Assessment of Resistance Development to KBP-7072 by Serial-Passage and Single-Step Mutational Selection

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Introduction

- KBP-7072 is a third-generation tetracycline (aminomethylcycline) antibacterial in clinical development for the treatment of acute bacterial skin and skin structure infection (ABSSSI), community-acquired bacterial pneumonia (CABP), and complicated intra-abdominal infection (cIAI).
- This study assessed the potential for resistance to KBP-7072 to develop in tetracycline-susceptible (S) and tetracycline-resistant (R) Acinetobacter baumannii, Escherichia coli, Staphylococcus aureus, and Streptococcus pneumoniae clinical isolates and quality control (QC) strains during single-step selection and 10-day serial-passage (multi-step selection) studies.

Methods and Materials

- Bacterial isolate identifications were confirmed by JMI Laboratories using matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany).
- Broth microdilution and agar dilution susceptibility testing was performed in triplicate according to Clinical and Laboratory Standards Institute (CLSI) M07 (2018) and M100 (2021) guidelines.
- The modal MIC values obtained became the baseline broth microdilution and agar dilution MIC values.
- American Type Culture Collection (ATCC) QC strains recommended by CLSI were incorporated into each testing event
- Single-step resistance testing was conducted using 4x, 8x, and 16x the baseline KBP-7072 agar dilution MIC value.
- Inoculated agar plates were incubated at 35°C in ambient atmosphere and examined for growth at 24 hours.
- Plates lacking visible colonies at 24 hours were incubated further and re-examined at 48 hours.
- Putative resistant colonies were counted and compared to the starting inoculum to calculate the single-step frequency of resistance.
- Representative colonies were selected from the agar plates demonstrating the highest KBP-7072 MIC values, transferred twice on drug-free agar plates, followed by confirmatory broth microdilution susceptibility testing against KBP-7072 and comparator agents.
- The modal broth microdilution KBP-7072 MIC value served as the baseline MIC for the serial-passage resistance testing
- Each strain was tested for susceptibility to KBP-7072 in 100 µL volumes using an initial starting inoculum of approximately 5 x 10⁵ CFU/mL.
- Subsequent daily passages utilized the entire well contents from the last well demonstrating visible growth from the previous day's test.
- This process was repeated for up to 10-daily transfers or until the KBP-7072 MIC value reached >64 mg/L.

Results

- respectively.

- (Table 1).

KBP-7072 single-step frequency of resistance values for S. aureus ATCC 29213 and S. aureus 1088771 (tetracyclineresistant) were very low at <9.4 x 10^{-10} and <8.6 x 10^{-10} ,

– No resistant mutants were recovered at 4x, 8x, or 16x the baseline KBP-7072 MIC value (Table 1)

KBP-7072 MIC values remained stable (no increase in MIC value) against S. aureus ATCC 29213 and increased 4-fold (highest MIC, 0.25 mg/L) for S. aureus 1088771 over the course of 10-daily serial passages (Figures 1–2).

Single-step frequency of resistance values for S. pneumoniae ATCC 49619 and S. pneumoniae 1119350 (penicillin- and tetracycline-resistant) ranged from >2.3 x 10^{-6} to <8.2 x 10^{-9} at 4x to 16x the baseline KBP-7072 MIC (Table 1).

• A \leq 4-fold increase in KBP-7072 MIC values (highest MIC, 0.03 mg/L) were observed following drug free passage and confirmatory MIC testing, indicating that the singlestep mutants obtained with S. pneumoniae ATCC 49619 (8x and 16x the baseline KBP-7072 MIC) and S. pneumoniae 1119350 (4x, 8x, and 16x the baseline KBP-7072 MIC) were not stably resistant (Table 1)

 KBP-7072 MIC values increased modestly (up to 4-fold; highest MIC, 0.03 mg/L) against S. pneumoniae ATCC 49619 and S. pneumoniae 1119350 following 10-daily serial passages (Figures 3–4).

KBP-7072 single-step frequency of resistance was low, ranging from 9.0 x 10⁻⁸ at 4x MIC to < 9.6 x 10⁻¹⁰ at 16x MIC for A. baumannii NCTC 13304 and from 2.7 x 10⁻⁷ at 4x MIC to <8.7 x 10⁻¹⁰ at 16x MIC for *A. baumannii* 1044704 (tetracycline-, levofloxacin-, and carbapenem-resistant)

In serial passage testing, KBP-7072 MIC values increased 8-fold (0.12 to 1 mg/L) for A. baumannii NCTC 13304 and 4-fold (0.5 to 2 mg/L) for A. baumannii 1044704 over the course of 10-daily serial passages (Figures 5–6).

- KBP-7072 MIC values remained elevated by \leq 8-fold against A. baumannii NCTC 13304 and A. baumannii 1044704 following drug-free passage and confirmatory MIC testing (data not shown).

– These mutants also demonstrated elevated MIC values to other tetracycline class agents (data not shown).

 Single-step frequency of resistance to KBP-7072 was very low (<8.3 x 10^{-10}) against *E. coli* ATCC 25922 and no resistant mutants were recovered (Table 1).

– Similarly, single-step frequency of resistance to KBP-7072 was low against a *E. coli* 1093450 (tetracycline- and levofloxacin-resistant; ESBL-phenotype), ranging from 1.6 x 10^{-7} at 4x MIC to <2.6 x 10^{-10} at 16x MIC (Table 1).

In serial passage testing, KBP-7072 MIC values increased 8-fold (0.12 to 1 mg/L) for *E. coli* ATCC 25922 and >128-fold (0.5 to >64 mg/L) for *E. coli* 1093450 over the course of 10-daily serial passages (Figures 7–8).

– KBP-7072 and tetracycline-class comparator agent MIC values remained elevated against *E. coli* following drug-free passage and confirmatory MIC testing (data not shown).







Table 1. KBP-7072 single-step frequency of resistance development

Isolate	KBP-7072 Agar dilution	Starting Inoculum (CFU/mL	Mutation frequency			
	MIC (mg/L)		Volume Plated (mL)	4x MIC	8x MIC	16 x
A. baumannii	0.12	1.0 x 10 ⁹	1 mL	9.0 x 10 ^{-8 a}	1.4 x 10 ⁻⁸	< 9.6 >
(NCTC 13304)			0.1 mL	9.6 x 10 ⁻⁹	<9.6 x 10 ⁻⁹	<9.6
A. baumannii	0.5	1.2 x 10 ⁹	1 mL	2.7 x 10 ⁻⁷	1.1 x 10 ⁻⁷	<8.7 >
(1044704)			0.1 mL	2.4 x 10 ⁻⁷	<8.7 x 10 ⁻⁹	<8.7
E. coli	0.12	1.2 x 10 ⁹	1 mL	<8.3 x 10 ⁻¹⁰	<8.3 x 10 ⁻¹⁰	<8.3 >
(ATCC 25922)			0.1 mL	<8.3 x 10 ⁻⁹	<8.3 x 10 ⁻⁹	<8.3
E. coli	0.5	3.8 x 10 ⁹	1 mL	2.1 x 10 ⁻⁸	5.2 x 10 ⁻¹⁰	<2.6 >
(1093450)			0.1 mL	1.6 x 10 ⁻⁷	<2.6 x 10 ⁻⁹	<2.6
S. aureus	0.12	1.1 x 10 ⁹	1 mL	<9.4 x 10 ⁻¹⁰	<9.4 x 10 ⁻¹⁰	< 9.4 >
(ATCC 29213)			0.1 mL	<9.4 x 10 ⁻⁹	<9.4 x 10 ⁻⁹	<9.4
S. aureus	0.12	1.2 x 10 ⁹	1 mL	<8.6 x 10 ⁻¹⁰	<8.6 x 10 ⁻¹⁰	<8.6 >
(1088771)			0.1 mL	<8.6 x 10 ⁻⁹	<8.6 x 10 ⁻⁹	<8.6
S. pneumoniae	0.008	1.2 x 10 ⁹	1 mL	>2.5 x 10 ^{-7 a}	>2.5 x 10 ^{-7 a}	>2.5 >
(ATCC 49619)			0.1 mL	>2.5 x 10 ^{-6 a}	8.2 x 10 ⁻⁹	<8.2
			0.01 mL	6.6 x 10 ⁻⁷	<8.2 x 10 ⁻⁸	<8.2
S. pneumoniae	0.015	1.3 x 10 ⁹	1 mL	>2.3 x 10 ^{-7 a}	>2.3 x 10 ^{-7 a}	>2.3 >
(1119350)			0.1 mL	>2.3 x 10 ^{-6 a}	>2.3 x 10 ^{-6 a}	>2.3 >
			0.01 mL	<7.6 x 10 ⁻⁸	<7.6 x 10 ⁻⁸	<7.6

Serial passage day



^a A hazy lawn of growth was observed (no individual colonies), therefore a value of 301 was used to estimate the frequency of resistance value.

Conclusions

- S. aureus KBP-7072 demonstrated a very low propensity for resistance development in single-step selection studies, and no resistant mutants were recovered. In serial passage testing, KBP-7072 MIC values remained unchanged or increased modestly (up to 4-fold; highest MIC, 0.25 mg/L) over the course of 10-daily serial passages.
- S. pneumoniae In single-step selection studies, KBP-7072 demonstrated a low rate of resistance development with no more than a 4-fold MIC increase (highest MIC, 0.03 mg/L) following drug free passage and confirmatory MIC testing. In serial passage testing, KBP-7072 MIC values increased modestly (2- to 4-fold; highest MIC, 0.03 mg/L) over the course of 10-daily passages.
- A. baumannii KBP-7072 demonstrated a low propensity for resistance development against A. baumannii in singlestep selection studies. In serial passage testing, KBP-7072 MIC values increased 4- to 8-fold (highest MIC, 2 mg/L) over the course of 10-daily serial passages. Putative resistant mutants from the single-step and serial passage studies against A. baumannii demonstrated stably elevated MIC values to KBP-7072 and other tetracycline class agents.
- *E. coli* In single-step selection studies, KBP-7072 demonstrated a low propensity for resistance development against *E. coli*. In serial passage testing, KBP-7072 MIC values increased 8- to >128-fold (highest MIC, >64 mg/L) over the course of 10-daily serial passages. Putative resistant mutants from the single-step and serial passage studies against E. coli demonstrated stably elevated MIC values to KBP-7072 and other tetracycline class agents.

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10^{-6 a} x 10⁻⁸

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