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In vitro activity of sulopenem and comparator agents against Enterobacterales and anaerobic clinical isolates collected during the SENTRY Antimicrobial Surveillance Program

Joshua M. Maher[1], Michael D. Huband[1]*, Christopher G. Blankers[1], Sailaja Puttagunta[2], Steven I. Aronin[2] and Mariana Castanheira [1]

[1]*JMI Laboratories, 345 Beaver Kreek Centre, Suite A, North Liberty, IA 52317, USA;* [2]*Iterum Therapeutics, Old Saybrook, CT 06475, USA*

*Corresponding author. E-mail: mike-huband@jmilabs.com
@jmilabs

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Objectives: Physicians must leverage several factors when making antibiotic therapy decisions, including route of administration and duration of therapy. Oral administration provides several potential advantages including increased accessibility, prevention of hospitalizations and earlier discharges. Sulopenem—a broad-spectrum, synthetic penem β-lactam agent—uniquely possesses both oral and IV formulations along with noted stability among antimicrobial-resistant subsets. This study evaluated the *in vitro* activity of sulopenem and comparator agents against contemporary Enterobacterales and anaerobic clinical isolates predominantly from patients with bloodstream, intra-abdominal and urinary tract infections.

Methods: A contemporary collection of 1647 Enterobacterales and 559 anaerobic isolates was assembled from medical centres in Europe and the USA. Isolates were susceptibility tested using the CLSI reference methods: broth microdilution for Enterobacterales and agar dilution for anaerobes.

Results: Sulopenem demonstrated potent *in vitro* antimicrobial activity ($MIC_{50/90}$, 0.03/0.25 mg/L) against Enterobacterales isolates regardless of infection type, inhibiting 99.2% of isolates at ≤1 mg/L. This activity was conserved against resistant phenotypes including ESBL-phenotype *Escherichia coli* ($MIC_{50/90}$, 0.03/0.06 mg/L) and ESBL-phenotype *Klebsiella pneumoniae* ($MIC_{50/90}$, 0.06/1 mg/L). Sulopenem maintained activity against ciprofloxacin-, nitrofurantoin- and trimethoprim/sulfamethoxazole-non-susceptible subsets ($MIC_{50/90}$, 0.03–0.06/0.12–0.5 mg/L). Against anaerobic isolates, sulopenem (98.9% inhibited at ≤4 mg/L) and meropenem [98.4% susceptible (CLSI)] were the most active compounds tested.

Conclusions: The potent *in vitro* activity of sulopenem against this large collection of recent Enterobacterales and anaerobic clinical isolates from multiple infection types supports its further clinical evaluation in the treatment of intra-abdominal and urinary tract infections.

Introduction

Infectious disease management relies on the selection of appropriate and effective antibiotic therapy. When making critical treatment decisions regarding antimicrobial therapy, physicians leverage multiple factors including accurate diagnosis, urgency of therapy, broad- or narrow-spectrum coverage, patient treatment outcomes and antimicrobial resistance.[1,2] To aid in the judicious use of these therapeutic agents, the appropriate route of administration and duration of therapy must be considered.[3,4] Although some clinical circumstances necessitate IV administration of antibiotics, oral administration provides several potential advantages, including increased treatment accessibility, decreased cost, prevention of hospitalizations and earlier discharges.[5–9] In situations where an equally effective and bioavailable oral antimicrobial agent is available, physicians should consider oral agents for outpatient treatment or as a potential step-down therapy.

Sulopenem (CP-70429)—a broad-spectrum, synthetic penem β-lactam antibiotic—uniquely possesses both oral and IV formulations.[10] Carbapenem antimicrobial agents including doripenem, ertapenem, imipenem and meropenem are currently

approved by the US FDA; however, these agents are only available in their parenteral formulation, requiring hospitalization or access to outpatient infusion services for their administration.[10] Oral administration of the prodrug sulopenem etzadroxil demonstrates increased absorption whereas IV administration of sulopenem (comprised of the stable *S* isomer) leads to significant active concentration in the urine.[11,12] With activity against both Gram-positive and -negative species, sulopenem inhibits peptidoglycan cross-linking thus preventing bacterial cell wall synthesis.[13,14] Notably, susceptibility reports for this compound indicate activity against fluoroquinolone-resistant, ESBL-producing and MDR Enterobacterales.[5,15–17] Sulopenem has recently completed several FDA Phase III clinical trials focused on treatment of uncomplicated urinary tract infection (UTI; NCT03354598), complicated UTI (cUTI; NCT03357614) and complicated additional intra-abdominal infection (IAI; NCT03358376).[18] An additional Phase III clinical trial is currently underway, evaluating sulopenem against amoxicillin/clavulanate for treatment of uncomplicated UTI in adult women (NCT05584657). This study evaluated the *in vitro* activity of sulopenem and comparator agents against contemporary Enterobacterales and anaerobic clinical isolates predominantly from patients with bloodstream infection (BSI), IAI and UTI.

Materials and methods

Bacterial isolates

A total of 1647 Enterobacterales and 559 anaerobic isolates were collected from medical centres in Europe and the USA between 2018 and 2020 as part of the SENTRY Antimicrobial Surveillance Program.[19] Enterobacterales isolates included primarily *Escherichia coli*, *Klebsiella* spp. and *Enterobacter cloacae* species complex isolates whereas anaerobic isolates were mainly *Bacteroides*, *Clostridium*, *Cutibacterium* and *Prevotella* genera. Carbapenem-resistant Enterobacterales (CRE) and ESBL-phenotype definitions were applied to Enterobacterales isolates using CLSI breakpoint criteria.[20] Bacterial identification was performed by MALDI-TOF MS using the MALDI Biotyper (Bruker Daltonics, Billerica, MS, USA) according to the manufacturer's instructions.

Susceptibility testing methods

Enterobacterales isolates were tested for antimicrobial susceptibility using CLSI reference broth microdilution methods[21] with frozen-form broth microdilution panels manufactured by JMI Laboratories. Anaerobic isolates were tested for antimicrobial susceptibility using CLSI reference agar dilution methods.[22] JMI Laboratories produced *Brucella* agar plates supplemented with haemin (5 µg), vitamin K1 (1 µg/mL) and laked sheep blood (5% v/v). *Clostridium septicum* isolate testing required the use of 12-well, non-treated microtitre plates due to the swarming nature of this species.[23] Specifically, 1 mL of molten agar containing the appropriate drug concentration was dispensed into each well to yield an agar depth of 3–4 mm, consistent with CLSI M07 methodology and equivalent to commercially available options.[24,25] All agar plates were reduced prior to use in a Bactron 600 anaerobe chamber (Sheldon Manufacturing, Cornelius, OR, USA) containing an atmosphere of 5% carbon dioxide, 5% hydrogen and 90% nitrogen.

Concurrent quality assurance testing utilized CLSI-recommended quality control strains including *Bacteroides fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, *Clostridioides difficile* ATCC 700057, *Eggerthella lenta* 43055 for testing anaerobic isolates, and *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 for testing Enterobacterales isolates. Concurrent bacterial colony counts monitored inoculum density throughout susceptibility testing. CLSI, FDA and EUCAST breakpoint criteria were utilized to determine susceptibility and resistance rates for comparator agents, where available.[20,26–28]

Results

Isolates and infection types

Enterobacterales isolates consisted of 983 *E. coli*, 347 *Klebsiella* spp., 110 *E. cloacae* species complex, 91 *Proteus mirabilis* and 116 other Enterobacterales species. These isolates were recovered from patients with BSI (23.5%; $n=387$), UTI (60.7%; $n=999$) and IAI (15.8%; $n=261$). Anaerobic isolates included 194 *Bacteroides* spp., 85 *Clostridium* spp., 77 *Cutibacterium* spp., 34 *Prevotella* spp. and 169 other Gram-positive and -negative anaerobic species. Anaerobic isolates were collected from patients with BSI (35.1%; $n=196$), skin and skin structure infections (32.2%; $n=180$), IAIs (5.0%; $n=28$), pneumonia in hospitalized patients (0.7%; $n=4$), UTIs (0.5%; $n=3$) and other infection types (26.5%; $n=148$ isolates).

Enterobacterales susceptibility testing results

Sulopenem demonstrated potent *in vitro* antibacterial activity ($MIC_{50/90}$, 0.03/0.25 mg/L) against 1647 Enterobacterales isolates regardless of infection type, inhibiting 99.2% of all Enterobacterales isolates at ≤1 mg/L, which is within the CLSI susceptible MIC breakpoint for doripenem, meropenem and imipenem against Enterobacterales and within the sulopenem clinical exposure levels (Table 1). The activity of sulopenem was comparable to other carbapenem agents, including ertapenem [98.3%/98.3% susceptible (S) (CLSI/EUCAST)], imipenem [94.7%/92.0% S (CLSI/EUCAST)] and meropenem [99.7%/99.8% S (CLSI/EUCAST)] (Table 2). Activity of other comparator agents against these Enterobacterales isolates was highest for amikacin [99.6%/99.0% S (CLSI/EUCAST)], gentamicin [91.1%/90.9% S (CLSI/EUCAST)], piperacillin/tazobactam [94.2%/91.7% S (CLSI/EUCAST)] and tigecycline [97.0% S (FDA)] (Table 2). Sulopenem inhibited 100.0% of *E. coli* isolates ($n=983$; $MIC_{50/90}$, 0.03/0.03 mg/L) and 98.8% of *Klebsiella* spp. isolates ($n=347$; $MIC_{50/90}$, 0.03/0.12 mg/L) at ≤1 mg/L. Sulopenem demonstrated potent *in vitro* activity against smaller Enterobacterales isolate subsets, including 29 *Citrobacter freundii* species complex ($MIC_{50/90}$, 0.06/0.12 mg/L; 100.0% inhibited at ≤0.5 mg/L), 110 *E. cloacae* species complex ($MIC_{50/90}$, 0.12/0.5 mg/L; 97.3% inhibited at ≤1 mg/L), 33 *Klebsiella aerogenes* ($MIC_{50/90}$, 0.12/0.25 mg/L; 100.0% inhibited at ≤1 mg/L), 41 *Klebsiella oxytoca* ($MIC_{50/90}$, 0.03/0.06 mg/L; 100.0% inhibited at ≤0.06 mg/L), 20 *Morganella morganii* ($MIC_{50/90}$, 1/1 mg/L; 100.0% inhibited at ≤1 mg/L), 91 *P. mirabilis* ($MIC_{50/90}$, 0.25/0.25 mg/L; 100.0% inhibited at ≤0.5 mg/L) and 14 *Providencia* spp. ($MIC_{50/90}$, 0.12/0.5 mg/L; 92.9% inhibited at ≤1 mg/L) isolates (Table 1). Sulopenem ($MIC_{50/90}$, 0.5/2 mg/L) inhibited 86.1% of *Serratia marcescens* ($n=36$) isolates at ≤1 mg/L (Table 1).

The activity of sulopenem was conserved against resistance phenotypes, including 170 ESBL-phenotype *E. coli* ($MIC_{50/90}$, 0.03/0.06 mg/L; 100.0% inhibited at ≤1 mg/L) and 49 ESBL-phenotype *K. pneumoniae* ($MIC_{50/90}$, 0.06/1 mg/L; 91.8% inhibited at ≤1 mg/L) (Table 1). Sulopenem exhibited elevated MIC values against four meropenem-non-susceptible (NS) *K. pneumoniae* (MIC_{50}, 16 mg/L; Table 2). Against four CRE

Table 1. Activity of sulopenem tested against 1647 Enterobacterales clinical isolates from the USA (2019)

	No. and cumulative % of isolates inhibited at MIC (mg/L) of:															mg/L	
Organism/organism group[a] (no. of isolates)	≤0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>[b]	MIC_{50}	MIC_{90}
Enterobacterales (1647)	0	2	192	938	216	120	94	44	28	7	2	1	1	0	2	0.03	0.25
	0.0	0.1	11.8	68.7	81.8	89.1	94.8	97.5	99.2	99.6	99.8	99.8	99.9	99.9	100.0		
Carbapenem-resistant (4)											0	1	1	0	2	16	
											0.0	25.0	50.0	50.0	100.0		
Ciprofloxacin-non-susceptible (405)	0	1	28	240	74	20	22	9	4	2	1	1	1	0	2	0.03	0.25
	0.0	0.2	7.2	66.4	84.7	89.6	95.1	97.3	98.3	98.8	99.0	99.3	99.5	99.5	100.0		
Nitrofurantoin-non-susceptible (547)	0	1	15	174	105	89	83	43	24	7	2	1	1	0	2	0.06	0.5
	0.0	0.2	2.9	34.7	53.9	70.2	85.4	93.2	97.6	98.9	99.3	99.5	99.6	99.6	100.0		
Trimethoprim/sulfamethoxazole-non-susceptible (434)	0	2	47	269	60	23	20	3	6	0	0	1	1	0	2	0.03	0.12
	0.0	0.5	11.3	73.3	87.1	92.4	97.0	97.7	99.1	99.1	99.1	99.3	99.5	99.5	100.0		
Escherichia coli (983)	0	2	180	703	79	11	5	0	3							0.03	0.03
	0.0	0.2	18.5	90.0	98.1	99.2	99.7	99.7	100.0								
Non-ESBL-phenotype (813)	0	1	175	582	47	7	1									0.03	0.03
	0.0	0.1	21.6	93.2	99.0	99.9	100.0										
ESBL-phenotype (170)	0	1	5	121	32	4	4	0	3							0.03	0.06
	0.0	0.6	3.5	74.7	93.5	95.9	98.2	98.2	100.0								
Meropenem-susceptible (≤1 mg/L) (982)	0	2	180	703	79	11	5	0	2							0.03	0.03
	0.0	0.2	18.5	90.1	98.2	99.3	99.8	99.8	100.0								
Meropenem-non-susceptible (>1 mg/L) (1)								0	1								
								0.0	100.0								
Klebsiella spp. (347)		0	6	199	95	31	5	5	2	0	0	1	1	0	2	0.03	0.12
		0.0	1.7	59.1	86.5	95.4	96.8	98.3	98.8	98.8	98.8	99.1	99.4	99.4	100.0		
K. pneumoniae (273)		0	6	174	68	14	3	3	1	0	0	1	1	0	2	0.03	0.06
		0.0	2.2	65.9	90.8	96.0	97.1	98.2	98.5	98.5	98.5	98.9	99.3	99.3	100.0		
Non-ESBL-phenotype (224)		0	5	157	49	11	2									0.03	0.06
		0.0	2.2	72.3	94.2	99.1	100.0										
ESBL-phenotype (49)		0	1	17	19	3	1	3	1	0	0	1	1	0	2	0.06	1
		0.0	2.0	36.7	75.5	81.6	83.7	89.8	91.8	91.8	91.8	93.9	95.9	95.9	100.0		
Meropenem-susceptible (≤1 mg/L) (269)		0	6	174	68	14	3	3	1							0.03	0.06
		0.0	2.2	66.9	92.2	97.4	98.5	99.6	100.0								
Meropenem-non-susceptible (>1 mg/L) (4)											0	1	1	0	2	16	
											0.0	25.0	50.0	50.0	100.0		
K. oxytoca (41)			0	23	18											0.03	0.06
			0.0	56.1	100.0												
K. aerogenes (33)			0	2	9	17	2	2	1							0.12	0.25
			0.0	6.1	33.3	84.8	90.9	97.0	100.0								
Proteus mirabilis (91)		0	1	1	11	32	42	4								0.25	0.25
		0.0	1.1	2.2	14.3	49.5	95.6	100.0									
Non-ESBL-phenotype (88)		0	1	1	10	31	41	4								0.25	0.25
		0.0	1.1	2.3	13.6	48.9	95.5	100.0									

Continued

Table 1. *Continued*

Organism/organism group[a] (no. of isolates)	No. and cumulative % of isolates inhibited at MIC (mg/L) of:															mg/L	
	≤0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>[b]	MIC_{50}	MIC_{90}
ESBL-phenotype (3)				0	1	1	1									0.12	
				0.0	33.3	66.7	100.0										
Enterobacter cloacae species complex (110)		0	1	14	22	29	25	9	7	3						0.12	0.5
		0.0	0.9	13.6	33.6	60.0	82.7	90.9	97.3	100.0							
Ceftazidime-susceptible (≤4 mg/L) (73)		0	1	14	22	19	11	5	1							0.06	0.25
		0.0	1.4	20.5	50.7	76.7	91.8	98.6	100.0								
Ceftazidime-non-susceptible (>4 mg/L) (37)					0	10	14	4	6	3						0.25	1
					0.0	27.0	64.9	75.7	91.9	100.0							
Morganella morganii (20)						0	1	7	12							1	1
						0.0	5.0	40.0	100.0								
Citrobacter koseri (9)		0	2	7												0.03	
		0.0	22.2	100.0													
Citrobacter freundii species complex (29)		0	2	12	7	6	1	1								0.06	0.12
		0.0	6.9	48.3	72.4	93.1	96.6	100.0									
Serratia marcescens (36)					0	2	9	17	3	3	2					0.5	2
					0.0	5.6	30.6	77.8	86.1	94.4	100.0						
Providencia spp. (14)					0	8	4	1	0	1						0.12	0.5
					0.0	57.1	85.7	92.9	92.9	100.0							
Other Enterobacterales (8)			0	2	2	1	2	0	1							0.06	
			0.0	25.0	50.0	62.5	87.5	87.5	100.0								

[a]Resistant, non-susceptible and ESBL phenotype distinctions are based on CLSI 2019 criteria.
[b]Greater than the highest concentration tested.

Table 2. Antimicrobial activity of sulopenem and comparator agents tested against 1647 Enterobacterales clinical isolates from the USA

			mg/L			CLSI[a]			EUCAST[a]		
Antimicrobial agent	Route of administration[b]	No. of isolates	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R	%S	%I	%R
Sulopenem	PO, IV	1647	0.03	0.25	0.008 to >32						
Ertapenem	IV, IM	1647	≤0.008	0.06	≤0.008 to >2	98.3	0.9	0.9	98.3		1.7
Imipenem	IV	1647	≤0.12	1	≤0.12 to >8	94.7	4.2	1.1	92.0	7.7	0.2
Meropenem	IV	1647	≤0.015	0.06	≤0.015 to >32	99.7	0.1	0.2	99.8	0.1	0.1
Amikacin	IV, IM	1647	2	4	≤0.25 to >32	99.6	0.2	0.1	99.0	0.6	0.4
Amoxicillin/clavulanic acid	PO	1647	4	>32	0.5 to >32	71.0	10.9	18.1	71.0[c]		29.0
									87.4[d]		12.6
Aztreonam	IV	1647	0.12	16	≤0.03 to >16	85.2	2.6	12.2	83.6	1.6	14.8
Cefepime	IV, IM	1647	0.06	8	≤0.008 to >256	87.9[e]	2.8	9.3	86.8	2.4	10.7
Ceftazidime	IV, IM	1647	0.25	16	0.03 to >32	86.5	1.9	11.6	82.5	3.9	13.5
Ceftriaxone	IV, IM	1647	≤0.06	>8	≤0.06 to >8	83.1	0.4	16.6	83.1	0.4	16.6
Cefuroxime	PO, IV, IM	1647	4	>64	≤0.5 to >64	55.9[f]	22.5	21.6			
						73.5[g]	4.9	21.6			
Ciprofloxacin	PO, IV	1645 [h]	≤0.03	>16	≤0.03 to >16	75.4	3.0	21.6	75.4	3.0	21.6
Gentamicin	IV, IM	1646[h]	0.5	2	≤0.12 to >16	91.1	0.3	8.6	90.9	0.2	8.9
Nitrofurantoin	PO	1647	32	>64	≤4 to >64	66.8	14.8	18.5			
Piperacillin/tazobactam	IV	1645[h]	2	8	≤0.06 to >128	94.2	2.2	3.6	91.7	2.5	5.8
Tetracycline	PO, IV	1646[h]	2	>16	≤0.5 to >16	68.0	1.9	30.1			
Tigecycline	IV	1645[h]	0.25	1	≤0.06 to 8	97.0[i]	2.7	0.4			
Trimethoprim/sulfamethoxazole	PO, IV	1642[h]	≤0.12	>16	≤0.12 to >16	73.6		26.4	73.6	0.4	26.0

Organisms include: *Citrobacter amalonaticus* (1), *Citrobacter farmeri* (1), *Citrobacter freundii* species complex (29), *Citrobacter koseri* (9), *Enterobacter cloacae* (38), *E. cloacae* species complex (72), *Escherichia coli* (983), *Escherichia marmotae* (1), *Klebsiella aerogenes* (33), *Klebsiella oxytoca* (41), *Klebsiella pneumoniae* (273), *Morganella morganii* (20), *Pantoea agglomerans* (1), *Proteus mirabilis* (91), *Proteus penneri* (2), *Proteus vulgaris* (1), *P. vulgaris* group (1), *Providencia alcalifaciens* (1), *Providencia rettgeri* (6), *Providencia stuartii* (7) and *Serratia marcescens* (36).

I, intermediate; IM, intramuscular; IV, intravenous; PO, oral; R, resistant; S, susceptible.

[a]Criteria as published by CLSI 2019 and EUCAST 2019.

[b]Route of administration published in M100 (32nd edition, 2022) Glossary II.

[c]Using other than uncomplicated urinary tract infection breakpoints.

[d]Using uncomplicated urinary tract infection-only breakpoints.

[e]Intermediate interpreted as susceptible-dose dependent.

[f]Using oral breakpoints.

[g]Using parenteral breakpoints.

[h]Missing isolates due to technical error (skipping).

[i]The US FDA breakpoints were published on 13 December 2017.

Table 3. Sulopenem and comparator agent agar dilution cumulative percent inhibition MIC results against 559 anaerobic isolates from the USA and Europe

	No. and cumulative % of isolates inhibited at MIC (mg/L) of:															
Organism/organism group (no. of isolates)	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>[a]	MIC_{50}	MIC_{90}
All anaerobes																
Sulopenem (559)	51	82	128	104	100	36	34	8	10	1	1			4	0.12	1
	9.1	23.8	46.7	65.3	83.2	89.6	95.7	97.1	98.9	99.1	99.3			100.0		
Clindamycin (559)		98	72	57	49	70	45	28	22					118	0.5	>4
		17.5	30.4	40.6	49.4	61.9	69.9	75.0	78.9					100.0		
Meropenem (559)	104	54	82	122	95	42	30	12	9	6				3	0.12	1
	18.6	28.3	42.9	64.8	81.8	89.3	94.6	96.8	98.4	99.5				100.0		
Metronidazole (559)			16	13	64	159	162	31	13	7	2			92	1	>16
			2.9	5.2	16.6	45.1	74.1	79.6	81.9	83.2	83.5			100.0		
Moxifloxacin (559)			9	46	170	131	46	60	22	33				42	0.5	8
			1.6	9.8	40.3	63.7	71.9	82.6	86.6	92.5				100.0		
Piperacillin/tazobactam (558[b])			178	52	69	69	39	34	25	29	31	18	4	10	0.25	16
			31.9	41.2	53.6	65.9	72.9	79.0	83.5	88.7	94.3	97.5	98.2	100.0		
Tigecycline (558[b])			102	99	98	76	86	43	28	17				9	0.25	2
			18.3	36.0	53.6	67.2	82.6	90.3	95.3	98.4				100.0		
Gram-negative anaerobes																
Sulopenem (287)	22	21	58	59	66	27	16	6	6	1	1			4	0.12	1
	7.7	15.0	35.2	55.7	78.7	88.2	93.7	95.8	97.9	98.3	98.6			100.0		
Clindamycin (287)		38	20	22	22	42	29	11	15					88	0.5	>4
		13.2	20.2	27.9	35.5	50.2	60.3	64.1	69.3					100.0		
Meropenem (287)	23	14	29	86	62	26	20	9	9	6				3	0.12	1
	8.0	12.9	23.0	53.0	74.6	83.6	90.6	93.7	96.9	99.0				100.0		
Metronidazole (287)			14	5	25	99	107	19	11	1	1			5	1	2
			4.9	6.6	15.3	49.8	87.1	93.7	97.6	97.9	98.3			100.0		
Moxifloxacin (287)			5	14	47	79	30	43	16	22				31	0.5	>8
			1.7	6.6	23.0	50.5	61.0	76.0	81.5	89.2				100.0		
Piperacillin/tazobactam (286[b])			62	14	29	39	25	26	24	26	20	8	3	10	0.5	16
			21.7	26.6	36.7	50.3	59.1	68.2	76.6	85.7	92.7	95.5	96.5	100.0		
Tigecycline (286[b])			26	24	43	60	68	23	18	16				8	0.5	4
			9.1	17.5	32.5	53.5	77.3	85.3	91.6	97.2				100.0		
Gram-positive anaerobes																
Sulopenem (272)	29	61	70	45	34	9	18	2	4						0.06	0.5
	10.7	33.1	58.8	75.4	87.9	91.2	97.8	98.5	100.0							
Clindamycin (272)		60	52	35	27	28	16	17	7					30	0.12	>4
		22.1	41.2	54.0	64.0	74.3	80.1	86.4	89.0					100.0		
Meropenem (272)	81	40	53	36	33	16	10	3							0.06	0.5
	29.8	44.5	63.9	77.2	89.3	95.2	98.9	100.0								
Metronidazole (272)			2	8	39	60	55	12	2	6	1			87	1	>16
			0.7	3.7	18.0	40.1	60.3	64.7	65.4	67.6	68.0			100.0		
Moxifloxacin (272)			4	32	123	52	16	17	6	11				11	0.25	4
			1.5	13.2	58.5	77.6	83.5	89.7	91.9	96.0				100.0		
Piperacillin/tazobactam (272)			116	38	40	30	14	8	1	3	11	10	1		0.12	2
			42.6	56.6	71.3	82.4	87.5	90.4	90.8	91.9	96.0	99.6	100.0			
Tigecycline (272)			76	75	55	16	18	20	10	1				1	0.12	2
			27.9	55.5	75.7	81.6	88.2	95.6	99.3	99.6				100.0		

Gram-negative organisms included: *Bacteroides caccae* (5), *Bacteroides fragilis* (109), *B. fragilis* group (3), *Bacteroides ovatus* (15), *B. ovatus/Bacteroides xylanisolvens* (2), *Bacteroides stercoris* (1), *Bacteroides thetaiotaomicron* (25), *B. thetaiotaomicron/Bacteroides faecis* (8), *Bacteroides uniformis* (7), *Bacteroides vulgatus* (18), *Fusobacterium necrophorum* (11), *Fusobacterium nucleatum* (13), *Fusobacterium periodonticum* (1), *Fusobacterium varium* (1), *Parabacteroides distasonis* (9), *Parabacteroides gordonii* (1), *Porphyromonas asaccharolytica* (1), *Porphyromonas somerae* (2), *Prevotella bergensis* (1), *Prevotella bivia* (5), *Prevotella buccae* (6), *Prevotella corporis* (1), *Prevotella denticola* (3), *Prevotella disiens* (2), *Prevotella intermedia* (4), *Prevotella loescheii* (1), *Prevotella melaninogenica* (4), *Prevotella nigrescens* (2), *Prevotella oris* (2), unspeciated *Bacteroides* (1), unspeciated *Fusobacterium* (2), unspeciated *Prevotella* (3), unspeciated *Veillonella* (3), *Veillonella atypica* (2), *Veillonella parvula* (12) and *Veillonella parvula* group (1).
Gram-positive organisms included: *Actinobaculum schaalii* (3), *Actinomyces canis* (1), *Actinomyces europaeus* (1), *Actinomyces naeslundii* (1), *Actinomyces neuii* (2), *Actinomyces odontolyticus* (4), *Actinomyces oris* (2), *Actinomyces radingae* (2), *Bifidobacterium breve* (2), *Bifidobacterium longum* (1), *Clostridium cochlearium* (1), *Clostridium innocuum* (3), *Clostridium perfringens* (65), *Clostridium ramosum* (4), *Clostridium septicum* (8), *Clostridium sporogenes* (2), *Clostridium tertium* (2), *Collinsella aerofaciens* (1), *Cutibacterium acnes* (73), *Cutibacterium avidum* (4), *Eggerthella lenta* (20), *Facklamia hominis* (1), *Finegoldia magna* (14), *Paeniclostridium sordellii* (2), *Parvimonas micra* (31), *Peptoniphilus asaccharolyticus* (1), *Peptoniphilus harei/indolicus* (7), *Peptostreptococcus anaerobius* (6), *Peptostreptococcus stomatis* (1), *Slackia exigua* (2), unspeciated *Actinomyces* (1), unspeciated *Peptoniphilus* (2), unspeciated *Peptostreptococcus* (1) and unspeciated *Propionibacterium* (1).
[a]Greater than the highest concentration tested.
[b]Missing isolate was an *F. necrophorum* isolate that died during susceptibility testing. This was indicated by no growth on the positive growth control agar poured alongside tigecycline and piperacillin/tazobactam, the last agents to be tested.

Table 4. Antimicrobial activity of sulopenem and comparator agents tested against anaerobic isolates from the USA and Europe

	mg/L			CLSI[a]			EUCAST[a]			FDA[a]		
Antimicrobial agent	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R	%S	%I	%R	%S	%I	%R
Sulopenem	0.12	1	≤0.015 to >16									
Clindamycin	0.5	>4	≤0.03 to >4	75.0	3.9	21.1	78.9		21.1	75.0[b]	3.9	21.1
Meropenem	0.12	1	≤0.015 to >8	98.4	1.1	0.5	96.8	2.7	0.5	98.4[b],c	1.1	0.5
Metronidazole	1	>16	≤0.06 to >16	83.2	0.3	16.5	81.9		18.1	83.2[b]	0.3	16.5
Moxifloxacin	0.5	8	≤0.06 to >8	82.6	4.0	13.4				82.6[b]	4.0	13.4
Piperacillin/tazobactam	0.25	16	≤0.06 to >64	94.3	3.9	1.8	88.7	5.6	5.7	94.3[b],c	3.9	1.8
Tigecycline	0.25	2	≤0.06 to >8							91.6	5.6	2.8

Organisms included: *Actinobaculum schaalii* (3), *Actinomyces canis* (1), *Actinomyces europaeus* (1), *Actinomyces naeslundii* (1), *Actinomyces neuii* (2), *Actinomyces odontolyticus* (4), *Actinomyces oris* (2), *Actinomyces radingae* (2), *Bacteroides caccae* (5), *Bacteroides fragilis* (109), *B. fragilis* group (3), *Bacteroides ovatus* (15), *Bacteroides ovatus/Bacteroides xylanisolvens* (2), *Bacteroides stercoris* (1), *Bacteroides thetaiotaomicron* (25), *Bacteroides thetaiotaomicron/Bacteroides faecis* (8), *Bacteroides uniformis* (7), *Bacteroides vulgatus* (18), *Bifidobacterium breve* (2), *Bifidobacterium longum* (1), *Clostridium cochlearium* (1), *Clostridium innocuum* (3), *Clostridium perfringens* (65), *Clostridium ramosum* (4), *Clostridium septicum* (8), *Clostridium sporogenes* (2), *Clostridium tertium* (2), *Collinsella aerofaciens* (1), *Cutibacterium acnes* (73), *Cutibacterium avidum* (4), *Eggerthella lenta* (20), *Facklamia hominis* (1), *Finegoldia magna* (14), *Fusobacterium necrophorum* (11), *Fusobacterium nucleatum* (13), *Fusobacterium periodonticum* (1), *Fusobacterium varium* (1), *Paeniclostridium sordellii* (2), *Parabacteroides distasonis* (9), *Parabacteroides gordonii* (1), *Parvimonas micra* (31), *Peptoniphilus asaccharolyticus* (1), *Peptoniphilus harei/indolicus* (7), *Peptostreptococcus anaerobius* (6), *Peptostreptococcus stomatis* (1), *Porphyromonas asaccharolytica* (1), *Porphyromonas somerae* (2), *Prevotella bergensis* (1), *Prevotella bivia* (5), *Prevotella buccae* (6), *Prevotella corporis* (1), *Prevotella denticola* (3), *Prevotella disiens* (2), *Prevotella intermedia* (4), *Prevotella loescheii* (1), *Prevotella melaninogenica* (4), *Prevotella nigrescens* (2), *Prevotella oris* (2), *Slackia exigua* (2), unspeciated *Actinomyces* (1), unspeciated *Bacteroides* (1), unspeciated *Fusobacterium* (2), unspeciated *Peptoniphilus* (2), unspeciated *Peptostreptococcus* (1), unspeciated *Prevotella* (3), unspeciated *Propionibacterium* (1), unspeciated *Veillonella* (3), *Veillonella atypica* (2), *Veillonella parvula* (12) and *V. parvula* group (1).

I, intermediate; R, resistant; S, susceptible.

[a]Criteria as published by CLSI (2022), EUCAST (2022) and the US FDA (2022).

[b]US FDA breakpoints were applied. The CLSI M100 standard was recognized.

[c]Using parenteral breakpoints.

isolates, tigecycline was the sole antimicrobial agent exhibiting activity at the current FDA breakpoint (MIC range, 0.5–2 mg/L; 100% S). Notably, sulopenem maintained potent *in vitro* antibacterial activity against Enterobacterales isolates NS to antimicrobial agents commonly used to treat UTI, including 405 ciprofloxacin-NS, 547 nitrofurantoin-NS and 434 trimethoprim/sulfamethoxazole-NS isolates with corresponding sulopenem $MIC_{50/90}$ values of 0.03/0.25 mg/L, 0.06/0.5 mg/L and 0.03/0.12 mg/L, respectively (Table 1).

Anaerobic isolate susceptibility testing results

Analogous to the activity observed against Enterobacterales, sulopenem demonstrated potent *in vitro* antibacterial activity ($MIC_{50/90}$, 0.12/1 mg/L) against 559 anaerobic isolates regardless of infection type, inhibiting 98.9% of all isolates at ≤4 mg/L, which is within the CLSI susceptible MIC breakpoint for ertapenem, meropenem and imipenem against anaerobes and within the sulopenem clinical exposure levels (Table 3). Sulopenem and meropenem demonstrated parallel activity against Gram-negative and -positive anaerobic species; sulopenem ($MIC_{50/90}$, 0.12/1 mg/L) inhibited 97.9% of Gram-negative anaerobic isolates ($n=287$) at ≤4 mg/L, equivalent to meropenem [$MIC_{50/90}$, 0.12/1 mg/L; 96.9%/93.7%/96.9% S (CLSI/EUCAST/FDA)] (Table 4). Likewise, sulopenem ($MIC_{50/90}$, 0.06/0.5 mg/L) inhibited 100.0% of Gram-positive anaerobic isolates at ≤4 mg/L, equivalent to meropenem [$MIC_{50/90}$, 0.06/0.5 mg/L; 100.0% S (CLSI/EUCAST/FDA)]. Additional comparator agent susceptibilities ranged from 75.0% (CLSI/FDA) for clindamycin to 94.3% (CLSI/FDA) for piperacillin/tazobactam. Of the anaerobic isolates tested, 25.0% (140/559) demonstrated clindamycin NS whereas 17.4% (97/559) demonstrated moxifloxacin NS; ≥96.9% of the anaerobic isolates in these resistant subgroups were inhibited by ≤4 mg/L of sulopenem.

Discussion

This study provides updated susceptibility data on the *in vitro* activity and spectrum of sulopenem against 1647 Enterobacterales and 559 anaerobic clinical isolates collected from 2018–2020 in Europe and the USA. Overall, sulopenem inhibited 99.2% of all Enterobacterales isolates at ≤1 mg/L. The activity of sulopenem ($MIC_{50/90}$, 0.03/0.03 mg/L), amoxicillin/clavulanate, meropenem and ciprofloxacin against *E. coli* isolates is corroborated by previously published susceptibility data.[10,29] Importantly, sulopenem maintained potent *in vitro* activity irrespective of ESBL-phenotype and fluoroquinolone-NS status. These data highlight that sulopenem is a potential option for treatment of UTI and IAI caused by resistant pathogens, a growing public health concern.[30] Currently, sulopenem has been evaluated in three Phase III clinical trials focused on efficacy of the oral sulopenem etzadroxil/probenecid formulation against UTI (NCT03354598), cUTI (NCT03357614) and IAI

(NCT03358376), with a fourth UTI-focused clinical trial underway (NCT05584657).[18,31,32] Recently, NCT03357614 and NCT03354 598 clinical trials were completed but narrowly missed the lower limit for demonstration of non-inferiority against ertapenem for treatment of cUTIs and ciprofloxacin for treatment of UTIs, respectively.[33,34] Of the Phase III trials completed to date, clinical exposures to sulopenem achieved were high enough to cover the MIC_{90} values summarized in this present study.

Limited reports on sulopenem activity are available for recent, clinically relevant anaerobic species. Data from previous studies by both Gootz *et al.*[13] and Ednie and Applebaum[35] gave comparable results to our study. Overall, the activity of sulopenem ($MIC_{50/90}$, 0.12/1 mg/L), clindamycin ($MIC_{50/90}$, 0.5/>4 mg/L), piperacillin/tazobactam ($MIC_{50/90}$, 0.25/16 mg/L) and metronidazole ($MIC_{50/90}$, 1/>16 mg/L) against anaerobic isolates agreed with published data.[35] Against all anaerobic isolates, sulopenem (98.9% inhibited at ≤4 mg/L) and meropenem [98.4% S (CLSI)] were the most active agents assessed. The potent *in vitro* activity of sulopenem suggests the potential of this agent to treat mixed anaerobic infections; however, additional data from complicated IAI clinical trials are needed.

In summary, sulopenem demonstrated potent *in vitro* antimicrobial activity against contemporary Enterobacterales isolates (including ESBL-phenotype strains) and Gram-positive and -negative anaerobic clinical isolates. These data indicate that oral sulopenem may be a potential treatment for uncomplicated UTI or as a step-down therapy for complicated UTI or IAI following IV treatment.

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Transparency declarations

JMI Laboratories contracted to perform services in 2021 for AbbVie Inc., Affinity Biosensors, AimMax Therapeutics, Inc., Alterity Therapeutics, Amicrobe, Inc., Arietis Pharma, Armata Pharmaceuticals, Inc., Astrellas Pharma Inc., Basilea Pharmaceutica AG, Becton, Dickinson and Company (BD), bioMérieux, Inc., Boost Biomes, Brass Dome Ventures Ltd, Bravos Biosciences, Bugworks Research Inc., Centers for Disease Control and Prevention, Cerba Research, Cidara Therapeutics, Cipla Ltd, ContraFect Corp., CXC7, DiamondV, Enveda Biosciences, Fedora Pharmaceuticals, Inc., Fimbrion Therapeutics, First Light Diagnostics, Forge Therapeutics, Inc., Fox Chase Cancer Center, GlaxoSmithKline plc (GSK), Harvard University, Institute for Clinical Pharmacodynamics (ICPD), International Health Management Associates (IHMA), Inc., Iterum Therapeutics plc, Janssen Research & Development, Johnson & Johnson, Kaleido Biosciences, Inc., Laboratory Specialists, Inc. (LSI), Meiji Seika Pharma Co., Ltd, Melinta Therapeutics, Menarini Group, Merck & Co., Inc., MicuRx Pharmaceuticals Inc., Mutabilis, Nabriva Therapeutics, National Institutes of Health, Novome Biotechnologies, Omnix Medical Ltd, Paratek Pharma, Pattern Bioscience, Pfizer Inc., Prokaryotics Inc., Pulmocide Ltd, QPEX Biopharma, Inc., Roche Holding AG, Roivant Sciences, SeLux Diagnostics, Inc., Shionogi Inc., Sinovent Pharmaceuticals, Inc., SNIPR Biome ApS, Spero Therapeutics, Summit Therapeutics, Inc., T2 Biosystems, TenNor Therapeutics, Thermo Fisher Scientific, University of Southern California, University of Wisconsin, USCAST, US Food and Drug Administration, Venatorx Pharmaceutics, Inc., Weill Cornell Medicine and Wockhardt Ltd. There are no speakers' bureaus or stock options to declare.

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