Low Propensity for Development of Spontaneous *In Vitro* Resistance to Tebipenem, Ertapenem, and Meropenem Among Enterobacterales Uropathogens

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INTRODUCTION

- Tebipenem, an oral carbapenem in development for treatment of complicated urinary tract infections and acute pyelonephritis, is active versus uropathogens which produce extended-spectrum beta-lactamases (ESBL) such as *Escherichia coli* and *Klebsiella pneumoniae*.
- Bacteria acquire resistance to antibiotics via multiple routes, including by chromosomal mutation. In the studies described herein the frequency of spontaneous resistance to tebipenem, meropenem and ertapenem among a panel of Enterobacterales isolates, including those resistant to clinically relevant antibacterials, was assessed using static and serial passage assays.
- Resistance to the challenge compounds was then confirmed via minimum inhibitory concentration (MIC) testing and characterized through whole genome sequencing (WGS). Relatedness of parent/mutant pairs was confirmed via multilocus sequence typing.

METHODS

- The static frequency of resistance (single-step, or FOR) to tebipenem (TBP), meropenem and ertapenem was assessed for a panel of twenty four Enterobacterales isolates including *E. coli* (n = 8), *K. pneumoniae* (n = 9), and other Enterobacterales (n = 7).
  - Approximately 10^8 CFU of isolates in mid-logarithmic phase growth were plated on agar containing 4 or 8 times the MIC of each carbapenem and incubated for 24h. Resistance was confirmed by MIC testing [1] and was defined as a ≥ four-fold increase in MIC.
- Serial passages were performed using two *E. coli* and two *K. pneumoniae* isolates, each of which included a wild type and ESBL+ isolate, through exposure to tebipenem, meropenem, or ertapenem over a 14-day period using broth microdilution methodologies.
- Whole genome sequencing was conducted on representative mutants from the static and serial passage studies and assembled sequences were aligned vs. β-lactamase-encoding genes, porins OmpF/OmpK35, OmpC/OmpK36, and OmpK37 (*K. pneumoniae* only) and penicillin binding proteins (PBPs) 1a, 1b, 2, and 3 and compared with parent isolates.

RESULTS

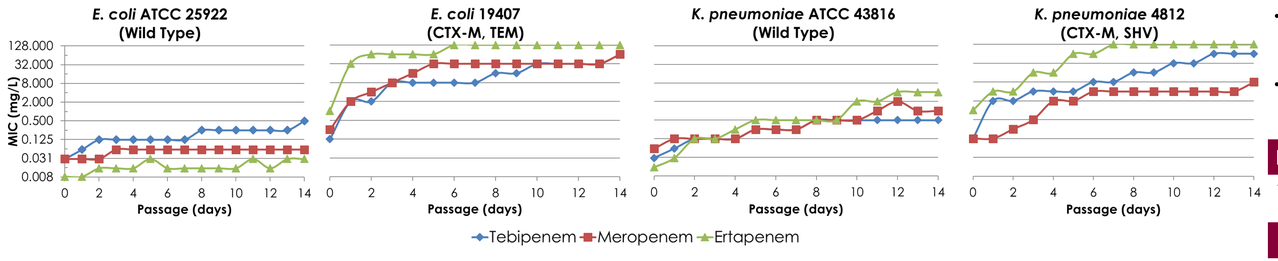
- FOR values for tebipenem, meropenem, and ertapenem at 4 to 8 times the MIC ranged from <6.87 × 10^{-9} to 5.54 × 10^{-5}, with similar results obtained for all species and genotypes, including ESBL-producing isolates and those with porin alterations (**Table 1).**
  - Mutants collected on tebipenem-containing agar plates demonstrated tebipenem MIC values of 0.12 to 2 mg/L with the highest MIC observed with parent *K. pneumoniae* isolate 25021 and 40031. Similarly, mutants selected on meropenem had meropenem MIC values of 0.125 to 4 mg/L and mutants selected on ertapenem had ertapenem MIC value of 0.125 to >8 mg/L.
- In the serial passage assays, MIC values for each agent vs. four isolates over the 14-day period were approximately five to eight 2-fold dilutions higher than baseline, resulting in similar MIC values to those observed in the FOR studies (0.12 to 2 mg/L) (**Figure 1**).
- Mutants isolated found in the presence of tebipenem, meropenem, and ertapenem had similarly decreased susceptibility to each compound.

RESULTS

Table 1. Tebipenem, meropenem and ertapenem baseline agar MIC, average frequency of resistance to each compound at 4 and 8 times the agar MIC at 24 hours

Isolate identifier	β-lactam resistance mechanism(s)	Tebipenem			Meropenem			Ertapenem		
		Baseline agar MIC (mg/L)	FOR at 4x MIC	FOR at 8x MIC	Baseline agar MIC (mg/L)	FOR at 4x MIC	FOR at 8x MIC	Baseline agar MIC (mg/L)	FOR at 4x MIC	FOR at 8x MIC
E. coli ATCC 25922	None	0.015	$7.11x10^{-7}$	$<2.42x10^{-9}$	0.015	$<1.98x10^{-9}$	$<1.98x10^{-9}$	0.008	$<2.59x10^{-9}$	$<2.59x10^{-9}$
E. coli 4643	CTX-M-15, OXA-1/30	0.015	$<1.57x10^{-9}$	$1.57x10^{-9}$	0.03	$<2.02x10^{-9}$	$<2.02x10^{-9}$	0.06	$1.83x10^{-7}$	$3.71x10^{-9}$
E. coli 13319	CTX-M-15, TEM-1, AcrAB-TolC, OmpC, OmpF	0.06	$4.64x10^{-8}$	$<4.55x10^{-9}$	0.03	$1.30x10^{-7}$	$4.69x10^{-9}$	0.25	$3.29x10^{-8}$	$<4.05x10^{-9}$
E. coli 21711	CTX-M-15, OXA-1/30, TEM-1	0.03	$1.29x10^{-8}$	$<3.64x10^{-9}$	0.03	$1.21x10^{-8}$	$<1.45x10^{-9}$	0.125	$9.02x10^{-9}$	$<1.19x10^{-9}$
E. coli 1801	CTX-M-15, AcrAB-TolC, OmpF	0.015	$2.31x10^{-8}$	$8.35x10^{-9}$	0.015	$1.81x10^{-8}$	$<6.04x10^{-9}$	0.06	$3.51x10^{-8}$	$<4.71x10^{-9}$
E. coli 30854	CTX-M-15, OmpC, TEM-1-like	0.03	$1.14x10^{-8}$	$<7.45x10^{-9}$	0.015	$3.11x10^{-7}$	$7.92x10^{-9}$	0.06	$3.64x10^{-7}$	$3.02x10^{-9}$
E. coli 39930	CTX-M-15, TEM-1, OXA-1/30, SHV-28, OmpC	0.015	$5.54x10^{-5}$	$5.66x10^{-5}$	0.015	$2.36x10^{-6}$	$5.21x10^{-7}$	0.06	$6.14x10^{-6}$	$2.31x10^{-6}$
E. coli 19407	CP_CTX-M_Group 1, CP_TEM_WT	0.5	$<6.45x10^{-9}$	$<6.45x10^{-9}$	0.125	$7.68x10^{-6}$	$7.92x10^{-6}$	0.5	$4.83x10^{-7}$	$4.42x10^{-7}$
K. pneumoniae ATCC 43816	SHV-1	0.015	$<1.71x10^{-9}$	$6.14x10^{-8}$	0.03	$<2.49x10^{-9}$	$<2.49x10^{-9}$	0.015	$<2.89x10^{-9}$	$<2.89x10^{-9}$
K. pneumoniae 25021	CTX-M-15, TEM-1, OXA-2	0.03	$8.31x10^{-7}$	$2.26x10^{-7}$	0.03	$5.81x10^{-7}$	$1.58x10^{-7}$	0.25	$3.50x10^{-8}$	$6.90x10^{-9}$
K. pneumoniae 40031	CTX-M-15, SHV-11	0.125	$2.03x10^{-6}$	$2.3x10^{-7}$	0.125	$5.41x10^{-7}$	$1.75x10^{-7}$	0.25	$3.94x10^{-7}$	$1.59x10^{-7}$
K. pneumoniae 50566	CTX-M_Group 1, SHV ESBL	0.03	$1.94x10^{-7}$	$1.93x10^{-7}$	0.03	$2.64x10^{-7}$	$1.22x10^{-7}$	0.125	$2.91x10^{-7}$	$1.28x10^{-7}$
K. pneumoniae 18823	CP_CTX-M_Group 1, SHV ESBL	0.015	$1.65x10^{-6}$	$1.95x10^{-7}$	0.03	$4.00x10^{-7}$	$1.81x10^{-7}$	0.06	$4.30x10^{-7}$	$3.28x10^{-7}$
K. pneumoniae 4812	CTX-M-15	0.06	$2.61x10^{-7}$	$1.56x10^{-7}$	0.125	$4.61x10^{-8}$	$2.34x10^{-8}$	0.25	$1.40x10^{-7}$	$9.01x10^{-8}$
K. pneumoniae 21904	CTX-M-15, OXA-1	0.03	$8.01x10^{-7}$	$2.13x10^{-7}$	0.03	$8.42x10^{-7}$	$2.80x10^{-7}$	0.125	$1.52x10^{-7}$	$4.65x10^{-8}$
K. pneumoniae 604	CTX-M-15, OXA-1/30, SHV-1, OmpK	0.06	$1.56x10^{-7}$	$8.45x10^{-8}$	0.06	$2.92x10^{-9}$	$<2.92x10^{-9}$	0.125	$1.00x10^{-8}$	$4.46x10^{-9}$
K. pneumoniae 934954	CTX-M-15, OXA-1, SHV-28, TEM-1	0.125	$5.36x10^{-8}$	$<1.40x10^{-9}$	0.125	$4.11x10^{-8}$	$2.35x10^{-9}$	0.5	$6.57x10^{-8}$	$1.40x10^{-9}$
P. mirabilis ATCC 29906	Wild type	0.015	$4.46x10^{-7}$	$5.63x10^{-8}$	0.03	$<2.04x10^{-9}$	$<2.04x10^{-9}$	0.008	$<3.70x10^{-9}$	$<3.70x10^{-9}$
P. mirabilis ATCC BAA-856	TEM-10	0.03	$1.78x10^{-6}$	$1.60x10^{-8}$	0.03	$4.01x10^{-7}$	$2.22x10^{-7}$	0.015	$5.54x10^{-7}$	$2.61x10^{-7}$
P. mirabilis ATCC BAA-2791	TEM+	0.06	$<1.68x10^{-9}$	$<1.68x10^{-9}$	0.06	$9.93x10^{-8}$	$5.72x10^{-8}$	0.015	$1.88x10^{-7}$	$1.60x10^{-7}$
C. freundii ATCC 8090	Wild type	0.015	$1.01x10^{-8}$	$<1.93x10^{-9}$	0.03	$<3.39x10^{-9}$	$<3.39x10^{-9}$	0.008	$<6.87x10^{-9}$	$<6.87x10^{-9}$
C. freundii ATCC BAA-2807	CTX-M-5	0.015	$8.70x10^{-7}$	$8.46x10^{-7}$	0.06	$<2.16x10^{-9}$	$<2.16x10^{-9}$	0.25	$<3.93x10^{-9}$	$<3.93x10^{-9}$
E. cloacae ATCC 13047	Wild type	0.03	$7.47x10^{-7}$	$<3.52x10^{-9}$	0.06	$<4.05x10^{-9}$	$<4.05x10^{-9}$	0.125	$3.00x10^{-8}$	$<5.04x10^{-9}$
E. cloacae AR Bank# 0073	cAmpC, CTX-M-9	0.015	$2.33x10^{-7}$	$3.47x10^{-8}$	0.03	$<1.97x10^{-9}$	$<1.97x10^{-9}$	0.06	$2.80x10^{-7}$	$7.35x10^{-8}$

Figure 1. Serial passage results for four Enterobacterales isolates versus tebipenem, meropenem and ertapenem, over the 14-day period



RESULTS

Table 2. Broth microdilution MIC values and molecular characterization of mutants with reduced susceptibility to tebipenem relative to the isogenic parent isolate

Parent isolate characteristics		Selection conditions[a]	Tebipenem MIC mg/L		Amino acid alterations
Identifier	β-lactam resistance mechanisms		Parent	Mutant	
E. coli ATCC 25922	None	Single-step, 8x TBP MIC	≤0.015	0.12	No alterations observed
		TBP serial passage, day 14	≤0.015	0.25	No alterations observed
E. coli 4643	CTX-M-15, OXA-1/30	Single-step, 4x TBP MIC	0.06	0.25	No alterations observed
E. coli 13319	CTX-M-15, TEM-1, AcrAB-TolC, OmpC, OmpF	Single-step, 4x TBP MIC	0.12	1	Multiple alterations in OmpC
E. coli 30854	CTX-M-15, OmpC, TEM-1-like	Single-step, 4x TBP MIC	0.03	0.5	Alterations in OmpC at 3' end
E. coli 19407	CTX-M-55, TEM-1	TBP serial passage, day 14	0.12	8	Multiple alterations in OmpC
K. pneumoniae ATCC 43816	SHV-1	Single-step, 4x TBP MIC	≤0.015	0.25	No alterations observed
		TBP serial passage, day 8	≤0.015	0.12	No alterations observed
K. pneumoniae 25021	CTX-M-15, TEM-1, OXA-1, SHV-28	Single-step, 8x TBP MIC	0.06	2	No alterations observed
K. pneumoniae 4812	CTX-M-15	Single-step, 8x TBP MIC	0.06	1	OmpK36 stop codon at AA125
		TBP serial passage, day 12	0.06	16	OmpK36 frame-shift alteration at AA124, premature stop codon at AA125
K. pneumoniae 604	CTX-M-15, OXA-1/30, SHV-1, OmpK	Single-step, 8x TBP MIC	0.12	2	OmpK36 ΔAA212-237

a. TBP=tebipenem

- Whole genome sequencing conducted with mutants selected during the single- and multi-step resistance studies identified alterations of OmpC in *E. coli* and its homologue OmpK36 in *K. pneumoniae* (**Table 2**).
- While no amino acid alterations on targeted proteins were detected in mutants derived from *E. coli* ATCC 25922 and 4643, factors which may affect susceptibility such as expression levels of OmpC, OmpF, other porins, efflux-pumps (eg. AcrAB-TolC), global regulators and β-lactamases such as chromosomally-encoded AmpC were not examined in this study.
- Similarly, while no amino acid alterations were observed in mutants derived from *K. pneumoniae* ATCC 43816 and 25021, expression levels of porins were not assessed in this study.

CONCLUSIONS

- These data suggest tebipenem has a low propensity for spontaneous resistance at 4 to 8 times the MIC *in vitro* and was similar to meropenem and ertapenem in both single-step and serial passage studies.
- Cross-resistance to tebipenem, meropenem, and ertapenem among mutants suggests common mechanisms contribute to elevated MIC values, including through reduced permeability.

REFERENCES

1. Clinical Laboratory Standards Institute. Methods for Dilutional Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 11th edition. CLSI supplement M07; 2022

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