β-lactamase characterization of baseline Enterobacterales pathogens from a Phase 3 trial of sulopenem for the treatment of uncomplicated urinary tract 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 efficacy, safety, and tolerability of oral sulopenem etzadroxil/probenecid versus ciprofloxacin in the treatment of adult women with uncomplicated urinary tract infection (uUTI). Here, the β-lactamase content of select Enterobacterales isolates recovered from patients enrolled in this trial 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 enrolled 1671 patients from 3 countries. The microbiologic-modified intent-to-treat (micro-MITT) population included 1071 patients with ≥10^5 CFU/mL of Enterobacterales (1058) or *S. saprophyticus* (13) in the baseline urine culture. 214 of the baseline Enterobacterales isolates from 193 (18.0%) micro-MITT patients met MIC screening criteria for β-lactamase content determination. Enterobacterales were comprised of 8 species, including *Escherichia coli* [80.4%] and *Klebsiella pneumoniae* [13.6%]. 38.3% (82/214) of Enterobacterales isolates harbored ≥2 β-lactamase-encoding genes. Most Enterobacterales (75.2%; 161/214) 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 (67.7%) or group 9 (29.2%). CTX-M-15 was identified in 56.1% of the isolates that were both quinolone non-susceptible and ESBL+.

Carbapenemase-encoding genes (*bla*OXA-48 & *bla*NDM-1) were noted in 6/214 Enterobacterales; these isolates were from Russia and Ukraine. 21 qualifying isolates tested negative for any resistance genes.

Conclusions
Uncomplicated urinary tract infection in adult females due to ESBL-producing Enterobacterales was common, while uUTI due to carbapenemase-producing Enterobacterales was uncommon, absent in patients from the United States. ESBL-producing isolates harboring CTX-M-15 were often resistant to fluoroquinolones, highlighting co-resistance and the need for new oral antibiotic options with broad-spectrum activity for the treatment of uUTI. Sulopenem demonstrated potent activity against multidrug resistant Enterobacterales including ESBL-producing strains and should be studied further for the treatment of uUTI.

INTRODUCTION

- SURE-1 (IT001-301) was a double-blind, double-dummy, Phase 3 randomized trial that enrolled 1671 ambulatory female adults with uncomplicated UTI (uUTI) and compared sulopenem etzadroxil/probenecid 500 mg/500 mg PO BID x 5 days to ciprofloxacin 250 mg PO BID x 3 days. The primary endpoint was overall (clinical + microbiologic) response in the microbiologic modified intent to treat (micro-MITT) population with baseline uropathogens either susceptible or resistant to the comparator, ciprofloxacin (micro-MITTS and micro-MITTR populations, respectively), at the Test-of-Cure (Day 12) 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-1 patients with uUTI

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	172	Russia	100
Klebsiella pneumoniae	29	United States	80
Enterobacter cloacae	4	Ukraine	34
Klebsiella aerogenes	4		
Citrobacter freundii	2		
Citrobacter koseri	1		
Morganella morganii	1		
Providencia stuartii	1		
Total	214	**Total**	214

RESULTS

Table 3: MIC results for sulopenem and comparators against β-lactamase producing strains of Enterobacterales, SURE-1 uUTI patients

		Number of isolate(s) at each MIC (mg/L) and cumulative %														
Organism	Agent[a]	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Enterobacterales (212)[b]	AMC								3 (1.4)*	5 (3.8)	43 (24.1)	66 (55.2)	45 (76.4)	50 (100)**		
	CZA					142 (67.0)*	31 (81.6)	9 (85.8)	14 (92.4)	13 (98.6)	0 (98.6)	1 (99.1)	2 (100)**			
	ETP						189 (89.1)*	7 (92.4)	3 (93.9)	4 (95.7)	3 (97.2)	3 (98.6)	3 (100)**			
	IPM					97 (45.7)*	75(81.1)	13 (87.3)	11 (92.4)	13 (98.6)	1 (99.1)	0 (99.1)	2 (100)**			
	MEM				183 (86.3)*	13 (92.4)	5 (94.8)	2 (95.7)	4 (97.6)	2 (98.6)	1 (99.1)	0 (99.1)	2 (100)**			
	TZP										138 (65.1)*	27 (77.8)	6 (80.7)	4 (82.5)	12 (88.2)	25 (100)
	SUL	2 (0.9)	49 (24.1)	70 (57.1)	47 (79.2)	19 (88.2)	10 (92.9)	7 (96.2)	1 (96.7)	1 (97.2)	3 (98.6)	1 (99.1)	0 (99.1)	2 (100.00)		
E. coli (170***)	AMC								1 (0.6)*	5 (3.5)	42 (28.2)	60 (63.5)	37 (85.3)	25 (100)**		
	CZA					128 (75.3)*	22 (88.2)	7 (92.3)	4 (94.7)	8 (99.4)	0 (99.4)	1 (100)**				
	ETP						166 (97.6)*	3 (99.4)	0 (99.4)	0 (99.4)	1 (100)**					
	IPM					92 (54.1)*	65 (92.3)	7 (96.5)	1 (97.1)	4 (99.4)	1 (100)**					
	MEM				165 (97.1)*	4 (99.4)	0 (99.4)	1 (100)**								
	TZP										125 (73.5)*	21 (85.9)	4 (88.2)	2 (89.4)	8 (94.1)	10 (100)
	SUL	2 (1.2)	49 (30.0)	67 (69.4)	35 (90.0)	11 (96.5)	5 (99.4)	0 (99.4)	1 (100)**							
K. pneumoniae (29****)	AMC								1 (3.4)*	0 (3.4)	1 (6.9)	6 (27.6)	5 (44.8)	16 (100)**		
	CZA					6 (20.7)*	7 (44.8)	2 (51.7)	8 (79.3)	4 (93.1)	0 (93.1)	0 (93.1)	2 (100.0)**			
	ETP						14 (48.3)*	2 (55.2)	3 (65.5)	2 (72.4)	2 (79.3)	3 (89.7)	3 (100.0)**			
	IPM					4 (13.8)*	9 (44.8)	4 (58.6)	8 (86.2)	2 (93.1)	0 (93.1)	0 (93.1)	2 (100.0)**			
	MEM				11 (37.9)*	7 (62.1)	1 (65.5)	1 (69.0)	4 (82.8)	2 (89.7)	1 (93.1)	0 (93.1)	2 (100.0)**			
	TZP										4 (13.8)*	4 (27.6)	2 (34.5)	2 (41.4)	4 (55.2)	13 (100.0)
	SUL			2 (6.9)	6 (27.6)	8 (55.2)	3 (65.5)	3 (75.9)	0 (75.9)	1 (79.3)	3 (89.7)	1 (93.1)	0 (93.1)	2 (100.0)**		
E. cloacae (4)	AMC												1 (25.0)	3 (100.0)**		
	CZA					1 (25.0)*	1 (50.0)	0 (50.0)	2 (100.0)							
	ETP						2 (50.0)*	0 (50.0)	0 (50.0)	2 (100.0)						
	IPM						1 (25.0)	2 (75.0)	0 (75.0)	1 (100.0)						
	MEM				2 (50.0)*	0 (50.0)	2 (100.0)									
	TZP										1 (25.0)*	1 (50.0)	0 (50.0)	0 (50.0)	0 (50.0)	2 (100.0)
	SUL				1 (25.0)	0 (25.0)	1 (50.0)	2 (100.0)								
Other (9)[c]	AMC								1 (11.1)*	0 (11.1)	0 (11.1)	0 (11.1)	2 (33.3)	6 (100.0)**		
	CZA					7 (77.8)*	1 (88.9)	0 (88.9)	0 (88.9)	1 (100.0)						
	ETP						7 (77.8)*	2 (100.0)								
	IPM					1 (11.1)*	0 (11.1)	0 (11.1)	2 (33.3)	6 (100.0)						
	MEM				5 (55.6)*	2 (77.8)	2 (100.0)									
	TZP										8 (88.9)*	1 (100.0)				
	SUL			1 (11.1)	5 (66.7)	0 (66.7)	1 (77.8)	2 (100.0)								

[a] AMC = amoxicillin/clavulanate; CZA = ceftazidime/avibactam; ETP = ertapenem; IPM = imipenem; MEM = meropenem; TZP = piperacillin/tazobactam; SUL = sulopenem
[B] Includes 172 *E. coli*, 29 *K. pneumoniae*, 4 *E. cloacae*, 4 *K. aerogenes*, 2 *C. freundii*, 1 *C. koseri*, 1 *M. morganii*, 1 *P. stuartii*
[C] Includes 4 *K. aerogenes*, 2 *C. freundii*, 1 *C. koseri*, 1 *M. morganii*, 1 *P. stuartii*
* Value represents number and percentage of isolates at or below given MIC; ** Value represents number and percentage of isolates at or above given MIC; ***E.coli = 172 isolates, two with missing MIC data; ****Includes 6 carbapenemase-producing isolates (CRE)

Table 5: Select characteristics of enzymes identified from isolates of SURE-1 patients with uUTI

Number of enzymes identified per qualifying isolate (N=214)					
	Number of enzymes identified				
	0	1	2	3	4
# isolates	21(9.8%)	112 (52.3%)	59 (27.6%)	20 (9.3%)	2 (0.9%)
Presence of CTX-M-type enzyme, alone or in combination (N=193)					
Category					
No CTX-M-type enzyme detected	CTX-M-type enzyme detected alone	CTX-M-type enzyme detected in combination with other enzyme(s)			
23 (11.9%)	97(50.3%)	73 (37.8%)			
Presence of CTX-M-15 enzyme in ESBL-positive isolates that were also quinolone non-susceptible, SURE-1 micro-MITT population					
69/123 (56.1%)					
Group type for isolates with a CTX-M-type enzyme (N=170)					
	CTX-M-1-type	CTX-M-9-type	Other CTX-M-type		
# isolates	118 (69.4%)	47 (27.6%)	5 (2.9%)		

Table 4: Summary of β-lactamase enzymes detected among baseline Enterobacterales, SURE-1 uUTI patients

Pathogen (No; % of all Enterobacterales)/ Results	No. of isolates	Pathogen (No; % of all Enterobacterales)/ Results	No. of isolates
E. coli (172; 80.4)		*K. pneumoniae* (continued)	
CMY-2	9	CTX-M-15; NDM-1*	1
CMY-2; CTX-M-15	1	CTX-M-15; OXA-48*; SHV-OSBL; TEM-OSBL	1
CMY-2; CTX-M-15; TEM-OSBL	4	CTX-M-15; SHV-OSBL	7
CMY-2; TEM-OSBL	2	CTX-M-15; SHV-OSBL; TEM-OSBL	8
CMY-2-TYPE; CTX-M-15; TEM-OSBL	1	CTX-M-27; SHV-OSBL	1
CTX-M-1	1	CTX-M-164; SHV-OSBL; TEM-OSBL	1
CTX-M-1-TYPE; TEM-OSBL	1	NDM-1*; SHV-OSBL	1
CTX-M-3	4	OXA-48*; SHV-OSBL	3
CTX-M-3; TEM-OSBL	2	SHV-ESBL; TEM-OSBL	1
CTX-M-14	9	SHV-OSBL; TEM-OSBL	1
CTX-M-14; TEM-OSBL	1	No acquired β-lactamases detected	1
CTX-M-15	50	*E. cloacae (4; 1.9)*	
CTX-M-15; TEM-OSBL	23	ACT-TYPE	1
CTX-M-27	26	ACT-TYPE; CTX-M-15; TEM-OSBL	2
CTX-M-27; TEM-OSBL	7	CTX-M-15; TEM-OSBL	1
CTX-M-27; TEM-TRUNC	1	*C. freundii (2; 0.9)*	
CTX-M-55	3	CMY-2-TYPE	1
CTX-M-55; TEM-OSBL	4	CMY-2-TYPE; CTX-M-15; TEM-OSBL	1
CTX-M-65; TEM-OSBL	1	*M. morganii (1; 0.5)*	
CTX-M-206	3	CTX-M-15; DHA-TYPE; TEM-OSBL	1
CTX-M-216	1	*K. aerogenes (4; 1.9)*	
TEM-OSBL	4	No acquired β-lactamases detected	4
No acquired β-lactamases detected	14	*C. koseri (1; 0.5)*	
K. pneumoniae (29; 13.6)		No acquired β-lactamases detected	1
CTX-M-3; SHV-OSBL; TEM-OSBL	1	*P. stuartii (1; 0.5)*	
CTX-M-14; SHV-OSBL; TEM-OSBL	1	No acquired β-lactamases detected	1
CTX-M-15; DHA-TYPE; SHV-OSBL; TEM-OSBL	1		

*NDM-1: Russia (2); OXA-48: Russia (2), Ukraine (2)

CONCLUSIONS

- Uncomplicated UTI in ambulatory adult females due to β-lactamase-producing Enterobacterales was common
- Uncomplicated UTI due to carbapenemase-producing Enterobacterales was uncommon, absent in patients from the United States
- ESBL-producing isolates harboring CTX-M-15 were often resistant to fluoroquinolones, highlighting co-resistance and the need for new oral antibiotic options with broad-spectrum activity for the treatment of uncomplicated UTI
- Sulopenem demonstrated potent activity against drug resistant Enterobacterales, including β-lactamase-producing strains and should be studied further for the treatment of uncomplicated UTI

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