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Introduction

Sulopenem (SLP), is an investigational thiopenem (β -lactam) available in both oral (sulopenem etzadroxil + probenecid) and parenteral (sulopenem) dosage forms. It is currently in development for the treatment of uncomplicated and complicated urinary tract infections, including infections caused by extended-spectrum β -lactamase (ESBL) producing and multidrug-resistant (MDR) Gram-negative bacilli.^{1,2} Orally, sulopenem-etzadroxil is combined with probenecid, and has a safety and efficacy profile similar to other penems and β -lactams.² Sulopenem is stable to renal dehydropeptidase I, unlike imipenem, and has been reported to be stable against hydrolytic attack by many β -lactamases, including ESBLs and AmpC enzymes which confer resistance to third-generation cephalosporins. The activity of sulopenem addresses several of the most urgent, serious, and concerning drug-resistant antimicrobial threats defined by the CDC, including ESBL-producing *Enterobacteriales*.

The current study assessed the *in vitro* activities of sulopenem and comparator antibacterial agents against clinical isolates of Gram-negative and Gram-positive pathogens isolated from urine and submitted by Canadian hospital laboratories to the CANWARD surveillance study from 2014 to 2020.

Materials and Methods

Bacterial Isolates: CANWARD is an ongoing, national, Health Canada partnered study assessing antimicrobial resistance patterns of pathogens causing infections in patients receiving care in hospitals across Canada.¹ Tertiary-care medical centres submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units.¹ From January 2014 through October 2020, each study site was asked to submit clinical isolates (consecutive, one per patient, per infection site) from inpatients and outpatients with respiratory, urine, wound, and bloodstream infections. The medical centres submitted "clinically significant" isolates from patients with a presumed infectious disease. Isolates were shipped on Amies semi-solid transport media to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), subcultured onto appropriate media, and stocked in skim milk at -80°C until minimum inhibitory concentration (MIC) testing was carried out. *E. coli* isolates were from the CANWARD surveillance study from the years 2014 through 2020. All other isolates were from 2016-2020 only. Putative AmpC phenotypes in *E. coli* were defined as an isolate where the ceftriaxone and/or ceftazidime MIC was ≥ 1 mg/L, the cefoxitin MIC was ≥ 32 mg/L, and the isolate tested ESBL-negative by the CLSI phenotypic confirmatory disk test (CLSI M100, 29th Ed., 2019).¹

Antimicrobial Susceptibilities: Following 2 subcultures from frozen stock, the *in vitro* activity of sulopenem and selected antimicrobials was determined by broth microdilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) (M07, 11th Ed., 2018) and MICs were interpreted using CLSI M100 (30th Ed., 2020). Antimicrobial agents were obtained as laboratory grade powders from their respective manufacturers. Stock solutions were prepared and dilutions made as described by CLSI. The MICs were determined using 96-well custom designed microtitre plates.^{1,2} These plates contained doubling antimicrobial dilutions in 100 μ l/well of cation adjusted Mueller-Hinton broth and inoculated to achieve a final concentration of approximately 5 x 10⁵ CFU/mL then incubated in ambient air for 24 hours prior to reading. Colony counts were performed periodically to confirm inocula. Quality control was performed using ATCC QC organisms including: *S. pneumoniae* 49619, *S. aureus* 29213, *E. faecalis* 29212, *E. coli* 25922, and *P. aeruginosa* 27853.

Table 1. In vitro activities of sulopenem and comparators versus Gram-negative bacilli

Organism (no. tested) / antimicrobial agent	MIC (μ g/mL)			% S	% I	% R
	50%	90%	Range			
Escherichia coli ALL (1125)						
Sulopenem	0.03	0.06	$\leq 0.008 - 4$	NA ^a	NA	NA
Meropenem	≤ 0.03	≤ 0.03	$\leq 0.03 - 1$	100	0	0
Ceftriaxone	≤ 0.25	16	$\leq 0.25 - > 64$	88.6	0.3	11.1
Amoxicillin/clavulanate	8	16	0.5 - > 32	77.9	16.7	5.4
TMP/SMX	≤ 0.12	> 8	$\leq 0.12 - > 8$	74.1	NA	25.9
Ciprofloxacin	≤ 0.06	> 16	$\leq 0.06 - > 16$	74.6	1.1	24.4
Nitrofurantoin	16	16	$\leq 0.5 - > 512$	97.6	1.2	1.2
Gentamicin	≤ 0.5	2	$\leq 0.5 - > 32$	91.6	0.3	8.1
Escherichia coli ESBL (113)						
Sulopenem	0.03	0.06	0.015 - 0.25	NA ^a	NA	NA
Meropenem	≤ 0.03	0.06	$\leq 0.03 - 0.25$	100	0	0
Ceftriaxone	> 64	> 64	1 - > 64	0.9	0.9	98.2
Amoxicillin/clavulanate	16	32	4 - > 32	47.9	36.5	15.6
TMP/SMX	> 8	> 8	$\leq 0.12 - > 8$	35.4	NA	64.6
Ciprofloxacin	> 16	> 16	$\leq 0.06 - > 16$	19.5	0.9	79.6
Nitrofurantoin	16	32	2 - 512	91.2	3.5	5.3
Gentamicin	≤ 0.5	> 32	$\leq 0.5 - > 32$	71.7	2.6	25.7
Escherichia coli AmpC (17)						
Sulopenem	0.06	0.25	0.015 - 4	NA ^a	NA	NA
Meropenem	≤ 0.03	0.06	$\leq 0.03 - 1$	100	0	0
Ceftriaxone	2	> 64	$\leq 0.25 - > 64$	41.2	11.7	47.1
Amoxicillin/clavulanate	> 32	> 32	8 - > 32	6.7	6.6	86.7
TMP/SMX	≤ 0.12	> 8	$\leq 0.12 - > 8$	70.6	NA	29.4
Ciprofloxacin	0.25	> 16	$\leq 0.06 - > 16$	58.8	5.9	35.3
Nitrofurantoin	16	64	8 - 256	88.2	5.9	5.9
Gentamicin	≤ 0.5	1	$\leq 0.5 - > 32$	94.1	0	5.9
Escherichia coli MDR^b (168)						
Sulopenem	0.03	0.06	0.015 - 4	NA ^a	NA	NA
Meropenem	≤ 0.03	0.06	$\leq 0.03 - 1$	100	0	0
Ceftriaxone	32	> 64	$\leq 0.25 - > 64$	44.0	0	56.0
Amoxicillin/clavulanate	16	32	4 - > 32	44.4	39.6	16
TMP/SMX	> 8	> 8	$\leq 0.12 - > 8$	10.1	NA	89.9
Ciprofloxacin	> 16	> 16	$\leq 0.06 - > 16$	12.5	0	87.5
Nitrofurantoin	16	32	$\leq 1 - 512$	90.5	4.1	5.4
Gentamicin	1	> 32	$\leq 0.5 - > 32$	64.3	0.6	35.1
Klebsiella pneumoniae ALL (171)						
Sulopenem	0.06	0.12	0.015 - 1	NA ^a	NA	NA
Meropenem	≤ 0.03	0.06	$\leq 0.03 - 0.5$	100	0	0
Ceftriaxone	≤ 0.25	> 64	$\leq 0.25 - > 64$	87.7	0	12.3
Amoxicillin/clavulanate	4	16	1 - > 32	85.0	8.5	6.5
TMP/SMX	≤ 0.12	> 8	$\leq 0.12 - > 8$	86.0	NA	14.0
Ciprofloxacin	≤ 0.06	2	$\leq 0.06 - > 16$	84.8	1.8	13.5
Nitrofurantoin	64	128	2 - > 512	36.8	41.5	21.6
Gentamicin	≤ 0.5	≤ 0.5	$\leq 0.5 - > 32$	97.1	0	2.9
Klebsiella pneumoniae ESBL (17)						
Sulopenem	0.06	0.12	0.03 - 1	NA ^a	NA	NA
Meropenem	0.06	0.12	$\leq 0.03 - 0.5$	100	0	0
Ceftriaxone	> 64	> 64	16 - > 64	0	0	100
Amoxicillin/clavulanate	16	32	8 - > 32	26.7	33.3	40.0
TMP/SMX	> 8	> 8	$\leq 0.12 - > 8$	11.8	NA	88.2
Ciprofloxacin	> 16	> 16	$\leq 0.06 - > 16$	23.5	5.9	70.6
Nitrofurantoin	64	512	32 - 512	17.6	53.0	29.4
Gentamicin	≤ 0.5	32	$\leq 0.5 - > 32$	76.5	0	23.5
Enterobacter cloacae (39)						
Sulopenem	0.12	0.5	0.03 - 4	NA ^a	NA	NA
Meropenem	0.06	0.12	$\leq 0.03 - 1$	100	0	0
Ceftriaxone	0.5	> 64	$\leq 0.25 - > 64$	59.0	0	41.0
Amoxicillin/clavulanate	> 32	> 32	8 - > 32	2.8	0	97.2
TMP/SMX	≤ 0.12	1	$\leq 0.12 - > 8$	92.3	NA	7.7
Ciprofloxacin	≤ 0.06	0.25	$\leq 0.06 - > 16$	92.3	2.6	5.1
Nitrofurantoin	64	128	4 - 256	30.8	43.6	25.6
Gentamicin	≤ 0.5	1	$\leq 0.5 - > 32$	97.4	0	2.6
Klebsiella oxytoca (32)						
Sulopenem	0.06	0.06	0.03 - 0.25	NA ^a	NA	NA
Meropenem	≤ 0.03	0.06	$\leq 0.03 - 0.12$	100	0	0
Ceftriaxone	≤ 0.25	≤ 0.25	$\leq 0.25 - 16$	90.6	3.1	6.3
Amoxicillin/clavulanate	4	8	1 - > 32	93.1	3.4	3.4
TMP/SMX	≤ 0.12	0.25	$\leq 0.12 - > 8$	93.8	NA	6.3
Ciprofloxacin	≤ 0.06	0.12	$\leq 0.06 - 0.25$	100	0	0
Nitrofurantoin	32	64	4 - 256	81.3	9.4	9.4
Gentamicin	≤ 0.5	1	$\leq 0.5 - > 32$	96.9	0	3.1

^a NA - not available.

^b MDR was defined as nonsusceptible to 3 agents from different antimicrobial classes (ceftriaxone, amoxicillin-clavulanate, TMP/SMX, nitrofurantoin, ciprofloxacin, and gentamicin).

^c Median MIC value

Results

Table 1. In vitro activities of sulopenem and comparators versus Gram-negative bacilli (Continued)

Organism (no. tested) / antimicrobial agent	MIC (μ g/mL)			% S	% I	% R
	50%	90%	Range			
Proteus mirabilis (70)						
Sulopenem	0.25	0.5	0.03 - 1	NA ^a	NA	NA
Meropenem	0.06	0.12	$\leq 0.03 - 0.25$	100	0	0
Ceftriaxone	≤ 0.25	≤ 0.25	$\leq 0.25 - 1$	100	0	0
Amoxicillin/clavulanate	1	4	0.5 - > 32	93.5	3.2	3.2
TMP/SMX	≤ 0.12	> 8	$\leq 0.12 - > 8$	72.9	NA	27.1
Ciprofloxacin	≤ 0.06	4	$\leq 0.06 - > 16$	78.6	0	21.4
Nitrofurantoin	128	128	64 - 256	0	21.4	78.6
Gentamicin	1	4	$\leq 0.5 - > 32$	90.0	1.4	8.6
Pseudomonas aeruginosa (70)						
Sulopenem	> 8	> 8	8 - > 8	NA ^a	NA	NA
Meropenem	1	4	$\leq 0.03 - > 32$	84.3	5.7	10.0
Ceftriaxone	64	> 64	4 - > 64	NA ^a	NA	NA
Amoxicillin/clavulanate	> 32	> 32	> 32 - > 32	NA ^a	NA	NA
TMP/SMX	8	> 8	1 - > 8	NA ^a	NA	NA
Ciprofloxacin	0.12	4	$\leq 0.06 - > 16$	81.4	2.9	15.7
Nitrofurantoin	> 512	> 512	> 512 - > 512	NA ^a	NA	NA
Gentamicin	1	4	$\leq 0.5 - > 32$	92.9	5.7	1.4

Table 2. In vitro activities of sulopenem and comparators versus Gram-positive cocci

Organism (no. tested) / antimicrobial agent	MIC (μ g/mL)			% S	% I	% R
	50%	90%	Range			
Staphylococcus aureus - MSSA (24)						
Sulopenem	0.06	0.25	0.06 - 0.25	NA ^a	NA	NA
Meropenem	0.12	0.25	0.12 - 0.5	NA ^a	NA	NA
Ceftriaxone	4	4	1 - 8	NA ^a	NA	NA
Amoxicillin/clavulanate	0.5	1	0.12 - 2	NA ^a	NA	NA
TMP/SMX	≤ 0.12	≤ 0.12	$\leq 0.12 - 0.5$	100	NA	0
Ciprofloxacin	0.25	4	0.12 - > 16	87.5	0	12.5
Nitrofurantoin	16	16	4 - 16	100	0	0
Gentamicin	≤ 0.5	≤ 0.5	$\leq 0.5 - 2$	100	0	0
Enterococcus faecalis (135)						
Sulopenem	8	8	1 - > 8	NA ^a	NA	NA
Meropenem	8	8	1 - > 32	NA ^a	NA	NA
Ceftriaxone	> 64	> 64	4 - > 64	NA ^a	NA	NA
Amoxicillin/clavulanate	0.5	1	0.25 - 2	NA ^a	NA	NA
TMP/SMX	≤ 0.12	> 8	$\leq 0.12 - > 8$	NA ^a	NA	NA
Ciprofloxacin	1	> 16	0.12 - > 16	63.0	12.6	24.4
Nitrofurantoin	8	16	2 - 128	99.3	0	0.7
Gentamicin	16	> 32	1 - > 32	NA ^a	NA	NA

MSSA - methicillin-susceptible *Staphylococcus aureus*; ^a NA - not available

Table 3. Distribution of sulopenem MICs versus Gram-negative organisms

Organism agent	Number of isolates for which the sulopenem MIC (μ g/ml) was:										
	≤ 0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
<i>E. coli</i> ALL	269	729	106	15	5						1
<i>E. coli</i> ESBL pos	13	57	36	4	3						
<i>E. coli</i> AmpC	3	3	4	5	1						1
<i>E. coli</i> MDR	26	97	36	5	3						1
<i>K. pneumoniae</i> ALL	4	71	76	18			2				
<i>K. pneumoniae</i> ESBL pos	4	9	3				1				
<i>E. cloacae</i>	10	5	9	8	6						1
<i>K. oxytoca</i>	13	17	1	1							
<i>P. mirabilis</i>	2	7	6	27	25	3					
<i>P. aeruginosa</i>										3	67

Table 4. Distribution of sulopenem