β-lactamase characterization of baseline Enterobacterales pathogens from a Phase 3 trial of sulopenem for the treatment of complicated 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 the efficacy, safety, and tolerability of intravenous (IV) sulopenem followed by oral sulopenem etzadroxil/probenecid versus IV ertapenem followed by ciprofloxacin or amoxicillin-clavulanate in the treatment of adults with complicated urinary tract infection (cUTI). 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 included 1395 patients from 13 countries. The microbiologic-modified intent-to-treat (micro-MITT) population included 884 patients with $\geq 10^5$ CFU/mL of Enterobacterales in the baseline urine culture. 371 of these baseline Enterobacterales isolates from 352 (39.8%) micro-MITT patients met the qualifying MIC screening criteria. Enterobacterales were comprised of 14 species, including *Escherichia coli* [52.0%], *Klebsiella pneumoniae* [25.1%], *Enterobacter cloacae* [8.4%], and *Proteus mirabilis* [7.0%]. A total of 50.7% (188/371) of Enterobacterales isolates harbored at least 2 β-lactamase-encoding genes. Most Enterobacterales (80.9%; 300/371) 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 (85.3%) or group 9 (14.7%).

Carbapenemase-encoding genes (*bla*NDM-1, *bla*OXA-48, *bla*VIM-1, *bla*NDM-5, *bla*OXA-244, and *bla*KPC-2) were noted in 5.9% (22/371) of Enterobacterales; these isolates were from Russia (10), Bulgaria (5), Ukraine (4), Georgia (2), and Croatia (1).

Conclusions
Complicated urinary tract infection in adults was due to Enterobacterales harboring ESBL- and carbapenemase-producing genes in 39.8% and 5.9% of isolates, respectively. Carbapenemase-producing Enterobacterales were concentrated in the Eastern European region. CTX-M, mainly group 1 and 9 enzymes, prevailed among cUTI 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 cUTI patients.

INTRODUCTION

- SURE-2 (IT001-302) was a double-blind, double-dummy, Phase 3 randomized trial that enrolled 1395 hospitalized adults with complicated UTI (cUTI) 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 BID or amoxicillin/clavulanate 875 mg BID, depending on baseline uropathogen susceptibility, to complete 7-10 days of therapy. The primary endpoint was overall (clinical + microbiologic) response in the microbiologic modified intent to treat (micro-MITT) population at the Test-of-Cure (Day 21) 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-2 patients with cUTI

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	193	Russia	124
Klebsiella pneumoniae	93	Bulgaria	74
Enterobacter cloacae	31	Ukraine	70
Proteus mirabilis	26	Georgia	44
Citrobacter freundii	8	Hungary	19
Morganella morganii	5	Latvia	14
Klebsiella aerogenes	3	Estonia	7
Klebsiella variicola	3	United States	5
Klebsiella oxytoca	3	Slovakia	5
Serratia marcescens	2	Croatia	4
Enterobacter xiangfangensis	1	Czech Republic	3
Providencia stuartii	1	Serbia	2
Raoultella ornitholytica	1	Poland	0
Raoultella planticola	1		
Total	371	**Total**	371

RESULTS

Table 3: MIC results for sulopenem and comparators against β-lactamase producing strains of Enterobacterales, SURE-2 cUTI patients

Organism	Agent[a]	Number of isolate(s) at each MIC (mg/L) and cumulative %														
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Enterobacterales (365***)[b]	AMC								5 (1.4)*	6 (3.0)	42 (14.5)	113 (45.5)	81 (67.7)	42 (79.2)	76 (100.0)**		
	CZA					187 (51.2)*	65 (69.0)	51 (83.0)	32 (91.8)	13 (95.3)	6 (97.0)	0 (97.0)	11 (100.0)**				
	ETP						277 (75.9)*	21 (81.6)	19 (86.8)	15 (91.0)	9 (93.4)	6 (95.1)	18 (100.0)**				
	IPM					111 (30.4)*	114 (61.6)	60 (78.1)	29 (86.0)	17 (90.7)	14 (94.5)	9 (97.0)	11 (100.0)**				
	MEM				279 (76.4)*	35 (86.0)	14 (89.9)	8 (92.0)	12 (95.3)	4 (96.4)	1 (96.7)	1 (97.0)	11 (100.0)**				
	TZP							26 (7.1)*	41 (18.4)	68 (37.0)	57 (52.6)	51 (66.6)	24 (73.1)	18 (78.1)	14 (81.9)	15 (86.0)	51 (100.0)
	SUL	1 (0.3)	79 (21.9)	101 (49.6)	52 (63.8)	43 (75.6)	33 (84.7)	24 (91.2)	12 (94.5)	5 (95.9)	3 (96.7)	0 (96.7)	1 (97.0)	11 (100.0)**			
E. coli (193)	AMC									3 (1.5)	33 (18.6)	86 (63.2)	46 (87.0)	10 (92.2)	15 (100.0)**		
	CZA					137 (71.0)*	34 (88.6)	15 (96.4)	3 (97.9)	2 (99.0)	0 (99.0)	0 (99.0)	2 (100.0)**				
	ETP						179 (92.7)*	8 (96.9)	3 (98.4)	0 (98.4)	0 (98.4)	0 (98.4)	3 (100.0)**				
	IPM					89 (46.1)*	78 (86.5)	21 (97.4)	2 (98.4)	1 (99.0)	1 (99.5)	0 (99.5)	1 (100.0)**				
	MEM				188 (97.4)*	1 (97.9)	0 (97.9)	1 (98.4)	1 (99.0)	0 (99.0)	0 (99.0)	0 (99.0)	2 (100.0)**				
	TZP							10 (5.2)*	33 (22.3)	57 (51.8)	42 (73.6)	27 (87.6)	9 (92.2)	6 (95.3)	2 (96.4)	5 (99.0)	2 (100.0)
	SUL	1 (0.5)	75 (39.4)	80 (80.8)	27 (94.8)	6 (97.9)	1 (98.4)	1 (99.0)	0 (99.0)	0 (99.0)	0 (99.0)	0 (99.0)	0 (99.0)	2 (100.0)**			
K. pneumoniae (92)	AMC									1 (1.1)	3 (4.3)	19 (25.0)	30 (57.6)	24 (83.7)	15 (100.0)**		
	CZA					17 (18.5)*	21 (41.3)	23 (66.3)	18 (85.9)	5 (91.3)	2 (93.5)	0 (93.5)	6 (100.0)**				
	ETP						42 (45.6)*	9 (55.4)	10 (66.3)	8 (75.0)	5 (80.4)	5 (85.9)	13 (100.0)**				
	IPM					18 (19.6)*	31 (53.3)	20 (75.5)	12 (88.0)	3 (91.3)	0 (91.3)	2 (93.5)	6 (100.0)**				
	MEM				46 (50.0)*	13 (64.1)	6 (70.6)	4 (75.0)	10 (85.9)	4 (90.2)	1 (91.3)	1 (92.4)	7 (100.0)**				
	TZP							1 (1.1)*	0 (1.1)	2 (3.3)	8 (12.0)	18 (31.5)	8 (40.2)	8 (48.9)	4 (53.3)	7 (60.9)	36 (100.0)
	SUL		4 (4.3)	18 (23.9)	16 (41.3)	16 (58.7)	11 (70.6)	8 (79.3)	7 (87.0)	2 (89.1)	2 (91.3)	0 (91.3)	1 (92.4)	7 (100.0)**			
E. cloacae (29)	AMC											1 (3.4)	0 (3.4)	5 (20.7)	23 (100.0)**		
	CZA					3 (10.3)*	4 (24.1)	6 (44.8)	8 (72.4)	3 (82.8)	3 (93.1)	0 (93.1)	2 (100.0)**				
	ETP						10 (34.5)*	3 (44.8)	3 (55.2)	7 (79.3)	3 (89.7)	1 (93.1)	2 (100.0)**				
	IPM					3 (10.3)	4 (24.1)	14 (72.4)	5 (89.7)	1 (93.1)	0 (93.1)	0 (93.1)	2 (100.0)**				
	MEM				11 (37.9)*	7 (62.1)	5 (79.3)	3 (89.7)	1 (93.1)	0 (93.1)	0 (93.1)	0 (93.1)	2 (100.0)**				
	TZP								1 (3.4)	2 (10.3)	3 (20.7)	1 (24.1)	4 (37.9)	3 (48.3)	3 (58.6)	2 (65.5)	10 (100.0)
	SUL			2 (6.9)	4 (20.7)	6 (41.4)	9 (72.4)	2 (79.3)	3 (89.7)	1 (93.1)	0 (93.1)	0 (93.1	0 (93.1)	2 (100.0)**			
P. mirabilis (25)	AMC								3 (12.0)*	2 (20.0)	5 (40.0)	5 (60.0)	3 (72.0)	1 (76.0)	6 (100.0)**		
	CZA					19 (76.0)*	1 (80.0)	1 (84.0)	1 (88.0)	1 (92.0)	1 (96.0)	0 (96.0)	1 (100.0)**				
	ETP						25 (100.0)*										
	IPM								3 (12.0)	6 (36.0)	11 (80.0)	4 (96.0)	1 (100.0)**				
	MEM				15 (60.0)*	8 (92.0)	2 (100.0)										
	TZP							10 (40.0)*	4 (56.0)	5 (76.0)	2 (84.0)	3 (96.0)	1 (100.0)				
	SUL					6 (24.0)	9 (60.0)	9 (96.0)	0 (96.0)	1 (100.0)							
Other (26)[c]	AMC								2 (7.7)*	0 (7.7)	1 (11.5)	2 (19.2)	2 (26.9)	2 (34.6)	17 (100.0)**		
	CZA					11 (42.3)*	5 (61.5)	6 (84.6)	2 (92.3)	2 (100.0)							
	ETP						21 (80.8)*	1 (84.6)	3 (96.1)	0 (96.1)	1 (100.0)						
	IPM					1 (3.8)*	1 (7.7)	5 (26.9)	7 (53.8)	6 (76.9)	2 (84.6)	3 (96.1)	1 (100.0)**				
	MEM				19 (73.1)*	6 (96.1)	1 (100.0)										
	TZP							5 (19.2)*	3 (30.8)	2 (38.5)	2 (46.1)	2 (53.8)	2 (61.5)	1 (65.4)	5 (84.6)	1 (88.5)	3 (100.0)
	SUL			1 (3.8)	5 (23.1)	9 (57.7)	3 (69.2)	4 (84.6)	2 (92.3)	1 (96.1)	1 (100.0)**						

[a] AMC = amoxicillin/clavulanate; CZA = ceftazidime/avibactam; ETP = ertapenem; IPM = imipenem; MEM = meropenem; TZP = piperacillin/tazobactam; SUL = sulopenem
[B] Includes 193 *E. coli*, 92 *K. pneumoniae*, 29 *E. cloacae*, 25 P. mirabilis, 8 *C. freundii*, 5 *M. morganii*, 3 *K. aerogenes*, 3 *K. variicola*, 3 *K. oxytoca*, 2 *S. marcescens*, 1 *E. xiangfangensis*, 1 *P. stuartii*, 1 *R. ornitholyticus*, 1 *R. planticola*
[C] Includes 8 *C. freundii*, 5 *M. morganii*, 3 *K. aerogenes*, 3 *K. variicola*, 3 *K. oxytoca*, 2 *S. marcescens*, 1 *E. xiangfangensis*, 1 *P. stuartii*, 1 *R. ornitholyticus*, 1 *R. planticola*
* Value represents number and percentage of isolates at or below given MIC; ** Value represents number and percentage of isolates at or above given MIC; *** Does not include Enterbacterales isolates with missing MIC data: *K. pneumoniae* (1), *E. cloacae* (3), *P. mirabilis* (1), *E. xiangfangensis* (1); Includes carbapenemase-producing isolates: *E. coli* (2), *K. pneumoniae* (12), *E. cloacae* (2), *P. mirabilis* (3)

Table 4: Summary of β-lactamase enzymes detected among baseline Enterobacterales, SURE-2 cUTI patients

Pathogen (No; % of all Enterobacterales)/ Results	No. of isolates	Pathogen (No; % of all Enterobacterales)/ Results	No. of isolates	Pathogen (No; % of all Enterobacterales)/ Results	No. of isolates
E. coli (193; 52.0)		CTX-M-15; CTX-M-9-TYPE; SHV-OSBL	1	CTX-M-15	1
CMY-2	4	CTX-M-15; KPC-2*; SHV-OSBL; TEM-OSBL	1	CTX-M-15; TEM-OSBL	7
CMY-2; CTX-M-15; TEM-OSBL**	1	CTX-M-15; NDM-1*; SHV-OSBL	2	CTX-M-55	2
CMY-2; TEM-OSBL	3	CTX-M-15; OXA-244*;SHV-OSBL; TEM-OSBL	2	CTX-M-55; TEM-OSBL	1
CMY-42	3	CTX-M-15; OXA-48*; SHV-OSBL	1	TEM-OSBL; VEB-16	1
CMY-42; CTX-M-15; NDM-5*	1	CTX-M-15; OXA-48*; SHV-OSBL; TEM-OSBL	1	No acquired β-lactamases detected	4
CTX-M-1	2	CTX-M-15; SHV-OSBL	17	*C. freundii (8; 2.2)*	
CTX-M-1; TEM-OSBL	1	CTX-M-15; SHV-OSBL; TEM-OSBL	42	CMY-2-TYPE	2
CTX-M-3	12	CTX-M-15; TEM-OSBL	1	CMY-2-TYPE; CTX-M-3; SHV-ESBL; TEM-OSBL	1
CTX-M-3; TEM-OSBL	4	CTX-M-27; SHV-OSBL	2	CMY-2-TYPE; CTX-M-15	2
CTX-M-3-15	1	CTX-M-55; NDM-5*; SHV-OSBL; TEM-OSBL	1	CMY-2-TYPE; CTX-M-15; TEM-OSBL	3
CTX-M-9-TYPE	3	CTX-M-55; SHV-OSBL	1	*M. morganii (5; 1.3)*	
CTX-M-14	6	CTX-M-65; SHV-OSBL; TEM-OSBL	1	CTX-M-3; DHA-TYPE	1
CTX-M-14; TEM-OSBL	4	DHA-1; SHV-OSBL	1	DHA-TYPE	4
CTX-M-15	81	NDM-1*; SHV-OSBL	1	*K. aerogenes (3; 0.8)*	
CTX-M-15; CTX-M-14	1	OXA-244*; SHV-OSBL; TEM-OSBL	1	No acquired β-lactamases detected	3
CTX-M-15; CTX-M-27	1	OXA-48*; SHV-OSBL	2	*K. variicola (3; 0.8)*	
CTX-M-15; NDM-1*	1	SHV-ESBL	1	CTX-M-15	1
CTX-M-15; TEM-OSBL	25	SHV-OSBL; TEM-OSBL	3	CTX-M-15; TEM-OSBL	2
CTX-M-27	16	*E. cloacae (31; 8.4)*		*K. oxytoca (3; 0.8)*	
CTX-M-27; TEM-OSBL	6	CTX-M-3	1	CTX-M-3	1
CTX-M-32	1	CTX-M-3; NDM-1*; TEM-OSBL	1	CTX-M-3; TEM-OSBL	1
CTX-M-55	1	CTX-M-3; TEM-OSBL	2	DHA-1	1
CTX-M-55; CTX-M-14	1	CTX-M-15	3	*S. marcescens (2; 0.5)*	
CTX-M-55; TEM-OSBL	2	CTX-M-15; CTX-M-2-TYPE	2	CTX-M-3; TEM-OSBL	1
SHV-ESBL; TEM-OSBL	1	CTX-M-15; NDM-1*; TEM-OSBL	1	TEM-OSBL	1
TEM-OSBL	5	CTX-M-15; NDM-5*; TEM-OSBL	1	*E. xiangfangensis (1; 0.3)*	
No acquired β-lactamases detected	6	CTX-M-15; TEM-OSBL	15	CTX-M-3; TEM-OSBL	1
K. pneumoniae (93; 25.1)		TEM-OSBL	1	*P. stuartii (1; 0.3)*	
CMY-2; NDM-1*; SHV-OSBL;TEM-OSBL	1	No acquired β-lactamases detected	4	TEM-OSBL	1
CMY-4; CTX-M-3; SHV-OSBL	1	*P. mirabilis (26; 7.0)*		*R. ornitholytica (1; 0.3)*	
CTX-M-3; SHV-OSBL; TEM-OSBL	3	CMY-2	1	No acquired β-lactamases detected	1
CTX-M-14; SHV-OSBL	1	CMY-99; SHV-ESBL; TEM-OSBL; VIM-1*	4	*R. planticola (1; 0.3)*	
CTX-M-14; SHV-OSBL; TEM-OSBL	1	CMY-99; TEM-OSBL	1	No acquired β-lactamases detected	1
CTX-M-15	4	CTX-M-9-TYPE	4		

*KPC-2: Russia (1); NDM-1: Russia (4), Ukraine (2), Bulgaria (1); NDM-5: Georgia (2), Russia (1); OXA-48: Ukraine (2), Russia (1), Croatia (1); OXA-244: Russia (3); VIM-1: Bulgaria (4)
** OSBL = original spectrum beta-lactamase

Table 5: Select characteristics of enzymes identified from isolates of SURE-2 patients with cUTI

	Number of enzymes identified per qualifying isolate (N=371)				
	Number of enzymes identified				
	0	1	2	3	4
# isolates	19(5.1%)	164 (44.2%)	116 (31.3%)	61 (16.4%)	11(3.0%)

Presence of CTX-M-type enzyme, alone or in combination (N=352)		
Category		
No CTX-M-type enzyme detected	CTX-M-type enzyme detected alone	CTX-M-type enzyme detected in combination with other enzyme(s)
43 (12.2%)	140 (39.8%)	169 (48.0%)

	Group type for isolates with a CTX-M-type enzyme (N=309)	
	CTX-M-1-type	CTX-M-9-type
# isolates	265 (85.8%)	44 (14.2%)

CONCLUSIONS

- Complicated urinary tract infection in hospitalized adults due to β-lactamase-producing Enterobacterales was common
- Complicated urinary tract 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 UTI Enterobacterales isolates that met screening criteria
- Novel oral antibiotics with potent activity against β-lactamase-producing organisms, such as sulopenem, are needed to facilitate early discharge for hospitalized complicated UTI patients

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