Zhao et al. BMC Infectious Diseases (2019) 19:508
https://doi.org/10.1186/s12879-019-4093-1

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

In vitro activities of Eravacycline against 336 isolates collected from 2012 to 2016 from 11 teaching hospitals in China

Chunjiang Zhao, Xiaojuan Wang, Yawei Zhang, Ruobing Wang, Qi Wang, Henan Li and Hui Wang*

Abstract

Background: In China multidrug-resistant bacteria pose a considerable threat to public health. Antimicrobial resistance has weakened the effectiveness of many medicines widely used today. Thus, discovering new antibacterial drugs is paramount in the effort to treat emerging drug-resistant bacteria.

Methods: Eravacycline, tigecycline and other clinical routine antibiotics were tested by reference broth microdilution method against 336 different strains collected from 11 teaching hospitals in China between 2012 and 2016. These isolates included *Enterobacteriaceae*, non-fermentative, *Staphylococcus* spp., *Enterococcus,* and a number of fastidious organisms. The strains involved in this study possess the most important drug resistance characteristics currently known in China. Drug resistant bacteria such as those producing extended spectrum β-lactamases (ESBL) and carbapenemases (KPC-2 and NDM-1), and those exhibiting colistin resistance (*mcr*-1) and tigecycline were included in this study. Additionally, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE), β-lactamase positive *Haemophilus influenzae*, and penicillin resistant *Streptococcus pneumoniae* (PRSP) were also included.

Results: Eravacycline exhibited good efficacy against all the strains tested, especially for organisms with ESBLs, carbapenemases, and *mcr*-1 gene compared with tigecycline and other antibiotics tested. The MIC values of eravacycline against carbapenemase producing *Enterobacteriaceae* and OXA-23-producing *A. baumannii* were much lower than the MIC values of other antibiotics. MRSA, VRE, β-lactamase positive *Haemophilus influenza*, and PRSP were sensitive to eravacycline in every strain tested. Furthermore, in most strains tested, the MICs of eravacycline were two to four-fold lower than the MICs of tigecycline.

Conclusions: Eravacycline has shown potent antibacterial activity against common and clinically important antibiotic-resistant pathogens. The MIC distribution of eravacycline was generally lower than that of tigecycline which demonstrates that this new drug is potentially more effective than the existing medications.

Keywords: Eravacycline, Tigecycline, Carbapenem resistant *Enterobacteriaceae* bacteria, *Acinetobacter baumannii*, Antibiotic resistance

* Correspondence: whuibj@163.com
Department of Clinical Laboratory, Peking University People's Hospital, Beijing 100044, China

Background

In China, microbial resistance to presently administered antimicrobial agents is increasing steadily owing to the emergence of novel resistance mechanisms in the microbes [1, 2]. Multidrug-resistant bacterium causes a considerable threat to public health. Antimicrobial resistance weakened the effectiveness of many medicines widely used today [3]. Thus discovering new antibacterial drugs are required to combat the threat of these emerging resistant bacteria. Eravacycline (TP-434 or 7-fluoro-9-pyrrolidinoacetamido-6-demethyl-6-deoxytetracycline) is a novel broad-spectrum synthetic tetracycline antibiotic being developed for the treatment of severe life-threatening infections, including those that are resistant to current broad-spectrum antibiotics [4]. Eravacycline has already been proven effective against some clinically important antibiotic-resistant pathogens, including gram-positive and gram-negative aerobic and anaerobic pathogens [5, 6]. Moreover, eravacycline was found to be safer and more effective than carbapenems in patients with complicated intra-abdominal infection (cIAI) during global phase 3 clinical trials (NCT01844856 and NCT02784704) [5, 7]. Additionally, there is a clinical development plan in place to introduce it into China to address bacterial drug resistance. The targets of eravacycline include complicated intra-abdominal infection (cIAI), complicated urinary tract infection (cUTI), and pulmonary infections caused by other susceptible pathogens. Tigecycline is a relatively new competing drug for eravacycline, imipenem, meropenem, and colistin in the treatment of carbapenem-resistant *Enterobacteriaceae.* The present study was designed to evaluate the in vitro activities of eravacycline against panels of clinical bacterial pathogens, with or without remarkable resistance factors, which were collected in recent years and were similar to pathogenic bacteria that this drug was designed to treat. This study was designed to prove the in-vitro efficacy of eravacycline (presented by minimum inhibitory concentration, MIC) against major target pathogens in China, which will be used to support further clinical development of eravacycline within China.

Methods

In the present study, a total of 336 different clinical isolates, were routinely collected from 11 teaching hospitals representing the south, north, northwest, east, and middle regions of mainland China between 2012 and 2016, and tested (list of the hospitals can be found in Additional file 1). After re-identification with the typical biochemical reaction of each organism, the strains were stored in a Microbank tube and placed in a refrigerator at − 80 degrees Celsius before test. All organisms and their associated drug resistance factors are detailed in Table 1. MIC measurements were performed via the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) M7-A9 (2012) [8]. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were utilized as quality controls in MIC testing of gram-negative bacteria. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were utilized as quality controls in MIC testing of gram-positive bacteria. *Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49247 and *Haemophilus influenzae* ATCC 49766 were used as quality controls during MIC testing of the fastidious organisms. Tigecycline, the major comparator for eravacycline, imipenem, meropenem and colistin to treat carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter baumannii*, were selected in the panel of antibiotics to be tested. We evaluated eravacycline with a gradient concentration of 0.002–16 mg/L against common clinical gram-negative bacilli, gram-positive cocci, and fastidious organisms collected from our previous studies [9–13], including *Enterobacteriaceae* (*Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae*), *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. Antibiotic solutions for susceptibility testing were freshly prepared according to the manual of CLSI [8]. A scatter plot of eravacycline versus tigecycline was drawn for each species of bacteria, to reveal the relationship between the two antibiotics in different organisms. All the results related to resistant genes were readily available, directly from our previous researches [12–14]. Statistical analyses and data visualization were done with R (version 3.4.4) and ggplot2 package (version 2.2.1).

Results

In vitro activity of eravacycline was evaluated against 336 strains of clinically significant species, with many exhibiting resistance factors (Table 1). In most of the strains tested, the MIC_{50} and MIC_{90} values for eravacycline were lower than that of tigecycline and other comparable antibiotics tested for each organism/phenotypic group. Furthermore, eravacycline was highly effective against all of the organisms tested, regardless of resistance factors.

For *Enterobacteriaceae* bacteria, the MIC values of eravacycline varied with the resistance characteristics, especially for *K. pneumoniae*. The MIC_{50} values of eravacycline against *E. cloacae* and *E. coli* were much lower than the values of other comparable drugs, especially in strains with resistance phenotypes (Table 2). For *K. pneumoniae*, the MIC distribution of eravacycline differed depending on the drug resistance features. *K. pneumoniae* strains which were ESBL-positive ($n = 10$),

Table 1 The strains involved in this study and antibiotic resistance characteristics of the strains

Group	Identification	Resistance features	Number
Enterobacteriaceae	*Klebsiella pneumoniae*	ESBL	10
		Tigecycline resistant	13
		kpc-2 positive	9
		NDM-1 positive	3
		mcr-1 positive	4
		Sensitive [a]	10
	Escherichia coli	ESBL	10
		mcr-1, NDM-5	5
		Carbapenem resistant	10
		Sensitive [a]	10
	Enterobacter cloacae	ESBL	6
		Carbapenem resistant	1
		Sensitive [a]	22
Non-fermentive	*Acinetobacter baumanii*	OXA-23 positive	21
		Tigecycline resistant	9
		Sensitive [a]	9
	Stenotrophomonas maltophilia	Sensitive [a]	29
Staphylococcus sp.	*Staphylococcus aureus*	MRSA	15
		MSSA	6
	Staphylococcus epidermidis	MRCoNS	10
		MSCoNS	10
	Staphylococcus haemolyticus	MRCoNS	8
		MSCoNS	1
	Staphylococcus hominis	MRCoNS	6
		MSCoNS	4
Enterococcus	*Enterococcus faecalis*	Sensitive [a]	10
	Enterococcus faecium	VRE	3
		Sensitive [a]	8
Fastidious	*Haemophilus influenzae*	β-lactamase negative	10
		β-lactamase positive	10
	Streptococcus pneumoniae	PRSP	10
		PSSP	10

[a]: Sensitive strains referred to strains do not have specific resistance characteristics such as ESBL, carbapenem resistance, polymyxin resistance and glycopeptide resistance

kpc-2-positive ($n = 9$) and NDM-1-positive ($n = 3$), had similar MIC distributions. The MIC_{50} value of eravacycline against strains with the above three resistance mechanisms is 0.5 mg/L, and the MIC90 values were 1 mg/L, 2 mg/L and 1 mg/L respectively.

K. pneumoniae strains resistant to tigecycline were susceptible to eravacycline at higher MIC_{50} values of 8 mg/L, while the MIC_{90} was equivalent to that of tigecycline at 16 mg/L. For *mcr-1* positive strains, the MIC_{50} of eravacycline was 1 mg/L compared with 16 mg/L for tigecycline, while the MIC_{90} of eravacycline and tigecycline was equivalent at 16 mg/L. The MIC_{50} (0.5 mg/L) and MIC_{90} (2 mg/L) values of eravacycline against carbapenem-resistant *K. pneumoniae,* were much lower than those of other antibiotics such as imipenem, meropenem, cephalosporins, and fluoroquinolones. The MIC distributions for *K. pneumoniae* of different resistant phenotypes to eravacycline, tigecycline, and other clinically common antibiotics are presented in Table 3.

MIC distributions for *A. baumannii* also varied by resistance characteristics. *A. baumannii* isolates were tigecycline resistant and showed slightly elevated MIC_{50} and MIC_{90} for eravacycline at 2 mg/L. OXA-23-producing *A. baumannii* isolates have a MIC_{50} of 1 mg/L and MIC_{90}

Table 2 MIC distribution of Eravacycline and relevant antibiotics against *E. coli* and *E. cloacae* of different resistance characteristics

Organism	Antibiotics	Carbapenem resistant [a]			ESBL			Sensitive [b]		
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
E.coli	Eravacycline	0.5	1	0.064–2	0.125	0.25	0.064–0.25	0.064	0.125	0.064–0.25
	Tigecycline	1	2	0.25–4	0.25	0.5	0.25–0.5	0.25	0.25	0.125–0.5
	Piperacillin/Tazobactam	256	256	2–256	2	8	1–256	1	2	0.5–2
	Cefoxitin	256	256	64–256	8	32	4–32	2	4	2–8
	Ceftazidime	256	256	0.5–256	32	64	16–128	0.064	0.25	0.064–0.25
	Cefoperazone/Sulbactam	256	256	8–256	16	32	8–256	0.25	1	0.064–4
	Ceftriaxone	256	256	2–256	256	256	64–256	0.032	0.064	0.016–0.064
	Cefotaxime	256	256	4–256	256	256	64–256	0.032	0.064	0.032–0.064
	Cefepime	64	256	0.25–256	32	64	8–128	0.016	0.032	0.016–0.064
	Ertapenem	32	32	16–32	0.125	0.25	0.016–1	0.016	0.016	0.016–0.016
	Imipenem	8	32	8–64	0.125	0.125	0.125–1	0.125	0.125	0.064–0.125
	Meropenem	8	32	4–32	0.032	0.064	0.016–0.064	0.016	0.016	0.016–0.016
	Amikacin	4	256	0.5–256	2	4	1–8	2	2	1–4
	Minocycline	8	16	0.5–16	1	8	0.5–16	1	2	0.5–8
	Ciprofloxacin	64	64	0.064–64	32	64	0.25–64	4	32	0.016–32
	Levofloxacin	16	64	0.125–128	16	32	0.5–64	8	8	0.032–16
	Moxifloxacin	16	32	0.5–64	16	32	0.5–64	8	16	0.032–16
E.cloacae	Eravacycline	0.5	0.5	0.5–0.5	0.25	0.5	0.125–0.5	0.5	0.5	0.125–1
	Tigecycline	2	2	2–2	1	1	0.125–2	0.5	2	0.5–2
	Piperacillin/Tazobactam	256	256	256–256	4	4	2–8	2	64	0.5–256
	Cefoxitin	256	256	256–256	8	32	4–256	256	256	64–256
	Ceftazidime	256	256	256–256	16	64	16–256	0.25	64	0.064–256
	Cefoperazone/Sulbactam	32	32	32–32	8	16	4–32	0.125	32	0.016–256
	Ceftriaxone	256	256	256–256	64	128	16–256	0.125	128	0.016–256
	Cefotaxime	256	256	256–256	64	128	16–256	0.125	256	0.016–256
	Cefepime	256	256	256–256	8	8	1–32	0.032	8	0.016–128
	Ertapenem	32	32	32–32	0.032	0.064	0.016–0.125	0.032	0.5	0.016–16
	Imipenem	32	32	32–32	0.25	0.25	0.125–0.25	0.25	1	0.125–2
	Meropenem	32	32	32–32	0.016	0.032	0.016–0.032	0.032	0.064	0.016–4
	Amikacin	256	256	256–256	1	2	1–8	1	2	0.5–256
	Minocycline	4	4	4–4	4	4	2–8	2	4	1–64
	Ciprofloxacin	64	64	64–64	2	32	0.25–64	0.032	4	0.016–64
	Levofloxacin	4	4	4–4	1	8	0.5–16	0.064	4	0.032–16
	Moxifloxacin	8	8	8–8	2	16	1–16	0.125	4	0.032–16

[a]: Of the 15 carbapenem resistant *E.coli*, 5 strains harbored mcr-1 and NDM-5 simultaneously
[b]: Sensitive strains referred to strains do not have ESBL and carbapenem resistance

of 2 mg/L for eravacycline, and these values were much lower than the MIC_{50} and MIC_{90} of tigecycline (4 mg/L, 4 mg/L), imipenem (64 mg/L, 64 mg/L), and meropenem (32 mg/L, 64 mg/L). The MIC distributions for *A. baumannii* with different resistant phenotypes to eravacycline, tigecycline, and other clinically relevant antibiotics such as imipenem, meropenem, and colistin are presented in Table 4.

For *S. maltophilia* there is no breakpoints available for tigecycline, the MIC distributions of tigecycline and eravacycline against *S. maltophilia* were evaluated. The MIC_{50} and MIC_{90} for eravacycline were both 1 mg/L, at the same time the MIC_{50} and MIC_{90} for tigecycline were 0.5 mg/L and 1 mg/L.

For *Staphylococcus* spp., the results indicated that MIC_{50} and MIC_{90} of eravacycline were 0.25 mg/L and 0.5 mg/L,

Table 3 MIC distribution of eravacycline and relevant antibiotics against *K. pneumoniae* of different resistance characteristics

Antibiotics	Sensitive, n=10			ESBL, n=10			*kpc*-2 positive, n=9			NDM-1 positive, n=3			*mcr*-1 positive, n=4			Tigecycline resistant, n=13		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Eravacycline	0.25	0.5	0.125-0.5	0.5	1	0.125-2	0.5	2	0.25-4	0.5	1	0.5-1	1	16	0.5-16	8	16	2-16
Tigecycline	0.5	1	0.5-2	1	4	0.5-4	1	4	0.125	1	2	1--2	16	16	2-16	8	16	8-16
Piperacillin/Tazobactam	2	4	2-4	4	256	2-256	256	256	256-256	256	256	256-256	4	4	4-4	16	32	4-32
Cefoxitin	4	8	2-16	8	16	2-32	256	256	64-256	256	256	256-256	8	8	2-8	32	64	8-128
Ceftazidime	0.125	0.25	0.125-0.25	64	256	16-256	64	256	32-256	256	256	256-256	1	1	0.125-1	1	64	0.5-64
Cefoperazone/Sulbactam	0.25	0.25	0.125-0.25	16	64	8-64	256	256	256-256	256	256	256-256	1	1	0.5-1	2	32	1-128
Ceftriaxone	0.064	0.064	0.032-0.125	256	256	64-256	256	256	16-256	256	256	256-256	0.064	0.125	0.032-0.125	0.25	256	0.064-256
Cefotaxime	0.032	0.125	0.032-0.125	256	256	64-256	256	256	32-256	256	256	256-256	0.125	0.125	0.032-0.125	0.5	128	0.125-256
Cefepime	0.032	0.064	0.032-0.064	32	64	4-128	64	256	32-256	128	256	128-256	2	2	0.032-2	2	64	0.125-64
Ertapenem	0.016	0.016	0.016-0.016	0.25	0.5	0.032-0.5	32	32	32-32	32	32	32-32	0.016	0.016	0.016-0.016	0.032	0.25	0.016-0.5
Imipenem	0.125	0.25	0.125-1	0.125	0.25	0.125-0.25	8	32	8-32	8	32	8-32	0.125	0.25	0.125-0.25	0.125	0.125	0.125-0.5
Meropenem	0.016	0.032	0.016-0.032	0.032	0.064	0.032-0.125	16	32	8-32	16	32	8-32	0.032	0.064	0.032-0.064	0.032	0.064	0.016-0.064
Colistin	0.25	0.25	0.125-0.25	0.25	0.25	0.125-0.25	0.25	0.25	0.125-0.25	0.25	0.25	0.125-0.25	32	64	16-64	0.25	32	0.125-32
Amikacin	1	1	0.5-1	1	4	0.5-32	1	256	0.5-256	2	2	1--2	1	1	1-1	1	2	0.5-256
Minocycline	2	4	2-8	16	32	2-32	32	32	4-32	32	32	4-32	16	32	16-32	32	128	16-256
Ciprofloxacin	0.016	0.032	0.016-0.25	2	64	0.016-64	32	64	16-64	64	64	64-64	32	32	0.032-32	32	64	0.25-64
Levofloxacin	0.064	0.125	0.064-0.5	2	16	0.064-64	16	64	16-64	32	32	16-32	16	16	0.064-16	8	32	0.5-64

[a]: Sensitive strains referred to strains do not have ESBL, carbapenem resistance and polymyxin resistance

Table 4 MIC distribution of Eravacycline and relevant antibiotics against *A. baumannii* of different resistance characteristics

Antibiotics	Sensitive [a], n = 9			OXA-23 positive, *n* = 21			Tigecycline resistant, n = 9		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Eravacycline	0.125	0.25	0.016–0.25	1	2	0.5–2	2	2	2–4
Tigecycline	0.25	0.5	0.25–0.5	4	4	4–8	8	8	8–8
Piperacillin/Tazobactam	2	4	0.016–8	256	256	256–256	256	256	256–256
Ceftazidime	2	8	0.125–32	256	256	64–256	256	256	256–256
Cefepime	1	4	0.032–32	64	256	32–256	256	256	128–256
Imipenem	0.125	1	0.125–1	64	64	16–64	64	64	64–128
Meropenem	0.032	1	0.016–1	32	64	16–64	64	64	32–128
Colistin	0.125	0.25	0.125–0.25	0.25	0.25	0.125–0.25	0.25	0.25	0.25–0.25
Amikacin	4	4	1–4	256	256	256–256	256	256	256–256
Minocycline	0.125	16	0.064–16	8	16	4–16	8	8	8–16
Ciprofloxacin	0.125	0.5	0.032–32	32	32	32–32	32	32	32–32
Levofloxacin	0.125	1	0.064–32	16	32	8–32	16	16	16–32

[a]: Sensitive strains referred to strains do not have carbapenem resistance and tigecycline resistance

respectively, for MRSA (methicillin-resistant *S. aureus*), for MSSA (methicillin-sensitive *S. aureus*) the MIC_{50} of eravacycline was as low as 0.064 mg/L, and MIC_{90} remained the same as that of MRSA. MIC_{50} and MIC_{90} of eravacycline for methicillin-resistant coagulase-negative *staphylococci* (MRCoNS) were 0.25 mg/L and 1 mg/L, respectively, and for MSCoNS (methicillin-sensitive coagulase-negative *staphylococci*) the values of eravacycline were lower at 0.016 mg/L and 0.25 mg/L, respectively. For other antibiotics, the values are presented in Table 5.

In the results obtained for *Enterococcus* spp. it was found that MIC_{50} and MIC_{90} of eravacycline for *E. faecalis* were both 0.032 mg/L. The MIC_{50} and MIC_{90} of eravacycline for *E. faecium* were 0.016 mg/L and 0.032 mg/L. For Vancomycin-Resistant *Enterococci* (VRE) strains, the MIC_{50} and MIC_{90} were identical with that of vancomycin-susceptible *E. faecium* strains. For other antibiotics, the values are presented in Table 6. In general, for gram-positive bacteria with varying resistance factors, eravacycline demonstrated substantial antibacterial activity.

Table 5 MIC distribution of Eravacycline and relevant antibiotics against *Staphylococcus. spp* of different resistance characteristics

Antibiotics	MRSA[a], *N* = 15		MSSA[b], *N* = 6			MRCoNS[c], *N* = 24				MSCoNS[d], N = 15		
MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}		Range	MIC_{50}	MIC_{90}	Range
Eravacycline	0.25	0.5	0.032–1	0.064	0.5	0.016–2	0.25	1	0.016–2	0.016	0.25	0.008–0.25
Tigecycline	0.25	0.5	0.125–0.5	0.25	0.25	0.125–0.25	0.25	0.5	0.125–0.5	0.125	0.25	0.064–0.25
Oxacillin	64	64	2–64	0.25	0.5	0.25–0.5	2	64	0.5–256	0.125	0.25	0.125–0.25
Cefoxitin	256	256	32–256	4	4	2–4	16	256	2–256	2	8	1–8
Vancomycin	1	1	0.5–1	0.5	0.5	0.5–0.5	1	2	0.5–2	0.5	1	0.25–1
Teicoplanin	2	2	0.5–2	0.5	0.5	0.5–1	2	4	0.064–8	0.5	2	0.125–2
Erythromycin	256	256	0.25–256	256	256	0.25–256	64	256	0.125–256	0.25	256	0.064–256
Minocycline	4	16	0.064–32	0.064	0.125	0.064–0.125	0.25	0.5	0.064–8	0.125	0.25	0.064–0.5
Ciprofloxacin	64	64	0.25–64	0.5	0.5	0.25–0.5	16	64	0.125–64	0.25	8	0.125–64
Levofloxacin	32	64	0.25–64	0.25	0.25	0.125–0.5	4	128	0.25–128	0.25	0.5	0.125–128
Moxifloxacin	8	16	0.016–32	0.032	0.064	0.016–0.064	1	16	0.064–32	0.064	1	0.032–16
Trimethoprim/Sulfamethoxazole	0.125	16	0.032–16	0.032	0.064	0.032–0.25	4	32	0.064–64	0.125	4	0.016–4
Chloramphenicol	8	8	4–32	8	8	4–64	4	8	2–64	4	4	2–8
Rifampin	256	256	0.004–256	0.008	0.016	0.004–0.016	0.008	256	0.004–256	0.008	0.016	0.004–0.016
Clindamycin	128	256	0.064–256	0.064	256	0.064–256	0.125	256	0.064–256	0.064	0.125	0.064–0.25
Linezolid	1	2	0.5–2	1	2	1–2	1	1	0.5–1	1	1	0.5–2

[a] Methicillin-resistant *Staphylococcus aureus*. [b] Methicillin- sensitive *Staphylococcus aureus*
[c] Methicillin-resistant coagulase-negative *staphylococci*. [d] Methicillin- sensitive coagulase-negative *staphylococci*

Table 6 MIC distribution of Eravacycline and relevant antibiotics against *Enterococci. spp* of different resistance characteristics

Antibiotics	*E.faecalis*, *n* = 10			*E.faecium*, *n* = 8			VRE[a], n = 3		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Eravacycline	0.032	0.032	0.016–0.125	0.016	0.032	0.008–0.064	0.016	0.032	0.008–0.032
Tigecycline	0.064	0.064	0.064–0.125	0.064	0.064	0.016–0.125	0.125	0.25	0.125–0.25
Ampicillin	1	8	1–8	64	64	4–64	64	64	64–64
Vancomycin	1	2	0.5–2	0.5	1	0.25–1	128	128	128–128
Teicoplanin	0.125	0.25	0.032–0.25	0.25	0.25	0.064–0.25	32	64	32–64
Erythromycin	1	256	0.25–256	256	256	0.016–256	0.125	256	0.125–256
Minocycline	16	16	0.064–16	0.032	16	0.032–16	0.064	16	0.064–16
Ciprofloxacin	2	32	0.5–64	64	64	4–64	64	64	64–64
Levofloxacin	2	64	1–64	64	128	1–128	64	64	64–64
Linezolid	1	2	1–2	1	1	0.5–1	1	1	1–1

[a] VRE referred to vancomycin-resistant *Enterococci*. All of the 3 VRE strains in this study were *E.faecium*

For fastidious strains, including 20 *S. pneumoniae* isolates and 20 *H. influenzae* isolates, eravacycline showed high antimicrobial activities against *S. pneumoniae* with MIC_{50} (0.008 mg/L) and MIC_{90} (0.008 mg/L), there was no difference with eravacycline distribution between PRSP (Penicillin-resistant *S. pneumoniae*) and PSSP (Penicillin-sensitive *S. pneumoniae*) strains (Table 7). For *H. influenzae* the MIC_{50} and MIC_{90} were 0.064 mg/L and 0.125 mg/L, and they were the same in both β-lactamase-positive and β-lactamase-negative strains (Table 8).

A jittered scatter plot was drawn using the MIC values of eravacycline and tigecycline involving all the strains tested. A clear pattern was found showing that most of the MIC values of tigecycline are higher than the corresponding MIC values of eravacycline (in many cases by 2 to 4 fold). For all of the clinical isolates tested, except for *Staphylococcus* spp. and *S. maltophilia,* more points are located above the diagonal y = x line, suggesting that eravacycline has lower MIC distribution than tigecycline (Fig. 1). For *Staphylococcus* spp. and *S. maltophilia* the points were distributed on both sides of the diagonal

Table 7 MIC distribution of Eravacycline and relevant antibiotics against *S.pneumoniae* of different resistance characteristics

Antibiotics	PSSP[a], n = 10			PRSP[b], n = 10		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Eravacycline	0.008	0.008	0.002–0.016	0.008	0.008	0.004–0.008
Tigecycline	0.016	0.016	0.008–0.016	0.016	0.016	0.016–0.016
Penicillin	0.016	0.016	0.016–0.032	4	4	4–4
Amoxicillin/Clavulanic acid	0.016	0.064	0.008–0.25	8	8	8–8
Cefuroxime	0.032	0.125	0.016–0.5	16	32	8–32
Cefaclor	1	2	1–4	256	256	128–256
Ceftriaxone	0.032	0.064	0.016–0.125	2	8	1–8
Erythromycin	8	32	0.5–256	256	256	128–256
Azithromycin	16	32	4–256	256	256	256–256
Clindamycin	0.125	128	0.032–256	256	256	128–256
Clarithromycin	2	32	0.25–256	256	256	256–256
Levofloxacin	1	1	0.25–32	1	1	1–1
Moxifloxacin	0.125	0.125	0.064–16	0.125	0.25	0.125–0.25
Trimethoprim/Sulfamethoxazole	4	8	0.064–8	8	16	4–32
Tetracycline	32	64	4–64	32	32	32–32
Chloramphenicol	4	8	1–16	4	4	4–4
Vancomycin	0.25	0.25	0.125–0.25	0.25	0.25	0.25–0.25

[a] *PSSP* Penicillin-sensitive *Streptococcus pneumoniae*
[b] *PRSP* Penicillin-resistant *Streptococcus pneumoniae*

Table 8 MIC distribution of Eravacycline and relevant antibiotics against *H. influenza* of different resistance characteristics

Antibiotics	β-lactamases negative, n = 10			β-lactamases positive, n = 10		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Eravacycline	0.064	0.125	0.064–0.125	0.064	0.125	0.032–0.125
Tigecycline	0.25	0.5	0.125–0.5	0.125	0.25	0.064–0.5
Ampicillin	0.125	0.5	0.125–1	16	64	0.064–64
Amoxicillin/Clavulanic acid	0.125	0.5	0.125–0.5	1	1	0.5–1
Penicillin	16	32	0.032–32	16	32	1–64
Cefaclor	2	8	0.5–8	4	16	1–32
Cefuroxime	1	2	0.25–4	1	4	0.25–16
Azithromycin	1	4	0.064–4	2	64	0.25–64
Clarithromycin	4	16	0.5–16	4	64	1–64
Levofloxacin	0.032	1	0.016–1	0.032	0.125	0.016–0.5
Moxifloxacin	0.032	1	0.016–1	0.032	0.25	0.016–0.5
Trimethoprim/Sulfamethoxazole	16	32	0.032–32	16	32	1–64
Tetracycline	1	4	0.064–4	2	64	0.25–64
Chloramphenicol	0.5	1	0.25–1	1	8	0.5–8

evenly, suggesting a comparable MIC distribution between eravacycline and tigecycline.

Discussion

As resistance to antibiotics grows worldwide, it becomes increasingly important to find new treatments for bacterial infections. In the present study, a new antibiotic eravacycline was compared to existing medications. Eravacycline demonstrated high in vitro activity against clinical isolates, including strains with specific resistant factors. Eravacycline was compared to a derivative of tigecycline, and in most cases presented with a lower MIC distribution for the majority of strains tested in this study. Since many years nosocomial pathogens, such as *Enterobacteriaceae* which are responsible for complicated intra-abdominal infection (cIAI) were increasing in frequency [15]. Moreover, cases of gram-positive cocci such as *S. aureus*, coagulase-negative *staphylococci,* and *enterococci,* the major causative organisms of complicated urinary tract infections (cUTI) were also increasing [16]. The emergence of multiple drug-resistant bacteria, such as Carbapenem resistant *Enterobacteriaceae* bacteria (CRE), Carbapenem-resistant *Acinetobacter baumannii* (CRAB) and Methicillin-resistant *Staphylococcus aureus* (MRSA), has compounded this problem significantly by increasing the difficulty of treatment, the proportion of failures, as well as the mortality rate of patients. Since Tigecycline and eravacycline belong to a different antibiotic class with a mechanism of action distinct from cephalosporins and carbapenem antibiotics, they can evade established resistance mechanisms of *Enterobacteriaceae* and exhibit higher efficacy against resistant bacteria. In this study, eravacycline showed high antibacterial activity against CRE strains, suggesting that eravacycline could be useful to treat complicated infections caused by CRE. Similarly, CRAB also shows resistance to antibiotics which were commonly used during the clinical practice. CRAB is the most notorious pathogen responsible for nosocomial infections in China at present [17–19]. This study found that the most effective drug for OXA-23 producing *A. baumannii* was colistin then eravacycline. Eravacycline also demonstrated high potency against OXA-23 producing *A. baumannii*, with a MIC_{50} of 1 mg/L which was much lower than other antibiotics, except for colistin. Similar to eravacycline in structure and mechanism, tigecycline has been widely utilized in China for many years, and tigecycline-resistant strains have also emerged with the increase in use of this antibiotic [20, 21]. In the present study, eravacycline also exhibited lower MIC distribution compared with tigecycline in tigecycline-resistant strains, suggesting that the mechanism which leads to tigecycline resistance does not inhibit the activity of eravacycline. Furthermore, high antibiotic potency against CRE and CRAB could make eravacycline a potential option to treat complex infections including respiratory and bloodstream infections. For *Staphylococcus* spp. the results were entirely different, with tigecycline values much lower than eravacycline. From the scatter plot we observed that the points are evenly distributed on both sides of the diagonal line (line: $y = x$). This may be either due to the combined effects of different resistance mechanisms, or potentially unknown resistance mechanisms. In addition, the total number of *Staphylococcus* spp. strains which were tested in this study was relatively small, which may cause random errors in the antibacterial activity of eravacycline. Thus, further validation utilizing different

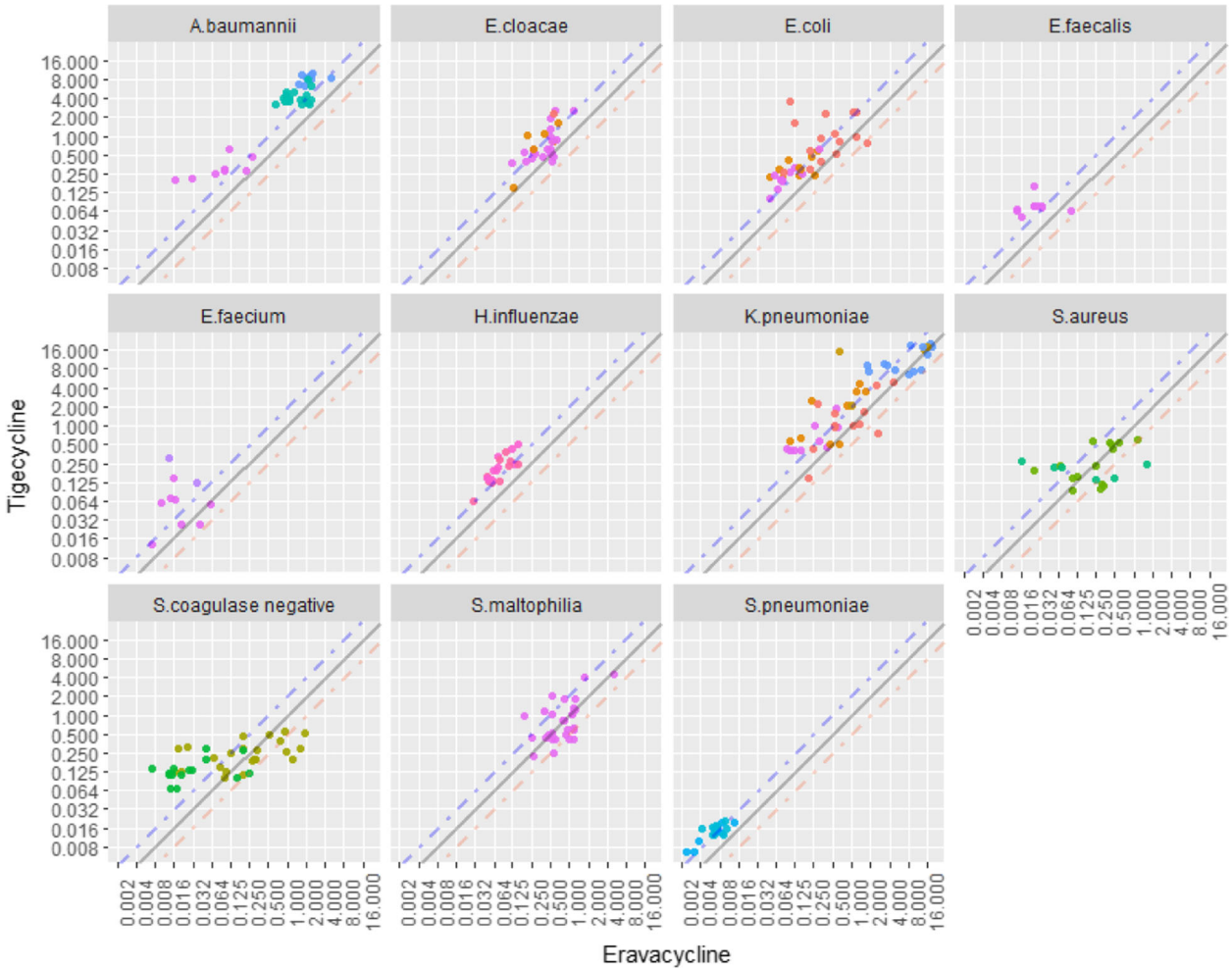

Fig. 1 Scatter plot of MIC values of tigecycline versus MIC value of eravacycline against various bacteria. Note: A tiny displacement was made to the points in this figure in order to avoid overlapping of the strains with the same eravacycline and tigecycline MIC values. This tiny displacement can ensure the actual distribution of all strains visible. The points on the grey solid line indicated the strains shared the identical eravacycline and tigecycline MIC values. The points above the blue dash line indicated that the MIC values of tigecycline were greater than twice than the MIC values of eravacycline. The points below the orange dash line indicated that the MIC values of eravacycline were greater than twice than the MIC

bacterial isolates is required. For fastidious strains, eravacycline demonstrated excellent potency despite resistance characteristics of the strains. From the scatter plot, we can see that although MIC values of eravacycline were generally lower than those of tigecycline, the MIC values of eravacycline were also rising with the MIC values of tigecycline proportionally, thus, we need to be alert to the possible cross-resistance potential of eravacycline and tigecycline, especially in strains with higher MIC values of tigecycline.

Limitation and suggestion

The clinical isolates tested were limited by country as they were exclusively collected in China and within this country, these isolates were only obtained from 11 teaching hospitals. No strains from other hospitals were utilized. Therefore, many different clinical isolates remain untested. Thus, it is important that researchers reproduce our work in other countries with different isolates in order to understand the full spectrum of this new antibiotics' efficacy. The results of this study show

that eravacycline has a positive application potential for the treatment of current drug-resistant bacterial infections. Considering the relatively small number of each organism and limited types of resistant phenotypes, the result of this study only partially represent the resistant phenotype encountered in real clinical practice, and additional studies are needed for a more comprehensive assessment of the antibacterial activity of eravacycline.

Conclusions

The results of this study proved that eravacycline possesses a broad spectrum of activity against a variety of gram-positive and gram-negative bacteria, including multi-drug resistant strains such as *A. baumannii* and carbapenem-resistant *Enterobacteriaceae.*

Additional file

Additional file 1: The list of committee and the institute to which it belongs for all hospitals that provided Administrative Consent to access or receive samples. This additional file list the committee (and the institute to which it belongs) for all hospitals that provided Administrative Consent to access or receive samples/data (DOCX 13 kb)

Abbreviations

CLSI: Clinical and Laboratory Standards Institute; CRAB: Carbapenem resistant *Acinetobacter baumannii*; CRE: Carbapenem resistant *Enterobacteriaceae*; cUTI: complicated urinary tract infections; ESBL: extended-spectrum-lactamases; MIC: minimum inhibitory concentration; MRSA: methicillin-resistant *Staphylococcus aureus*; MSCoNS: Methicillin- sensitive coagulase-negative *staphylococci*; PCR: polymerase chain reaction; PRSP: penicillin resistant *Streptococcus pneumoniae*; VRE: Vancomycin-resistant *enterococci*

Acknowledgements

Not Applicable.

Authors' contributions

HW, CZ conceived and designed experiments. CZ, XW, YZ, RW, QW and HL performed antibiotic susceptibility testing. HW, CZ wrote the manuscript. CZ performed the data processing and data visualization. All authors read and approved the final manuscript.

Funding

No funding was obtained for this study.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Study protocols were reviewed and granted by the Ethical Committee of Peking University People's Hospital (No. 2017PHB163). For the hospitals participated, administrative permissions to access the raw samples were granted by the Research Department of the hospitals participated.

Consent for publication

Not applicable as no human subjects.

Competing interests

The authors declare that they have no competing interests.

Received: 12 September 2018 Accepted: 15 May 2019
Published online: 10 June 2019

References

1. Yang Y, Song W, Lin H, Wang W, Du L, Xing W. Antibiotics and antibiotic resistance genes in global lakes: a review and meta-analysis. Environ Int. 2018;116:60–73. https://doi.org/10.1016/j.envint.2018.04.011.
2. Qiao M, Ying G-G, Singer AC, Zhu Y-G. Review of antibiotic resistance in China and its environment. Environ Int. 2018;110:160–72. https://doi.org/10.1016/j.envint.2017.10.016.
3. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48:1–12. https://doi.org/10.1086/595011.
4. Grossman TH, Murphy TM, Slee AM, Lofland D, Sutcliffe JA. Eravacycline (TP-434) is efficacious in animal models of infection. Antimicrob Agents Chemother. 2015;59:2567–71. https://doi.org/10.1128/AAC.04354-14.
5. Solomkin JS, Ramesh MK, Cesnauskas G, Novikovs N, Stefanova P, Sutcliffe JA, et al. Phase 2, randomized, double-blind study of the efficacy and safety of two dose regimens of eravacycline versus ertapenem for adult community-acquired complicated intra-abdominal infections. Antimicrob Agents Chemother. 2014;58:1847–54. https://doi.org/10.1128/AAC.01614-13.
6. Sutcliffe JA, O'Brien W, Fyfe C, Grossman TH. Antibacterial activity of Eravacycline (TP-434), a novel Fluorocycline, against hospital and community pathogens. Antimicrob Agents Chemother. 2013;57:5548–58. https://doi.org/10.1128/AAC.01288-13.
7. Solomkin J, Evans D, Slepavicius A, Lee P, Marsh A, Tsai L, et al. Assessing the efficacy and safety of Eravacycline vs Ertapenem in complicated intra-abdominal infections in the investigating gram-negative infections treated with Eravacycline (IGNITE 1) trial: a randomized clinical trial. JAMA Surg. 2017;152:224–32. https://doi.org/10.1001/jamasurg.2016.4237.
8. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests f or Bacteria That Grow Aerobically; Approved St andard. 9th ed. CLSI doc M07-A9. Wayne: Clin Lab Stand Inst; 2012.
9. Zhang Y, Zhao C, Wang Q, Wang X, Chen H, Li H, et al. High prevalence of Hypervirulent Klebsiella pneumoniae infection in China: geographic distribution, clinical characteristics, and antimicrobial resistance. Antimicrob Agents Chemother. 2016;60:6115–20. https://doi.org/10.1128/AAC.01127-16.
10. Wang X, Chen H, Zhang Y, Wang Q, Zhao C, Li H, et al. Genetic characterisation of clinical Klebsiella pneumoniae isolates with reduced susceptibility to tigecycline: role of the global regulator RamA and its local repressor RamR. Int J Antimicrob Agents. 2015;45:635–40. https://doi.org/10.1016/j.ijantimicag.2014.12.022.
11. Zhang Y, Zeng J, Liu W, Zhao F, Hu Z, Zhao C, et al. Emergence of a hypervirulent carbapenem-resistant Klebsiella pneumoniae isolate from clinical infections in China. J Inf Secur. 2015;71:553–60. https://doi.org/10.1016/j.jinf.2015.07.010.
12. Wang X, Zhang F, Zhao C, Wang Z, Nichols WW, Testa R, et al. In vitro activities of ceftazidime-avibactam and aztreonam-avibactam against 372 gram-negative bacilli collected in 2011 and 2012 from 11 teaching hospitals in China. Antimicrob Agents Chemother. 2014;58:1774–8. https://doi.org/10.1128/AAC.02123-13.
13. Wang X, Xu X, Li Z, Chen H, Wang Q, Yang P, et al. An outbreak of a nosocomial NDM-1-producing Klebsiella pneumoniae ST147 at a teaching hospital in mainland China. Microb Drug Resist. 2014;20:144–9. https://doi.org/10.1089/mdr.2013.0100.
14. Wang Q, Wang X, Wang J, Ouyang P, Jin C, Wang R, et al. Phenotypic and Genotypic Characterization of Carbapenem-resistant Enterobacteriaceae: Data From a Longitudinal Large-scale CRE Study in China (2012–2016). Clin Infect Dis. 2018;67(suppl_2):S196–205. https://doi.org/10.1093/cid/ciy660.
15. Sartelli M, Catena F, Ansaloni L, Coccolini F, Corbella D, Moore EE, et al. Complicated intra-abdominal infections worldwide: the definitive data of the CIAOW study. World J Emerg Surg. 2014;9:37. https://doi.org/10.1186/1749-7922-9-37.
16. Pallett A, Hand K. Complicated urinary tract infections: practical solutions for the treatment of multiresistant gram-negative bacteria. J Antimicrob Chemother. 2010;65(Suppl 3):iii25–33. https://doi.org/10.1093/jac/dkq298.
17. Wang J, Hu J, Harbarth S, Pittet D, Zhou M, Zingg W. Burden of healthcare-associated infections in China: results of the 2015 point prevalence survey in dong Guan City. J Hosp Infect. 2017;96:132–8. https://doi.org/10.1016/j.jhin.2017.02.014.

18. Gong Y, Shen X, Huang G, Zhang C, Luo X, Yin S, et al. Epidemiology and resistance features of Acinetobacter baumannii isolates from the ward environment and patients in the burn ICU of a Chinese hospital. J Microbiol. 2016;54:551–8. https://doi.org/10.1007/s12275-016-6146-0.
19. Yuan X, Liu T, Wu D, Wan Q. Epidemiology, susceptibility, and risk factors for acquisition of MDR/XDR gram-negative bacteria among kidney transplant recipients with urinary tract infections. Infect Drug Resist. 2018;11:707–15. https://doi.org/10.2147/IDR.S163979.
20. Deng M, Zhu M-H, Li J-J, Bi S, Sheng Z-K, Hu F-S, et al. Molecular epidemiology and mechanisms of tigecycline resistance in clinical isolates of Acinetobacter baumannii from a Chinese university hospital. Antimicrob Agents Chemother. 2014;58:297–303. https://doi.org/10.1128/AAC.01727-13.
21. Du X, He F, Shi Q, Zhao F, Xu J, Fu Y, et al. The rapid emergence of Tigecycline resistance in blaKPC-2 harboring Klebsiella pneumoniae, as mediated in vivo by mutation in tetA during Tigecycline treatment. Front Microbiol. 2018;9:648. https://doi.org/10.3389/fmicb.2018.00648.

Publisher's Note