

# 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)

Chih-Cheng Lai,<sup>1</sup> Ying-Sheng Chen,<sup>2</sup> Nan-Yao Lee,<sup>3</sup> Hung-Jen Tang,<sup>4,5</sup> Susan Shin-Jung Lee,<sup>6,7</sup> Chin-Fu Lin,<sup>8</sup> Po-Liang Lu,<sup>9-11</sup> Jiunn-Jong Wu,<sup>12</sup> Wen-Chien Ko,<sup>13</sup> Wen-Sen Lee,<sup>14</sup> Po-Ren Hsueh<sup>15,16</sup>

<sup>1</sup>Department of Intensive Care Medicine, Chi Mei Medical Center, Liuying, Taiwan; <sup>2</sup>Division of Infectious Diseases, Department of Internal Medicine, Cardinal Tien Hospital, New Taipei City, Taiwan; <sup>3</sup>Department of Internal Medicine, National Cheng Kung University Medical College and Hospital, Tainan, Taiwan; <sup>4</sup>Department of Internal Medicine, Chi Mei Medical Center, Tainan, Taiwan; <sup>5</sup>Department of Health and Nutrition, Chia Nan University of Pharmacy and Science, Tainan, Taiwan; <sup>6</sup>Department of Internal Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; <sup>7</sup>Faculty of Medicine, School of Medicine, National Yang Ming University, Taipei, Taiwan; <sup>8</sup>Department of Pathology and Laboratory Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; <sup>9</sup>Department of Internal Medicine, Kaohsiung Medical University Hospital, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; <sup>10</sup>Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; <sup>11</sup>Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; <sup>12</sup>Department of Biotechnology and Laboratory Science in Medicine, National Yang-Ming University, Taipei, Taiwan; <sup>13</sup>Department of Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan; <sup>14</sup>Division of Infectious Diseases, Department of Internal Medicine, Wan Fang Medical Center, Taipei Medical University, Taipei, Taiwan; <sup>15</sup>Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan; <sup>16</sup>Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

Correspondence: Po-Ren Hsueh  
Departments of Laboratory Medicine and Internal Medicine, National Taiwan University College of Medicine, National Taiwan University Hospital, 7 Chung Shan South Road, Taipei 100, Taiwan  
Email hsporen@ntu.edu.tw

**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*<sub>KPC</sub> and *bla*<sub>OXA48</sub>-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*<sub>KPC</sub> and *bla*<sub>OXA48</sub>-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.<sup>1,2</sup> 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.<sup>3,4</sup> Moreover, the increasing number of multidrug-resistant organisms (MDROs) that cause health-care-acquired infections in the ICU complicates this

condition further.<sup>5–7</sup> 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.<sup>8–10</sup> In addition to carbapenem, MDR GNB can also develop resistance to colistin, which is one of the limited antibiotic choices for MDRO infections.<sup>11</sup> Several resistance mechanisms, including extended-spectrum  $\beta$ -lactamases, such as the *ampC* gene, carbapenemase genes, and *mcr* genes, are reported to be responsible for carbapenem and colistin resistance.<sup>11,12</sup> 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.<sup>13–15</sup> 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.<sup>5,6,16–19</sup>

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  $-70^{\circ}\text{C}$  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).<sup>20</sup> 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.<sup>20</sup> *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  $\leq 2$  mg/L) and non-WT (NWT; MICs  $\geq 4$  mg/L) based on their susceptibility to colistin. For *P. aeruginosa* and *A. baumannii* complex isolates, MICs of  $\leq 2$  and  $\geq 4$  mg/L for colistin are identified as susceptible and resistant, respectively.<sup>17</sup> 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.<sup>20</sup>

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  $\pm 1$  log<sub>2</sub> 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 (%)				
	<i>E. coli</i> (n=121)	<i>K. pneumoniae</i> (n=137)	<i>P. aeruginosa</i> (n=128)	<i>A. baumannii</i> complex (n=138)	<i>C. freundii</i> (n=10)	<i>E. cloacae</i> (n=51)	<i>S. marcescens</i> (n=42)		<i>P. mirabilis</i> (n=38)	<i>M. morgani</i> (n=13)	<i>B. cepacia</i> (n=19)	<i>S. maltophilia</i> (n=61)
<b>Hospital (location within Taiwan)</b>												
NTUH (N)	20	20	20	20	5	10	10	10	4	10	10	139 (18.3)
TMWFFH (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)
CMC (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)
<b>Clinical sources</b>												
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 <sup>a</sup>	2	2	4	1	—	—	—	2	2	—	—	13 (1.7)

**Note:** <sup>a</sup>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; TMWFFH, 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. morgani*, *Morganella morgani*; *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*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, and *bla*<sub>OXA48</sub>-like, among the carbapenem-insusceptible Enterobacteriaceae.<sup>15,16</sup>

## 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.<sup>21</sup>

## 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.<sup>6</sup> 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. morgani* 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 <sub>50</sub>	MIC <sub>90</sub>	S (%)	I (%)	R (%)
<b><i>Escherichia coli</i> (121)</b>						
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
<b><i>Klebsiella pneumoniae</i> (137)</b>						
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
<b><i>Enterobacter cloacae</i> (51)</b>						
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 <sub>50</sub>	MIC <sub>90</sub>	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
<b><i>Serratia marcescens</i> (42)</b>						
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
<b><i>Proteus mirabilis</i> (38)</b>						
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
<b><i>Morganella morganii</i> (13)</b>						
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 <sub>50</sub>	MIC <sub>90</sub>	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
<b>Citrobacter freundii (10)</b>						
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
<b>Pseudomonas aeruginosa (128)</b>						
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)
<b>Acinetobacter baumannii complex (138)</b>						
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 <sub>50</sub>	MIC <sub>90</sub>	S (%)	I (%)	R (%)
<b><i>Stenotrophomonas maltophilia</i> (61)</b>						
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
<b><i>Burkholderia cepacia</i> (19)</b>						
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.

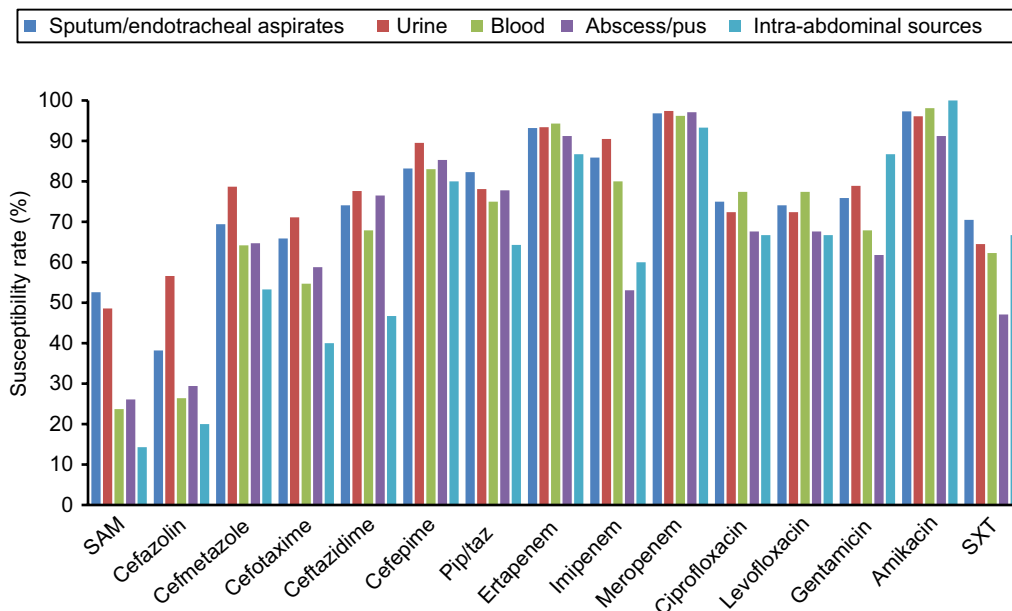


Figure 1 Antibiotic-susceptibility rate of Enterobacteriaceae according to source of isolation.

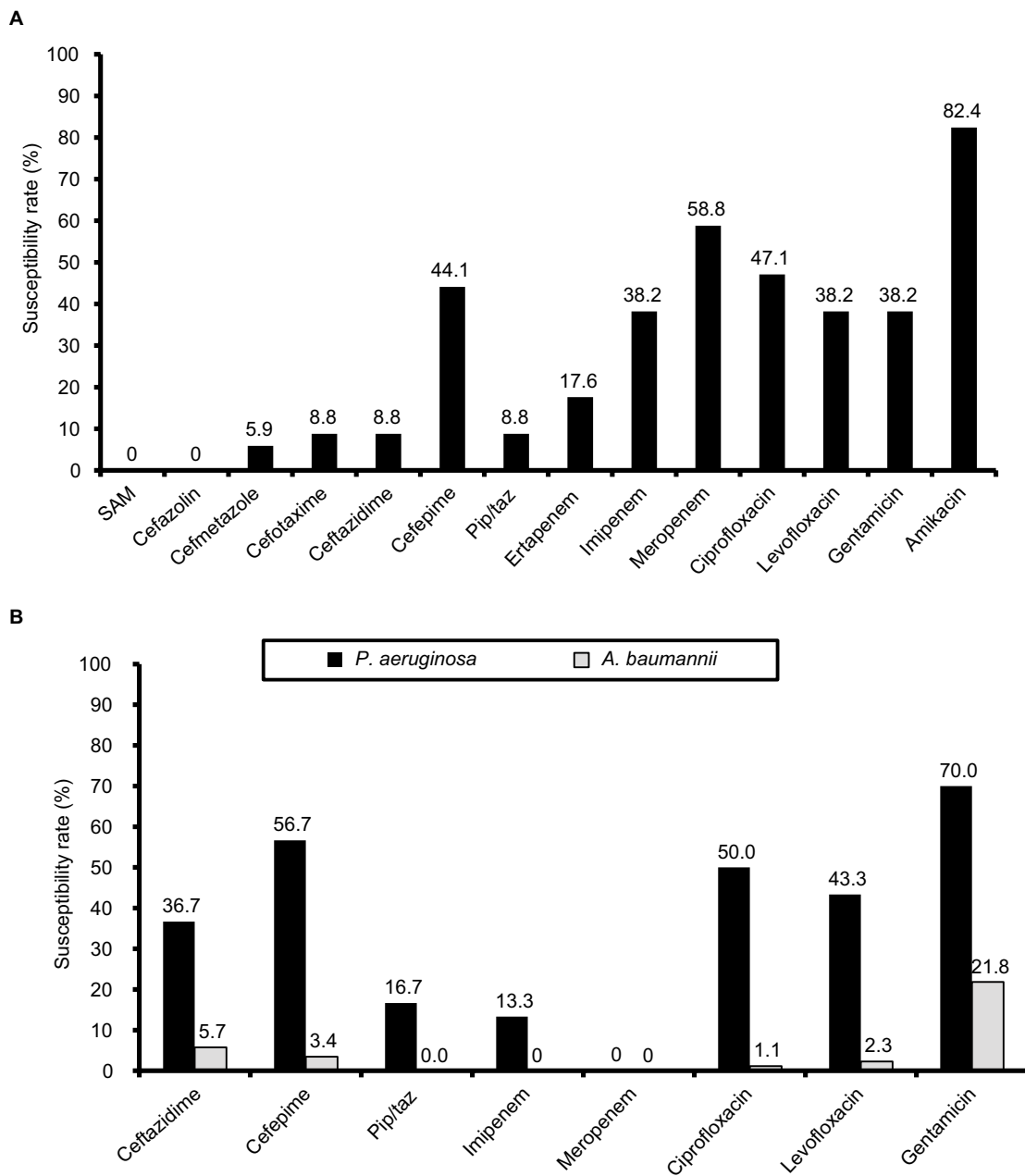
Abbreviations: SAM, sulbactam–ampicillin; Pip/taz, piperacillin–tazobactam; SXT, sulfamethoxazole–trimethoprim.

carbapenemases using the Xpert Carba-R. Furthermore, three and one *K. pneumoniae* isolates were found to carry the resistance genes *bla*<sub>KPC</sub> and *bla*<sub>OXA48</sub>, respectively. The three *K. pneumoniae* isolates carrying *bla*<sub>KPC</sub> were resistant to most of the other antibiotics, but the *K. pneumoniae* isolate carrying *bla*<sub>OXA48</sub> 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  $\leq 0.5$  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-I genes among colistin-NWT isolates

In total, 111 (25.7%) Enterobacteriaceae isolates were identified as colistin NWT (MIC  $\geq 4$  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

	<i>K. pneumoniae</i> isolates (MIC, mg/L)				<i>E. cloacae</i> isolates (MIC, mg/L)	
	KP1	KP2	KP3	KP4	EC1	EC2
Source	Blood	Drainage	CSF	Surgical wound	Sputum	Urine
Resistant gene	<i>bla</i> <sub>KPC</sub>	<i>bla</i> <sub>KPC</sub>	<i>bla</i> <sub>KPC</sub>	<i>bla</i> <sub>OXA48</sub>	<i>mcr-1</i>	<i>mcr-1</i>
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. morgani* (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<sup>6</sup> 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

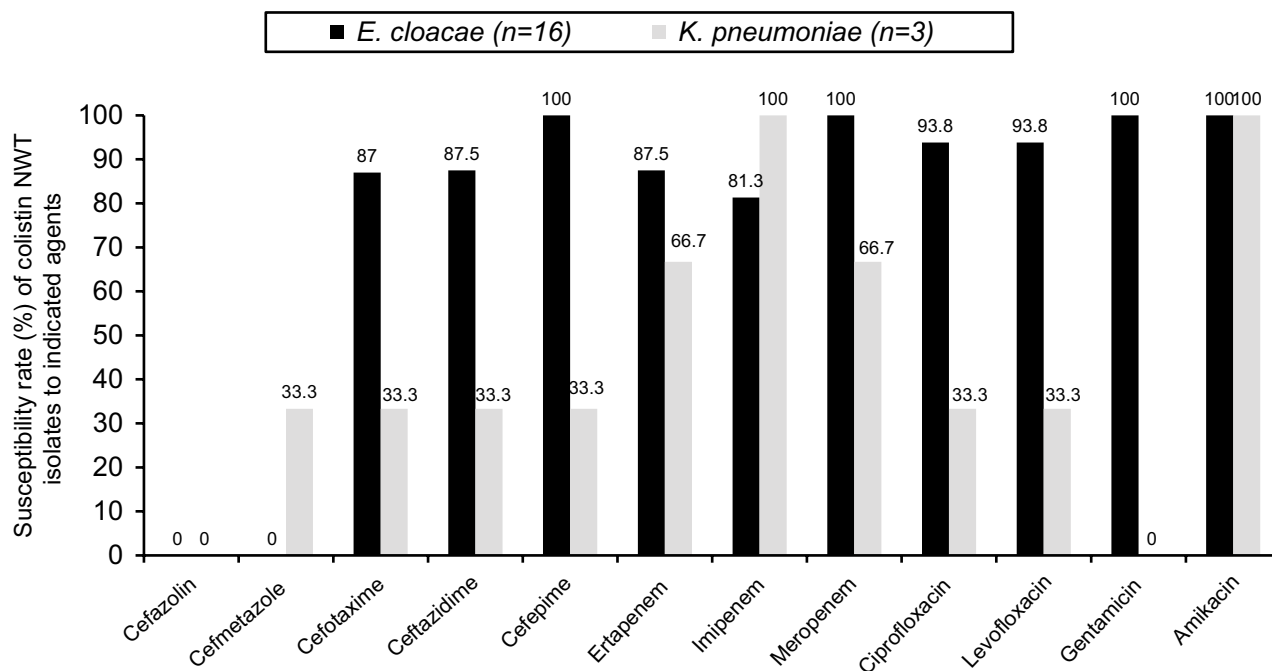
Based on the surveillance of common GNB in the ICU, including Enterobacteriaceae and NFGNB, we have several significant findings. First, the prevalence of carbapenem-insusceptible Enterobacteriaceae was found to be 9.4%. For *P. aeruginosa* and *A. baumannii* complex, carbapenem-insusceptibility rates 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

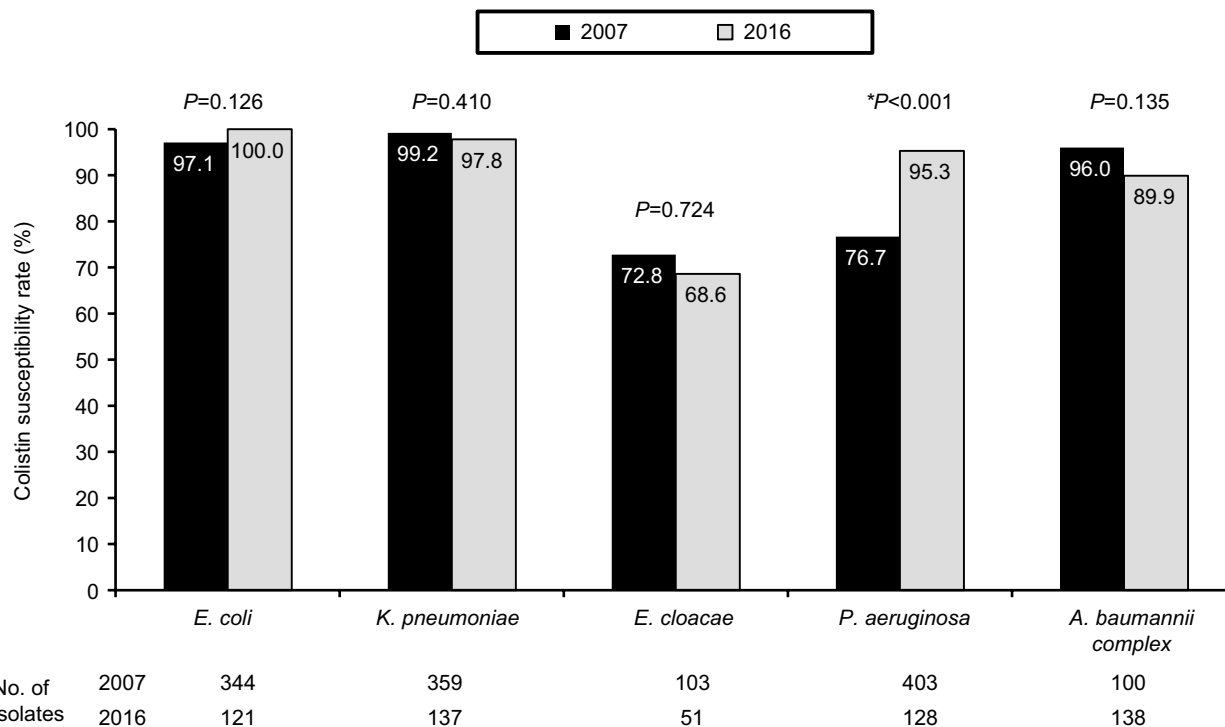
Organism (total isolates)	Method	Isolates with indicated colistin MICs (mg/L) determined by two methods, n (%)										Agreement <sup>a</sup>		Isolates with VME, <sup>b</sup> n (%)
		≤0.5	1	2	4	8	16	≥32	Essential	Categorical				
<i>Escherichia coli</i> (121)	BMD	14 (11.7)	103 (85.8)	3 (2.5)	0	0	0	0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

**Notes:** <sup>a</sup>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; <sup>b</sup>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*.



**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<sup>6</sup> 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.<sup>22–25</sup> 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.<sup>22,26,27</sup> 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.<sup>22,27</sup>

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  $\leq 0.5$  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,<sup>28</sup> 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.<sup>29</sup> 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).<sup>6</sup> 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).<sup>6</sup> 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*<sub>KPC</sub> 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,<sup>30</sup> where 13 (21.7%) of the 60 carbapenem-insusceptible *K. pneumoniae* in ICUs in 2012 harbored *bla*<sub>KPC</sub>. In fact, no *bla*<sub>KPC</sub> gene was detected in Enterobacteriaceae isolates from the previous SMART from ICUs in Taiwan before 2011.<sup>5</sup> This suggests the emergence of *bla*<sub>KPC</sub> 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*<sub>KPC</sub> and *bla*<sub>OXA48</sub> 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.

## References

- Halpern NA, Goldman DA, Tan KS, Pastores SM. Trends in critical care beds and use among population groups and Medicare and Medicaid beneficiaries in the United States: 2000–2010. *Crit Care Med*. 2016;44(8):1490–1499.
- Lai CC, Ho CH, Chang CL, et al. Critical care medicine in Taiwan from 1997 to 2013 under national health insurance. *J Thorac Dis*. 2018;10(8):4957–4965.
- Navoa-Ng JA, Berba R, Galapia YA, et al. Device-associated infections rates in adult, pediatric, and neonatal intensive care units of hospitals in the Philippines: international nosocomial infection control Consortium (INICC) findings. *Am J Infect Control*. 2011;39(7):548–554.
- Tao L, Hu B, Rosenthal VD, Gao X, He L. Device-associated infection rates in 398 intensive care units in Shanghai, China: international nosocomial infection control Consortium (INICC) findings. *Int J Infect Dis*. 2011;15(11):e774–e780.
- Jean SS, Hsueh PR, Lee WS, et al. Carbapenem susceptibilities and non-susceptibility concordance to different carbapenems amongst clinically important gram-negative bacteria isolated from intensive care units in Taiwan: results from the surveillance of multicentre antimicrobial resistance in Taiwan (SMART) in 2009. *Int J Antimicrob Agents*. 2013;41(5):457–462.
- Jean SS, Lee WS, Yu KW, et al. Rates of susceptibility of carbapenems, ceftibiprole, and colistin against clinically important bacteria collected from intensive care units in 2007: results from the surveillance of multicenter antimicrobial resistance in Taiwan (SMART). *J Microbiol Immunol Infect*. 2016;49(6):969–976.
- Lee YL, Chen YS, Toh HS, et al. Antimicrobial susceptibility of pathogens isolated from patients with complicated intra-abdominal infections at five medical centers in Taiwan that continuously participated in the study for monitoring antimicrobial resistance trends (SMART) from 2006 to 2010. *Int J Antimicrob Agents*. 2012;40(Suppl):S29–S36.
- Tsao LH, Hsin CY, Liu HY, et al. Risk factors for healthcare-associated infection caused by carbapenem-resistant *Pseudomonas aeruginosa*. *J Microbiol Immunol Infect*. 2018;51(3):359–366.

9. Chen CH, Kuo HY, Hsu PJ, et al. Clonal spread of carbapenem-resistant *Acinetobacter baumannii* across a community hospital and its affiliated long-term care facilities: A cross sectional study. *J Microbiol Immunol Infect*. 2018;51(3):377–384.
10. Lee CM, Lai CC, Chiang HT, et al. Presence of multidrug-resistant organisms in the residents and environments of long-term care facilities in Taiwan. *J Microbiol Immunol Infect*. 2017;50(2):133–144.
11. Liu YY, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism *MCR-1* in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis*. 2016;16(2):161–168.
12. Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. *Clin Microbiol Rev*. 2018;31(2):e00079-17.
13. Can F, Menekse S, Ispir P, et al. Impact of the ST101 clone on fatality among patients with colistin-resistant *Klebsiella pneumoniae* infection. *J Antimicrob Chemother*. 2018;73(5):1235–1241.
14. Ripabelli G, Tamburro M, Guerrizio G, et al. Tracking multidrug-resistant *Klebsiella pneumoniae* from an Italian hospital: molecular epidemiology and surveillance by PFGE, RAPD and PCR-Based resistance genes prevalence. *Nat Microbiol*. 2018;75(8):977–987.
15. Shen Y, Zhou H, Xu J, et al. Anthropogenic and environmental factors associated with high incidence of *mcr-1* carriage in humans across China. *Nat Microbiol*. 2018;3(9):1054–1062.
16. Hung MN, Hsueh PR, Chang HT, et al. In vitro activities of various piperacillin and sulbactam combinations against bacterial pathogens isolated from Intensive Care Units in Taiwan: SMART 2004 programme data. *Int J Antimicrob Agents*. 2007;29(2):145–152.
17. Jean SS, Hsueh PR, Lee WS, et al. Nationwide surveillance of antimicrobial resistance among non-fermentative gram-negative bacteria in intensive care units in Taiwan: SMART programme data 2005. *Int J Antimicrob Agents*. 2009;33(3):266–271.
18. Jean SS, Hsueh PR, Lee WS, et al. In vitro activities of doripenem and other carbapenems against clinically important bacteria isolated in intensive care units: nationwide data from the SMART programme. *Eur J Clin Microbiol Infect Dis*. 2010;29(4):471–475.
19. Jean SS, Lee WS, Bai KJ, et al. Relationship between the distribution of cefepime minimum inhibitory concentrations and detection of extended-spectrum  $\beta$ -lactamase production among clinically important Enterobacteriaceae isolates obtained from patients in intensive care units in Taiwan: results from the surveillance of multicenter antimicrobial resistance in Taiwan (SMART) in 2007. *J Microbiol Immunol Infect*. 2015;48(1):85–91.
20. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. 28th Informational Supplement. M100-ED28. Wayne, PA: CLSI; 2018.
21. Rebelo AR, Bortolaia V, Kjeldgaard JS, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* for surveillance purposes. *Euro Surveill*. 2018;23(6).
22. Lai CC, Lin YT, Lin YT, et al. Clinical characteristics of patients with bacteraemia due to the emergence of *mcr-1*-harboring Enterobacteriaceae in humans and pigs in Taiwan. *Int J Antimicrob Agents*. 2018;52(5): 651–657.
23. Quan J, Li X, Chen Y, et al. Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: a multicentre longitudinal study. *Lancet Infect Dis*. 2017;17(4): 400–410.
24. Wang Y, Tian GB, Zhang R, et al. Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study. *Lancet Infect Dis*. 2017;17(4):390–399.
25. Kocsis B, Kádár B, Tóth Ákos, Fullár A, Szabó D. *MgrB* variants in colistin-susceptible and colistin-resistant *Klebsiella pneumoniae* ST258. *J Microbiol Immunol Infect*. 2017;50(5):735–736.
26. Corbella M, Mariani B, Ferrari C, et al. Three cases of *mcr-1*-positive colistin-resistant *Escherichia coli* bloodstream infections in Italy, August 2016 to January 2017. *Euro Surveill*. 2017;22(16):30517.
27. Nordmann P, Lienhard R, Kieffer N, Clerc O, Poirel L. Plasmid-Mediated Colistin-Resistant *Escherichia coli* in Bacteremia in Switzerland. *Clin Infect Dis*. 2016;62(10):1322–1323.
28. Lo-Ten-Foe JR, de Smet AM, Diederer BM, Kluytmans JA, van Keulen PH. Comparative evaluation of the VITEK 2, disk diffusion, estest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* strains. *Antimicrob Agents Chemother*. 2007;51(10):3726–3730.
29. Sader HS, Farrell DJ, Flamm RK, Jones RN. Antimicrobial susceptibility of gram-negative organisms isolated from patients hospitalized in intensive care units in United States and European hospitals (2009–2011). *Diagn Microbiol Infect Dis*. 2014;78(4):443–448.
30. Chang YY, Chuang YC, Siu LK, et al. Clinical features of patients with carbapenem nonsusceptible *Klebsiella pneumoniae* and *Escherichia coli* in intensive care units: a nationwide multicenter study in Taiwan. *J Microbiol Immunol Infect*. 2015;48(2):219–225.

## Infection and Drug Resistance

### Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic

resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>

Dovepress