Journal of Microbiology, Immunology and Infection 56 (2023) 358–366

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

In vitro susceptibility of common Enterobacterales to eravacycline in Taiwan

Chun-Fu Huang [a,b], Jann-Tay Wang [b], Yu-Chung Chuang [b,*], Wang-Huei Sheng [b], Yee-Chun Chen [b]

[a] *Department of Internal Medicine, National Taiwan University Hospital Yunlin Branch, Yunlin County, Taiwan*
[b] *Department of Internal Medicine, National Taiwan University Hospital, Taipei City, Taiwan*

Received 8 March 2022; received in revised form 6 September 2022; accepted 26 September 2022
Available online 2 October 2022

KEYWORDS
Carbapenem-resistant *Enterobacterales*;
Enterobacterales;
Eravacycline;
In vitro susceptibility

Abstract *Background:* New tetracycline derivatives exhibit broad-spectrum antimicrobial activities. This study aimed to assess the *in vitro* activity of eravacycline against common *Enterobacterales*.
Methods: Clinical *Enterobacterales* isolates were collected between 2017 and 2021. The minimum inhibitory concentration (MIC) was determined using a broth microdilution test.
Results: We identified *Klebsiella pneumoniae* (n = 300), *Escherichia coli* (n = 300), *Klebsiella oxytoca* (n = 100), *Enterobacter cloacae* complex (n = 100), *Citrobacter freundii* (n = 100), and *Proteus mirabilis* (n = 100). All *P. mirabilis* strains were resistant to eravacycline. Excluding *P. mirabilis*, the susceptibility rates to eravacycline, omadacycline, and tigecycline were 75.2%, 66.9%, and 73%, respectively. The MIC_{50} and MIC_{90} (mg/L) of eravacycline were 0.5 and 4 for *K. pneumoniae*, 0.5 and 1 for *E. coli*, 0.5 and 1 for *K. oxytoca*, 0.5 and 2 for *E. cloacae* complex, and 0.25 and 1 for *C. freundii*. In cefotaxime non-susceptible and meropenem susceptible *Enterobacterales*, excluding *P. mirabilis*, the susceptibility rates of eravacycline, omadacycline, and tigecycline were 69.7%, 57.1%, and 66.2%. We found decreased susceptibility rates of three new tetracycline derivatives against meropenem non-susceptible *Enterobacterales* (eravacycline: 47.1%, omadacycline: 39.4%, and tigecycline: 39.4%). Eravacycline showed a high susceptibility rate against cefotaxime non-susceptible and meropenem susceptible *K. oxytoca* (100%), *C. freundii* (93.2%), *E. coli* (85.9%), and meropenem non-susceptible *E. coli* (100%).
Conclusion: This study provides the MIC and susceptibility rate of eravacycline for common *Enterobacterales*. Eravacycline could be a therapeutic choice for cefotaxime non-susceptible or meropenem non-susceptible *Enterobacterales*, especially *K. oxytoca*, *C. freundii*, and *E. coli*.

* Corresponding author. Department of Internal Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei 100, Taiwan.
E-mail address: weischuang@gmail.com (Y.-C. Chuang).

https://doi.org/10.1016/j.jmii.2022.09.009

Introduction

Enterobacterales are important normal flora and are common causes of community- or healthcare-related infection.[1] Multiple drug-resistant bacteria are a substantial threat associated with morbidity and mortality worldwide.[2] Carbapenem is considered the last resort of treatment for multidrug-resistant *Enterobacterales*.[3] However, carbapenem-resistant *Enterobacterales* are spreading rapidly, especially through carbapenemase-expressing plasmids. In addition, an increasing prevalence of carbapenem-resistant *Enterobacterales*, especially in long-term care units, is found around the world,[4,5] which were listed as a critical priority by the World Health Organization in 2016.[2] New tetracycline derivatives have broad-spectrum activity against gram-positive and gram-negative bacteria and anaerobes, including a series of antibiotic-resistant bacteria, and are listed as a potential choice of antibiotics against infection by carbapenem-resistant *Enterobacterales* under the guidance of the Infectious Diseases Society of America.[6] Tigecycline was approved for the treatment of complicated skin and skin structure infections and complicated intra-abdominal infections by the U.S. Food and Drug Administration (FDA) in 2005.[7] Two new tetracycline derivatives, omadacycline and eravacycline, were approved by the U.S. FDA in 2018.[8,9] Omadacycline was the first regimen for new oral and intravenous tetracycline derivatives. A previous study demonstrated the *in vitro* activity of omadacycline against a broad spectrum of gram-positive microorganisms, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and penicillin-resistant *Streptococcus pneumoniae*.[10] Omadacycline are indicated for community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections. Eravacycline, a synthetic fluorocycline antibiotic, comprises of the tetracycline core scaffold with modifications in the tetracycline *D* ring.[9] The clinical studies IGNITE1 and IGNITE4 showed that eravacycline is a potential antimicrobial agent in complicated intra-abdominal infection.[11,12] Additionally, eravacycline has been indicated for complicated intra-abdominal infections.

Drug susceptibility to eravacycline has been widely established in Western countries.[13—15] However, studies on drug susceptibility to eravacycline in Taiwan are limited to antimicrobial-resistant microorganisms.[16] Therefore, this study aimed to assess the *in vitro* antimicrobial activity of eravacycline against clinical isolates of common *Enterobacterales* in Taiwan.

Methods

This cohort study was conducted at the National Taiwan University Hospital (NTUH), a 2200-bed medical center located in Taipei City, which provides both primary and tertiary care. This study adhered with the principles of the Declaration of Helsinki.

Bacterial isolates

One thousand clinical isolates, namely *Escherichia coli* (n = 300), *Enterobacter cloacae* complex (n = 100), *Klebsiella pneumoniae* (n = 300), *Klebsiella oxytoca* (n = 100), *Citrobacter freundii* (n = 100), and *Proteus mirabilis* (n = 100) were collected at the NTUH from 2017 to 2021. All *K. pneumoniae*, *E. coli*, *E. cloacae* complex, and *P. mirabilis* isolates were collected from blood samples. *K. oxytoca* and *C. freundii* were also collected from blood samples (82 blood samples from *K. oxytoca* and 73 blood samples from *C. freundii)*. Other isolates were obtained from non-blood samples, including sputum, urine, bile, ascites, anal swabs, throat swabs, and skin pus. All the isolates were collected from different patients. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker BioTyper; Bruker Daltonics, Bremen, Germany) was used to identify the isolates. Initial screening for carbapenem non-susceptibility of all isolates was conducted using Vitek 2 (bioMérieux, Inc, Hazelwood, MO, USA). The carbapenems tested were ertapenem, imipenem-cilastatin, and meropenem. We defined the bacteria as carbapenem non-susceptible strains if the susceptibility to any carbapenem was intermediate or resistant. We used a modified carbapenem inactivation method (mCIM) test for the phenotypic detection of carbapenemase in all carbapenem-non-susceptible *Enterobacterales* according to the Clinical and Laboratory Standards Institute (CLSI).[17] Among the carbapenem-non-susceptible *Enterobacterales* with the carbapenemase-producing phenotype, carbapenemase genes, including *bla*-KPC, *bla*-NDM, *bla*-OXA-48, *bla*-IMP, and *bla*-VIM, were detected using a PCR Amplification Kit with Takara Taq (TAKARA, Kyoto, Japan).[18,19]

Antimicrobial agents, susceptibility testing, and minimum inhibitory concentration (MIC) interpretative criteria

The antimicrobial agents selected for testing were eravacycline, omadacycline, tigecycline, meropenem, and cefotaxime. The MIC of the antimicrobial agents was determined using the broth microdilution method according to the CLSI 2021.[17] The interpretation breakpoints were based on the CLSI (cefotaxime and meropenem),[17] EUCAST (eravacycline and tigecycline),[20] and U.S. FDA (omadacycline).[21] Quality control was performed according to the CLSI, using *E. coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *S. aureus* ATCC29213.

Statistical analyses

A two-sample test of proportions was used to compare the susceptibility rates of the different antimicrobial agents. A two-sided P-value of <0.05 was considered statistically significant. Spearman's correlation coefficient was used to

analyze the correlation between the MICs of different antimicrobial agents. We defined a strong correlation as rho >0.7, moderate correlation as rho 0.40—0.69, and weak correlation as 0 < rho <0.39.[22] Cohen's kappa coefficient was used to evaluate the susceptibility agreement among the three new tetracycline derivatives. We interpreted Cohen's kappa coefficient as slight (0—0.2), fair (0.21—0.4), moderate (0.41—0.6), substantial (0.61—0.8), and almost perfect agreement (0.81—1).[23] Data were analyzed using Stata software (version 14; StataCorp, College Station, TX, USA).

Results

A total of 1000 clinical isolates of *K. pneumoniae* (n = 300), *E. coli* (n = 300), *K. oxytoca* (n = 100), *E. cloacae* complex (n = 100), *C. freundii* (n = 100), and *P. mirabilis* (n = 100) were collected at the NTUH from 2017 to 2021. All isolates of *K. pneumoniae*, *E. coli*, *E. cloacae* complex, and *P. mirabilis* were collected from the blood samples. *K. oxytoca* and *C. freundii* were mainly isolated from blood samples (82 and 73 isolates, respectively), whereas others were isolated from the sputum, urine, bile, ascites, skin pus samples, throat swabs, and anal swabs. The sputum, urine, bile, ascites, and skin pus samples corresponded with pneumonia, urinary tract infection, biliary tract infection, peritonitis, and skin and soft tissue infection, respectively. One throat swab (one isolate of *K. oxytoca*) was collected from the oral ulcer. Three anal swabs (two isolates of *K. oxytoca* and one isolate of *C. freundii*) were collected from surveillance cultures in the intensive care units. All the isolates were collected from different patients.

According to the results of Vitek 2, 152 carbapenem non-susceptible clinical isolates were found within 1000 clinical isolates, namely *E. coli* (n = 12), *E. cloacae* complex (n = 25), *K. pneumoniae* (n = 60), *K. oxytoca* (n = 34), and *C. freundii* (n = 21). All *P. mirabilis* isolates were susceptible to carbapenems. A total of 96 clinical isolates were classified as carbapenemase-producing *Enterobacterales* using the mCIM test. There were 91 carbapenemase-producing isolates with identified carbapenemase genotypes were identified. The number and species of carbapenemase-producing *Enterobacterales* are summarized in Table 1. One isolate of *C. freundii* carried *bla*-NDM and *bla*-IMP. Five clinical carbapenemase-producing isolates, namely, three isolates of *K. pneumoniae*, one isolate of *E. cloacae* complex, and one isolate of *C. freundii*, failed to identify with the carbapenemase genotype and were excluded from Table 1.

The MIC distributions of the isolates are summarized in Table 2. In a total of 1000 *Enterobacterales* isolates, the susceptibility rates of eravacycline, omadacycline, and tigecycline were 67.7%, 60.2%, and 65.7%, respectively. All *P. mirabilis* isolates were resistant to new tetracycline derivatives. Because *P. mirabilis* exhibits intrinsically reduced susceptibility to tetracycline and its derivative,[13,24,25] we might not use new tetracycline derivatives for the treatment of *P. mirabilis* associated infections. Therefore, we excluded *P. mirabilis* from the susceptibility analysis to avoid underestimation of the susceptibility rates. The susceptibility rates of the 900 clinical isolates to eravacycline, omadacycline, and tigecycline were 75.2%, 66.9%, and 73%, respectively.

Among the *Enterobacterales*, excluding *P. mirabilis*, the susceptibility rates were significantly higher for eravacycline than for omadacycline ($P < 0.001$) but were similar to tigecycline ($P = 0.35$). The susceptibility rates of *E. coli* (92.3% versus 73.7%, $P < 0.001$) and *C. freundii* (89% versus 74%, $P = 0.006$) to eravacycline were significantly higher than those of omadacycline. The susceptibility agreements between eravacycline and tigecycline (kappa 0.76, $P < 0.001$) and tigecycline and omadacycline (kappa 0.63, $P < 0.001$). The susceptibility agreement between eravacycline and omadacycline was moderate (kappa 0.58, $P < 0.001$). The MIC ranges of eravacycline were 0.125—16 mg/L for *K. pneumoniae*, 0.125—4 mg/L for *E. coli*, 0.125—2 mg/L for *K. oxytoca*, 0.25—8 mg/L for *E. cloacae* complex, and 0.125—4 mg/L for *C. freundii*. The susceptibility rates of the different *Enterobacterales* to eravacycline ranged from 52.3 to 92.3%. The MIC_{50} and MIC_{90} of eravacycline were 0.5 mg/L and 4 mg/L for *K. pneumoniae*, 0.5 mg/L and 1 mg/L for *E. coli*, 0.5 mg/L and 1 mg/L for *K. oxytoca*, 0.5 mg/L and 2 mg/L for *E. cloacae* complex, and 0.25 mg/L and 1 mg/L for *C. freundii*, respectively. The MIC distribution results of the selected 900 isolates were strongly correlated between eravacycline and tigecycline (rho = 0.73, $P < 0.001$, Fig. 1A) and moderately correlated between eravacycline and omadacycline (rho = 0.67, $P < 0.002$, Fig. 1B).

Among the cefotaxime non-susceptible and meropenem susceptible isolates (n = 343), the susceptibility rates to eravacycline, omadacycline, and tigecycline were 69.7%, 57.1%, and 66.2%, respectively (Table 3). The MIC ranges of eravacycline were 0.25—16 mg/L for *K. pneumoniae*, 0.125—4 mg/L for *E. coli*, 0.25—0.5 mg/L for *K. oxytoca*, 0.25—8 mg/L for *E. cloacae* complex, and 0.125—4 mg/L for *C. freundii*. The susceptibility rates around different species ranged from 41.2 to 100% and were relatively low in *K. pneumoniae* (41.2%) and *the E. cloacae* complex (54.5%).

Table 1 The numbers and species of carbapenemase-producing *Enterobacterales* with identified carbapenemase genotypes.

Carbapenemase-producing *Enterobacterales* (numbers)	*bla*-KPC isolates (numbers)	*bla*-NDM isolates (numbers)	*bla*-OXA-48 isolates (numbers)	*bla*-IMP isolates (numbers)	*bla*-VIM isolates (numbers)
K. pneumoniae (34)	22	0	7	3	2
E. coli (3)	0	0	1	1	1
K. oxytoca (31)	1	4	0	6	20
E. cloacae complex (8)	0	4	0	4	0
C. freundii (15)[a]	2	4	0	10	0

[a] One isolate of carbapenemase-producing *C. freundii* carried *bla*-NDM and *bla*-IMP.

Table 2 Drug susceptibility and minimum inhibitory concentration distributions of the included *Enterobacterales*.

Pathogen (numbers)	Eravacycline Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]	Omadacycline Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]	Tigecycline Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]	Meropenem Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]	Cefotaxime Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]
K. pneumoniae (300)	52.3% (157) 0.125–16 [0.5, 4]	52.7% (158)1 to >32 [4, 32]	48.7% (146)0.25–16 [1, 4]	85% (255)≤0.03 to >16 [≤0.03, 8]	53% (158)≤0.06 to >32 [0.5, >32]
E. coli (300)	92.3% (277) 0.125–4 [0.5, 1]	73.7% (221)1 to >32 [4, 8]	91.7% (275) 0.06–2 [0.25, 0.5]	98.7% (296)≤0.03 to >16 [≤0.03, ≤0.03]	54% (162)≤0.06 to >32 [0.125, >32]
K. oxytoca (100)	90% (90) 0.125–2 [0.5, 1]	83% (83)2–16 [4, 8]	85% (85)0.25–2 [0.5, 1]	68% (68)≤0.03 to >16 [≤0.03, 16]	56% (56)≤0.06 to >32 [0.125, >32]
E. cloacae complex (100)	64% (64) 0.25–8 [0.5, 2]	66% (66)2–32 [4, 16]	66% (66)0.25–8 [0.5, 2]	93% (93)≤0.03 to >16 [0.06, 1]	38% (38)≤0.06 to >32 [32, >32]
C. freundii (100)	89% (89) 0.125–4 [0.25, 1]	74% (74)2 to >32 [4, 16]	85% (85)0.125–4 [0.25, 1]	84% (84)≤0.03 to >16 [≤0.03, 4]	40% (40)≤0.06 to >32 [32, >32]
P. mirabilis (100)	0% (0)2–16 [4, 4]	0% (0)>32 [>32, >32]	0% (0)2 to >16 [4, 8]	100% (100)0.06–1100 [0.125, 0.25]	86% (86)≤0.06 to >32 [<0.06, 8]
Enterobacterales, excluding *P. mirabilis* (900)	75.2% (677) 0.125–16 [0.5, 1]	66.9% (602)1 to >32 [4, 16]	73% (657)0.125–8 [0.5, 2]	88.4% (796)≤0.03 to >16 [≤0.03, 2]	50.4% (454)≤0.06 to >32 [1, >32]
All *Enterobacterales* (1000)	67.7% (677) 0.125–16 [0.5, 1]	60.2% (602)1 to >32 [4, 16]	65.7% (657)0.125 to >16 [0.5, 4]	89.6% (896)≤0.03 to >16 [≤0.03, 2]	54% (540)<0.06 to >32 [0.25, >32]

[a] N: numbers.
[b] mg/L.
MIC: minimum inhibitory concentration.

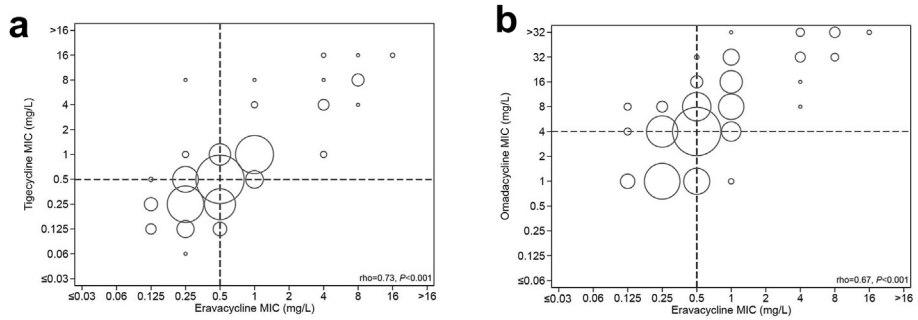

Figure 1. Bubble plot comparing the MIC distribution of (A) eravacycline versus tigecycline and (B) eravacycline versus omadacycline in all the isolates. *P. mirabilis* was excluded from this analysis. The bubble sizes indicate the isolates' numbers.

Table 3 Drug susceptibility and minimum inhibitory concentration distributions of cefotaxime non-susceptible and meropenem susceptible *Enterobacterales*.

Pathogen (numbers)	Eravacycline Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]	Omadacycline Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]	Tigecycline Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]
K. pneumoniae (97)	41.2% (40) 0.25—16 [1, 8]	37.1% (36) 1 to >32 [8, 32]	30.9% (30) 0.25—16 [1, 4]
E. coli (135)	85.9% (116) 0.125—4 [0.5, 1]	60.7% (82) 1 to >32 [4, 8]	86.7% (117) 0.125—2 [0.25, 1]
K. oxytoca (12)	100% (12) 0.25—0.5 [0.25, 0.5]	58.3% (7) 2—16 [4, 16]	100% (12) 0.25—0.5 [0.5, 0.5]
E. cloacae complex (55)	54.5% (30) 0.25—8 [0.5, 2]	61.8% (34) 2—32 [4, 16]	54.5% (30) 0.25—8 [0.5, 2]
C. freundii (44)	93.2% (41) 0.125—4 [0.25, 0.5]	84.1% (37) 2—32 [4, 8]	86.4% (38) 0.125—2 [0.25, 1]
Enterobacterales, excluding *P. mirabilis* (343)	69.7% (239) 0.125—16 [0.5, 2]	57.1% (196) 1 to >32 [4, 16]	66.2% (227) 0.125—16 [0.5, 2]

[a] N: numbers.
[b] mg/L.
MIC: minimum inhibitory concentration.

The susceptibility rates of cefotaxime non-susceptible and meropenem-susceptible *C. freundii*, *E. coli*, and *K. oxytoca* to eravacycline were 92.3%, 85.9%, and 100%, respectively. The MIC_{50} and MIC_{90} of eravacycline were 1 mg/L and 8 mg/L for *K. pneumoniae*, 0.5 mg/L and 1 mg/L for *E. coli*, 0.25 mg/L and 0.5 mg/L for *K. oxytoca*, 0.5 mg/L and 2 mg/L for *E. cloacae* complex, and 0.25 mg/L and 0.5 mg/L for *C. freundii*, respectively.

Among the meropenem non-susceptible isolates (n = 104), susceptibility rates to eravacycline, omadacycline, and tigecycline were 47.1%, 39.4%, and 39.4%, respectively (Table 4). The susceptibility rates were similar between eravacycline and tigecycline among meropenem non-susceptible isolates ($P=0.26$). The MIC ranges of eravacycline were 0.125—16 mg/L for *K. pneumoniae*, 0.25—0.5 mg/L for *E. coli*, 0.25—1 for *K. oxytoca*, 0.5—2 mg/L for *E. cloacae* complex, and 0.25—4 mg/L for *C. freundii*. The susceptibility rates ranged from 20 to 100% and dramatically decreased in *K. pneumoniae* (20%), *E. cloacae* complex (42.9%), and *C. freundii* (56.3%). We observed

Table 4 Drug susceptibility and minimum inhibitory concentration distributions of the meropenem non-susceptible isolates.

Pathogen (numbers)	Eravacycline Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]	Omadacycline Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]	Tigecycline Susceptibility (N[a]) MIC[b] range [MIC_{50}, MIC_{90}]
K. pneumoniae (45)	20% (9)0.125—16 [2, 8]	20% (9)1 to >32 [16, >32]	13.3% (6)0.25—16 [2, 8]
E. coli (4)	100% (4)0.25—0.5 [0.5, 0.5]	75% (3)2—8 [2, 8]	100% (4)0.25—0.5 [0.25, 0.5]
K. oxytoca (32)	75% (24)0.25—1 [0.5, 1]	65.6% (21)2—16 [8, 16]	56.3 (18)0.25—2 [0.5, 2]
E. cloacae complex (7)	42.9% (3)0.5—2 [1, 2]	28.6% (2)2—32 [8, 32]	57.1% (4)0.25—4 [0.5, 2]
C. freundii (16)	56.3% (9)0.25—4 [0.5, 2]	37.5% (6)2 to >32 [8, 32]	56.3% (9)0.25—4 [0.5, 4]
Enterobacterales (104)	47.1% (49)0.125—16 [1, 4]	39.4% (41)1 to >32 [8, >32]	39.4% (41)0.25—16 [1, 8]

[a] N: numbers.
[b] mg/L.
MIC: minimum inhibitory concentration.

relatively high susceptibility rates to meropenem non-susceptible *K. oxytoca* (75%) and *E. coli* (100%). The MIC_{50} and MIC_{90} of eravacycline were 2 mg/L and 8 mg/L for *K. pneumoniae*, 0.5 mg/L and 0.5 mg/L for *E. coli*, 0.5 mg/L and 1 mg/L for *K. oxytoca*, 1 mg/L and 2 mg/L for *E. cloacae* complex, and 0.5 mg/L and 2 mg/L for *C. freundii*, respectively (Table 4). The MIC distribution of 104 meropenem non-susceptible isolates was strongly correlated between eravacycline and tigecycline (rho = 0.87, $P < 0.001$, Fig. 2A) and between eravacycline and omadacycline (rho = 0.77, $P < 0.001$, Fig. 2B). The MIC distribution results of the 96 carbapenemase-producing isolates were strongly correlated between eravacycline and tigecycline (rho = 0.82, $P < 0.001$, Fig. 3A) and between eravacycline and omadacycline (rho = 0.78, $P < 0.001$, Fig. 3B).

In different genotypes of carbapenemase species, the susceptibility rates of KPC-, NDM-, IMP-, VIM-, and OXA-48-producing species to eravacycline were 28%, 41.7%, 45.8%, 87%, and 25%, respectively. The corresponding tigecycline results were 12%, 41.7%, 41.7%, 78.3%, and 37.5%, respectively.

Discussion

In our study, we evaluated the susceptibility of common *Enterobacterales* to new tetracycline derivatives in Taiwan. We also compared the *in vitro* susceptibilities to eravacycline, omadacycline, and tigecycline. Strongly correlated MIC distributions were observed for eravacycline and tigecycline. All *P. mirabilis* isolates were resistant to new tetracycline derivatives. Among the common *Enterobacterales*, excluding *P. mirabilis*, the susceptibility rate to eravacycline was significantly higher than that to omadacycline (75.2% vs. 66.9%, $P < 0.01$) and similar to tigecycline (75.2% vs. 73%, $P = 0.35$). Cohen's kappa test also showed substantial agreement of susceptibility between eravacycline and tigecycline (kappa 0.76, $P < 0.001$) and between tigecycline and omadacycline (kappa 0.63, $P < 0.001$). Cohen's kappa test showed a moderate agreement in susceptibility between eravacycline and omadacycline (kappa 0.58, $P < 0.001$). In all 104 meropenem non-susceptible *Enterobacterales*, the susceptibility rate to eravacycline was higher than that to omadacycline and tigecycline but was not significantly different (47.1% vs. 39.4%, $P = 0.26$).

To compare the effects of eravacycline against *Enterobacterales*. We performed Spearman's correlation tests for MICs between three new tetracycline derivatives, including eravacycline versus omadacycline and eravacycline versus tigecycline. The MIC distribution results of the selected isolates were strongly correlated between eravacycline and tigecycline, and moderately correlated between eravacycline and omadacycline. These results imply that the activity of eravacycline might be more similar to tigecycline than omadacycline. However, further clinical studies are warranted to apply these *in vitro* findings in clinical practice.

In a global surveillance study, including Taiwan, eravacycline showed high susceptibility (EUCAST breakpoint ≤0.5 mg/L) rates against gram-negative *Enterobacterales* (92.6%), including *Klebsiella* species (90.6%), *Enterobacter* species (89.6%), *Citrobacter* species (94.6%), and *E. coli* (98.8%).[13] In another study conducted in Taiwan from 2017 to 2020, new tetracycline derivatives, including

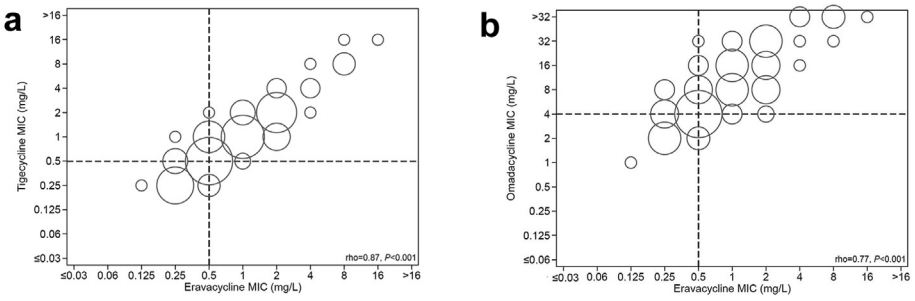

Figure 2. Bubble plot comparing the MIC distribution of (A) eravacycline versus tigecycline and (B) eravacycline versus omadacycline in meropenem non-susceptible isolates. The bubble sizes indicate the isolates' numbers.

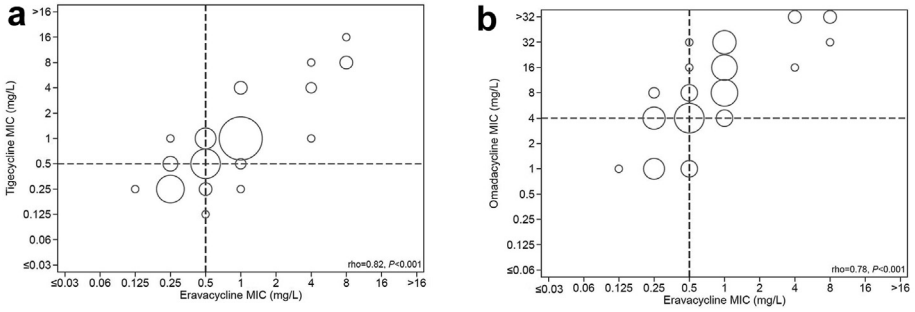

Figure 3. Bubble plot comparing the MIC distribution of (A) eravacycline versus tigecycline and (B) eravacycline versus omadacycline in carbapenemase-producing isolates. The bubble sizes indicate the isolates' numbers.

eravacycline, omadacycline, and tigecycline, also showed high susceptibility rates against carbapenem-resistant *E. coli* (96.2%, 92.3%, and 100%, respectively). The susceptibility rates of carbapenem-resistant *K. pneumoniae* to eravacycline, omadacycline, and tigecycline were relatively low (84%, 56.6%, and 93.2%, respectively). Despite all the new tetracycline derivatives having *in vitro* activity against gram-positive and gram-negative microorganisms, eravacycline had good activity against multidrug-resistant *Enterobacterales* and *Acinetobacter baumannii* and was preferred as a more effective regimen against gram-negative microorganisms and broad-spectrum beta-lactamase-producing bacteria compared to omadacycline.[26] Omadacycline is less effective against carbapenem-resistant *K. pneumoniae* than eravacycline.[16] Additionally, our study showed that eravacycline might exhibit a higher susceptibility rate against *Enterobacterales*, especially *E. coli* and *C. freundii,* than omadacycline. The C7 and C9 substituents contributed to the differences in structure between the new tetracycline derivatives, which might also result in different antimicrobial activity.[27]

In our study, the susceptibility rate of *E. coli* to eravacycline was similar to that in previous studies; however, the susceptibility rates of *K. pneumoniae* (52.3%) and *E. cloacae* complex (64%) to eravacycline were dramatically lower than those in previous studies. In our study, tigecycline also showed lower susceptibility rates against *K. pneumoniae* (48.7%) and the *E. cloacae* complex (66%). This may be explained by the different study settings used. First, the isolates were collected from 16 hospitals in Taiwan in the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) study.[16] In this study, isolates were collected from a tertiary medical center. The prevalence of multidrug resistance is high in NTUH.[28] Despite eravacycline-evading tetracycline-specific efflux pumps, it remains vulnerable to multidrug efflux pumps and tetracycline-degrading enzyme.[29] Second, the MICs of eravacycline, omadacycline, and tigecycline were determined using the Sensititre microbroth dilution method in the SMART study.[16] In our study, the MICs of the tested antibiotics were determined using the broth microdilution method according to the CLSI. The tigecycline MIC of automated antimicrobial susceptibility testing may differ from that of the broth microdilution test.[30,31] Using automated antimicrobial susceptibility testing, lower and unacceptable essential and categorical agreement rates were obtained for isolates belonging to species other than *E. coli*.[32] In another study in Taiwan, the susceptibility rate of imipenem-non-susceptible *K. pneumoniae* to eravacycline was 36.8%, which is similar to that of our study. The MICs of the tested antimicrobial agents were determined using the broth microdilution test in that study.[33] The difference in the antimicrobial susceptibility test could be the reason for the different results compared to those of previous studies.

The susceptibility rates to eravacycline, omadacycline, and tigecycline decreased in the cefotaxime non-susceptible isolates and meropenem non-susceptible isolates; however, the MIC distributions were similar. The mechanism of tetracyclines involves the inhibition of protein synthesis by binding to the 30 S ribosomal subunit of the target bacteria.[34] Common mechanisms of resistance are efflux pump production, ribosomal protection, and enzymatic inactivation of tetracyclines.[35] Carbapenemase is the most common mechanism of carbapenem resistance in *Enterobacterales*. In a study on the *in vitro* antimicrobial activity against carbapenemase-producing *K. pneumoniae* conducted in Greece, the susceptibility rates to tigecycline and eravacycline were 80.5% and 66.2%, respectively. Among KPC-, NDM-, VIM-, and OXA-48 carbapenemase-producing *K. pneumoniae*, the susceptibility rates to tigecycline were similar (80—81.8%), but those to eravacycline were very different (36.4—68.7%). OXA-48-producing *K. pneumoniae* has the lowest susceptibility to eravacycline.[36] Our study also noted the poor potency of eravacycline against KPC- and OXA-48-producing *Enterobacterales*. The susceptibility rates of the KPC- and OXA-48-producing species to eravacycline were only 28% and 25%, respectively. Although eravacycline was not hydrolyzed by carbapenemase, the coexistence of carbapenemase and efflux pump might explain the susceptibility rate of carbapenemase-producing microorganisms.[14,37,38] We found that the susceptibility rates of new tetracycline derivatives to specific carbapenemase-producing *Enterobacterales*, such as KPC-, NDM-, IMP-, and OXA-48 producing strains were unsatisfactory. Although new tetracycline derivatives were not hydrolyzed by carbapenemase, the coexistence of carbapenemase and efflux pump might explain the susceptibility rate of carbapenemase-producing microorganisms. However, our study could not confirm this hypothesis since we did not test for the existence of the efflux pump. The mechanism of differences in the susceptibility rates of new tetracycline derivatives among different carbapenem-resistant *Enterobacterales* and carbapenemase-producing *Enterobacterales* is unclear and warrants further research.

Our study has several limitations. First, this study was conducted based on a single tertiary medical care experience and could not present the environment in Taiwan. Second, to estimate the bloodstream infection isolates collected to determine the *in vitro* activity of eravacycline against true infection, the severity of bloodstream infection might be more severe than that of complicated intra-abdominal infections. We might have underestimated the susceptibility of complicated intra-abdominal infections to eravacycline. Thirdly, we collected six common species of *Enterobacterales*, but several pathogenic microorganisms were excluded from the study. Fourth, the number of some isolates, such as meropenem non-susceptible *E. coli*, was small, and the susceptibility rates could be affected by bias. Fifth, the underlying resistance mechanisms of new tetracycline derivatives were not explored in the present study. Finally, this study was designed as an *in vitro* drug-susceptibility test. The clinical efficacy of new tetracycline derivatives should be established in clinical trials.

Conclusion

This study showed drug susceptibility to new tetracycline derivatives, including eravacycline, omadacycline, and tigecycline. Susceptibility rates to eravacycline and tigecycline were similar. Although there were no obvious differences in MIC distributions among carbapenemase-producing *Enterobacterales*, susceptibility to eravacycline decreased in cefotaxime- and meropenem-non-susceptible

Enterobacterales, especially *K. pneumoniae* and *E. cloacae* complex. In other species, such as cefotaxime non-susceptible and meropenem-susceptible *C. freundii*, *E. coli*, and *K. oxytoca* or meropenem non-susceptible *E. coli*, relatively high susceptibility rates of eravacycline were still observed and could be a potential choice of therapy. Antimicrobial susceptibility tests provide important information for the treatment of infections, particularly drug-resistant microorganism-related infections. This study presents antimicrobial susceptibility data for new tetracycline derivatives in Taiwan.

Funding

This study was partially supported by the Ministry of Science and Technology, Taiwan (110-2628-B-002-059, 110-2326-B-002-003-MY3, and 109-2314-B-002-235-MY3), Ministry of Health and Welfare, Taiwan (MOHW110-TDU-B-211-124002), and National Taiwan University Hospital (110-S4901). The funding sources had no role in the design of the study; collection, analysis, and interpretation of data; or in writing the manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgments

The authors thank the Third Core Facility at NTUH for technical assistance and facility support.

References

1. Tilahun M, Kassa Y, Gedefie A, Ashagire M. Emerging carbapenem-resistant *Enterobacterales* infection, its epidemiology and novel treatment options: a review. *Infect Drug Resist* 2021;**14**:4363—74.
2. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**:318—27.
3. El-Gamal MI, Brahim I, Hisham N, Aladdin R, Mohammed H, Bahaaeldin A. Recent updates of carbapenem antibiotics. *Eur J Med Chem* 2017;**131**:185—95.
4. Potter RF, D'Souza AW, Dantas G. The rapid spread of carbapenem-resistant Enterobacterales. *Drug Resist Updates* 2016;**29**:30—46.
5. Chen HY, Jean SS, Lee YL, Lu MC, Ko WC, Liu PY, et al. Carbapenem-resistant *Enterobacterales* in long-term care facilities: a global and narrative review. *Front Cell Infect Microbiol* 2021;**11**:601968.
6. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases society of America guidance on the treatment of extended-spectrum beta-lactamase producing *Enterobacterales* (ESBL-E), carbapenem-resistant *Enterobacterales* (CRE), and Pseudomonas aeruginosa with difficult-to-treat resistance (DTR-P. aeruginosa). *Clin Infect Dis* 2021;**72**:e169—83.
7. Pankey GA. *Tigecycline*. *J Antimicrob Chemother* 2005;**56**:470—80.
8. Gallagher JC. Omadacycline: a modernized tetracycline. *Clin Infect Dis* 2019;**69**:S1—5.
9. Scott LJ. Eravacycline: a review in complicated intra-abdominal infections. *Drugs* 2019;**79**:315—24.
10. Macone AB, Caruso BK, Leahy RG, Donatelli J, Weir S. In vitro and in vivo antibacterial activities of omadacycline, a novel aminomethylcycline. *Antimicrob Agents Chemother* 2014;**58**:1127—35.
11. Solomkin JS, Gardovskis J, Lawrence K, Montravers P, Sway A, Evans D, et al. IGNITE4: results of a phase 3, randomized, multicenter, prospective trial of eravacycline vs meropenem in the treatment of complicated intraabdominal infections. *Clin Infect Dis* 2019;**69**:921—9.
12. Felice VG, Efimova E, Izmailyan S, Napolitano LM, Chopra T. Efficacy and tolerability of eravacycline in bacteremic patients with complicated intra-abdominal infection: a pooled analysis from the IGNITE1 and IGNITE4 studies. *Surg Infect* 2021;**22**:556—61.
13. Morrissey I, Olesky M, Hawser S, Lob SH, Karlowsky JA, Corey GR, et al. In vitro activity of eravacycline against gram-negative bacilli isolated in clinical laboratories worldwide from 2013 to 2017. *Antimicrob Agents Chemother* 2020;**64**:16999.e1719.
14. Livermore DM, Mushtaq S, Warner M, Woodford N. In vitro activity of eravacycline against carbapenem-resistant *Enterobacterales* and acinetobacter baumannii. *Antimicrob Agents Chemother* 2016;**60**:3840—4.
15. Clark JA, Kulengowski B, Burgess DS. In vitro activity of eravacycline compared with tigecycline against carbapenem-resistant Enterobacterales. *Int J Antimicrob Agents* 2020;**56**:106178.
16. Lee YL, Ko WC, Lee WS, Lu PL, Chen YH, Cheng SH, et al. *In vitro* activity of cefiderocol, cefepime/zidebactam, cefepime/enmetazobactam, omadacycline, eravacycline and other comparative agents against carbapenem-nonsusceptible *Enterobacterales*: results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) in 2017-2020. *Int J Antimicrob Agents* 2021;**58**:106377.
17. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing: 31th informational supplement, M100S*. Wayne, PA: CLSI; 2021.
18. Paul D, Dhar Chanda D, Maurya AP, Mishra S, Chakravarty A, Sharma GD, et al. Co-carriage of blaKPC-2 and blaNDM-1 in clinical isolates of Pseudomonas aeruginosa associated with hospital infections from India. *PLoS One* 2015;**10**:e0145823.
19. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011;**70**:119—23.
20. European Committee on Antimicrobial Susceptibility Testing. *Breakpoint tables for interpretation of MICs and zone diameters, version 11.0*. EUCAST; 2021.
21. Us Food and Drug Administration. *Omadacycline—injection products. FDA- identified interpretive criteria*. Silver Spring, MD: FDA; 2019. p. 18.
22. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. *Anesth Analg* 2018;**126**:1763—8.
23. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;**33**:159—74.
24. Schaffer JN, Pearson MM. *Proteus mirabilis* and urinary tract infections. *Microbiol Spectr* 2015;**3**:10—1128.
25. Visalli MA, Murphy E, Projan SJ, Bradford PA. AcrAB multidrug efflux pump is associated with reduced levels of susceptibility to tigecycline (GAR-936) in. *Proteus mirabilis*. *Antimicrob Agents Chemother* 2003;**47**:665—9.
26. Grossman TH. Tetracycline antibiotics and resistance. *Cold Spring Harb. Perspect. Med* 2016;**6**:a025387.

27. Rusu A, Lorena BE. The development of third-generation tetracycline antibiotics and new perspectives. *Pharmaceutics* 2021;**13**:2085.
28. Chuang YC, Chen YC, Chang SC, Sun CC, Chang YY, Chen ML, et al. Secular trends of healthcare-associated infections at a teaching hospital in Taiwan, 1981-2007. *J Hosp Infect* 2010;**76**: 143—9.
29. Alosaimy S, Abdul-Mutakabbir JC, Kebriaei R, Jorgensen SCJ, Rybak MJ. Evaluation of eravacycline: a novel fluorocycline. *Pharmacotherapy* 2020;**40**:221—38.
30. Stone ND, O'Hara CM, Williams PP, McGowan Jr JE, Tenover FC. Comparison of disk diffusion, VITEK 2, and broth microdilution antimicrobial susceptibility test results for unusual species of Enterobacterales. *J Clin Microbiol* 2007;**45**:340—6.
31. Yin D, Guo Y, Li M, Wu W, Tang J, Liu Y, et al. Performance of VITEK 2, E-test, Kirby-Bauer disk diffusion, and modified Kirby-Bauer disk diffusion compared to reference broth microdilution for testing tigecycline susceptibility of carbapenem-resistant *K. pneumoniae* and A. baumannii in a multicenter study in China. *Eur J Clin Microbiol Infect Dis* 2021;**40**:1149—54.
32. Huang TD, Berhin C, Bogaerts P, Glupczynski Y. In vitro susceptibility of multidrug-resistant *Enterobacterales* clinical isolates to tigecycline. *J Antimicrob Chemother* 2012;**67**: 2696—9.
33. Kuo SC, Wang YC, Tan MC, Huang WC, Shiau YR, Wang HY, et al. In vitro activity of imipenem/relebactam, meropenem/vaborbactam, ceftazidime/avibactam, cefepime/zidebactam and other novel antibiotics against imipenem-non-susceptible Gram-negative bacilli from Taiwan. *J Antimicrob Chemother* 2021;**76**:2071—8.
34. Roberts MC. Tetracycline therapy: update. *Clin Infect Dis* 2003; **36**:462—7.
35. Munita JM, Arias CA. Mechanisms of antibiotic resistance. *Microbiol Spectr* 2016;**4**:10—1128.
36. Maraki S, Mavromanolaki VE, Magkafouraki E, Moraitis P, Stafylaki D, Kasimati A, et al. Epidemiology and in vitro activity of ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, eravacycline, plazomicin, and comparators against Greek carbapenemase-producing Klebsiella pneumoniae isolates. *Infection* 2022;**50**:467—74.
37. He F, Fu Y, Chen Q, Ruan Z, Hua X, Zhou H, et al. Tigecycline susceptibility and the role of efflux pumps in tigecycline resistance in KPC-producing Klebsiella pneumoniae. *PLoS One* 2015;**10**:e0119064.
38. Jia W, Li C, Zhang H, Li G, Liu X, Wei J. Prevalence of genes of OXA-23 carbapenemase and AdeABC efflux pump associated with multidrug resistance of acinetobacter baumannii isolates in the ICU of a comprehensive hospital of northwestern China. *Int J Environ Res Publ Health* 2015;**12**:10079—92.