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In vitro activity of a novel aminomethylcycline antibacterial (KBP-7072), a third-generation tetracycline, against clinical isolates with molecularly characterized tetracycline resistance mechanisms

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Objectives: This study evaluated the *in vitro* activity of KBP-7072 against 413 contemporary surveillance isolates, including subsets with known tetracycline resistance genes.

Materials: In total, 105 *Klebsiella pneumoniae* (51 tetracycline resistant), 103 *Escherichia coli* (52 tetracycline resistant), 103 *Staphylococcus aureus* (51 tetracycline resistant) and 102 *Streptococcus pneumoniae* (51 tetracycline resistant) isolates were included. These isolates were tested by broth microdilution using fresh media. CLSI/EUCAST breakpoints were applied, except for tigecycline and omadacycline, which used FDA criteria.

Results: KBP-7072 (MIC_{50}, 0.06 mg/L), tigecycline (MIC_{50}, 0.12 and 0.25 mg/L) and omadacycline (MIC_{50}, 0.12 and 0.5 mg/L) showed similar MIC_{50}s for tetracycline-susceptible and -resistant *S. aureus*. Other tetracycline comparators had their MIC_{50} increased 64- to 256-fold by *tet*. For *S. pneumoniae*, KBP-7072 ($MIC_{50/90}$, ≤0.015/0.03 mg/L) showed the lowest MICs, which remained unchanged for tetracycline-susceptible or -resistant isolates [mostly *tet*(M)]. Similar MICs were observed for omadacycline ($MIC_{50/90}$, 0.03–0.06/0.06 mg/L) and tigecycline ($MIC_{50/90}$, 0.03/0.03 mg/L) in the *S. pneumoniae* population. Tetracycline-susceptible and -resistant *E. coli* [94.2% *tet*(A)/*tet*(B)], KBP-7072 (MIC_{90}, 0.25 and 1 mg/L, respectively) and tigecycline (MIC_{90}, 0.25 and 0.5 mg/L) showed similar MIC_{90}s. KBP-7072 ($MIC_{50/90}$, 0.25/0.5 mg/L) and tigecycline ($MIC_{50/90}$, 0.5/0.5 mg/L) had the lowest MIC for tetracycline-susceptible *K. pneumoniae*. The MIC for KBP-7072 ($MIC_{50/90}$, 1/4 mg/L) and tigecycline ($MIC_{50/90}$, 1/2 mg/L) increased 2- to 8-fold for tetracycline-resistant *K. pneumoniae*, which mostly produced Tet(A).

Conclusions: KBP-7072 activity was minimally affected by the presence of acquired tetracycline genes.

Introduction

Antimicrobial resistance (AMR) among Gram-positive cocci (GPC) and Gram-negative bacilli (GNB) is now a well-recognized impediment to medical progress.[1,2] Selection of bacterial pathogens with intrinsic or acquired mechanisms of resistance (MOR) to antimicrobial agents is facilitated by excessive and frequently inappropriate use of broad-spectrum antibacterial agents to the extent that entire classes of agents have been rendered useless clinically.[1–5] The threat of AMR to healthcare has been exacerbated recently by widespread use of antimicrobial therapies as part of the package of clinical care for coronavirus disease 2019 (COVID-19).[6]

Increasing rates of resistance to β-lactams, fluoroquinolones, macrolides and older tetracyclines (doxycycline, minocycline and tetracycline) in recent years among *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli* and *Klebsiella pneumoniae* pose serious challenges for providing effective treatment of community- and hospital-associated infections.[1–3,7–9] *S. aureus*, *E. coli* and *Klebsiella* spp. represent the top three causes of healthcare-associated infections (HAI)[2] and *S. pneumoniae* is a leading cause of community-acquired bacterial pneumonia (CABP).[10] The increase in infections caused by MDR (resistant to one or more agents in at least three different classes of agents) bacteria poses significant problems for individuals with serious and potentially life-threatening infections given the few available and effective therapeutic options.[1,2,4]

The tetracycline class of antimicrobial agents has been in use clinically for more than 60 years.[3,5,11] These agents continue to be

used clinically for treatment of a variety of GPC and GNB bacterial infections, including those intracellular pathogens as well as protozoans.[3,5,11] The broad use of the tetracycline class of agents has resulted in the development of resistance to the older members, especially tetracycline and doxycycline, by genes that are often associated with mobile genetic elements.[3,5,11] Tetracycline resistance in both GPC and GNB is mostly due to the acquisition of genes encoding efflux, ribosomal protection proteins and tetracycline-inactivating enzymes, but target site alterations and the overexpression of intrinsic efflux pumps do also occur.[3,12,13]

Semisynthetic derivatives of tetracycline, such as omadacycline, tigecycline and eravacycline with improved potency against MDR GPC and GNB, including against bacteria with acquired tetracycline-resistance genes, have been approved for clinical use.[3] Most recently, an additional aminomethylcycline, KBP-7072, has been developed and is undergoing early-stage clinical development for the treatment of acute bacterial skin and skin structure infection (ABSSSI), CABP and complicated intraabdominal infections (cIAI).[14] Modifications at the C-9 position provide activity even in the presence of ribosomal protection proteins and efflux pump mechanisms.[14] Whereas eravacycline, omadacycline and tigecycline have been extensively investigated,[3] little has been published regarding the *in vitro* spectrum and potency of KBP-7072, including strains with defined tetracycline-resistance mechanisms. The present study investigated the *in vitro* activity of KBP-7072 against a subset of key target pathogens (*S. aureus, S. pneumoniae, E. coli* and *K. pneumoniae*) that were molecularly characterized for acquired tetracycline-resistance mechanisms. A comparative analysis of newer-generation tetracycline derivatives (omadacycline and tigecycline) with older-generation tetracyclines (doxycycline, minocycline and tetracycline) is also provided.

Materials and methods

Bacterial isolates

A global collection of 413 tetracycline-resistant and -susceptible isolates recovered from various infections in 119 medical centres located in 34 countries during the SENTRY Surveillance Program for 2015–19 were included (Table S1, available as Supplementary data at *JAC-AMR* Online).[7–9] Tetracycline-resistant isolates (205) were previously selected for genome sequencing and screened for various resistance mechanisms, and were known to carry acquired tetracycline-resistance genes. A similar number of tetracycline-susceptible isolates (208) were also selected. The collection of isolates included: 103 *S. aureus* (51 tetracycline resistant; MIC ≥16 mg/L), 102 *S. pneumoniae* (51 tetracycline resistant; MIC ≥4 mg/L), 103 *E. coli* (52 tetracycline resistant; MIC ≥16 mg/L) and 105 *K. pneumoniae* (51 tetracycline resistant; MIC ≥16 mg/L). Bacterial isolate identification was confirmed by standard algorithms supported by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and genome sequencing.

Antimicrobial susceptibility testing

Isolates were tested for susceptibility to KBP-7072, doxycycline, minocycline, omadacycline, tetracycline and tigecycline by broth microdilution following CLSI M07[15] guidelines. Results were interpreted using CLSI,[16] EUCAST[17] and FDA[18] breakpoint criteria. Frozen-form broth microdilution panels were manufactured by JMI Laboratories (North Liberty, IA, USA) and contained fresh CAMHB (2.5%–5% lysed horse blood added for testing streptococci). KBP-7072 and omadacycline were provided by KBP Biosciences Co. Ltd (Jinan, China). Quality assurance was performed by concurrently testing CLSI-recommended quality control reference strains (*S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619).

Characterization of resistance mechanisms by next-generation sequencing

Tetracycline-resistant isolates had their total genomic DNA extracted by the fully automated Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, OH, USA), which was used as input material for library construction. DNA libraries were prepared using the Nextera™ library construction protocol (Illumina, San Diego, CA, USA) following the manufacturer's instructions and were sequenced on MiSeq Sequencer platforms at JMI Laboratories. FASTQ format sequencing files for each sample set were assembled independently using *de novo* assembler SPAdes 3.11.1. In-house software was applied to align the assembled sequences against a curated database containing known tetracycline-resistance genes with the purpose of screening.[19,20]

Results and discussion

The geographic distribution of tetracycline-resistant isolates and tetracycline-resistance genes associated with each of the species included in this study is detailed in Table S1. Table 1 presents cumulative numbers and percentages of isolates inhibited by KBP-7072. KBP-7072 showed MIC_{50} and MIC_{90} values of 0.06 and 0.5 mg/L, respectively, for *S. aureus* and inhibited all isolates at ≤1 mg/L, including the tetracycline-resistant subset (Table 1). Overall, KBP-7072 had an MIC_{50} value of 0.06 mg/L for tetracycline-resistant *S. aureus*. The MIC_{50} values for KBP-7072 varied between 0.25 mg/L for *tet*(M)-carrying isolates and 0.06 mg/L when tested against other genotypes (Table 1). In comparison, KBP-7072 MIC_{90} values (MIC_{90}, 0.5 mg/L) for tetracycline-resistant *S. aureus* increased 4-fold when compared with tetracycline-susceptible (MIC_{90}, 0.12 mg/L) isolates, although MIC values did not exceed 1 mg/L for any isolate. In addition, KBP-7072 (MIC_{50}, 0.06 mg/L) showed the lowest MIC_{50} value, followed by tigecycline (MIC_{50}, 0.12 and 0.25 mg/L) and omadacycline (MIC_{50}, 0.12 and 0.5 mg/L) for tetracycline-susceptible and -resistant *S. aureus*, respectively. Other tetracycline comparators had their MIC_{50} values increased 64- to 256-fold by *tet* genes (Table 2).

For *S. pneumoniae*, KBP-7072 ($MIC_{50/90}$, ≤0.015/0.03 mg/L) showed the lowest MIC results, which remained unchanged when comparing results obtained against tetracycline-susceptible and -resistant isolates [mostly *tet*(M)] (Table 1). Similar MIC results were observed for omadacycline ($MIC_{50/90}$, 0.03–0.06/0.06 mg/L) and tigecycline ($MIC_{50/90}$, 0.03/0.03 mg/L) when tested against tetracycline-susceptible and -resistant *S. pneumoniae* populations, respectively (Table 2).

KBP-7072 had MIC_{50} values of 0.12 mg/L and 0.25 mg/L when tested against tetracycline-susceptible and -resistant *E. coli*, respectively (Table 1). KBP-7072 (MIC_{90}, 0.25 and 1 mg/L, respectively) and tigecycline (MIC_{90}, 0.25 and 0.5 mg/L) showed similar MIC_{90} values for these *E. coli* groups, as well as for tetracycline-susceptible *K. pneumoniae* (KBP-7072 $MIC_{50/90}$, 0.25/0.5 mg/L and tigecycline $MIC_{50/90}$, 0.5/0.5 mg/L) (Table 2). In contrast, the MIC for both KBP-7072 ($MIC_{50/90}$, 1/4 mg/L) and tigecycline ($MIC_{50/90}$, 1/2 mg/L) increased 2- to 8-fold for tetracycline-resistant *K. pneumoniae*, which mostly produced Tet(A) (Table 2).

The tetracycline-resistance mechanisms observed here represent those encoded by common genes carried by GPC and GNB,

Table 1. Antimicrobial activity of KBP-7072 tested against the main organism groups and characterized subsets

	Number (cumulative %) of isolates inhibited at MIC (mg/L) of:											
Organism/organism group/genotype (no. of isolates)	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	MIC_{50}	MIC_{90}
S. aureus (103)		11 (10.7)	56 (65.0)	13 (77.7)	7 (84.5)	14 (98.1)	2 (100.0)				0.06	0.5
Tetracycline susceptible (52)		7 (13.5)	33 (76.9)	9 (94.2)	1 (96.2)	2 (100.0)					0.06	0.12
Tetracycline resistant (51)		4 (7.8)	23 (52.9)	4 (60.8)	6 (72.5)	12 (96.1)	2 (100.0)				0.06	0.5
tet(M) (29)		2 (6.9)	5 (24.1)	2 (31.0)	6 (51.7)	12 (93.1)	2 (100.0)				0.25	0.5
tet(K) (15)		1 (6.7)	12 (86.7)	2 (100.0)							0.06	0.12
tet(K) + *tet*(M) (5)			5 (100.0)								0.06	—
Other[a] (2)		1 (50.0)	1 (100.0)								0.06	—
S. pneumoniae (102)	72 (70.6)	30 (100.0)									≤0.015	0.03
Tetracycline susceptible (51)	31 (60.8)	20 (100.0)									≤0.015	0.03
Tetracycline resistant (51)	41 (80.4)	10 (100.0)									≤0.015	0.03
tet(M) (50)	40 (80.0)	10 (100.0)									≤0.015	0.03
tet(32) (1)	1 (100.0)										—	—
E. coli (103)			3 (2.9)	57 (58.3)	27 (84.5)	9 (93.2)	7 (100.0)				0.12	0.5
Tetracycline susceptible (51)			3 (5.9)	39 (82.4)	9 (100.0)						0.12	0.25
Tetracycline resistant (52)				18 (34.6)	18 (69.2)	9 (86.5)	7 (100.0)				0.25	1
tet(A) (20)				5 (25.0)	7 (60.0)	3 (100.0)					0.25	0.5
tet(A) + *tet*(B) (8)				1 (12.5)	4 (62.5)	1 (75.0)	2 (100.0)				0.25	—
tet(B) (21)				9 (42.9)	9 (85.7)	1 (90.5)	2 (100.0)				0.25	0.5
tet(D) (3)				3 (100.0)							0.12	—
K. pneumoniae (105)			1 (1.0)	8 (8.6)	41 (47.6)	22 (68.6)	19 (86.7)	7 (93.3)	5 (98.1)	2 (100.0)	0.5	2
Tetracycline susceptible (54)			1 (1.9)	8 (16.7)	39 (88.9)	6 (100.0)					0.25	0.5
Tetracycline resistant (51)					2 (3.9)	16 (35.3)	19 (72.5)	7 (86.3)	5 (96.1)	2 (100.0)	1	4
tet(A) (40)					2 (5.0)	14 (40.0)	16 (80.0)	4 (90.0)	2 (95.0)	2 (100.0)	1	2
tet(A) + *tet*(B) (2)								2 (100.0)			2	—
tet(A) + *tet*(G) (2)							1 (50.0)	1 (100.0)			1	—
tet(D) (5)						2 (40.0)	1 (60.0)	0 (60.0)	2 (100.0)		1	—
tet(G) (2)							1 (50.0)	0 (50.0)	1 (100.0)		1	—

[a]Contains 1 isolate with *tet*(L) and 1 isolate with *tet*(L)/*tet*(M).

Table 2. *In vitro* activity of old and new generation tetracycline agents

	MIC_{50}/MIC_{90}, mg/L (% susceptible by CLSI/EUCAST[a])					
Organism (*n*)	KBP-7072	doxycycline	minocycline	omadacycline	tetracycline	tigecycline
S. aureus						
TET-S (52)	0.06/0.12	0.12/0.25 (100.0/100.0)	0.12/0.25 (100.0/98.1)	0.12/0.25 (94.2/–)	0.25/0.5 (100.0/96.2)	0.12/0.25 (98.1/98.1)
TET-R[b] (51)	0.06/0.5	8/8 (29.4/11.8)	8/16 (43.1/29.4)	0.5/2 (58.8/–)	64/64 (0.0/0.0)	0.25/0.5 (90.2/90.2)
S. pneumoniae						
TET-S (51)	≤0.015/0.03	0.12/0.12 (100.0/100.0)	0.12/0.12 (–/100.0)	0.03/0.06 (100.0/–)	0.25/0.25 (100.0/100.0)	0.03/0.03 (100.0/–)
TET-R[c] (51)	≤0.015/0.03	4/16 (0.0/3.9)	8/16 (–/2.0)	0.06/0.06 (100.0/–)	32/64 (0.0/0.0)	0.03/0.03 (100.0/–)
E. coli						
TET-S (51)	0.12/0.25	1/2 (100.0/–)	1/1 (100.0/–)	0.5/1 (–/–)	1/2 (100.0/–)	0.12/0.25 (100.0/100.0)
TET-R[d] (52)	0.25/1	32/>32 (5.8/–)	8/32 (42.3/–)	1/4 (–/–)	>64/>64 (0.0/–)	0.25/0.5 (100.0/98.1)
K. pneumoniae						
TET-S (54)	0.25/0.5	1/2 (100.0/–)	1/2 (100.0/–)	1/2 (100.0/–)	1/2 (100.0/–)	0.5/0.5 (100.0/–)
TET-R[e] (51)	1/4	16/>32 (0.0/–)	4/>32 (52.9/–)	4/16 (54.9/–)	>64/>64 (0.0/–)	1/2 (92.2/–)

TET, tetracycline; S, susceptible; R, resistant.
[a]CLSI and EUCAST breakpoints were applied. FDA breakpoint interpretive criteria were used for tigecycline and omadacycline, with susceptibility shown in place of CLSI.
[b]Contains 15 *tet*(K), 5 *tet*(K)/*tet*(M), 1 *tet*(L), 29 *tet*(M) and 1 *tet*(L)/*tet*(M) (see Table S1).
[c]All isolates carried *tet*(M), except for 1 strain with a *tet*(32).
[d]Contains 20 *tet*(A), 8 *tet*(A)/*tet*(B), 21 *tet*(B) and 3 *tet*(D).
[e]Contains 40 *tet*(A), 2 *tet*(A)/*tet*(B), 2 *tet*(A)/*tet*(G), 5 *tet*(D) and 2 *tet*(G).

although the presence of the overexpression of intrinsic efflux pumps and target site alterations were not investigated, which could be considered as a study limitation. In addition, the isolates included in this study were initially selected for molecular characterization for reasons other than the screening of tetracycline-resistance genes. Thus, isolates and their respective resistance genes are exclusively presented here for the purpose of *in vitro* activity analysis and do not represent their actual occurrences in these populations. Moreover, isolates from the Latin American and Asia-Pacific regions were underrepresented. Further analyses are required to complete this *in vitro* assessment of activity for KBP-7072. Based on MIC_{90} values, KBP-7072 was active *in vitro* against tetracycline-susceptible and -resistant isolates with potencies remaining somehow stable between each respective group. This stable MIC profile observed for KBP-7072 between tetracycline-susceptible and -resistant isolates resembled that of tigecycline. Thus, this stable *in vitro* activity of KBP-7072 in the presence of various *tet* genes warrants further development of this investigational agent as an additional option for the treatment of infections caused by MDR GPC and GNB.

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Supplementary data

Table S1 is available as Supplementary data at *JAC-AMR* Online

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