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) 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 (MIC50, 0.06 mg/L), tigecycline (MIC50, 0.12 and 0.25 mg/L) and omadacycline (MIC 50, 0.12 and 0.5 mg/L) showed similar MIC 50s for tetracycline-susceptible and -resistant S. aureus. Other tetracycline comparators had their MIC50 increased 64- to 256-fold by tet. For S. pneumoniae, KBP-7072 (MIC50/90, ≤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 (MIC50/90, 0.03–0.06/0.06 mg/L) and tigecycline (MIC50/90, 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 (MIC90, 0.25 and 1 mg/L, respectively) and tigecycline (MIC90, 0.25 and 0.5 mg/L) showed similar MIC90s. KBP-7072 (MIC50/90, 0.25/1 mg/L) and tigecycline (MIC50/90, 0.5/0.5 mg/L) had the lowest MIC for tetracycline-susceptible K. pneumoniae. The MIC for KBP-7072 (MIC50/90, 1/4 mg/L) and tigecycline (MIC50/90, 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)5 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 protozoa.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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).\textsuperscript{14} Modifications at the C-9 position provide activity even in the presence of ribosomal protection proteins and efflux pump mechanisms.\textsuperscript{14} Whereas eravacycline, omadacycline and tigecycline have been extensively investigated,\textsuperscript{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).\textsuperscript{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, tigecycline and tetracycline by broth microdilution following CLSI M07\textsuperscript{15} guidelines. Results were interpreted using CLSI,\textsuperscript{16} EUCAST\textsuperscript{17} and FDA\textsuperscript{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\textsuperscript{19} 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.\textsuperscript{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\textsubscript{50} and MIC\textsubscript{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\textsubscript{50} value of 0.06 mg/L for tetracycline-resistant S. aureus. The MIC\textsubscript{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\textsubscript{50} values (MIC\textsubscript{50}, 0.5 mg/L) for tetracycline-resistant S. aureus increased 4-fold when compared with tetracycline-susceptible (MIC\textsubscript{50}, 0.12 mg/L) isolates, although MIC values did not exceed 1 mg/L for any isolate. In addition, KBP-7072 (MIC\textsubscript{50}, 0.06 mg/L) showed the lowest MIC\textsubscript{50} value, followed by tigecycline (MIC\textsubscript{50}, 0.12 and 0.25 mg/L) and omadacycline (MIC\textsubscript{50}, 0.12 and 0.5 mg/L) for tetracycline-susceptible and -resistant S. aureus, respectively. Other tetracycline comparators had their MIC\textsubscript{50} values increased 64- to 256-fold by tet genes (Table 2).

For S. pneumoniae, KBP-7072 (MIC\textsubscript{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\textsubscript{50/90}, 0.03–0.06/0.06 mg/L) and tigecycline (MIC\textsubscript{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\textsubscript{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\textsubscript{50/90} 0.25 and 1 mg/L, respectively) and tigecycline (MIC\textsubscript{50/90}, 0.25 and 0.5 mg/L) showed similar MIC\textsubscript{50} values for these E. coli groups, as well as for tetracycline-susceptible K. pneumoniae (KBP-7072 MIC\textsubscript{50/90}, 0.25/0.5 mg/L and tigecycline MIC\textsubscript{50/90}, 0.5/0.5 mg/L) (Table 2). In contrast, the MIC for both KBP-7072 (MIC\textsubscript{50/90} 1/4 mg/L) and tigecycline (MIC\textsubscript{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,
| 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) Tetracycline susceptible (52) | 11 (10.7) | 56 (55.0) | 13 (77.7) | 7 (84.5) | 14 (98.1) | 2 (100.0) | | | | | 0.06 | 0.5 |
| Tetracycline resistant (51) | | 7 (13.5) | 33 (76.9) | 9 (94.2) | 1 (96.2) | 2 (100.0) | | | | | 0.06 | 0.12 |
| tet(M) (29) | 4 (7.8) | 23 (52.9) | 4 (60.8) | 6 (72.5) | 12 (96.1) | 2 (100.0) | | | | | 0.06 | 0.5 |
| tet(K) (15) | 2 (6.9) | 5 (24.1) | 2 (31.0) | 6 (51.7) | 12 (93.1) | 2 (100.0) | | | | | 0.25 | 0.5 |
| tet(K) + tet(M) (5) | 1 (6.7) | 12 (86.7) | 2 (100.0) | | | | | | | | 0.06 | 0.12 |
| Other* (2) | 1 (50.0) | | | | | | | | | | 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) | | | | | | | | — | — |
| 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 (40.0) | 22 (68.6) | 19 (68.7) | 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) | | | | | | | | | | — | — |
| 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 | — |

*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

| Organism (n) | KBP-7072 | doxycycline | minocycline | omadacycline | tetracycline | tigecycline |
|--------------|----------|-------------|-------------|--------------|--------------|-------------|
| S. aureus    |          |             |             |              |              |             |
| TET-S (52)   | 0.06/0.12| 0.12/0.25   | 0.12/0.25   | 0.12/0.25    | 0.25/0.5     | 0.12/0.25   |
| TET-R (51)   | 0.06/0.5 | 8/8 (29.4/11.8) | 8/16 (43.1/29.4) | 0.5/2 (58.8/--) | 32/64 (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 (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 (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/-) | 0.5/0.5 (100.0/--) | 1/2 (92.2/-) |
| TET-R (51)   | 1/4 | 16/>32 (0.0/-) | 4/>32 (52.9/-) | 4/16 (54.9/-) | >64/>64 (0.0/-) |           |

TET, tetracycline; S, susceptible; R, resistant.

aCLSI and EUCAST breakpoints were applied. FDA breakpoint interpretive criteria were used for tigecycline and omadacycline, with susceptibility shown in place of CLSI.
bContains 15 tet(K), 5 tet(A)/tet(M), 1 tet(L), 29 tet(M) and 1 tet(L)/tet(M) (see Table S1). cAll isolates carried tet(M), except for 1 strain with a tet(32). dContains 20 tet(A), 8 tet(A)/tet(B), 21 tet(B) and 3 tet(D). eContains 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 MIC90 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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