Antimicrobial activities of ceftazidime–avibactam, ceftolozane–tazobactam, and other agents against *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolated from intensive care units in Taiwan: results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan in 2016

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**Objective:** The aim of this study was to investigate the in vitro antimicrobial susceptibilities of clinically important Gram-negative bacteria from seven intensive care units in Taiwan in 2016.

**Materials and methods:** In total, 300 non-duplicate isolates of *Escherichia coli* (*n* = 100), *Klebsiella pneumoniae* (*n* = 100), and *Pseudomonas aeruginosa* (*n* = 100) collected from 300 patients were studied. The minimum inhibitory concentrations (MICs) of these isolates to antimicrobial agents were determined using the broth microdilution method. Carbapenemase-encoding genes (*bla*mcr*, *bla*VIM, *bla*GES, *bla*KPC, and *bla*OXA-48-like) were studied for the isolates that were not susceptible to any carbapenems. Sequencing analysis of the *mcr* genes (*mcr*1–5) was conducted for all isolates with colistin MICs ≥ 2 mg/L.

**Results:** Ertapenem non-susceptibility was detected in 3% (*n* = 3) *E. coli* and 12% (*n* = 12) *K. pneumoniae* isolates. The susceptibility rates of imipenem, ceftazidime–avibactam (CAZ–AVB), and ceftolozane–tazobactam (CLZ–TAZ) were 99%, 99%, and 88%, respectively, for *E. coli*, 91%, 100%, and 80%, respectively, for *K. pneumoniae*, and 66%, 91%, and 93%, respectively, for *P. aeruginosa*. Carbapenemase-encoding genes were not detected in *E. coli*, detected in four (33.3%) *K. pneumoniae* isolates that were not susceptible to ertapenem (three harboring *bla*KPC and one harboring *bla*OXA-48-like), and were not detected in *P. aeruginosa* isolates that were not susceptible to imipenem. One *K. pneumoniae* isolate was resistant to colistin (MIC 4 mg/L) and negative for *mcr* genes.

**Conclusion:** CAZ–AVB exhibited excellent activity against carbapenem-resistant *Enterobacteriaceae*, and CLZ–TAZ exhibited good activity against imipenem-resistant *P. aeruginosa*.

**Keywords:** carbapenem resistance, second-generation, β-lactam, β-lactamase inhibitor combinations, carbapenemase-encoding genes, *mcr*

**Introduction**
Carbapenemase-producing bacteria, especially *Enterobacteriaceae* (Carbapenemase-producing *Enterobacteriaceae*, CPE), are emerging worldwide and causing significant morbidity and mortality.1–6 In early 2000, carbapenem resistance was mostly reported...
in *Pseudomonas aeruginosa* (mediated by the *bla*<sub> OprM</sub>, *bla*<sub> VIM</sub>, and *bla*<sub> SM</sub> carbapenemases) and *Acinetobacter baumannii* (mediated by the *bla*<sub> OXA-23</sub>, *bla*<sub> OXA-24</sub>, and *bla*<sub> OXA-58</sub> carbapenemases).<sup>7,8</sup> Currently, *Klebsiella pneumoniae* carbapenemase (*bla*<sub>KPC</sub>) and New Delhi metallo-β-lactamase (*bla*<sub>NDM</sub>) have become matters of primary concern as carbapenem resistance has spread from non-fermenters (nosocomial opportunistic pathogens) to *Enterobacteriaceae*, which can easily disseminate and cause infections in the community.<sup>9,10</sup> The major risk factors for patients infected with carbapenem-resistant Gram-negative bacteria (GNB) are serious underlying illness, long-term care facility residence, and exposure to carbapenem.<sup>11–13</sup>

Owing to limited treatment options, colistin has become the main antimicrobial agent, either alone or in combination with other drugs.<sup>3–5</sup> However, high failure rates have been noted with colistin treatment in previous reviews.<sup>13–5</sup> New β-lactam combination agents, including ceftazidime–avibactam (CAZ–AVB) and ceftolozane–tazobactam (CLZ–TAZ), exhibit potent in vitro activities against CPE and possess the potential to replace colistin.<sup>14,15</sup> A recently published case series of patients with CPE infections who were treated with these two new agents demonstrated the superior efficacies of CAZ–AVB and CLZ–TAZ compared to that of colistin.<sup>16–18</sup> However, the susceptibility rates of bacteria to these new agents vary among countries due to different resistance mechanisms,<sup>1,5,15,19</sup> and the British guidelines recommend performing molecular typing for carbapenemases for selecting the most suitable agent for CPE treatment.<sup>4</sup> Although determination of the molecular mechanisms of carbapenem resistance is difficult, several new methods for accomplishing this have recently become available.<sup>20</sup>

Antimicrobial resistance among clinically important bacteria collected from intensive care units (ICUs) which were assessed for >10 years in Taiwan<sup>2</sup> and increase in carbapenem resistance among *Enterobacteriaceae* and *P. aeruginosa* have been noted.<sup>21</sup> The purpose of this study was to delineate the in vitro antibacterial activities of CAZ–AVB and CLZ–TAZ against *Escherichia coli*, *K. pneumoniae*, and *P. aeruginosa* isolates collected from ICUs in Taiwan.

### Materials and methods

#### Collection of isolates

Three hundred consecutive, non-duplicate *E. coli* (n=100), *K. pneumoniae* (n=100), and *P. aeruginosa* (n=100) isolates were collected from various clinical specimens of 300 patients in ICUs at seven major teaching hospitals in Taiwan (two in the northern part, one in the middle part, and four in the southern part of Taiwan) from January 1, 2016, to December 31, 2016 (Table 1). The majority of these isolates were recovered from sputum/endotracheal

| Source | No. of isolates | E. coli (n=100) | K. pneumoniae (n=100) | P. aeruginosa (n=100) | No. (%) of isolates (n=300) |
|--------|----------------|----------------|----------------------|----------------------|---------------------------|
| Hospital (location within Taiwan) | | | | | |
| NTUH (N) | 18 | 15 | 16 | 49 (16.3) |
| TMWFH (N) | 1 | 13 | 8 | 22 (7.3) |
| VGH-Taichung (M) | 17 | 15 | 15 | 47 (15.7) |
| CMMC (S) | 16 | 14 | 15 | 45 (15.0) |
| NCKUH (S) | 16 | 15 | 16 | 47 (15.7) |
| KMUH (S) | 16 | 14 | 15 | 45 (15.0) |
| VGH-Kaohsiung (S) | 16 | 14 | 15 | 45 (15.0) |
| Clinical sources | | | | | |
| Sputum/endotracheal aspirates | 36 | 68 | 77 | 181 (60.3) |
| Urine | 34 | 15 | 6 | 55 (18.3) |
| Blood | 13 | 10 | 9 | 32 (10.7) |
| Pus/wound | 8 | 3 | 5 | 16 (5.3) |
| Ascites | 6 | – | 1 | 7 (2.3) |
| Abscess fluids | 2 | 3 | 2 | 7 (2.3) |
| Bile | 1 | – | – | 1 (0.3) |
| Cerebrospinal fluid | – | 1 | – | 1 (0.3) |

**Abbreviations:** CMMC, Chi Mei Medical Center; E. coli, *Escherichia coli*; ICUs, intensive care units; KMUH, Kaohsiung Medical University Hospital; K. pneumoniae, Klebsiella pneumoniae; M, middle; N, northern; NCKUH, National Cheng Kung University Hospital; NTUH, National Taiwan University Hospital; P. aeruginosa, *Pseudomonas aeruginosa*; S, southern; TMWFH, Taipei Municipal Wan-Fang Hospital; VGH-Kaohsiung, Kaohsiung Veterans General Hospital; VGH-Taichung, Taichung Veterans General Hospital.
aspirates (n=181, 60.3%), urine (n=55, 18.3%), and blood (n=32, 10.7%) samples of the ICU patients (Table 1). The institutional review board of the National Taiwan University Hospital (201512064RSB) approved this study and waived the requirement for written informed consent. The ethical committees waived the need for informed consent because limited private health information was collected and this research involved minimal risk to the subjects.

### Antimicrobial susceptibility testing

In this study, the broth microdilution method with Sensititre™ Gram-negative minimum inhibitory concentration (MIC) plates (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the MICs of the evaluated antibiotics. *E. coli* determine the MICs of the evaluated antibiotics 25922 and *P. aeruginosa* ATCC 27853 were used for quality control on each testing day. The MIC break points recommended by the Clinical and Laboratory Standards Institute (CLSI) in 2018 were used to define the susceptibility of the isolates. For *E. coli* and *K. pneumoniae* isolates, MICs of ≤8/4 and ≥16/4 mg/L for CAZ–AVB are identified as susceptible and resistant, respectively, whereas MICs of ≤2/4, 4/4, and ≥8/4 mg/L for CLZ–TAZ are classified as susceptible, intermediate, and resistant, respectively, in the CLSI guidelines. For *P. aeruginosa* isolates, MICs of ≤8/4 and ≥16/4 mg/L for CAZ–AVB are identified as susceptible and resistant, respectively, and those of ≤4/4, 8/4, and ≥16/4 mg/L for CLZ–TAZ are classified as susceptible, intermediate, and resistant, respectively, in the CLSI guidelines. For *E. coli* and *K. pneumoniae* isolates, no CLSI MIC break points for colistin and tigecycline for defining susceptibilities are recommended. However, the CLSI defines the susceptibility of *E. coli* and *K. pneumoniae* isolates to colistin as wild type (WT; MICs of ≤2 mg/L) and non-WT (MICs of ≥4 mg/L). For *P. aeruginosa* isolates, MICs of ≤2 and ≥4 mg/L for colistin are identified as susceptible and resistant, respectively. For defining the susceptibility of *E. coli* and *K. pneumoniae* isolates to tigecycline, MICs of ≤1 and >2 mg/L recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were adopted for defining susceptibility and resistance, respectively.

### Detection of carbapenemases

For *E. coli* and *K. pneumoniae* isolates displaying non-susceptibility to any carbapenem agents (ertapenem, imipenem, meropenem, or doripenem) and for *P. aeruginosa* isolates exhibiting non-susceptibility to imipenem, meropenem, or doripenem, the Xpert® Carba-R assay (Cepheid, Sunnyvale, CA, USA) was used to detect the carbapenemase-encoding alleles, including *bla*<sub>ESK</sub>, *bla*<sub>IMI</sub>, *bla*<sub>IMP</sub>, *bla*<sub>IMI</sub>, and *bla*<sub>OXA-48-like</sub>. CPE and carbapenemase-producing *P. aeruginosa* isolates were defined as *Enterobacteriaceae* or *P. aeruginosa*, respectively, harboring genes encoding any carbapenemase.

### Detection of mcr-1 to mcr-5

PCR amplification of the whole-cell DNA of the isolates with colistin MICs of ≥2 mg/L was performed using previously described primers specific for *mcr-1, mcr-2, mcr-3, mcr-4,* and *mcr-5,* and the amplification products were sequenced.

### Statistical analyses

To compare the antimicrobial susceptibility between imipenem-susceptible and non-susceptible *P. aeruginosa* isolates, Pearson’s chi-squared test or Fisher’s exact test were used. Two-tailed *P*-values of <0.05 were considered to indicate significant differences. The analysis was performed using SPSS Version 17 (SPSS Inc., Chicago, IL, USA).

### Results

#### Antimicrobial susceptibilities of the isolates

The MIC ranges of CLZ–TAZ and CAZ–AVB for *E. coli* ATCC 25922 were 0.25–0.5 and 0.12–0.25 mg/L, respectively, whereas those for *P. aeruginosa* ATCC 27853 were 0.25–0.5 and 1–4 mg/L, respectively. The MIC ranges of the other agents tested against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were within the MIC ranges recommended by the CLSI.

Table 2 summarizes the susceptibilities of *CLZ–TAZ, CAZ–AVB,* and other antimicrobial agents against the 300 isolates of *E. coli, K. pneumoniae,* and *P. aeruginosa.* Overall, amikacin exhibited excellent activity (≥96%) against all isolates tested. The observed rates of non-susceptibility to ertapenem were 3% among *E. coli* and 12% among *K. pneumoniae* isolates. The rates of susceptibility to imipenem were 99% for *E. coli,* 91% for *K. pneumoniae,* and 66% for *P. aeruginosa.* All the *E. coli* isolates were inhibited by 0.5 mg/L tigecycline. In contrast, 89% of the *K. pneumoniae* isolates were inhibited by 1 mg/L tigecycline (susceptible based on the EUCAST criteria), whereas eight and three isolates exhibited MICs of 2 mg/L (intermediate by the EUCAST criteria) and 4 mg/L (resistant by the EUCAST criteria), respectively. All *P. aeruginosa* isolates were susceptible to colistin (MICs of ≤2 mg/L) and all *E. coli* isolates were inhibited by 0.5 mg/L colistin (all WT isolates). Five of the *P. aeruginosa* isolates exhibited colistin MICs of 2 mg/L. Among the *K. pneumoniae* isolates, 99%.
Table 2 In vitro susceptibilities of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* isolates collected from patients admitted to the ICUs of seven major teaching hospitals across Taiwan in 2016 to 19 antimicrobial agents

| Bacterial species (isolate no.) and antimicrobial agent tested | MIC (mg/L) | % of indicated susceptibility |
|---------------------------------------------------------------|------------|------------------------------|
|                                                               | Range      | MIC<sub>50</sub> | MIC<sub>90</sub> | Susceptible | Intermediate | Resistant |
| *E. coli* (n=100)                                             |            |                |                |             |              |           |
| CLZ–TAZ                                                       | 0.12–64    | 0.5            | 4              | 88          | 3            | 9         |
| CAZ–AVB                                                       | ≤0.06–16   | 0.12           | 0.5            | 99          | NA           | 1         |
| Ampicillin                                                    | 2–64       | >64            | >64            | 15          | 0            | 85        |
| Cefazolin                                                     | 1–64       | >64            | >64            | 21          | 11           | 68        |
| Cefoxitin                                                    | 4–64       | 16             | >64            | 47          | 17           | 36        |
| Ceftriaxone                                                  | ≤0.12–>64  | 8              | >64            | 47          | 0            | 53        |
| Ceftazidine                                                  | ≤0.12–256  | 2              | 64             | 55          | 11           | 34        |
| Cefepime                                                      | ≤0.12–64   | 0.25           | >64            | 63          | 8            | 29        |
| Amoxicillin–clavulanate                                      | 2–64       | 16             | 64             | 46          | 20           | 34        |
| Cefoperazone–sulbactam                                       | 0.12–64    | 4              | 32             | NA          | 100          | NA        |
| Piperacillin–tazobactam                                      | 1–128      | 4              | 32             | 88          | 0            | 8         |
| Ertapenem                                                    | ≤0.06–8    | ≤0.06          | 0.25           | 97          | 2            | 1         |
| Meropenem                                                    | ≤0.06–1    | ≤0.06          | ≤0.06          | 100         | 0            | 0         |
| Imipenem                                                     | ≤0.06–2    | 0.12           | 0.25           | 99          | 1            | 0         |
| Doripenem                                                    | ≤0.06–1    | ≤0.06          | ≤0.06          | 100         | 0            | 0         |
| Ciprofloxacin                                                | ≤0.06–64   | 0.5            | 64             | 61          | 0            | 39        |
| Levofoxacin                                                  | ≤0.06–64   | 0.5            | 32             | 62          | 0            | 38        |
| Amikacin                                                     | 0.5–64     | 2              | 4              | 99          | 0            | 1         |
| Tigecycline                                                  | ≤0.12–0.5  | ≤0.12          | 0.25           | NA          | NA           | NA        |
| Colistin                                                     | ≤0.12–0.5  | 0.25           | 0.25           | 100 (WT), 0 (NWT) |              |           |
| *K. pneumoniae* (n=100)                                      |            |                |                |             |              |           |
| CLZ–TAZ                                                       | 0.12–64    | 0.5            | 64             | 80          | 3            | 17        |
| CAZ–AVB                                                       | ≤0.06–8    | 0.25           | 1              | 100         | NA           | 0         |
| Ampicillin                                                    | 16–64      | >64            | >64            | 0           | 7            | 93        |
| Cefazolin                                                     | 1–64       | 2              | >64            | 55          | 2            | 43        |
| Cefoxitin                                                    | 4–64       | 8              | >64            | 64          | 2            | 34        |
| Ceftriaxone                                                  | ≤0.12–>64  | ≤0.12          | >64            | 72          | 1            | 27        |
| Ceftazidine                                                  | ≤0.06–256  | 0.5            | 256            | 66          | 3            | 31        |
| Cefepime                                                     | ≤0.06–64   | ≤0.12          | 64             | 78          | 5            | 17        |
| Amoxicillin–clavulanate                                      | 2–64       | 4              | 64             | 63          | 7            | 30        |
| Cefoperazone–sulbactam                                       | 0.25–64    | 0.5            | 64             | NA          | NA           | NA        |
| Piperacillin–tazobactam                                      | 2–128      | 4              | 128            | 77          | 7            | 16        |
| Ertapenem                                                    | ≤0.06–64   | ≤0.06          | 0.12           | 92          | 1            | 7         |
| Meropenem                                                    | ≤0.06–64   | ≤0.06          | 0.12           | 92          | 1            | 7         |
| Imipenem                                                     | 0.12–64    | 0.25           | 1              | 91          | 3            | 6         |
| Doripenem                                                    | ≤0.06–64   | ≤0.06          | 0.12           | 92          | 1            | 7         |
| Ciprofloxacin                                                | ≤0.06–64   | ≤0.06          | 64             | 71          | 1            | 28        |
| Levofoxacin                                                  | ≤0.06–64   | ≤0.06          | 32             | 73          | 2            | 25        |
| Amikacin                                                     | 0.25–64    | 1              | 2              | 96          | 0            | 4         |
| Tigecycline                                                  | 0.25–4     | 0.25           | 2              | NA          | NA           | NA        |
| Colistin                                                     | ≤0.12–4    | 0.25           | 0.25           | 99 (WT), 1 (NWT) |              |           |
| *P. aeruginosa* (n=100)                                      |            |                |                |             |              |           |
| CLZ–TAZ                                                       | 0.25–64    | 1              | 4              | 93          | 5            | 2         |
| CAZ–AVB                                                       | 1–64       | 2              | 8              | 91          | NA           | 9         |
| Ampicillin                                                    | 64–64      | >64            | >64            | NA          | NA           | NA        |
| Cefazolin                                                     | >64        | >64            | >64            | NA          | NA           | NA        |
| Cefoxitin                                                    | >64        | >64            | >64            | NA          | NA           | NA        |
| Ceftriaxone                                                  | 4–64       | >64            | >64            | NA          | NA           | NA        |
| Ceftazidine                                                  | 1–256      | 4              | 128            | 71          | 7            | 22        |
| Cefepime                                                     | 0.25–64    | 4              | 32             | 73          | 14           | 13        |

(Continued)
were inhibited by 2 mg/L colistin, whereas one exhibited colistin MIC of 4 mg/L (ie, non-WT).

The rates of susceptibility to CAZ–A VB were 99% for E. coli, 100% for K. pneumoniae, and 91% for P. aeruginosa. The MIC of the E. coli isolate resistant to CAZ–A VB was 16/4 mg/L. The rates of susceptibility to CLZ–TAZ were 88% for E. coli, 80% for K. pneumoniae, and 93% for P. aeruginosa.

Table 2 (Continued)

| Bacterial species (isolate no.) and antimicrobial agent tested | MIC (mg/L) | % of indicated susceptibility |
|---------------------------------------------------------------|------------|------------------------------|
|                                                               | Range      | MIC<sub>50</sub> | MIC<sub>90</sub> | Susceptible | Intermediate | Resistant |
| Cefoperazone–sulbactam                                        | 0.5->64    | 8               | 64             | NA          | NA           | NA        |
| Piperacillin–tazobactam                                       | 0.25->128  | 8               | >128           | 66          | 11           | 23        |
| Ertapenem                                                     | 0.5->64    | 8               | 64             | NA          | NA           | NA        |
| Meropenem                                                     | ≤0.06->64  | 0.5             | 8              | 77          | 7            | 16        |
| Imipenem                                                      | 0.5->64    | 2               | 16             | 66          | 12           | 22        |
| Doripenem                                                     | ≤0.06->64  | 0.5             | 8              | 77          | 9            | 14        |
| Ciprofloxacin                                                 | ≤0.06->64  | 0.12            | 16             | 79          | 1            | 20        |
| Levofloxacin                                                  | ≤0.06->64  | 0.5             | 16             | 76          | 4            | 20        |
| Amikacin                                                     | 1->64      | 2               | 4              | 99          | 0            | 1         |
| Tigecycline                                                  | 0.5->32    | 8               | 16             | NA          | NA           | NA        |
| Colistin                                                      | 0.5–2      | 8               | 1              | 1           | 100          | 19        |

Note: The MICs were interpreted based on the criteria of the 2018 CLSI.<sup>18</sup> Abbreviations: CAZ–AVB, ceftazidime–avibactam; CLSI, Clinical and Laboratory Standards Institute; CLZ–TAZ, ceftolozane–tazobactam; E. coli, Escherichia coli; ICUs, intensive care units; K. pneumoniae, Klebsiella pneumoniae; MICs, minimum inhibitory concentrations; NA, non-applicable; NWT, non-WT; P. aeruginosa, Pseudomonas aeruginosa; WT, wild type.

Discussion

New β-lactam combination agents, including CAZ–AVB and CLZ–TAZ, have been approved in 2015 and 2014, respectively, for use in cases of urinary tract infections and intra-abdominal infections based on the results of randomized controlled trials.<sup>14</sup> However, the increasing prevalence of carbapenem-resistant pathogens is a major hurdle for effective treatment using these two agents. In a recently published evaluation of CLZ–TAZ for the treatment of serious infections (51% pneumonia) caused by carbapenem-resistant P. aeruginosa,<sup>15</sup> the successful treatment rate was 74% on average (70% for monotherapy and 87% for combination therapy with another active agent). Treatment failure was associated with isolate MICs ≥8 mg/L. Similarly, a multicenter evaluation of CAZ–AVB against CPE showed significant reduction...
in all-cause hospital mortality compared to colistin. In that study, 37% patients were treated with CAZ–AVB monotherapy, whereas only 6% patients were treated with colistin monotherapy. A pooled analysis of CAZ–AVB Phase III clinical trials indicated that its efficacy was comparable to that of carbapenems used for critical infections, including nosocomial pneumonia caused by multidrug-resistant pathogens. An increase in the use of the new β-lactamase inhibitors can be anticipated, although careful susceptibility testing and surveillance studies are warranted as the rates and mechanisms of resistance vary among regions and resistance may develop during treatment.

In this surveillance study of isolates collected from patients admitted to seven ICUs in Taiwan in 2016, the non-susceptibility rate of E. coli isolates to ertapenem (3%) was lower than that to ceftriaxone (53%) and ceftepime (37%). For K. pneumoniae, 12% isolates were not susceptible to ertapenem, and carbapenemase-encoding genes were detected in four isolates (three isolates harboring blaKPC and one harboring blaOXA-48-like). CAZ–AVB exhibited excellent in vitro activities against E. coli (99%) and K. pneumoniae (100%) isolates, whereas CTL–TAZ exhibited lower activities (88% and 80%, respectively). These observations imply that CAZ–AVB can be confidently recommended as an empirical and definitive treatment for infections caused by Enterobacteriaceae in the ICU setting in Taiwan.

For P. aeruginosa, the imipenem susceptibility rate was only 66%. In this study, carbapenem non-susceptibility was associated with decreased susceptibility to other antimicrobial agents tested with the exception of amikacin and colistin. The overall susceptibility rate of CLZ–TAZ was 93% for all P. aeruginosa isolates, but decreased to 85% for imipenem non-susceptible isolates, whereas the corresponding rates for CAZ–AVB were 91% and 79%, respectively. Nevertheless, the rates of bacterial susceptibility to these new two β-lactamase inhibitors were higher than those to other current drugs, including ceftepime and piperacillin–tazobactam. It is surprising that the Xpert® Carba-R assay detected no carbapenemase-encoding genes among the carbapenem non-susceptible P. aeruginosa. According to previous studies on carbapenem-resistant P. aeruginosa in Taiwan, the overproduction of active efflux pump and OprD polymorphisms were the major mechanisms of resistance, which might explain the negative results.

In the present study, colistin and amikacin were active in vitro against the three tested major pathogenic bacteria. Compared to other antimicrobial agents in Taiwan, the use of aminoglycosides is gradually decreasing owing to the possibility of developing nephrotoxicity as a side effect. In a previous 9-year study between 2003 and 2011, the mean consumption of amikacin (defined daily dose per 1,000 patient-days) was 8, compared to 56 for extended-spectrum

![Figure 1](https://www.dovepress.com/)

**Figure 1** Comparison of the susceptibilities to seven selected agents between imipenem-susceptible and imipenem-non-susceptible P. aeruginosa isolates.

**Notes:** Pearson’s chi-squared test or Fisher’s exact test was used. Two-tailed P-values of <0.05 were considered to indicate significant differences.

**Abbreviations:** CAZ, ceftriaxone; CAZ–AVB, ceftazidime–avibactam; CIP, ciprofloxacin; CLZ–TAZ, ceftolozane–tazobactam; FEP, ceftepime; LVX, levofloxacin; P. aeruginosa, Pseudomonas aeruginosa; TZP, piperacillin–tazobactam.
cephalosporins, 17 for piperacillin–tazobactam, 22.4 for carbapenems (with an increase each year), and 52 for quinolone. However, the emergence of resistant microorganisms warrants further clinical study on the role of aminoglycosides, which have been proposed to be useful as part of combination therapies or high-dose monotherapy. Meanwhile, although tigecycline has maintained a high susceptibility rate against Enterobacteriaceae for >10 years since its first use, it should be applied cautiously and be limited to the treatment of intra-abdominal and soft tissue infections according to the British guidelines.

Our study had several limitations. First, CAZ–AVB and CLZ–TAZ combinations were tested against only a limited number of drug-resistant, β-lactamase-harboring isolates. Second, we did not perform the in vitro susceptibilities of some oral carbapenems (tebipenem and faropenem), ceftolozane, and other new β-lactam combination agents, including diazabicyclooctane-based inhibitors and boronic acid-based inhibitor such as vaborbactam (meropenem–vaborbactam). The current Sensititre format does not include these agents, and we cannot obtain or purchase the standard powders of these agents for in-house broth microdilution study. Furthermore, these agents have not yet been launched in Taiwan now, and most of them will not be available in the near future in this country.

Conclusion
CAZ–AVB and CLZ–TAZ, which have not yet been launched in Taiwan, have high susceptibility rates against E. coli, K. pneumoniae, and P. aeruginosa isolates collected from ICUs in Taiwan. With the recent publication of a case series showing their clinical superiority to colistin, these two agents may be used instead of the current colistin-based treatment for carbapenem-resistant pathogens in the near future.

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References
1. Tzouvelekis LS, Markogiannakis A, Psychogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev. 2012;25(4):682–707.
2. Jean SS, Lee WS, Yu KW, et al. Rates of susceptibility of carbapenems, ceftobiprole, 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.
3. Li J, Nation RL, Turnidge JD, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis. 2006;6(9):589–601.
4. Paul M, Daikos GL, Durante-Mangoni E, et al. Colistin alone versus colistin plus meropenem for treatment of severe infections caused by carbapenem-resistant Gram-negative bacteria: a randomized controlled trial. Lancet Infect Dis. 2018;18(4):391–400.
5. Hawkey PM, Warren RE, Livermore DM, et al. Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for antimicrobial Chemotherapy/Healthcare infection Society/British infection association joint working Party. J Antimicrob Chemother. 2018;73(Suppl 3):ii12–ii78.
6. Lee CH, Su TY, Ye JJ, et al. Risk factors and clinical significance of bacteremia caused by Pseudomonas aeruginosa resistant only to carbapenems. J Microbiol Immunol Infect. 2017;50(5):677–683.
7. Livermore DM, Woodford N. Carbapenemases: a problem in waiting? Curr Opin Microbiol. 2000;3(5):489–495.
8. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect. 2006;12(9):826–836.
9. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis. 2009;9(4):228–236.
10. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10(9):597–602.
11. Ting SW, Lee CH, Liu JW. Risk factors and outcomes for the acquisition of carbapenem-resistant Gram-negative Bacillus bacteremia: a retrospective propensity-matched case control study. J Microbiol Immunol Infect. 2018;51(5):621–628.
12. Tsao LH, Hsin CY, Liu HY, Chuang HC, Chen LY, Lee YJ. Risk factors for healthcare-associated infection caused by carbapenem-resistant Pseudomonas aeruginosa. J Microbiol Immunol Infect. 2018;51(3):359–366.
13. 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.
14. van Duijn D, Bonomano RA. Cefazidime–avibactam and ceftolozane/tazobactam: second-generation β-lactam/β-lactamase inhibitor combinations. Clin Infect Dis. 2016;63(2):234–241.
15. Sader HS, Castanheira M, Shortridge D, Mendes RE, Flamm RK. Antimicrobial activity of Cefazidime–Avibactam tested against multidrug-resistant Enterobacteriaceae and Pseudomonas aeruginosa isolates from U.S. medical centers, 2013 to 2016. Antimicrob Agents Chemother. 2017;61(11):e01045–17.
16. Munita JM, Aitken SL, Miller WR, et al. Multicenter evaluation of ceftolozane/tazobactam for serious infections caused by carbapenem-resistant Pseudomonas aeruginosa. Clin Infect Dis. 2017;65(1):158–161.
17. Shields RK, Nguyen MH, Chen L, et al. Cefazidime–avibactam is superior to other treatment regimens against carbapenem-resistant Klebsiella pneumoniae bacteremia. Antimicrob Agents Chemother. 2017;61(8):e00883–17.
18. van Duijn D, Lok JJ, Earley M, et al. Colistin versus ceftazidime–avibactam in the treatment of infections due to carbapenem-resistant Enterobacteriaceae. Clin Infect Dis. 2018;66(2):163–171.
19. Katchanov J, Asar L, Klupp EM, et al. Carbapenem-resistant Gram-negative pathogens in a German University medical Center: prevalence, clinical implications and the role of novel β-lactam/β-lactamase inhibitor combinations. PLoS One. 2018;13(4):e0195757.
20. Lutgring JD, Limbago BM. The problem of carbapenemase-producing-carbapenem-resistant-Enterobacteriaceae detection. J Clin Microbiol. 2016;54(3):529–534.
21. 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.
22. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Eighth Informational Supplement M100-S28. Wayne, PA: CLSI; 2018.
23. European Committee on Antimicrobial Susceptibility Testing [webpage on the Internet]. Breakpoint Tables for Interpretation of MICs and Zone Diameters (version 8.0). Available from: http://www.eucast.org/clinical_breakpoints/. Accessed September 9, 2018.
24. Jean SS, Lu MC, Shi ZY , et al. In vitro activity of ceftazidime-avibactam, Ceftolozane-Tazobactam, and other comparable agents against clinically important Gram-negative bacilli: results from the 2017 surveillance of multicenter antimicrobial resistance in Taiwan (SMART). Infect Drug Resist. 2018;11:1983–1992.
25. Stone GG, Newell P, Gasink LB, et al. Clinical activity of ceftazidime/avibactam against MDR Enterobacteriaceae and Pseudomonas aeruginosa: pooled data from the ceftazidime/avibactam Phase III clinical trial programme. J Antimicrob Chemother. 2018;73(9):2519–2523.
26. Kao CY, Chen SS, Hung KH, et al. Overproduction of active efflux pump and variations of OprD dominate in imipenem-resistant Pseudomonas aeruginosa isolated from patients with bloodstream infections in Taiwan. BMC Microbiol. 2016;16(1):107.
27. Shu JC, Kuo AJ, Su LH, et al. Development of carbapenem resistance in Pseudomonas aeruginosa is associated with OprD polymorphisms, particularly the amino acid substitution at codon 170. J Antimicrob Chemother. 2017;72(9):2489–2495.
28. Lee HS, Loh YY, Lee JI, Liu CS, Chu C. Antimicrobial consumption and resistance in five Gram-negative bacterial species in a hospital from 2003 to 2011. J Microbiol Immunol Infect. 2015;48(6):647–654.
29. Gálvez R, Luengo C, Cornejo R, et al. Higher than recommended amikacin loading doses achieve pharmacokinetic targets without associated toxicity. Int J Antimicrob Agents. 2011;38(2):146–151.
30. Sadeghi K, Hamishehkar H, Najmeddin F, et al. High-dose amikacin for achieving serum target levels in critically ill elderly patients. Infect Drug Resist. 2018;11:223–228.
31. Chen YH, Lu PL, Huang CH, et al. Trends in the susceptibility of clinically important resistant bacteria to tigecycline: results from the tigecycline in vitro surveillance in Taiwan study, 2006 to 2010. Antimicrob Agents Chemother. 2012;56(3):1452–1457.