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Multi-Locus Sequence Typing of *Escherichia coli* Isolates from a Phase 2 Complicated Intra-Abdominal Trial for Eravacycline

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Revised Abstract

Background. The novel broad spectrum fluorocycline, eravacycline, and comparator ertapenem were tested in a phase 2 clinical trial assessing efficacy in the treatment of community-acquired complicated intra-abdominal infections (cIAIs) with sites in Bulgaria, India, Latvia, Lithuania, Romania, and the United States. Single *Escherichia coli* clonal groups, i.e., ST131, can be responsible for significant antimicrobial-resistant infections globally. The strain typing of *E. coli* isolates was done to determine the frequency of *E. coli* ST131 in patients with cIAIs in this trial.

Methods. A total of two hundred and twelve baseline isolates were collected from one hundred and nineteen patients, with *E. coli* as the most frequent isolate, comprising 44.3% (n=94) of all bacterial isolates in the microbiologically modified intent-to-treat population. Of these *E. coli*, eighty-eight individual isolates were available for evaluation of antimicrobial profile, resistance gene characterization, and multi-locus sequence type (MLST). Standard PCR methodology was used to amplify specific regions from seven independent loci: *adk, fumC, gyrB, icd, mdh, purA* and *recA* using the Environmental Research Institute at University College, Cork (ERI-UCC) database primers. Sequencing was performed by Genewiz (Cambridge, MA) and MLST analysis was done through the ERI-UCC *E. coli* database. Antibiotic-resistant isolates identified by testing in microtiter broth according to CLSI guidelines were also characterized by PCR analysis for tetracycline and extended spectrum β-lactamase (ESBL) genes.

Results. A total of 40 diverse sequence types were identified with the largest contributions from ST10 (n=7), ST73 (n= 13), ST131 (n=5), ST648 (n=5), ST59 (n=4), ST58 (n=3), ST69 (N=3), ST95 (N=3), ST167 (N=3), and two isolates each of ST205, ST223, ST227, ST405, ST410, ST493, and ST657. There were three isolates with sequences that did not appear to match known sequence types. A total of 17 isolates exhibited ESBL and levofloxacin-resistant phenotypes with ESBL resistance genes identified from CTX-M (n=9), OXA (n=10), CMY (n=9), TEM (n=14), and NDM (n=1) families, while 30 isolates exhibited tetracycline resistance associated with the presence of *tet*(A) or *tet*(B) genes.

Conclusions. *E. coli* was the predominant pathogen in the phase 2 trial. MLST analysis confirmed that the *E. coli* strains were heterogeneous and that common pathogenic isolates of *E. coli* from strain types ST131, ST95, ST69, ST10, ST648, and ST73 accounted for at least 40.9% of the *E. coli* isolates.

Background

Eravacycline (TP-434) has been shown to be effective against a majority of Gram-negative multidrug-resistant (MDR) pathogens in preclinical studies[1]. Eravacycline is being developed as a broad spectrum intravenous (IV) antibiotic with potential for oral step-down for empiric treatment of severe and life-threatening bacterial infections. It has the potential to be used as a once-daily IV monotherapy capable of treating MDR Gram-negative pathogens and its efficacy was demonstrated in a recent phase 2 trial for the treatment of complicated intra-abdominal infections[2,3]. Eravacycline also offers potent, broad spectrum coverage of other serious and MDR Gram-positive, anaerobic, and atypical pathogens.

Tetraphase is continuing to evaluate eravacycline's differentiated profile in two phase 3 studies to assess its use in the treatment of complicated intra-abdominal infections (cIAIs) and complicated urinary tract infections (cUTIs).

The current study aims to further identify and characterize drug-resistant isolates of *E.coli* from patients treated with eravacycline or ertapenem in a recent cIAI phase 2 trial. *E. coli* isolates were analyzed by multi-locus sequence typing (MLST) using the guidelines, primers and the online database from the Environmental Research Institute at University College, Cork (ERI-UCC) [4] .

Methods

Bacterial isolates from this study were collected by Eurofins-Medinet as part of the eravacycline cIAI phase 2 trial, TP-434-P2-cIAI-1. Antibacterial profiles of isolates were performed by Eurofins and confirmed at Tetraphase Pharmaceuticals according to the CLSI guidelines [5]. Isolates exhibiting drug resistant profiles were screened by PCR for common tetracycline, cephalosporin, and carbapenem resistance markers as appropriate.

Bacterial stocks for PCR were prepared by suspending 3-5 colonies of each isolate in 500 µL of DNase/Rnase-free water and boiling for 5 minutes. PCR reactions were run using a standard reaction protocol with Epicentre Failsafe PCR systems (Illumina, Madison, WI) and 2 µL of DNA template prepared as described above. Primers for tetracycline resistance and ESBL genes were designed in-house or used as described in previous published sources [6, 7, 8] and ordered through Operon (Eurofins-MWG, Huntsville, AL).

To further characterize isolates of *E.coli*, multi-locus sequence typing (MLST) was performed according to parameters outlined by the ERI-UCC *E.coli* database[4] using primers for *adk, fumC, gyrB, icd, mdh, purA* and *recA* genes synthesized by Operon. All sequencing reactions were performed by Genewiz, Inc (Cambridge, MA). Alignments and translations for confirming tetracycline and ESBL genes were done using publically available search databases at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi), Expasy (http://web.expasy.org/translate/), Uniprot (http://www.uniprot.org/blast/), and EMBL (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Results

Figure 1 – Eravacycline Phase 2 Baseline Species Distribution

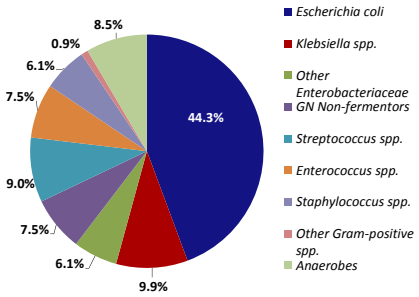

Figure 2 – *E. coli* Sequence Type Distribution

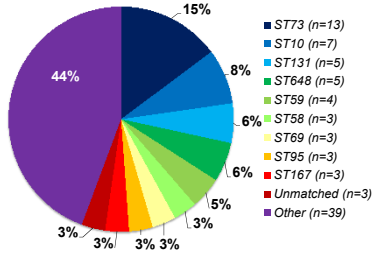

Table 2 – Geographic Distribution of Multidrug Resistant Isolates

Sequence Type	Resistance Profile	Source Country
10	Tet Gen	India
46	Gen Pip/Taz Lev	Lithuania
90	Tet Gen Lev	Romania
131	Tet Cef Lev	Romania
162	Tet Gen Lev	Bulgaria
167	Tet Cef Pip/Taz Lev	India
167	Tet Gen Cef Lev	India
167	Tet Cef Pip/Taz Lev	India
205	Cef Pip/Taz Lev	India
205	Cef Pip/Taz Lev	India
361	Tet Cef Pip/Taz Lev	India
405	Tet Cef Lev	India
405	Tet Gen Cef Pip/Taz Lev	India
410	Cef Pip/Taz Lev	India
410	Cef Pip/Taz Lev	India
493	Tet Pip/Taz	Romania
617	Cef Lev	India
648	Tet Cef Pip/Taz Lev Ert	India
648	Tet Lev	India
648	Tet Pip/Taz Lev	India
648	Tet Gen Cef Pip/Taz Lev	India
648	Tet Gen Cef Pip/Taz Lev	India

Table 1 – Susceptibility and Molecular Profiles of Most Prevalent Sequence Types

Antibiotic (MIC range in µg/mL for 88 *E. coli* isolates)	MIC Ranges (µg/mL) and Representation of ESBL and Tetracycline-Resistant Genes in Sequence Type Subsets								
	ST73 (n=13)	**ST10 (n=7)**	**ST131 (n=5)**	**ST648 (n=5)**	**ST59 (n=4)**	**ST58 (n=3)**	**ST69 (n=3)**	**ST95 (n=3)**	**ST167 (n=3)**
Eravacycline (0.12 – 1)	0.12 - 0.25	0.12 - 0.25	0.12 - 0.25	0.25	0.12 - 0.25	0.25 - 0.5	0.25 - 0.5	0.12	0.12 - 0.5
Ertapenem (≤0.002 - >16)	≤0.002 - 0.004	0.004 -0.008	0.004 - 0.015	0.008 - >16	0.004 -0.008	0.004	≤0.002 - 0.004	0.004	0.015 - 0.5
Tetracycline (0.5 - >32)	0.5 - >32	0.5 - >32	1 - >32	>32	1 - >32	1	>32	1	>32
Gentamicin (0.25 - >8)	0.25 - 1	0.5 - >8	0.5 - 2	0.5 - >8	0.5 - 2	0.5 - 1	0.5 - 2	0.25 - 1	0.5 - >8
Ceftazidime (≤0.03 - >64)	0.06 - 0.12	0.06 - 0.12	0.06 - 4	0.12 - >64	0.06 - 0.12	0.12	0.06 - 0.12	0.06 - 0.12	32 - >64
Cefotaxime (≤0.015 - >32)	≤0.015 - 0.06	≤0.015 - 0.06	0.03 - 32	0.12 - >32	0.03	0.03 - 0.06	0.03 - 0.06	0.06	>32
Piperacillin/Tazobactam (≤0.5 - >64)	≤0.5 - 2	≤0.5 - 2	1 - 2	4 - >64	1	1 - 2	≤0.5 - 1	1 - 2	1 - >64
Levofloxacin (0.015 - >4)	0.015 - 0.25	0.015 - 0.25	0.03 - >4	>4	0.015 - 0.03	0.03 - 0.06	0.03	0.03 - 0.25	>4
ESBL or tet-gene positive isolates	*tet*(B) (n=1)	bla_{TEM} (n=1) *tet*(A) (n=2)	bla_{CTX-M} (n=1) *tet*(A) (n=1) *tet*(B) (n=1)	bla_{TEM} (n=2) bla_{CTX-M} (n=3) bla_{CMY} (n=1) bla_{OXA} (n=5) bla_{NDM-7} (n=1) *tet*(B) (n=5)	bla_{TEM} (n=1) *tet*(A) (n=1)		bla_{TEM} (n=3) *tet*(A) (n=1) *tet*(B) (n=2)		bla_{CTX-M} (n=3) bla_{CMY} (n=2) bla_{OXA} (n=3) *tet*(A) (n=2)
Country	Bulgaria (n=2) India (n=7) Latvia (n=3) Lithuania (n=1)	Bulgaria (n=2) India (n=2) Lithuania (n=2) Romania (n=1)	Bulgaria (n=1) Latvia (n=2) Lithuania (n=1) Romania (n=1)	India (n=5)	Latvia (n=2) Lithuania (n=1) Romania (n=1)	India (n=1) Latvia (n=1) Lithuania (n=1)	Lithuania (n=3)	Lithuania (n=3)	India (n=3)

Conclusions

- The largest share of isolates from the eravacycline phase 2 cIAI trial were *E. coli.*
- The majority of multidrug-resistant isolates was ST648 (22.7%) from India.
- Multi-locus sequence typing of these isolates revealed a highly diverse population representing more than 40 unique sequence types with ST131 representing only 5.7% of the phase 2 isolates.
- Screening of isolates by PCR revealed a variety of tetracycline resistance genes, β-lactamase, and extended spectrum β-lactamase genes.
- The $MIC_{50/90}$ values of eravacycline were 0.25/0.5 µg/mL regardless of resistance phenotype and consistent with the values obtained with an even larger set of isolates (n=445[1]).
- Eravacycline was potent and efficacious across all sequence types and MDR phenotypes.

References

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