates were screened for

Table 2 Antimicrobial susceptibility of Gram-negative bacteria isolated from patients admitted to intensive care units of seven main teaching hospitals in Taiwan in 2016

Organism and agents tested	MIC (mg/L)			Isolates, n (%)		
	Range	MIC_{50}	MIC_{90}	S (%)	I (%)	R (%)
***Escherichia coli* (121)**						
Ampicillin–sulbactam	≤2–≥32	≥32	≥32	35 (28.9)	12 (9.9)	74 (61.2)
Cefazolin	≤4–≥64	≥64	≥64	51 (42.1)	—	70 (57.9)
Cefmetazole	≤1–≥64	≤1	32	101 (83.5)	10 (8.3)	10 (8.3)
Cefotaxime	≤1–≥64	≤1	≥64	64 (52.9)	1 (0.8)	56 (46.3)
Ceftazidime	≤1–≥64	≤1	≥64	84 (69.4)	1 (0.8)	36 (29.8)
Cefepime	≤1–≥64	≤1	≥64	101 (83.5)	5 (4.1)	15 (12.4)
Piperacillin–tazobactam	≤4–≥128	≤4	≥128	93 (76.9)	14 (11.6)	14 (11.6)
Ertapenem	≤0.5–≥8	≤0.5	≤0.5	118 (97.5)	1 (0.8)	2 (1.7)
Imipenem	≤0.25–4	≤0.25	≤0.25	120 (99.2)	0	1 (0.8)
Meropenem	≤0.25–4	≤0.25	≤0.25	120 (99.2)	0	1 (0.8)
Ciprofloxacin	≤0.25–≥4	0.5	≥4	74 (61.2)	1 (0.8)	46 (38.0)
Levofloxacin	≤0.12–≥8	1	≥8	75 (62.0)	1 (0.8)	45 (37.2)
Gentamicin	≤1–≥16	≤1	≥16	91 (75.2)	1 (0.8)	29 (24.0)
Amikacin	≤2–16	≤2	4	121 (100)	0	0
Trimethoprim–sulfamethoxazole	≤1–≥16	≤1	≥16	73 (60.3)	—	48 (39.7)
Tigecycline	≤0.5–4	≤0.5	≤0.5	NA	NA	NA
Colistin	≤0.5	≤0.5	≤0.5	NA	NA	NA
Colistin-BMD	0.5–2	1	1	NA	NA	NA
***Klebsiella pneumoniae* (137)**						
Ampicillin–sulbactam	≤2–≥32	8	≥32	77 (56.2)	5 (3.6)	55 (40.1)
Cefazolin	≤4–≥64	≤4	≥64	0	84 (61.3)	53 (38.7)
Cefmetazole	≤1–≥64	≤1	≥64	102 (74.5)	16 (11.7)	19 (13.9)
Cefotaxime	≤1–≥64	≤1	≥64	96 (70.1)	6 (4.4)	35 (25.5)
Ceftazidime	≤1–≥64	≤1	≥64	98 (71.5)	6 (4.4)	33 (24.1)
Cefepime	≤1–≥64	≤1	32	117 (85.4)	2 (1.5)	18 (13.1)
Piperacillin–tazobactam	≤4–≥128	≤4	≥128	97 (70.8)	13 (9.5)	27 (19.7)
Ertapenem	≤0.5–≥8	≤0.5	≤0.5	124 (90.5)	5 (3.6)	8 (5.8)
Imipenem	≤0.25–≥16	≤0.25	1	125 (91.2)	8 (5.8)	4 (2.9)
Meropenem	≤0.25–≥16	≤0.25	≤0.25	130 (94.9)	0	7 (5.1)
Ciprofloxacin	≤0.25–≥4	≤0.25	≥4	107 (78.1)	1 (0.7)	29 (21.2)
Levofloxacin	≤0.12–≥8	≤0.12	≥8	105 (76.6)	4 (2.9)	28 (20.4)
Gentamicin	≤1–≥16	≤1	≥16	97 (70.8)	9 (6.6)	31 (22.6)
Amikacin	≤2–≥64	≤2	≤2	132 (96.4)	0	5 (3.6)
Trimethoprim–sulfamethoxazole	≤1–≥16	≤1	≥16	89 (65.0)	—	48 (35.0)
Tigecycline	≤0.5–≥8	≤0.5	≥8	NA	NA	NA
Colistin	≤0.5–≥16	≤0.5	≤0.5	NA	NA	NA
Colistin BMD	0.5–16	1	1	NA	NA	NA
***Enterobacter cloacae* (51)**						
Cefazolin	≤4–≥64	≥64	≥64	1 (2.0)		50 (98.0)
Cefmetazole	2–≥64	≥64	≥64	2 (3.9)	3 (5.9)	46 (90.2)
Cefotaxime	≤1–≥64	≤1	≥64	30 (58.8)	0	21 (41.2)
Ceftazidime	≤1–≥64	≤1	≥64	31 (60.8)	0	20 (39.2)
Cefepime	≤1–≥64	≤1	16	39 (76.5)	5 (9.8)	7 (13.7)
Ertapenem	≤0.5–≥8	≤0.5	4	40 (78.4)	2 (3.9)	9 (17.6)
Imipenem	≤0.25–≥16	0.5	2	42 (82.4)	4 (7.8)	5 (9.8)
Meropenem	≤0.25–≥16	≤0.25	1	47 (92.2)	1 (2.0)	3 (5.9)
Ciprofloxacin	≤0.25–≥4	≤0.25	≥4	41 (80.4)	2 (3.9)	8 (15.7)
Levofloxacin	≤0.12–≥8	≤0.12	≥8	41 (80.4)	2 (3.9)	8 (15.7)
Gentamicin	≤1–≥16	≤1	≥16	43 (84.3)	0	8 (15.7)
Amikacin	≤2–32	≤2	4	49 (96.1)	2 (3.9)	0

(*Continued*)

Table 2 (*Continued*)

Organism and agents tested	MIC (mg/L)			Isolates, n (%)		
	Range	MIC_{50}	MIC_{90}	S (%)	I (%)	R (%)
Trimethoprim–sulfamethoxazole	≤1–≥16	≤1	≥16	38 (74.5)	—	13 (25.5)
Tigecycline	≤0.5–≥8	1	≥8	NA	NA	NA
Colistin	≤0.5–≥16	≤0.5	≤0.5	NA	NA	NA
Colistin BMD	0.5–>32	1	>32	NA	NA	NA
***Serratia marcescens* (42)**						
Cefazolin	≥64–≥64	≥64	≥64	0	0	42 (100)
Cefmetazole	4–≥64	8	≥64	35 (83.3)	1 (2.4)	6 (14.3)
Cefotaxime	≤1–≥64	≤1	32	28 (66.7)	2 (4.8)	12 (28.6)
Ceftazidime	≤1–≥64	≤1	≥64	37 (88.1)	0	5 (11.9)
Cefepime	≤1–32	≤1	8	35 (83.3)	5 (11.9)	2 (4.8)
Piperacillin–tazobactam	≤4–≥128	≤4	16	39 (92.9)	0	3 (7.1)
Ertapenem	≤0.5	≤0.5	≤0.5	42 (100)	0	0
Meropenem	≤0.25	≤0.25	≤0.25	42 (100)	0	0
Ciprofloxacin	≤0.25–≥4	≤0.25	≥4	36 (85.7)	0	6 (14.3)
Levofloxacin	≤0.12–≥8	≤0.12	≥8	34 (81.0)	3 (7.1)	5 (11.9)
Gentamicin	≤1–≥16	≤1	8	35 (83.3)	3 (7.1)	4 (9.5)
Amikacin	≤2–≥64	≤2	8	40 (95.2)	0	2 (4.8)
Trimethoprim–sulfamethoxazole	≤1–≥16	≤1	≤1	40 (95.2)	—	2 (4.8)
Tigecycline	≤0.5–≥8	1	≥8	NA	NA	NA
Colistin BMD	1–>32	>32	>32	NA	NA	NA
***Proteus mirabilis* (38)**						
Ampicillin–sulbactam	≤2–≥32	4	≥32	20 (52.6)	7 (18.4)	11 (28.9)
Cefazolin	≤4–≥64	≤4	≥64	19 (50)		19 (50)
Cefmetazole	≤1–≥64	2	4	37 (97.4)	0	1 (2.6)
Cefotaxime	≤1–≥64	≤1	8	30 (78.9)	0	8 (21.1)
Ceftazidime	≤1–≥64	≤1	4	35 (92.1)	0	3 (7.9)
Cefepime	≤1–≥64	≤1	4	33 (86.8)	3 (7.9)	2 (5.3)
Piperacillin–tazobactam	≤4–16	≤4	≤4	38 (100)	0	0
Ertapenem	≤0.5–≥8	≤0.5	≤0.5	36 (94.7)	1 (2.6)	1 (2.6)
Imipenem	≤0.25–≥16	4	8	2 (5.3)	14 (36.8)	22 (57.9)
Meropenem	≤0.25–8	≤0.25	1	37 (97.4)	0	1 (2.6)
Ciprofloxacin	≤0.25–≥4	≤0.25	≥4	24 (63.2)	5 (13.2)	9 (23.7)
Levofloxacin	≤0.12–≥8	0.5	≥8	26 (68.4)	6 (15.8)	6 (15.8)
Gentamicin	≤1–≥16	≤1	≥16	20 (52.6)	6 (15.8)	12 (31.6)
Amikacin	≤2–≥64	≤2	8	34 (89.5)	1 (2.6)	3 (7.9)
Trimethoprim–sulfamethoxazole	≤1–≥16	≥16	≥16	11 (28.9)	—	27 (71.1)
Tigecycline	1–≥8	4	4	NA	NA	NA
Colistin BMD	>32	>32	>32	NA	NA	NA
***Morganella morganii* (13)**						
Ampicillin–sulbactam	16–≥32	≥32	≥32	0	1 (7.7)	12 (92.3)
Cefazolin	≥64–≥64	≥64	≥64	0	0	13 (100)
Cefmetazole (12)	8–≥64	8	32	10 (83.3)	1 (8.3)	1 (8.3)
Cefotaxime	≤1–≥64	≤1	≥64	8 (61.5)	0	5 (38.5)
Ceftazidime	≤1–≥64	≤1	≥64	9 (69.2)	0	4 (30.8)
Cefepime	≤1–≥64	≤1	8	11 (84.6)	1 (7.7)	1 (7.7)
Piperacillin–tazobactam	≤4–≥128	≤4	≤4	12 (92.3)	0	1 (7.7)
Ertapenem	≤0.5–≤0.5	≤0.5	≤0.5	13 (100)	0	0
Imipenem	≤0.25–8	2	8	3 (23.1)	4 (30.8)	6 (46.2)
Meropenem	≤0.25–1	≤0.25	1	13 (100)	0	0
Ciprofloxacin	≤0.25–≥4	≤0.25	≥4	11 (84.6)	0	2 (15.4)
Levofloxacin	≤0.12–≥8	≤0.12	≥8	11 (84.6)	0	2 (15.4)
Gentamicin	≤1–≥16	≤1	≥16	9 (69.2)	1 (7.7)	3 (23.1)

(*Continued*)

Table 2 (*Continued*)

Organism and agents tested	MIC (mg/L)			Isolates, n (%)		
	Range	MIC_{50}	MIC_{90}	S (%)	I (%)	R (%)
Amikacin	≤2–≥64	≤2	4	12 (92.3)	0	1 (7.7)
Trimethoprim–sulfamethoxazole	≤1–≥16	≤20	≥320	9 (69.2)	—	4 (30.8)
Tigecycline	≤0.5–≥8	1	4	NA	NA	NA
Colistin	≥16	≥16	≥16	NA	NA	NA
Colistin BMD	>32	>32	>32	NA	NA	NA
***Citrobacter freundii* (10)**						
Cefazolin	≥64	≥64	≥64	0	0	10 (100)
Cefmetazole	32–≥64	32	≥64	0	5 (50)	5 (50)
Cefotaxime	≤1–≥64	≤1	≥64	5 (50)	0	5 (50)
Ceftazidime	≤1–≥64	2	≥64	5 (50)	0	5 (50)
Cefepime	≤1–4	≤1	2	9 (90)	1 (10)	0
Piperacillin–tazobactam	≤4–≥128	32	≥128	4 (40)	3 (30)	3 (30)
Ertapenem	≤0.5–4	≤0.5	≤0.5	9 (90)	0	1 (10)
Imipenem	≤0.25–≥16	0.5	≥16	8 (80)	0	2 (20)
Meropenem	≤0.25–≥16	≤0.25	≥16	8 (80)	0	2 (20)
Ciprofloxacin	≤0.25–2	≤0.25	1	9 (90)	1 (10)	0
Levofloxacin	≤0.12–4	0.5	4	8 (80)	2 (20)	0
Gentamicin	≤1–≥16	≤1	≥16	8 (80)	0	2 (20)
Amikacin	≤2	≤2	≤2	10 (100)	0	0
Trimethoprim–sulfamethoxazole	≤1	≤1	≤1	10 (100)	—	0
Tigecycline	≤0.5–1	≤0.5	1	NA	NA	NA
Colistin	≤0.5	≤0.5	≤0.5	NA	NA	NA
Colistin BMD	0.5–1	1	1	NA	NA	NA
***Pseudomonas aeruginosa* (128)**						
Ceftazidime	≤1–≥64	4	32	89 (69.5)	23 (18.0)	16 (12.5)
Cefepime	≤1–≥64	2	16	107 (83.6)	11 (8.6)	10 (7.8)
Piperacillin–tazobactam	≤4–≥128	8	≥128	74 (57.8)	18 (14.1)	36 (28.1)
Imipenem	≤0.25–≥16	2	≥16	102 (79.7)	0	26 (20.3)
Meropenem	≤0.25–≥16	0.5	≥16	98 (76.6)	5 (3.9)	25 (19.5)
Ciprofloxacin	≤0.25–≥4	≤0.25	≥4	105 (82.0)	4 (3.1)	19 (14.8)
Levofloxacin	≤0.12–≥8	1	≥8	97 (75.8)	10 (7.8)	21 (16.4)
Gentamicin	≤1–≥16	≤1	4	117 (91.4)	5 (3.9)	6 (4.7)
Amikacin	≤2–≥64	≤2	4	127 (99.2)	0	1 (0.8)
Trimethoprim–sulfamethoxazole	≤1–≥16	8	≥16	NA	NA	NA
Tigecycline	≤0.5–≥8	≥8	≥8	NA	NA	NA
Colistin	≤0.5	≤0.5	≤0.5	128 (100)	—	0
Colistin BMD	1–8	2	2	122 (95.3)	—	6 (4.7)
***Acinetobacter baumannii* complex (138)**						
Ampicillin–sulbactam	≤2–≥32	16	≥32	61 (44.2)	18 (13.0)	59 (42.8)
Cefotaxime	≤1–≥64	≥64	≥64	42 (30.4)	22 (15.9)	74 (53.6)
Ceftazidime	≤1–≥64	≥64	≥64	47 (34.1)	18 (13.0)	73 (52.9)
Cefepime	≤1–≥64	≥64	≥64	48 (34.8)	2 (1.4)	88 (63.8)
Piperacillin–tazobactam	≤4–≥128	≥128	≥128	44 (31.9)	0	94 (68.1)
Imipenem	≤0.25–≥16	≥16	≥16	51 (37.0)	1 (0.7)	86 (62.3)
Meropenem	≤0.25–≥16	≥16	≥16	51 (37.0)	2 (1.4)	85 (61.6)
Ciprofloxacin	≤0.25–≥4	≥4	≥4	44 (31.9)	0	94 (68.1)
Levofloxacin	≤0.12–≥8	4	≥8	45 (32.6)	30 (21.7)	63 (45.7)
Gentamicin	≤1–≥16	≥16	≥16	65 (47.1)	3 (2.2)	70 (50.7)
Trimethoprim–sulfamethoxazole	≤1–≥16	8	≥16	56 (40.6)	—	82 (59.4)
Tigecycline	≤0.5–≥8	1	4	NA	NA	NA
Colistin	≤0.5–2	0.5	0.5	138 (100)	—	0
Colistin BMD	0.5–16	2	2	124 (89.9)	—	14 (10.1)

(*Continued*)

Table 2 (*Continued*)

Organism and agents tested	MIC (mg/L)			Isolates, n (%)		
	Range	MIC_{50}	MIC_{90}	S (%)	I (%)	R (%)
***Stenotrophomonas maltophilia* (61)**						
Levofloxacin	0.25–≥8	1	≥8	47 (77.0)	4 (6.6)	10 (16.4)
Trimethoprim–sulfamethoxazole	≤1–≥16	≤1	≥16	43 (70.5)	—	18 (29.5)
Colistin BMD (60)	2–>32	>32	>32	NA	NA	NA
***Burkholderia cepacia* (19)**						
Ceftazidime	2–4	4	4	19 (100)	0	0
Cefepime	2–32	8	32	NA	NA	NA
Piperacillin–tazobactam	≥128	≥128	≥128	NA	NA	NA
Imipenem	≥16	≥16	≥16	NA	NA	NA
Meropenem	1–4	4	4	19 (100)	0	0
Ciprofloxacin	1–≥4	2	≥4	NA	NA	NA
Levofloxacin	1–≥8	4	4	6 (31.6)	11 (57.9)	2 (10.5)
Gentamicin	≥16	≥16	≥16	NA	NA	NA
Amikacin	≥64	≥64	≥64	NA	NA	NA
Trimethoprim–sulfamethoxazole	≤1–4	≤1	≤1	18 (94.7)	—	1 (5.3)
Tigecycline	2–≥8	≥8	≥8	NA	NA	NA
Colistin	≥16	≥16	≥16	NA	NA	NA
Colistin BMD	>32	>32	>32	NA	NA	NA

Abbreviations: BMD, broth microdilution; MIC, minimum inhibitory concentration; NA, not available; I, intermediate; R, resistant; S, susceptible.

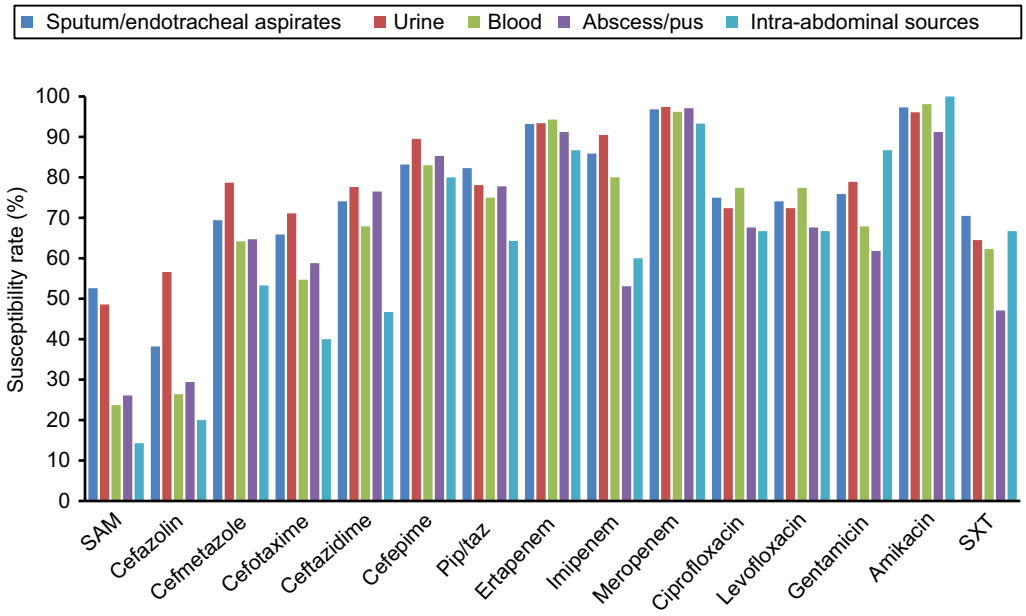


Figure 1 Antibiotic-susceptibility rate of Enterobacteriaceae according to source of isolation.
Abbreviations: SAM, sulbactam–ampicillin; Pip/taz, piperacillin–tazobactam; SXT, sulfamethoxazole–trimethoprim.

carbapenemases using the Xpert Carba-R. Furthermore, three and one *K. pneumoniae* isolates were found to carry the resistance genes bla_{KPC} and bla_{OXA48}, respectively. The three *K. pneumoniae* isolates carrying bla_{KPC} were resistant to most of the other antibiotics, but the *K. pneumoniae* isolate carrying bla_{OXA48} was susceptible to most of the antibiotics (Table 3). All these four clinical isolates were colistin WT.

MIC distribution of colistin determined by two susceptibility methods

Distribution of colistin MICs for isolates tested using BMD Vitek 2 are shown in Table 4. For some species (*P. mirabilis*, *S. marcescens*, and *S. maltophilia*), data retrieved from Vitek 2 are not available. In general, MIC values determined using BMD were higher than those obtained by the Vitek 2.

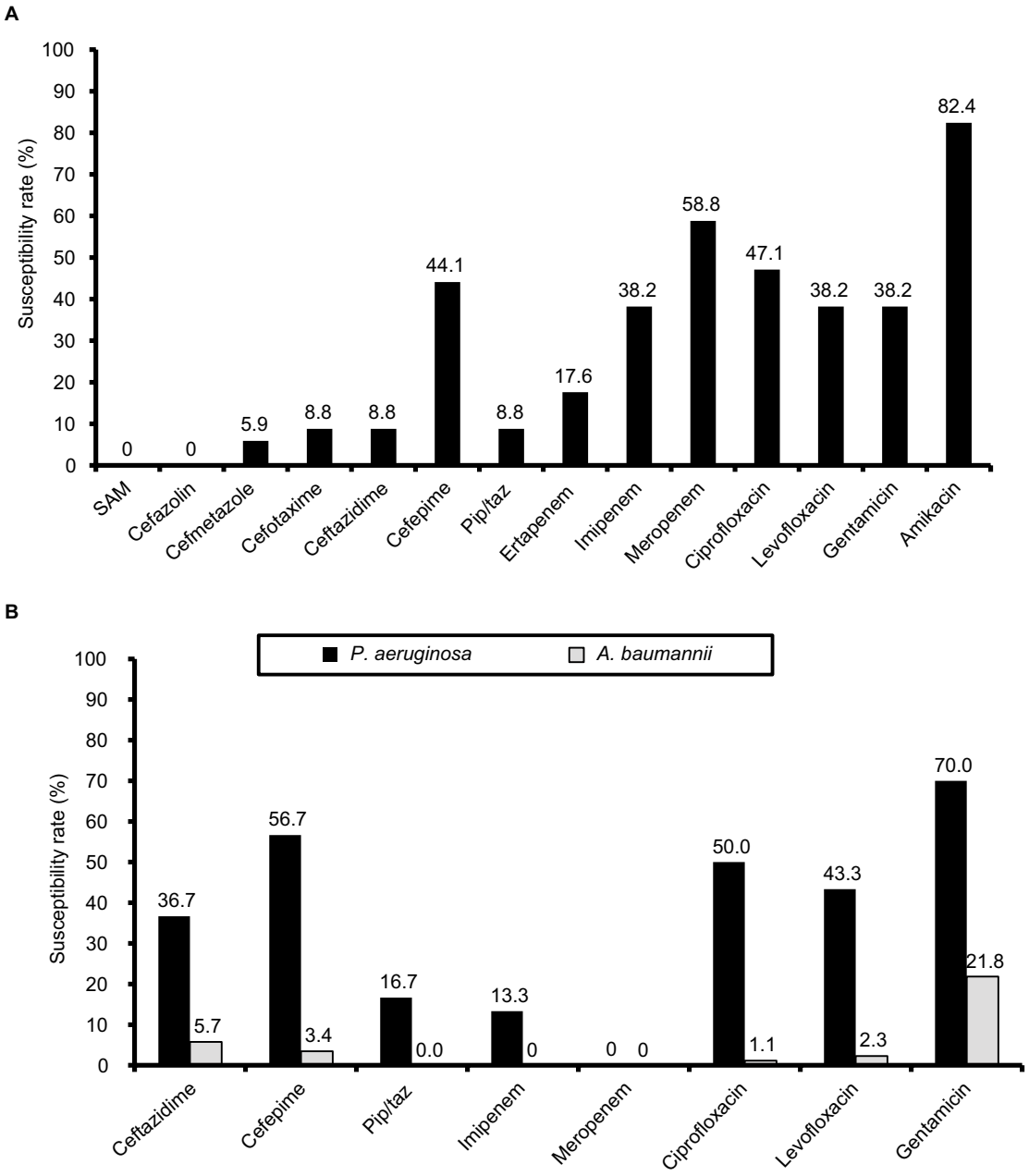


Figure 2 Antibiotic-susceptibility rates.
Notes: (**A**) Carbapenem-insusceptible Enterobacteriaceae; (**B**) *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex.
Abbreviations: SAM, sulbactam–ampicillin; Pip/taz, piperacillin–tazobactam.

When colistin MIC was measured using the Vitek 2 and not by BMD, MICs ≤0.5 were noted for colistin NWT among 14 (87.5%) of 16 *E. cloacae* and two (66.7%) of the three *K. pneumoniae* isolates (Table 4). Two *E. cloacae* and one *K. pneumoniae* isolate showed colistin MIC >16 using the Vitek 2 (Table 4). VMEs between the two methods were found to be 0 for *E. coli*, 66.7% for *K. pneumoniae*, 87.5% for *E. cloacae*, 100% for *P. aeruginosa*, and 100% for *A. baumannii* complex isolates (Table 4).

mcr-1 genes among colistin-NWT isolates

In total, 111 (25.7%) Enterobacteriaceae isolates were identified as colistin NWT (MIC ≥4 mg/L). In addition to bacterial species with inherent colistin resistance, such as *P. mirabilis*

Table 3 Antimicrobial susceptibility of four carbapenem-insusceptible *Klebsiella pneumoniae* isolates harboring carbapenemase genes, and two *mcr-1*-carrying *Enterobacter cloacae* isolates to selected agents

	***K. pneumoniae* isolates (MIC, mg/L)**				***E. cloacae* isolates (MIC, mg/L)**	
	KP1	**KP2**	**KP3**	**KP4**	**EC1**	**EC2**
Source	Blood	Drainage	CSF	Surgical wound	Sputum	Urine
Resistant gene	bla_{KPC}	bla_{KPC}	bla_{KPC}	bla_{OXA48}	*mcr-1*	*mcr-1*
Ampicillin–sulbactam	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	—	—
Cefazolin	≥64 (R)	≥64 (R)	≥64 (R)	4 (I)	≥64 (R)	≥64 (R)
Cefmetazole	≥64 (R)	≥64 (R)	≥64 (R)	1 (S)	≥64 (R)	≥64 (R)
Cefotaxime	≥64 (R)	≥64 (R)	≥64 (R)	1 (S)	≤1 (S)	≤1 (S)
Ceftazidime	≥64 (R)	≥64 (R)	≥64 (R)	1 (S)	≤1 (S)	≤1 (S)
Cefepime	≥64 (R)	≥64 (R)	≥64 (R)	1 (S)	≤1 (S)	≤1 (S)
Piperacillin–tazobactam	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	—	—
Ertapenem	≥8 (R)	≥8 (R)	≥8 (R)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)
Imipenem	≥16 (R)	≥16 (R)	≥16 (R)	2 (R)	1 (S)	≤0.25 (S)
Meropenem	≥16 (R)	≥16 (R)	≥16 (R)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)
Ciprofloxacin	≥4 (R)	≥4 (R)	≥4 (R)	1 (S)	≤0.25 (S)	≤0.25 (S)
Levofloxacin	≥8 (R)	≥8 (R)	≥8 (R)	1 (S)	≤0.12 (S)	≤0.12 (S)
Gentamicin	1 (S)	≥16 (R)	≥16 (R)	≤1 (S)	≤1 (S)	≤1 (S)
Amikacin	2 (S)	≥64 (R)	4 (S)	≤2 (S)	≤2 (S)	≤2 (S)
Tigecycline	4 (S)	≥8 (R)	1 (S)	≤0.5 (S)	2 (S)	1 (S)
Colistin	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)
Colistin BMD	1 (S)	0.5 (S)	1 (S)	1 (S)	32 (R)	32 (R)

Abbreviations: MIC, minimum inhibitory concentration; I, intermediate; R, resistant; S, susceptible; BMD, broth microdilution; CSF, cerebrospinal fluid.

(100%, 38 of 38), *M. morganii* (100%, 14 of 14), and *S. marcescens* (95.2%, 40 of 42), colistin NWT isolates were found in 31.4% (16 of 51) of *E. cloacae* and 2.2% (three of 137) of *K. pneumoniae*. In contrast, all the *E. coli* (n=121) isolates were WT. Except cefazolin and cefmetazole, most antibiotics exhibited good in vitro activity against the 16 *E. cloacae* isolates that belonged to colistin NWT (Figure 3). We further compared rates of WT susceptibility to colistin among selected isolates from patients admitted to ICUs of the hospitals in a previous study[6] in 2007 and the present work in 2016 (Figure 4). The colistin-susceptibility rate of *P. aeruginosa* was higher in 2016 than that in 2007 ($P<0.001$), but no significant difference was found among other pathogens: *E. coli*, *K. pneumoniae*, *E. cloacae* and *A. baumannii* complex (all $P>0.05$, Figure 4). The gene *mcr-1* was detected only in two (1.8%) *E. cloacae* isolates among the colistin NWT isolates that had a colistin MIC of 32 mg/L. In addition to cefazolin and cefmetazole, these two *E. cloacae* isolates harboring *mcr-1* were susceptible to all other antibiotics (Table 3).

With regard to NFGNB isolates, the colistin-resistance rate was highest for *B. cepacia* (100%, 19 of 19) and *S. maltophilia* (96.7%, 58 of 60), followed by *A. baumannii* complex isolates (10.1%, 14 of 138) and *P. aeruginosa* (4.7%, six of 128).

Discussion

Based on the surveillance of common GNB in the ICU, including Enterobacteriaceae and NFGNB, we have several significant findings. First, the prevalence of carbapenem-insusceptible Enterobacteriaceae was found to be 9.4%. For *P. aeruginosa* and *A. baumannii* complex, carbapenem-insusceptibility rates were ~23% and 63%, respectively. Furthermore, we found that the carbapenem-resistance rate of Enterobacteriaceae varied depending on the site of infection. The imipenem-resistance rate was much higher for isolates from abscess/pus and intra-abdominal sources than from other sources. Moreover, the in vitro activity of the three carbapenems against carbapenem-insusceptible Enterobacteriaceae was different: meropenem demonstrated greater potency compared to ertapenem and imipenem. More than 40% ertapenem- or imipenem-insusceptible Enterobacteriaceae remained susceptible to meropenem. In addition to carbapenem, most of the other antibiotics exhibited poor in vitro activity against carbapenem-insusceptible Enterobacteriaceae. The only exception was amikacin, which demonstrated a susceptibility rate >80% against carbapenem-insusceptible Enterobacteriaceae. All these findings helped us recognize the resistance burden and antibiotic-resistance patterns of carbapenem-insusceptible Enterobacteriaceae.

Table 4 Distribution of MICs for isolates tested against colistin using the reference BMD method (recommended by the CLSI) and Vitek 2

Organism (total isolates)	Method	Isolates with indicated colistin MICs (mg/L) determined by two methods, n (%)							Agreement[a]		Isolates with VME,[b] n (%)
		″0.5	1	2	4	8	16	≥32	Essential	Categorical	
Escherichia coli (121)	BMD	14 (11.7)	103 (85.8)	3 (2.5)	0	0	0	0	118 (97.5)	121 (100)	0
	Vitek 2	120 (100)	0	0	0	0	0	0			
Klebsiella pneumoniae (137)	BMD	6 (4.4)	124 (90.5)	4 (2.9)	1 (0.7)	0	2 (1.5)	0	131 (95.6)	135 (98.5)	2 (66.7)
	Vitek 2	136 (99.3)	0	0	0	0	1 (0.7)	0			
Proteus mirabilis (38)	BMD	0	0	0	0	0	0	38 (100)	NA	NA	NA
Morganella morganii (12)	BMD	0	0	0	0	0	0	12 (100)	12 (100)	NA	0
	Vitek 2	0	0	0	0	0	12 (100)	0			
Citrobacter freundii (10)	BMD	2 (20.0)	8 (80.0)	0	0	0	0	0	10 (100)	NA	0
	Vitek 2	10 (100)	0	0	0	0	0	0			
Enterobacter cloacae (51)	BMD	1 (2.0)	32 (62.7)	2 (3.9)	0	1 (2.0)	1 (2.0)	14 (27.5)	35 (68.6)	37 (72.5)	14 (87.5)
	Vitek 2	49 (96.1)	0	0	0	0	2 (3.9)	0			
Serratia marcescens (42)	BMD	0	1 (2.4)	1 (2.4)	0	0	0	40 (95.2)	NA	NA	NA
Pseudomonas aeruginosa (128)	BMD	0	14 (10.9)	108 (84.4)	4 (3.1)	2 (1.6)	0	0	14 (10.9)	122 (95.3)	6 (100)
	Vitek 2	128 (100)	0	0	0	0	0	0			
Acinetobacter baumannii complex (138)	BMD	2 (1.4)	60 (43.5)	62 (44.9)	7 (5.1)	5 (3.6)	2 (1.4)	0	62 (44.9)	124 (89.9)	14 (100)
	Vitek 2	133 (96.4)	3 (2.2)	2 (1.4)	0	0	0	0			
Burkholderia cepacia (19)	BMD	0	0	0	0	0	0	19 (100)	19 (100)	NA	0
	Vitek 2	0	0	0	0	0	19 (100)	0			
Stenotrophomonas maltophilia (61)	BMD	0	0	2 (3.3)	3 (4.9)	2 (3.3)	8 (13.1)	45 (73.8)	NA	NA	NA

Notes: [a]Categorical agreement between susceptibility-testing methods measured as percentage of isolates with concordant test results when determining susceptible or WT and resistant or NWT to colistin; [b]major errors for Vitek 2 defined as colistin-resistant or NWT isolate determined using BMD method interpreted as colistin-susceptible or WT isolate.

Abbreviations: MICs, minimum inhibitory concentrations; BMD, broth microdilution; CLSI, Clinical and Laboratory Standards Institute; VME, very major error; NA, not available; WT, wild type; NWT, non-WT.

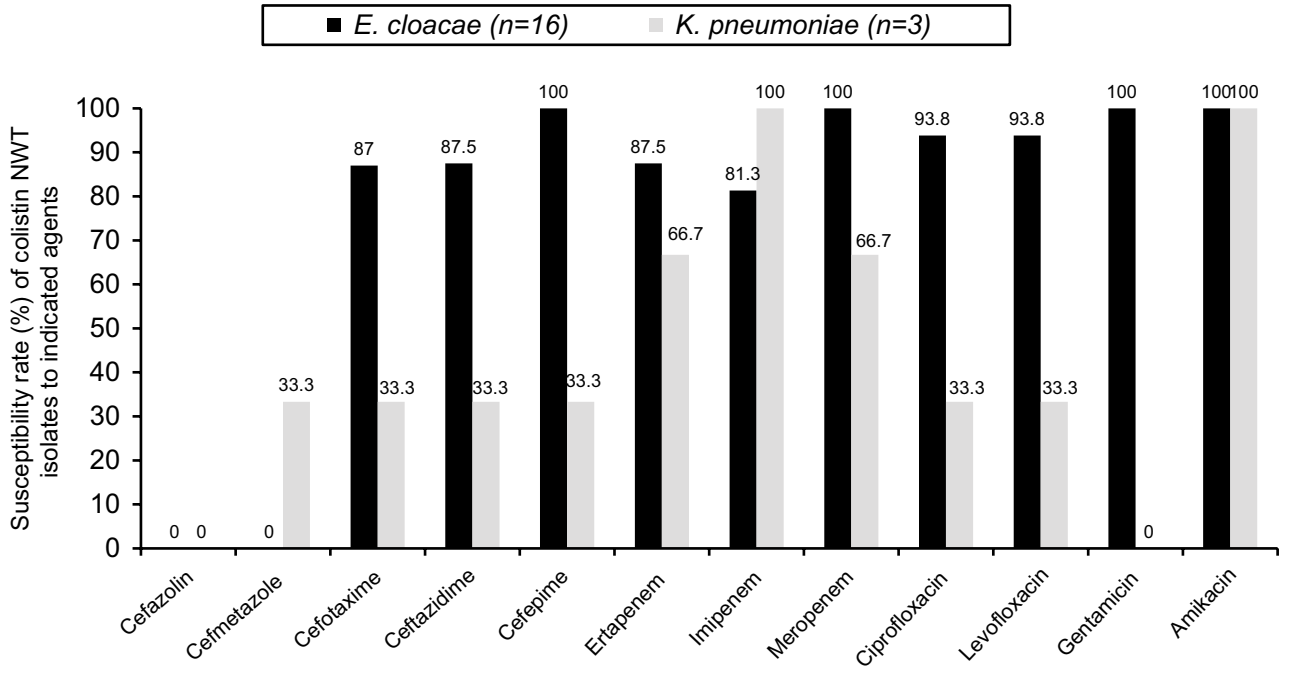
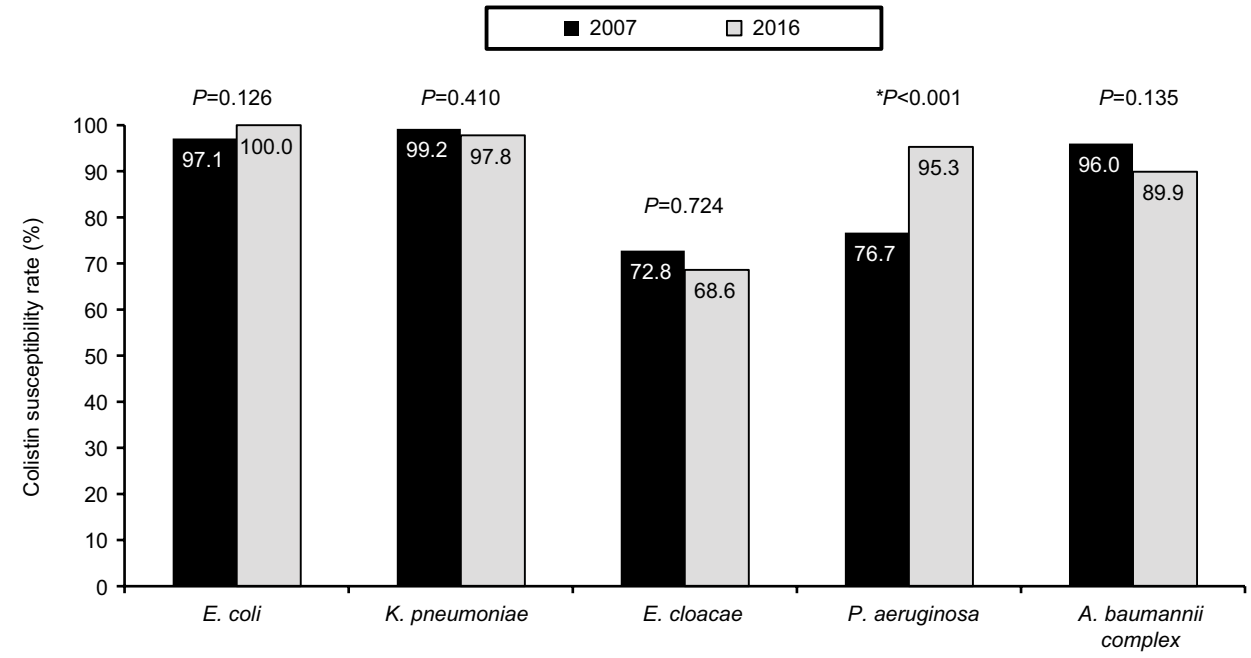


Figure 3 Antibiotic-susceptibility rates of colistin non-wild-type (NWT) *Enterobacter cloacae* and *Klebsiella pneumoniae*.



No. of isolates	2007	344	359	103	403	100
	2016	121	137	51	128	138

Figure 4 Rates of wild-type susceptibility to colistin among selected isolates from patients admitted to intensive care units of seven major teaching hospitals in Taiwan in 2007[6] and 2016.
Notes: *Significant difference in susceptibility.

These data will provide clinicians with useful information regarding the use of empiric antibiotics in ICUs.

Colistin may be the drug of last resort for MDROs, including carbapenem-resistant organisms. However, the emergence of colistin resistance and *mcr-1*-encoding plasmid-mediated colistin resistance has been found among Enterobacteriaceae worldwide.[22–25] In this study, rates of NWT-colistin susceptibility were 31.4% and 2.2% for *E. cloacae* and *K. pneumoniae*, respectively. In contrast, all the *E. coli* isolates belonged to WT colistin. Moreover, two isolates of *E. cloacae* were found to harbor the *mcr-1* gene. For these colistin NWT Enterobacteriaceae, including the two carrying the *mcr-1* gene, other broad-spectrum antibiotics showed good in vitro activities.[22,26,27] Although the susceptibility profiles of colistin NWT Enterobacteriaceae to the other classes of antibiotics were favorable, regular surveillance is warranted to monitor the development of other resistance mechanisms among colistin NWT Enterobacteriaceae, which may help in determining resistance to broad-spectrum antibiotics.[22,27]

In this study, NWT Enterobacteriaceae were confirmed by BMD and compared with the results of the Vitek 2. We found that 87.5% (14 of 16) of NWT *E. cloacae* and 66.7% (two of three) of NWT *K. pneumoniae* isolates had MICs ≤0.5 mg/L using the Vitek 2. For colistin, a VME between the two susceptibility methods, ie, Vitek 2 and BMD, was found to be 87.5% for *E. cloacae* and 100% for both *P. aeruginosa* and *A. baumannii* complex isolates. All these findings are consistent with previous studies,[28] and indicate that the Vitek 2 is a low-sensitivity tool to identify NWT.

We also found that the colistin-insusceptibility rates of *P. aeruginosa* and *A. baumannii* complex were 4.7% and 10.1%, respectively. This finding is consistent with a previous study in European hospitals between 2009 and 2011, where colistin exhibited good activity against *P. aeruginosa* strains, with 99.4% susceptibility rate in ICU patients.[29] In contrast, the colistin-insusceptibility rate of *P. aeruginosa* in this surveillance was significantly lower compared to that observed in the previous SMART in 2007, where the colistin-insusceptibility rate of *P. aeruginosa* was 23.3% (94 of 403, Figure 4).[6] As for other bacterial species tested, differences in insusceptibility (*A. baumannii* complex) or NWT to colistin and susceptibility to the carbapenems tested were not statistically significant (Figure 4).[6] This comparison suggests that the resistance rate of *P. aeruginosa* seems to decrease with time in Taiwan. However, continuous monitoring of colistin resistance is still needed to investigate the secular trend.

In this surveillance, three *K. pneumoniae* isolates harbored bla_{KPC}, and their prevalence was 16.7% among the 18 carbapenem-insusceptible *K. pneumoniae* isolates. This prevalence was similar to that found in the previous study,[30] where 13 (21.7%) of the 60 carbapenem-insusceptible *K. pneumoniae* in ICUs in 2012 harbored bla_{KPC}. In fact, no bla_{KPC} gene was detected in Enterobacteriaceae isolates from the previous SMART from ICUs in Taiwan before 2011.[5] This suggests the emergence of bla_{KPC} among carbapenem-insusceptible *K. pneumoniae* in the ICUs of Taiwan.

Conclusion

Although carbapenem demonstrated good in vitro activity against most of the Enterobacteriaceae isolates from ICUs, 8.7% of Enterobacteriaceae isolates were not susceptible to carbapenem. Among these carbapenem-insusceptible Enterobacteriaceae isolates, the carbapenemase genes bla_{KPC} and bla_{OXA48} were found in *K. pneumoniae* isolates. About a quarter of Enterobacteriaceae isolates were identified as colistin NWT, but the gene *mcr-1* was detected in only two *E. cloacae* isolates among the colistin-NWT isolates. Colistin MICs determined by the Vitek 2 were not reliable, especially for the *E. cloacae* and *A. baumannii* complex isolates.

Disclosure

The authors report no conflicts of interest in this work.

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