Molecular Epidemiology of *Escherichia coli* Causing Urinary Tract Infections in United States and *In vitro* Activity of Tebipenem, Including against Strain Lineage and Resistant Subsets (2018–2020)

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Introduction

- Urinary tract infections (UTIs) are among the most common bacterial infections and the great majority of these infections are caused by *Escherichia coli*.
- The treatment of UTIs has become more complex due to the increase of resistance, and approximately 20% and 25% of *E. coli* responsible for UTI in the United States are not susceptible to fluoroquinolones and trimethoprim-sulfamethoxazole, respectively (see poster 1057).
- The increase in antimicrobial resistance among isolates of *E. coli* has been driven mostly by the dissemination of the extraintestinal lineage belonging to sequence type (ST) 131. In general, these isolates are characterized by:
  - Serotype O25:H4
  - Fluoroquinolone resistance due to double mutations in GyrA, and
  - Carry the extended-spectrum β-lactamase (ESBL) $bla_{CTX\text{-}M\text{-}15}$
- Additional antimicrobial agents for guided and empirical treatment of UTI are urgently needed, especially oral bioavailable options. Tebipenem is an oral carbapenem in clinical development for treating complicated UTIs and acute pyelonephritis.
- This study investigates the epidemiology of *E. coli* causing UTI in patients hospitalized in the US and the activity of tebipenem and comparator agents against various subsets.

Materials and Methods

Bacterial organisms

- A total of 2,395 *E. coli* collected from 58 medical centers in 9 US Census Divisions were recovered from urine samples during the 2018–2020 STEWARD Surveillance Program and included in the study.
- Bacterial identification was confirmed by standard algorithms supported by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany).

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution following the Clinical and Laboratory Standards Institute (CLSI) M07 (2018) guidelines.
- Frozen-form broth microdilution panels were manufactured by JMI Laboratories (North Liberty, IA, USA) and contained cation-adjusted Mueller-Hinton broth.
- Quality assurance was performed by sterility checks, colony counts, and testing CLSI-recommended quality control reference strains.
- Isolates displaying MIC results of ≥2 mg/L for ceftazidime, aztreonam, and/or ceftriaxone were selected for molecular screening of extended-spectrum β-lactamase (ESBL), plasmid-encoded AmpC, oxacillinase and carbapenemase genes, and epidemiology typing (MLST).

Screening of β-lactamase genes

- Selected isolates had 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 3.11.0. An in-house software was applied to align the assembled sequences against a comprehensive in-house database containing known β-lactamase genes.

Epidemiology typing

- Multilocus sequence typing (MLST) was performed by extracting a defined set of 7 housekeeping gene fragments (~500 bp).
  - Each fragment was compared to known allelic variants for each locus (housekeeping gene) on the MLST website (PubMLST, https://pubmlst.org).
- An allele sharing 100% genetic identity with a known variant received a numeric designation.
  - A 7-number sequence (1 for each housekeeping gene) formed an allelic profile, defined as sequence type (ST).
  - Isolates containing alleles that did not match an existing sequence in the MLST database were submitted/deposited for allele and/or ST assignments.

Results

- A total of 15.0% (360/2,035) of *E. coli* met the MIC criteria for screening of β-lactamases and most isolates (74.7%; 269/360) carried $bla_{CTX\text{-}M}$ (Table 1).
  - $bla_{CTX\text{-}M}$ associated with group 1 comprised the majority of genes (59.1%; 159/269), whereas the remaining $bla_{CTX\text{-}M}$ belonged to group 9 (40.5%; 109/269) (Table 1).
  - One isolate carried $bla_{CTX\text{-}M}$ genes ($bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}27}$) associated with both groups.
  - bla_{CMY} (33/360; 9.2%) was the most common cephalosporinase, followed by bla_{DHA} (7/360; 1.9%).
  - Other genes were comprised of $bla_{KPC\text{-}2}$ (1) or $bla_{SHV\text{-}12}$ (1) alone. Among 56 isolates ESBL, plasmid AmpC, or carbapenemase genes were not detected.
- A total of 55 ST types were noted in isolates that met the MIC criteria for screening of β-lactamases, with most isolates belonging to ST131 (53.1%; 191/360).
  - However, 3 isolates were single-locus variants (ST2279 and ST8671) of ST131; therefore comprising 53.9% (194/360) associated with clonal complex 131.
  - 25 (6.9%) and 23 (6.4%) isolates belonged to ST38 and ST1193, respectively, followed by STs represented by 8 or fewer isolates each (data not shown).
  - Among ST131, 55.2% (107/194) carried $bla_{CTX\text{-}M\text{-}15}$ and 35.1% (68/194) had genes associated with group 9 (9 isolates carrying $bla_{CTX\text{-}M\text{-}14}$ and 59 carrying $bla_{CTX\text{-}M\text{-}27}$).
  - 4 ST131 isolates carried $bla_{CMY\text{-}2}$ and another 15 isolates did not show any ESBL, plasmid AmpC, or carbapenemase genes.
- Tebipenem had MIC_{50} and MIC_{90} results of 0.015 mg/L and 0.015 mg/L, respectively, against non-ESBL isolates (Table 1). Similar MIC_{50} and MIC_{90} results (0.015 mg/L and 0.03 mg/L, respectively) were obtained against most subsets.
- Various parenteral agents were active (>90% susceptible) against the non-ESBL subset (Table 2).
  - However, oral options such as amoxicillin-clavulanate (86.6% susceptible), cefuroxime (74.2% susceptible), levofloxacin (84.2% susceptible), and trimethoprim-sulfamethoxazole (75.1% susceptible) showed limited activity (74.2%–86.6% susceptible).
  - Susceptibility rates (≤50.5% susceptible) for these oral agents were even lower against the ESBL and ST131 subsets.
- Carbapenem agents (93.8%–100% susceptible) and piperacillin-tazobactam (93.8%–93.9% susceptible) were active against the subset of isolates that met the MIC criteria for screening of β-lactamases and the ST131 subset (Table 2).

Conclusions

- In general half of the isolates that met the MIC criteria for screening of β-lactamases and caused UTI in the US belong to ST131.
  - These isolates used to carry mostly $bla_{CTX\text{-}M\text{-}15}$. However, an additional subset carrying $bla_{CTX\text{-}M}$ genes associated with group 9 were almost half of ST131 isolates.
- Carbapenem agents, including tebipenem, showed consistent *in vitro* activity against all subsets, regardless of resistance genotype or lineage. In addition, the tebipenem potency based on MIC_{90} (0.03 mg/L) against ESBL and ST131 isolates was greater than other oral options (MIC_{90}, >4 mg/L) for treating UTI caused by *E. coli*.
- The continued expansion of the ST131 lineage poses a complex scenario for antimicrobial treatment of UTI in the US. These data support the clinical development of tebipenem as a convenient oral treatment option for UTI caused by *E. coli* in the US.

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Table 1. Antimicrobial activity of tebipenem and ertapenem tested against *E. coli* isolates included in this study

Organism/organism group (no. of isolates)		No. and cumulative % of isolates inhibited at MIC (mg/L) of: ≤0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	MIC_{50}	MIC_{90}
Non-ESBL (2,035)	Tebipenem	5 (0.2)	588 (29.1)	1,351 (95.5)	72 (99.1)	14 (99.8)	4 (>99.9)	1 (100.0)					0.015	0.015
	Ertapenem		1,654 (86.7)	220 (98.2)	19 (99.2)	12 (99.8)	2 (99.9)	1 (100.0)					≤0.008	0.015
ESBL[a] (360)	Tebipenem		24 (6.7)	247 (75.3)	63 (92.8)	15 (96.9)	6 (98.6)	3 (99.4)	1 (99.7)	0 (99.7)	0 (99.7)	1[a] (100.0)	0.015	0.03
	Ertapenem		34 (9.7)	107 (40.2)	101 (68.9)	58 (85.5)	26 (92.9)	10 (95.7)	6 (97.4)	7 (99.4)	2 (100.0)		0.03	0.12
$bla_{CTX\text{-}M}$ (269)	Tebipenem		20 (7.4)	195 (79.9)	40 (94.8)	9 (98.1)	4 (99.6)	1 (100.0)					0.015	0.03
	Ertapenem		23 (8.8)	86 (41.6)	75 (70.2)	45 (87.4)	17 (93.9)	8 (96.9)	5 (98.9)	3 (100.0)			0.03	0.12
$bla_{CTX\text{-}M}$-group 1[b] (159)	Tebipenem		6 (3.8)	116 (76.7)	28 (94.3)	5 (97.5)	3 (99.4)	1 (100.0)					0.015	0.03
	Ertapenem		6 (4.0)	27 (21.9)	53 (57.0)	38 (82.1)	14 (91.4)	7 (96.0)	4 (98.7)	2 (100.0)			0.03	0.12
$bla_{CTX\text{-}M}$-group 9[c] (109)	Tebipenem		14 (12.8)	78 (84.4)	12 (95.4)	4 (99.1)	1 (100.0)						0.015	0.03
	Ertapenem		17 (15.6)	58 (68.8)	21 (88.1)	7 (94.5)	3 (97.2)	1 (98.2)	1 (99.1)	1 (100.0)			0.015	0.06
bla_{CMY}[d] (33)	Tebipenem		1 (3.0)	16 (51.5)	13 (90.9)	1 (93.9)	1 (97.0)	0 (97.0)	1 (100.0)				0.015	0.03
	Ertapenem		2 (6.2)	4 (18.8)	8 (43.8)	10 (75.0)	5 (90.6)	0 (90.6)	0 (90.6)	2 (96.9)	1 (100.0)		0.06	0.12
bla_{DHA} (7)	Tebipenem			2 (28.6)	2 (57.1)	2 (85.7)	1 (100.0)						0.03	—
	Ertapenem				3 (42.9)	2 (71.4)	1 (85.7)	0 (85.7)	0 (85.7)	1 (100.0)			0.06	—
Other[e] (56)	Tebipenem		3 (5.4)	36 (69.6)	10 (87.5)	4 (94.6)	1 (96.4)	2 (100.0)					0.015	0.06
	Ertapenem		8 (14.5)	19 (49.1)	15 (76.4)	2 (80.0)	5 (89.1)	2 (92.7)	1 (94.5)	3 (100.0)			0.03	0.25
ST131[f] (194)	Tebipenem		4 (2.1)	153 (80.9)	30 (96.4)	4 (98.5)	1 (99.0)	2 (100.0)					0.015	0.03
	Ertapenem		11 (5.8)	63 (38.9)	57 (68.9)	37 (88.4)	13 (95.3)	4 (97.4)	3 (98.9)	2 (100.0)			0.03	0.12
Non-ST131 (166)	Tebipenem		20 (12.0)	94 (68.7)	33 (88.6)	11 (95.2)	5 (98.2)	1 (98.8)	1 (99.4)	0 (99.4)	0 (99.4)	1 (100.0)	0.015	0.06
	Ertapenem		23 (14.3)	44 (41.6)	44 (68.9)	21 (82.0)	13 (90.1)	6 (93.8)	3 (95.7)	5 (98.8)	2 (100.0)		0.03	0.12

[a] Defined here as isolates displaying MIC results of ≥2 mg/L for ceftazidime, aztreonam, and/or ceftriaxone. A single isolate carried $bla_{KPC\text{-}2}$ and tebipenem and ertapenem MIC of 4 mg/L and 2 mg/L were obtained against this isolate, respectively. ESBL isolates may contain multiple genes and may be present in more than 1 subset.
[b] Represented by $bla_{CTX\text{-}M\text{-}15}$, except for 9 isolates carrying $bla_{CTX\text{-}M\text{-}55}$ and 1 isolate with $bla_{CTX\text{-}M\text{-}32}$.
[c] Represented by $bla_{CTX\text{-}M\text{-}14}$ (21), $bla_{CTX\text{-}M\text{-}27}$ (87), and $bla_{CTX\text{-}M\text{-}65}$ (1).
[d] Includes CMY-2, CMY-4, CMY-42, and CMY-102 encoding genes. Five isolates also carried $bla_{CTX\text{-}M}$, whereas 1 isolate also had a bla_{SHV}.
[e] Includes isolates that met the MIC criteria for screening of β-lactamases, where ESBL, plasmid AmpC, or carbapenemase genes were not detected.
[f] Includes ST131 and single-locus variants ST2279 (1 isolate) and ST8671 (2 isolates).

Table 2. Antimicrobial activity of tebipenem and comparator agents tested against *E. coli* and subsets

Antimicrobial agent	MIC (mg/L) 50%	90%	Range	CLSI[a] %S	%I	%R
Non-ESBL (2,035)						
Tebipenem	0.015	0.015	≤0.004 to 0.25	NA	NA	NA
Ertapenem	≤0.008	0.015	≤0.008 to 0.25	100.0	0.0	0.0
Meropenem	≤0.015	0.03	≤0.015 to 0.12	100.0	0.0	0.0
Imipenem	≤0.12	≤0.12	≤0.12 to 1	100.0	0.0	0.0
Ampicillin	4	>32	1 to >32	55.3	0.0	44.7
Amoxicillin-clavulanic acid	4	16	≤0.25 to >32	86.6	10.9	2.5
Aztreonam	0.12	0.25	≤0.03 to 1	100.0	0.0	0.0
Cefazolin	2	8	≤0.5 to >32	96.4[b] 96.4[c]	— —	3.6 3.6
Ceftazidime	0.12	0.25	0.03 to 1	100.0	0.0	0.0
Ceftriaxone	≤0.06	0.12	≤0.06 to 1	100.0	0.0	0.0
Cefuroxime	4	8	≤0.5 to 32	74.2[b] 95.3[c]	25.2 4.1	0.5 0.5
Levofloxacin	0.03	8	≤0.015 to >32	84.2	1.2	14.6
Nitrofurantoin	16	32	≤4 to >64	97.9	0.9	1.2
Piperacillin-tazobactam	2	4	≤0.06 to >128	98.9	0.3	0.8
Trimethoprim-sulfamethoxazole	≤0.12	>4	≤0.12 to >4	75.1	—	24.9
ESBL (360)						
Tebipenem	0.015	0.03	0.008 to 4	NA	NA	NA
Ertapenem	0.03	0.12	≤0.008 to 2	97.4	2.0	0.6
Meropenem	0.03	0.03	≤0.015 to 2	99.7	0.3	0.0
Imipenem	≤0.12	0.25	≤0.12 to 4	99.4	0.3	0.3
Ampicillin	>32		>32 to >32	0.0	0.0	100.0
Amoxicillin-clavulanic acid	16	32	2 to >32	47.2	30.6	22.2
Aztreonam	16	>16	0.12 to >16	18.9	17.2	63.9
Cefazolin	>32	>32	8 to >32	0.6[b] 0.6[c]	— —	99.4 99.4
Ceftazidime	16	>32	0.25 to >32	28.6	16.9	54.4
Ceftriaxone	>8	>8	0.12 to >8	6.4	1.4	92.2
Cefuroxime	>64	>64	8 to >64	0.0[b] 1.7[c]	4.5 2.8	95.5 95.5
Levofloxacin	8	32	≤0.015 to >32	26.1	2.5	71.4
Nitrofurantoin	16	32	≤4 to >64	90.6	3.7	5.7
Piperacillin-tazobactam	4	16	≤0.06 to >128	93.9	4.2	1.9
Trimethoprim-sulfamethoxazole	>4	>4	≤0.12 to >4	35.8	—	64.2
ST131 (194)						
Tebipenem	0.015	0.03	0.008 to 0.25	NA	NA	NA
Ertapenem	0.03	0.12	≤0.008 to 1	98.9	1.1	0.0
Meropenem	0.03	0.03	≤0.015 to 0.5	100.0	0.0	0.0
Imipenem	≤0.12	≤0.12	≤0.12 to 0.5	100.0	0.0	0.0
Ampicillin	>32		>32 to >32	0.0	0.0	100.0
Amoxicillin-clavulanic acid	8	16	4 to >32	50.5	40.7	8.8
Aztreonam	>16	>16	0.25 to >16	11.3	18.6	70.1
Cefazolin	>32	>32	32 to >32	0.0[b] 0.0[c]	— —	100.0 100.0
Ceftazidime	16	>32	0.5 to >32	24.2	19.1	56.7
Ceftriaxone	>8	>8	0.12 to >8	0.5	1.0	98.5
Cefuroxime	>64	>64	8 to >64	0.0[b] 0.5[c]	0.5 0.0	99.5 99.5
Levofloxacin	16	32	0.06 to >32	8.8	1.5	89.7
Nitrofurantoin	16	64	≤4 to >64	89.5	2.6	7.9
Piperacillin-tazobactam	4	16	0.5 to 128	93.8	5.2	1.0
Trimethoprim-sulfamethoxazole	>4	>4	≤0.12 to >4	34.0	—	66.0

ESBL, extended-spectrum β-lactamase and defined here as isolates displaying MIC results of ≥2 mg/L for ceftazidime, aztreonam, and/or ceftriaxone. A single isolate carried $bla_{KPC\text{-}2}$ and tebipenem and ertapenem MIC of 4 mg/L and 2 mg/L were obtained against this isolate, respectively.
[a] Criteria as published by CLSI (2021); NA, not applicable; "—", not available.
[b] Using oral breakpoints.
[c] Using parenteral breakpoints.

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