β-lactamase characterization of baseline Enterobacterales pathogens from a Phase 3 trial of sulopenem for the treatment of complicated intraabdominal infection

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ABSTRACT

Background
Between 2018-2020, a Phase 3, randomized, multi-center, double-blind, double-dummy trial was conducted to determine the efficacy, safety, and tolerability of intravenous (IV) sulopenem followed by oral sulopenem etzadroxil/probenecid versus IV ertapenem followed by ciprofloxacin and metronidazole or amoxicillin-clavulanate in the treatment of adults with complicated intra-abdominal infection (cIAI). Here, the β-lactamase content of select Enterobacterales isolates recovered from patients enrolled in these trials was characterized.

Methods
Enterobacterales isolates with MIC values of >1µg/mL for ceftriaxone, imipenem, meropenem, and/or ertapenem were screened for the presence of *bla* encoding ESBLs, AmpC β-lactamases, and carbapenemases by multiplex PCR. Detected genes were sequenced and the enzyme variant deduced by comparison to NCBI databases.

Results
The trial included 670 patients from 11 countries. The microbiologic-modified intent-to-treat (micro-MITT) population included 515 patients with ≥1 gram-negative pathogen identified from intraabdominal site or blood at study entry, including 637 Enterobacterales. 115 baseline Enterobacterales isolates from 95 (18.4%) micro-MITT patients met the qualifying MIC screening criteria. Enterobacterales were comprised of 13 species, including *Escherichia coli* [47.0%], *Klebsiella pneumoniae* [20.9%], and *Enterobacter cloacae* [13.0%]. 47.0% (54/115) of Enterobacterales isolates harbored ≥2 β-lactamase-encoding genes. Most Enterobacterales (66.1%; 76/115) carried *bla*CTX-M alone or in combination with other ESBL/pAmpC/carbapenemases and/or narrow-spectrum enzymes. The CTX-M-encoding genes were predominantly from group 1 (85.5%) or group 9 (14.5%).

Carbapenemase-encoding genes (*bla*NDM-1, *bla*OXA-48, *bla*OXA-232, *bla*VIM-1, and *bla*KPC-2) were noted in 11.3% (13/115) of Enterobacterales; these isolates were from Ukraine (7), Russia (3), Bulgaria (2), and Georgia (1).

Conclusions
Complicated intraabdominal infection in adults was due to Enterobacterales harboring ESBL- and carbapenemase-producing genes in 18.0% and 11.3% of isolates, respectively. Carbapenemase-producing Enterobacterales were concentrated in the Eastern European region. CTX-M, mainly group 1 and 9 enzymes, prevailed among cIAI Enterobacterales isolates that met the screening criteria. Novel oral antibiotics with potent activity against ESBL-producing organisms, such as sulopenem, are needed to facilitate early discharge for hospitalized IAI patients.

INTRODUCTION

- SURE-3 (IT001-303) was a double-blind, double-dummy, Phase 3 randomized trial that enrolled 674 hospitalized adults with complicated intraabdominal infection (cIAI) and compared sulopenem 1000 mg IV once daily x 5 days followed by oral sulopenem BID to complete 7-10 days of therapy, or ertapenem 1000 mg IV once daily x 5 days followed by oral ciprofloxacin 500 mg PO BID along with metronidazole 500 mg PPO four times daily or amoxicillin/clavulanate 875 mg BID, depending on baseline pathogen susceptibility, to complete 7-10 days of therapy. The primary endpoint was clinical response in the microbiologic modified intent to treat (micro-MITT) population at the Test-of-Cure (Day 28) Visit.

- The study presented here reports the characterization of β-lactamase content among baseline Enterobacterales isolates that met the predefined MIC criteria for *bla* encoding extended-spectrum β-lactamase (ESBL), AmpC β-lactamases, and carbapenemases

METHODS

- All Enterobacterales isolates were evaluated by MIC threshold to determine if β-lactamase screening was warranted.
- Qualifying MIC thresholds and specific β–lactamase enzymes screened for by multiplex PCR are outlined in Table 1.
- All detected *bla* genes were amplified with extragenic primers and sequenced in their entirety and compared to databases maintained at NCBI to determine the variant, with the exception of:
  - SHV and TEM
    - $_{bla}$TEM and $_{bla}$SHV were screened by limited sequencing to identify genes encoding TEM-type ad SHV-type enzymes containing amino acid substitutions common to ESBLs at the following positions:
      - SHV a.a. 146, 179, 238, 240; TEM a.a. 104, 164, 238, 240
        - Based on the presence/absence of these signature amino acids, TEM and SHV enzyme variants were reported as –ESBL or –OSBL (original spectrum β-lactamase)
  - Chromosomal AmpC genes intrinsic to particular species
    - ACT/MIR detected in *Enterobacter* spp.,
    - CMY detected in *Citrobacter* spp.,
    - ACC in *Hafnia alvei*,
    - DHA detected in *Morganella morganii*

RESULTS

Table 1: β-lactamase testing qualifications and specific enzymes screened for in Enterobacterales pathogens recovered from SURE-3 patients with cIAI

Screening Qualifications

- Ceftriaxone MIC >1 µg/mL,
- Imipenem MIC >1 µg/mL (*Proteus* species, *Providencia* species, *M. morganii* MIC >4 µg/mL),
- Meropenem MIC >1 µg/mL, **OR**
- Ertapenem MIC >1 µg/mL

Qualifying Isolates Screened for by Multiplex PCR:

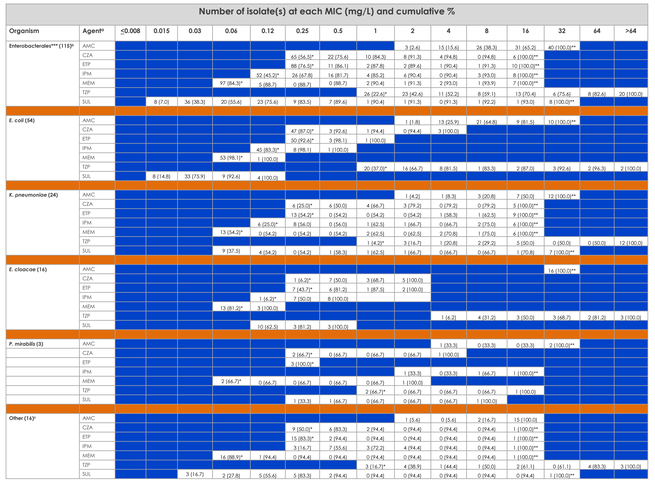
- *bla* encoding ESBLs
  - TEM, SHV, CTX-Ms (5 subtypes), GES, VEB, PER
- AmpC β-lactamases
  - ACC, ACT, CMY, DHA, FOX, MIR, MOX
- Carbapenemases
  - KPC, OXA-48 group, IMP, VIM, NDM, SPM, GIM

Table 2: Baseline Enterobacterales isolates meeting MIC screening criteria

Species	Number of Isolates	Region	Number of Isolates
Escherichia coli	54	Ukraine	48
Klebsiella pneumoniae	24	Bulgaria	29
Enterobacter cloacae	15	Russia	20
Citrobacter freundii	5	Georgia	8
Serratia marcescens	3	Latvia	4
Enterobacter, non-speciated	3	Estonia	4
Proteus mirabilis	3	Hungary	2
Citrobacter braakii	2	United States	0
Klebsiella aerogenes	2	Serbia	0
Klebsiella variicola	1	Poland	0
Citrobacter koseri	1	Czech Republic	0
Providencia stuartii	1		
Raoultella ornitholytica	1		
Total	115	**Total**	115

RESULTS

Table 3: MIC results for sulopenem and comparators against β-lactamase producing strains of Enterobacterales, SURE-3 cIAI patients



[a] AMC = amoxicillin/clavulanate; CZA = ceftazidime/avibactam; ETP = ertapenem; IPM = imipenem; MEM = meropenem; TZP = piperacillin/tazobactam; SUL = sulopenem
[b] Includes 54 *E. coli*, 24 *K. pneumoniae*, 16 *E. cloacae*, 3 *P. mirabilis*, 2 *C. braakii*, 2 *K. aerogenes*, 1 *K. variicola*, 1 *C. koseri*, 1 *P. stuartii*, 1 *Raoultella ornitholytica*
[c] Includes 2 *C. braakii*, 2 *K. aerogenes*, 1 *K. variicola*, 1 *C. koseri*, 1 *P. stuartii*, 1 *Raoultella ornitholytica*
* Value represents number and percentage of isolates at or below given MIC; ** Value represents number and percentage of isolates at or above given MIC; ***Includes carbapenemase-producing isolates: *K. pneumoniae* (9), *P. mirabilis* (1), *P. stuartii* (1)

Table 5: Select characteristics of enzymes identified from isolates of SURE-3 patients with cIAI

Number of enzymes identified per qualifying isolate (N=115)

	Number of enzymes identified				
	0	1	2	3	4
# isolates	20 (17.4%)	41 (35.7%)	32 (27.8%)	17 (14.8%)	5 (4.3%)

Presence of CTX-M-type enzyme, alone or in combination (N=95)

Category		
No CTX-M-type enzyme detected	CTX-M-type enzyme detected alone	CTX-M-type enzyme detected in combination with other enzyme(s)
18 (18.9%)	29(30.5%)	48 (50.5%)

Group type for isolates with a CTX-M-type enzyme (N=77)

	CTX-M-1-type	CTX-M-9-type
# isolates	66 (85.7%)	11 (14.3%)

Table 4: Summary of β-lactamase enzymes detected among baseline Enterobacterales, SURE-3 cIAI patients

Pathogen (No; % of all Enterobacterales)/ Results	No. of isolates	Pathogen (No; % of all Enterobacterales)/ Results	No. of isolates
E. coli (54; 47.0)		CTX-M-15	2
CMY-2	3	CTX-M-15; TEM-OSBL	9
CMY-2; TEM-OSBL**	2	No acquired β-lactamases detected	4
CTX-M-1	1	*C. freundii (5; 4.3)*	
CTX-M-3; TEM-OSBL	1	CMY-2-TYPE	1
CTX-M-9-TYPE; TEM-OSBL	1	CMY-2-TYPE; CTX-M-15; SHV-ESBL; TEM-OSBL	1
CTX-M-14; TEM-OSBL	2	CMY-2-TYPE; SHV-ESBL	1
CTX-M-15	16	No acquired β-lactamases detected	2
CTX-M-15; TEM-OSBL	9	Enterobacter, non-speciated (3; 2.6)	
CTX-M-27	7	CTX-M-15	1
CTX-M-27; TEM-OSBL	1	No acquired β-lactamases detected	1
TEM-OSBL	4	*S. marcescens (3; 2.6)*	
No acquired β-lactamases detected	7	CTX-M-15; SHV-ESBL; TEM-OSBL	2
K. pneumoniae (24; 20.9)		CTX-M-15; TEM-OSBL	1
CTX-M-3; SHV-OSBL; TEM-OSBL	1	*P. mirabilis (3; 2.6)*	
CTX-M-15; KPC-2*; SHV-OSBL; TEM-OSBL	1	CMY-2	1
CYX-M-15; NDM-1*; OXA-232*; SHV-OSBL	1	CMY-99; SHV-ESBL; TEM-OSBL; VIM-1*	1
CTX-M-15; NDM-1*; SHV-OSBL	1	No acquired β-lactamases detected	1
CTX-M-15; OXA-232*; SHV-OSBL	1	*K. aerogenes (2; 1.7)*	
CTX-M-15; OXA-48*; SHV-OSBL	1	No acquired β-lactamases detected	2
CTX-M-15; OXA-48*; SHV-OSBL; TEM-OSBL	1	*C. braakii (2; 1.7)*	
CTX-M-15; SHV-OSBL	3	No acquired β-lactamases detected	2
CTX-M-15; SHV-OSBL; TEM-OSBL	9	*C. koseri (1; 0.9)*	
CTX-M-55; NDM-1*; SHV-OSBL	1	CTX-M-1	1
NDM-1*	1	*P. stuartii (1; 0.9)*	
NDM-1*; SHV-OSBL	1	CTX-M-15; NDM-1*; OXA-48*	1
SHV-OSBL	1	*K. variicola (1; 0.9)*	
SHV-OSBL; TEM-OSBL	1	SHV-ESBL	1
E. cloacae (15; 13.0)		*R. ornitholytica (1; 0.9)*	
CTX-M-3	1	No acquired β-lactamases detected	1

*KPC-2: Bulgaria (2); NDM-1: Ukraine (5), Russia (1); OXA-48: Russia (2), Georgia (1), OXA-232: Ukraine (2); VIM-1: Bulgaria (1)
** OSBL = original spectrum beta-lactamase

CONCLUSIONS

- Complicated intraabdominal infection in hospitalized adults due to β-lactamase-producing Enterobacterales was common
- Complicated intraabdominal infection due to carbapenemase-producing Enterobacterales was less common
  - Carbapenemase-producing Enterobacterales were concentrated in the Eastern European region
- CTX-M, exclusively group 1 and group 9 enzymes, prevailed among complicated intraabdominal infection Enterobacterales isolates that met the screening criteria
- Novel oral antibiotics with potent activity against β-lactamase-producing organisms, such as sulopenem, are needed to facilitate early discharge for hospitalized intraabdominal infection patients.