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Antimicrobial Activity of Gepotidacin Tested against *Escherichia coli* and *Staphylococcus saprophyticus* Isolates Causing Urinary Tract Infections in Medical Centers Worldwide (2019 to 2020)

S. J. Ryan Arends,[a] Deborah Butler,[a] Nicole Scangarella-Oman,[a] Mariana Castanheira,[a] Rodrigo E. Mendes[a]

[a]JMI Laboratories, North Liberty, Iowa, USA

ABSTRACT The *in vitro* activities of gepotidacin and comparator agents against 3,560 *Escherichia coli* and 344 *Staphylococcus saprophyticus* collected from female (81.1%) and male (18.9%) patients with urinary tract infections (UTIs) in a global prospective surveillance program in 2019 to 2020 were determined. Isolates collected from 92 medical centers in 25 countries, including the United States, Europe, Latin America, and Japan, were tested for susceptibility by reference methods in a central monitoring laboratory. Gepotidacin inhibited 98.0% (3,488/3,560 isolates) of *E. coli* and 100% (344/344 isolates) of *S. saprophyticus* at gepotidacin concentrations of ≤4 μg/mL and ≤0.25 μg/mL, respectively. This activity was largely unaffected with isolates that demonstrated resistance phenotypes to other oral standard-of-care antibiotics, including amoxicillin-clavulanic acid, cephalosporins, fluoroquinolones, fosfomycin, nitrofurantoin, and trimethoprim-sulfamethoxazole. Gepotidacin also inhibited 94.3% (581/616 isolates) of *E. coli* isolates with an extended-spectrum β-lactamase-producing phenotype, 97.2% (1,085/1,129 isolates) of *E. coli* isolates resistant to ciprofloxacin, 96.1% (874/899) of *E. coli* isolates resistant to trimethoprim-sulfamethoxazole, and 96.3% (235/244 isolates) of multidrug-resistant *E. coli* isolates at gepotidacin concentrations of ≤4 μg/mL. In summary, gepotidacin demonstrated potent activity against a large collection of contemporary UTI *E. coli* and *S. saprophyticus* strains collected from patients worldwide. These data support the further clinical development of gepotidacin as a potential treatment option for patients with uncomplicated UTIs.

KEYWORDS ESBL, oral, resistance, urinary tract infection

Escherichia coli is the most common pathogen causing urinary tract infections (UTIs) (1–3). Historically, these infections have been treated with oral antibiotics, including trimethoprim-sulfamethoxazole, cephalosporins, and fluoroquinolones. However, the prevalence of isolates resistant to fluoroquinolones or trimethoprim-sulfamethoxazole has increased, as has the number of isolates displaying extended-spectrum β-lactamase (ESBL)-producing phenotypes. This scenario has precluded the use of many of the aforementioned oral agents for the empirical and guided treatment of UTIs (4). This increase in ESBL-producing isolates is due in part to the rapid clonal expansion of sequence type 131 (ST131) *E. coli* isolates, especially in the nosocomial setting (5). ESBL-producing *E. coli* strains, including ST131 isolates, are often coresistant to other agents used to treat UTIs, such as fluoroquinolones, leading to the recommendation of older antibiotics for the treatment of UTIs (6). Current first-line treatment options include amoxicillin, amoxicillin-clavulanate, fosfomycin, nitrofurantoin, the amdinocillin prodrug pivmecillinam, and trimethoprim-sulfamethoxazole.

Gepotidacin is a novel, bactericidal, first-in-class triazaacenaphthylene antibacterial that inhibits DNA gyrase and topoisomerase IV by a distinct mechanism of action, which confers activity against most strains of target pathogens, such as *E. coli*, *Staphylococcus saprophyticus*, and *Neisseria gonorrhoeae*, including those that are resistant to current antibiotics

Address correspondence to Rodrigo E. Mendes, rodrigo-mendes@jmilabs.com.

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(7, 8). Gepotidacin represents a future option for oral treatment of uncomplicated UTIs (uUTIs), defined as UTIs among premenopausal, nonpregnant women with no known urological abnormalities or comorbidities (6). Gepotidacin possesses oral bioavailability (9) and has completed to phase 3 clinical studies (ClinicalTrials.gov registration numbers NCT04020341, NCT04187144, and NCT04010539) for the treatment of uUTIs and urogenital gonorrhea (10–14). In addition, pharmacokinetic (PK)/pharmacodynamic (PD) studies and potential effects of gepotidacin on the gut microbiome have been reported (15–17).

Prior studies of gepotidacin established *in vitro* activity for the most common UTI target pathogens (18–21); however, this study prospectively monitored the *in vitro* activity of gepotidacin and comparator agents against a contemporary collection of *E. coli* and *S. saprophyticus* strains recovered from UTIs. The *in vitro* activity of gepotidacin against these isolates, as well as against subsets displaying resistance to other agents, is discussed.

RESULTS

The gepotidacin MIC_{50} and MIC_{90} values were both 2 μg/mL against 3,560 *E. coli* isolates, with 98.0% of the isolates being inhibited at gepotidacin concentrations of $\leq$4 μg/mL (Table 1). The rates of susceptibility to amoxicillin-clavulanate (MIC_{50}, 8 μg/mL; MIC_{90}, 16 μg/mL), cefadroxil, ciprofloxacin (MIC_{50}, 0.015 μg/mL; MIC_{90}, $>$4 μg/mL), and trimethoprim-sulfamethoxazole (MIC_{50}, $\leq$0.12 μg/mL; MIC_{90}, $>$4 μg/mL) were 79.6%, 82.5%, 72.5%, and 68.2%, respectively (Table 2). Higher rates of susceptibility to fosfomycin (MIC_{50}, 0.5 μg/mL; MIC_{90}, 1 μg/mL; 99.0% susceptible using Clinical and Laboratory Standards Institute [CLSI] guidelines and 97.7% susceptible using European Committee on Antimicrobial Susceptibility Testing [EUCAST] guidelines), amdinocillin (MIC_{50}, 0.5 μg/mL; MIC_{90}, 4 μg/mL; 94.1% susceptible), nitrofurantoin (MIC_{50}, 16 μg/mL; MIC_{90}, 32 μg/mL; 97.3% susceptible using CLSI guidelines and 98.7% susceptible using EUCAST guidelines), and nitroxoline (99.9% susceptible) were seen for all *E. coli* isolates. Further stratification, based on collection setting, gender, and/or culture source, rates of susceptibility to comparator agents for countries from which at least 30 isolates were collected can be found in Tables S1 and S2 in the supplemental material for *E. coli* and *S. saprophyticus*, respectively.

Identical gepotidacin MIC_{50} and MIC_{90} values (MIC_{50}, 2 μg/mL; MIC_{90}, 4 μg/mL; 94.5% to 97.2% of isolates inhibited at $\leq$4 μg/mL) were observed among subsets of *E. coli* resistant to amoxicillin-clavulanate, ciprofloxacin, amdinocillin, nitrofurantoin, or trimethoprim-sulfamethoxazole. Only the fosfomycin-resistant isolates (n = 25) had a different gepotidacin MIC_{90} value of 8 μg/mL, with 84.0% of gepotidacin MIC values being $\leq$4 μg/mL (Table 1).

An ESBL-producing phenotype was observed in 616 (17.3%) of 3,560 *E. coli* isolates tested. Gepotidacin (MIC_{50}, 2 μg/mL; MIC_{90}, 4 μg/mL) activity against these isolates remained comparable to that for non-ESBL-producing *E. coli* isolates (MIC_{50}, 2 μg/mL; MIC_{90}, 2 μg/mL) (Table 1). Amoxicillin-clavulanate (MIC_{50}, 16 μg/mL; MIC_{90}, 32 μg/mL), cefadroxil, ciprofloxacin (MIC_{50}, $>$4 μg/mL; MIC_{90}, $>$4 μg/mL), and trimethoprim-sulfamethoxazole (MIC_{50}, $>$4 μg/mL; MIC_{90}, $>$4 μg/mL) had susceptibility rates of 49.1%, 3.7%, 21.5%, and 39.8%, respectively, against ESBL-producing *E. coli* isolates. However, the numbers of observed isolates susceptible to fosfomycin (96.6% using CLSI guidelines and 95.1% using EUCAST guidelines), amdinocillin (96.8%), nitrofurantoin (92.7% using CLSI guidelines and 96.4% using EUCAST guidelines), and nitroxoline (100%) remained high (Table 2).

Of all tested *E. coli* isolates, 899 (25.3%) and 1,129 (31.7%) were resistant to ciprofloxacin and trimethoprim-sulfamethoxazole, respectively (Table 2). The gepotidacin MIC_{50} and MIC_{90} values for these resistant populations were 2 and 4 μg/mL, respectively, similar to those for their respective susceptible population counterparts (MIC_{50}, 2 μg/mL; MIC_{90}, 2 μg/mL) (data not shown). Amoxicillin-clavulanate, cefadroxil, ciprofloxacin, and trimethoprim-sulfamethoxazole susceptibility rates were $<$70% for these resistant subsets, while fosfomycin, amdinocillin, nitrofurantoin, and nitroxoline susceptibility rates were $>$90% (Table 2).

A total of 244 *E. coli* isolates (6.9%) had a multidrug-resistant (MDR) phenotype. Gepotidacin activities for MDR (MIC_{50}, 2 μg/mL; MIC_{90}, 4 μg/mL) and non-MDR (MIC_{50}, 2 μg/mL; MIC_{90}, 2 μg/mL) isolates were similar (Table 1). Analogous to the data for ESBL-producing isolates, low susceptibility rates were seen for amoxicillin-clavulanate (MIC_{50}, 16 μg/mL;

TABLE 1 Antimicrobial activity of gepotidacin tested against *E. coli* and *S. saprophyticus* isolates collected worldwide (2019 to 2020)

Organism type[a] (no. of isolates)/Phenotype	No. of isolates (cumulative %) with gepotidacin MIC (mg/L) of:											MIC_{50}	MIC_{90}
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32		
E. coli (3,560)	3 (0.1)	4 (0.2)	10 (0.5)	30 (1.3)	190 (6.7)	1,217 (40.8)	1,779 (90.8)	255 (98.0)	48 (99.3)	19 (99.9)	5 (100.0)	2	2
Amoxicillin-clavulanate-resistant (201)			0 (0.0)	3 (1.5)	12 (7.5)	50 (32.3)	94 (79.1)	31 (94.5)	4 (96.5)	5 (99.0)	2 (100.0)	2	4
Ciprofloxacin-resistant (899)	3 (0.3)	2 (0.6)	6 (1.2)	23 (3.8)	100 (14.9)	311 (49.5)	337 (87.0)	92 (97.2)	15 (98.9)	8 (99.8)	2 (100.0)	2	4
Fosfomycin-resistant (25)				0 (0.0)	3 (12.0)	7 (40.0)	7 (68.0)	4 (84.0)	3 (96.0)	1 (100.0)		2	8
Mecillinam-resistant (151)	1 (0.7)	0 (0.7)	0 (0.7)	3 (2.6)	8 (7.9)	39 (33.8)	78 (85.4)	17 (96.7)	3 (98.7)	2 (100.0)		2	4
Nitrofurantoin-resistant (46)			0 (0.0)	1 (2.2)	1 (4.3)	11 (28.3)	24 (80.4)	7 (95.7)	1 (97.8)	0 (97.8)	1 (100.0)	2	4
Trimethoprim-sulfamethoxazole-resistant (1,129)	2 (0.2)	0 (0.2)	5 (0.6)	12 (1.7)	87 (9.4)	420 (46.6)	468 (88.0)	91 (96.1)	31 (98.8)	9 (99.6)	4 (100.0)	2	4
Non-ESBL-producing (2,944)	1 (<0.1)	3 (0.1)	7 (0.4)	25 (1.2)	141 (6.0)	1,016 (40.5)	1,534 (92.6)	180 (98.7)	28 (99.7)	8 (>99.9)	1 (100.0)	2	2
ESBL-producing (616)	2 (0.3)	1 (0.5)	3 (1.0)	5 (1.8)	49 (9.7)	201 (42.4)	245 (82.1)	75 (94.3)	20 (97.6)	11 (99.4)	4 (100.0)	2	4
Non-MDR (3,316)	1 (<0.1)	4 (0.2)	9 (0.4)	27 (1.2)	175 (6.5)	1,133 (40.7)	1,686 (91.5)	218 (98.1)	42 (99.4)	18 (99.9)	3 (100.0)	2	2
MDR (244)	2 (0.8)	0 (0.8)	1 (1.2)	3 (2.5)	15 (8.6)	84 (43.0)	93 (81.1)	37 (96.3)	6 (98.8)	1 (99.2)	2 (100.0)	2	4
Outpatient (2,301)	0 (0.0)	3 (0.1)	6 (0.4)	15 (1.0)	122 (6.3)	763 (39.5)	1,182 (90.9)	169 (98.2)	31 (99.6)	8 (99.9)	2 (100.0)	2	2
Inpatient (1,158)	1 (0.1)	1 (0.2)	4 (0.5)	15 (1.8)	61 (7.1)	418 (43.2)	548 (90.5)	84 (97.8)	13 (98.9)	10 (99.7)	3 (100.0)	2	2
S. saprophyticus (344)	3 (0.9)	276 (81.1)	64 (99.7)	1 (100.0)								0.06	0.12

[a]Resistant phenotypes determined according to CLSI interpretive criteria.

TABLE 2 Activity of gepotidacin and comparator antimicrobial agents tested against *E. coli* and selected subsets

Isolate type and antimicrobial agent (no. of isolates tested)/Phenotype	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Result with CLSI guidelines (%)[a]		Result with EUCAST guidelines (%)[a]	
			Sensitive	Resistant	Sensitive	Resistant
All *E. coli* isolates (3,560)						
Gepotidacin	2	2				
Ciprofloxacin	0.015	>4	72.5	25.3	72.5	25.3
Amikacin	4	8	99.5	0.3	98.2[b]	1.8
Amoxicillin-clavulanic acid[c]	8	16	79.6	5.7		
Cefadroxil	[d]				82.5[e]	17.5
Ceftriaxone	≤0.06	>8	83.7	16.1	83.7[f]	16.3
Fosfomycin	0.5	1	99.0[g]	0.7	97.7[h]	2.3
Mecillinam	0.5	4	94.1[g]	4.2	94.1[h]	5.9
Nitrofurantoin	16	32	97.3	1.3	98.7[e]	1.3
Nitroxoline	[d]				99.9[e]	0.1
Piperacillin-tazobactam[c]	2	8	94.7	2.8	94.7	5.3
Trimethoprim-sulfamethoxazole[c]	≤0.12	>4	68.2	31.8	68.2	31.3
ESBL-producing isolates (616)						
Gepotidacin	2	4				
Ciprofloxacin	>4	>4	21.5	74.6	21.5	74.6
Amikacin	4	8	97.9	1.1	91.7[b]	8.3
Amoxicillin-clavulanic acid[c]	16	32	49.1	19.7		
Cefadroxil	[d]				3.7[e]	96.3
Ceftriaxone	>8	>8	6.0	93.2	6.0[f]	94
Fosfomycin	0.5	2	96.6[g]	2.9	95.1[h]	4.9
Mecillinam	1	4	96.8[g]	2.3	96.8[h]	3.2
Nitrofurantoin	16	32	92.7	3.6	96.4[e]	3.6
Nitroxoline	[d]				100.0[e]	0.0
Piperacillin-tazobactam[c]	4	16	81.4	9.0	81.4	18.6
Trimethoprim-sulfamethoxazole[c]	>4	>4	39.8	60.2	39.8	59.5
Ciprofloxacin-resistant isolates (899)						
Gepotidacin	2	4				
Ciprofloxacin	>4	>4	0.0	100.0	0.0	100.0
Amikacin	4	8	98.4	0.8	93.8[b]	6.2
Amoxicillin-clavulanic acid[c]	8	16	60.0	9.6		
Cefadroxil	[d]				49.7[e]	50.3
Ceftriaxone	4	>8	49.6	50.2	49.6[f]	50.4
Fosfomycin	0.5	2	97.1[g]	2.2	95.2[h]	4.8
Mecillinam	1	4	94.7[g]	4.1	94.7[h]	5.3
Nitrofurantoin	16	32	93.5	3.7	96.3[e]	3.7
Nitroxoline	[d]				99.9[e]	0.1
Piperacillin-tazobactam[c]	4	16	85.5	7.2	85.5	14.5
Trimethoprim-sulfamethoxazole[c]	>4	>4	44.8	55.2	44.8	54.6
Trimethoprim-sulfamethoxazole-resistant isolates (1,129)						
Gepotidacin	2	4				
Ciprofloxacin	0.25	>4	51.7	49.3	51.7	43.9
Amikacin	4	8	98.7	0.8	95.7[b]	4.3
Amoxicillin-clavulanic acid[c]	8	16	64.2	8.7		
Cefadroxil	[d]				67.5[e]	32.5
Ceftriaxone	≤0.06	>8	68.3	31.4	68.3[f]	31.7
Fosfomycin	0.5	2	98.2[g]	1.5	96.3[h]	3.7
Mecillinam	1	8	90.8[g]	6.7	90.8[h]	9.2
Nitrofurantoin	16	32	95.1	2.3	97.7[e]	2.3
Nitroxoline	[d]				99.9[e]	0.1
Piperacillin-tazobactam[c]	2	16	88.8	6.2	88.8	11.2
Trimethoprim-sulfamethoxazole[c]	>4	>4	0.0	100.0	0.0	98.7
MDR isolates (244)						
Gepotidacin	2	4				
Ciprofloxacin	>4	>4	0.4	95.9	0.4	95.9
Amikacin	4	16	93.4	3.7	79.1[b]	20.9

(Continued on next page)

TABLE 2 (Continued)

Isolate type and antimicrobial agent (no. of isolates tested)/Phenotype	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Result with CLSI guidelines (%)[a]		Result with EUCAST guidelines (%)[a]	
			Sensitive	Resistant	Sensitive	Resistant
Amoxicillin-clavulanic acid[c]	16	32	20.5	24.2		
Cefadroxil	[d]				7.8[e]	92.2
Ceftriaxone	>8	>8	5.3	5.3	5.3[f]	94.7
Fosfomycin	0.5	8	93.4[g]	5.7	91.8[h]	8.2
Mecillinam	1	8	95.1[g]	3.3	95.1[h]	4.9
Nitrofurantoin	16	64	89.8	6.6	93.4[e]	6.6
Nitroxoline	[d]				100.0[e]	0.0
Piperacillin-tazobactam[c]	8	64	53.5	22.2	53.5	46.5
Trimethoprim-sulfamethoxazole[c]	>4	>4	29.9	70.1	29.9	69.7

[a]Criteria published by CLSI (29) and EUCAST (31). Blank fields indicate no interpretive criteria, with the exception of amoxicillin-clavulanic acid (due to the 2:1 ratio, only CLSI criteria were applied).
[b]For UTIs.
[c]Amoxicillin-clavulanic acid was tested at a 2:1 ratio, piperacillin-tazobactam was tested with tazobactam at a fixed concentration of 4 μg/mL, and trimethoprim-sulfamethoxazole was tested at a 1:19 ratio.
[d]Susceptibility testing by disk diffusion; MICs were not determined.
[e]Breakpoints for uUTIs.
[f]Breakpoints for infections other than meningitis.
[g]Tested by agar dilution; UTI breakpoints.
[h]Tested by agar dilution; breakpoints for oral treatment of uUTIs.

MIC_{90}, 32 μg/mL; 20.5% susceptible), cefadroxil (7.8% susceptible), ciprofloxacin (MIC_{50}, >4 μg/mL; MIC_{90}, >4 μg/mL; 0.4% susceptible), and trimethoprim-sulfamethoxazole (MIC_{50}, >4 μg/mL; MIC_{90}, >4 μg/mL; 29.9% susceptible) against MDR isolates. Against these isolates, susceptibility rates of >90% were observed for fosfomycin (93.4% using CLSI guidelines and 91.8% using EUCAST guidelines), amdinocillin (95.1%), nitrofurantoin (89.8% using CLSI guidelines and 93.4% using EUCAST guidelines), and nitroxoline (100.0%) (Table 2).

Isolates were stratified into outpatient and inpatient subsets based on the medical service line provided by the participating sites. Gepotidacin was active against the outpatient isolates (MIC_{50}, 2 μg/mL; MIC_{90}, 2 μg/mL) and inhibited 98.2% of *E. coli* isolates at ≤4 μg/mL (Table 1). Similar results were observed for gepotidacin against the inpatient isolates (MIC_{50}, 2 μg/mL; MIC_{90}, 2 μg/mL; 97.8% of isolates with MICs of ≤4 μg/mL). For some of the comparator agents, greater rates of susceptibility were seen for outpatient isolates, compared with the inpatient subset (amoxicillin-clavulanic acid: outpatient, 81.6%; inpatient, 75.4%; cefadroxil: outpatient, 85.3%; inpatient, 76.5%; cefazolin: outpatient, 83.0%; inpatient, 74.4%; ceftriaxone: outpatient, 86.5%; inpatient, 78.1%; ciprofloxacin: outpatient, 76.3%; inpatient, 64.4%; trimethoprim-sulfamethoxazole: outpatient, 70.5%; inpatient, 63.6%) (data not shown). The percentages of isolates susceptible to fosfomycin, amdinocillin, nitrofurantoin, and nitroxoline showed little difference (<1.0%) between outpatient and inpatient populations (data not shown).

Gepotidacin MIC_{50} and MIC_{90} values against 344 *S. saprophyticus* isolates were 0.06 and 0.12 μg/mL, respectively, and all observed gepotidacin MIC values were ≤0.25 μg/mL (Table 1). Most agents tested were active against this species, with susceptibility rates of >90% for trimethoprim-sulfamethoxazole (MIC_{50}, ≤0.5 μg/mL; MIC_{90}, ≤0.5 μg/mL; 97.1% susceptible), ciprofloxacin (MIC_{50}, 0.25 μg/mL; MIC_{90}, 0.5 μg/mL; 99.4% susceptible), nitrofurantoin (MIC_{50}, 16 μg/mL; MIC_{90}, 16 μg/mL; 100.0% susceptible), and vancomycin (MIC_{50}, 1 μg/mL; MIC_{90}, 2 μg/mL; 100.0% susceptible) (Table 3).

DISCUSSION

UTIs remain a common global health problem. Increasing resistance to oral agents, including cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole, have limited their use as empirical treatment (4). Current oral first-line empirical options for treating uUTIs include fosfomycin, nitrofurantoin, and amdinocillim (6). The data from this large collection of recent *E. coli* isolates from UTIs support these treatment options, as the proportions of all *E. coli* isolates that were susceptible to ciprofloxacin, cefadroxil, and

TABLE 3 Activity of gepotidacin and comparator antimicrobial agents tested against *S. saprophyticus* ($n = 344$)

Antimicrobial agent	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Result with CLSI guidelines (%)[a]		Result with EUCAST guidelines (%)[a]	
			Sensitive	Resistant	Sensitive	Resistant
Gepotidacin	0.06	0.12				
Ciprofloxacin	0.25	0.5	99.4	0.3	99.4[b]	0.6
Fosfomycin	128	>256				
Nitrofurantoin	16	16	100.0	0.0	100.0[c]	0.0
Penicillin	0.25	0.5	3.5	96.5		
Trimethoprim-sulfamethoxazole[d]	≤0.5	≤0.5	97.1	2.9	97.1	1.7
Vancomycin	1	2	100.0	0.0	100.0	0.0

[a]Criteria published by CLSI (29) and EUCAST (31). Blank fields indicate no interpretive criteria.
[b]Defined as susceptible, with increased exposure.
[c]Breakpoints for uUTIs.
[d]Trimethoprim-sulfamethoxazole was tested at a 1:19 ratio.

trimethoprim-sulfamethoxazole were smaller (68.2% to 82.5%) than those for fosfomycin, amdinocillin, nitrofurantoin, and nitroxoline (94.1% to 99.9%). The contrast between these two drug sets was even more evident when the susceptibility rates against ESBL-producing and MDR isolates were compared. Against both ESBL-producing and MDR isolates, limited activity and low susceptibility rates (<50% susceptible) were seen for amoxicillin-clavulanate, cefadroxil, ciprofloxacin, and trimethoprim-sulfamethoxazole, while susceptibility rates of >90% were observed for fosfomycin, amdinocillin, nitrofurantoin, and nitroxoline.

The *in vitro* activities of fosfomycin, amdinocillin, nitrofurantoin, and nitroxoline against UTI *E. coli* strains, regardless of phenotype, have renewed interest in these old agents as oral options for treating UTIs. Nitrofurantoin is widely available and was approved by the U.S. FDA in 1954, and nitroxoline has been in clinical use in western European countries since 1962. Both agents have limitations, such as lack of PK and PD data, mainly bacteriostatic activity, and limited commercial availability (for nitroxoline) (22). Fosfomycin was introduced in Europe in the 1970s and was approved by the U.S. FDA in 1996 for single-dose treatment of uUTIs caused by *E. coli* or *Enterococcus faecalis* (23). Although fosfomycin is active *in vitro*, it has been reported that older clinical trial studies might have overestimated the clinical efficacy of fosfomycin (24); furthermore, a higher clinical cure rate with nitrofurantoin, compared with fosfomycin, has been reported (25). Finally, amdinocillin has been used for many decades for uUTIs in Nordic European countries and has shown *in vitro* stability against CTX-M-producing *E. coli* strains. However, clinical efficacy studies with these MDR isolates are lacking (26). Despite the potent *in vitro* activity shown by these older agents, these various limitations demonstrate the need for the clinical development of new agents (27).

Gepotidacin is currently under clinical development for the treatment of uUTIs and urogenital gonorrhea. In summary, gepotidacin demonstrated potent *in vitro* activity against a large global collection of contemporary *E. coli* isolates causing UTIs, inhibiting 98.0% of all *E. coli* isolates at MIC values of ≤4 μg/mL. Gepotidacin retained this activity against both ESBL-producing and MDR subsets, with 94.3% and 96.3%, respectively, of gepotidacin MIC values being ≤4 μg/mL. When tested against many subsets of drug-resistant *E. coli* phenotypes, gepotidacin maintained similar MIC_{50} and MIC_{90} values (2 and 4 μg/mL, respectively), with the single exception of fosfomycin-resistant *E. coli* strains, for which the gepotidacin MIC_{90} value was one doubling dilution higher at 8 μg/mL. However, this difference may be a result of the small sample size ($n = 25$). Of note, gepotidacin retained activity against isolates that were resistant to current first-line agents for uUTIs, with MIC values of ≤4 μg/mL for 84.0%, 96.7%, 95.7%, and 96.1% of isolates that were resistant to fosfomycin, amdinocillin, nitrofurantoin, and trimethoprim-sulfamethoxazole, respectively. Previous studies demonstrated that the gepotidacin concentration in urine after administration of 1,500 mg twice a day had a maximum value of 580 μg/mL between doses on day 1 and 920 μg/mL on day 4. Also, the steady-state total trough levels remained above 4 μg/mL within 12 h (15). These PK parameters indicate that the gepotidacin concentration in urine during the dosing

interval remains above the MIC values for 98.0% of the *E. coli* isolates tested here, including resistant subsets.

Finally, gepotidacin (MIC_{100}, 0.25 μg/mL) also demonstrated potent *in vitro* activity against contemporary *S. saprophyticus* isolates, against which older agents, such as fosfomycin, amdinocillin, and nitroxoline, lack activity. These *in vitro* data provide recent information and benchmark for gepotidacin activity prior to its clinical approval and use for treating uUTIs. As resistance to current therapy options continues to increase, these data support further clinical development of gepotidacin as a potential new agent for the treatment of uUTIs.

MATERIALS AND METHODS

Bacterial isolates. A total of 3,560 *E. coli* isolates and 344 *S. saprophyticus* isolates were collected from 92 medical centers in 25 countries in 2019 to 2020 as part of the SENTRY Antimicrobial Surveillance Program. The geographic distribution of isolates included the United States (all nine U.S. Census Divisions, 45 medical centers) (2,176 isolates [55.7% overall]), Europe (17 countries, 34 medical centers) (1,252 isolates [32.1% overall]), Latin America (6 countries, 9 medical centers) (249 isolates [6.4% overall]), and Japan (4 medical centers) (227 isolates [5.8% overall]). All isolates were cultured from urine or urethral catheter samples and deemed responsible for UTI based on local criteria. Only 1 isolate per patient per infection episode was included in this study. Isolates were collected from both female (81.1%) and male (18.9%) patients. Most isolates (68.4%) were recovered from samples that had been collected from patients associated with medical service lines representing outpatient treatment, including ambulatory/outpatient, family practice, or emergency room services. Other isolates (31.6%) were cultured from patients in medical service lines suggestive of hospitalized individuals. Species identification was confirmed by standard biochemical tests and, where necessary, the matrix-assisted laser desorption ionization (MALDI) Biotyper (Bruker Daltonics, Billerica, MA, USA) according to the manufacturer's instructions.

Susceptibility testing. The broth microdilution method was performed according to CLSI methods to determine susceptibility to gepotidacin and its comparator agents (28). Susceptibility to amoxicillin-clavulanate was tested at the CLSI-recommended 2:1 ratio. Susceptibility to amdinocillin and fosfomycin was determined by reference agar dilution following recommendations made by the CLSI in the M07 (28) and M100 (29) documents. The testing medium utilized was Mueller-Hinton agar, and fosfomycin testing included supplementation with glucose-6-phosphate at a final concentration of 25 μg/mL. Susceptibility to the comparators nitroxoline (30 μg) and cefadroxil (30 μg) was determined by disk diffusion following the CLSI M02 and M100 guidelines (29, 30). Disk inhibition zones and MIC values were validated by concurrently testing CLSI- and/or EUCAST-recommended quality control (QC) reference strains ATCC 25922, ATCC 27853, ATCC 29213, and ATCC 35218. All QC results were within published ranges (29). CLSI (29) and EUCAST (31) susceptibility interpretive criteria were used to determine susceptibility/resistance percentages for comparator agents. A single value was reported when susceptibility breakpoints agreed between CLSI and EUCAST guidelines (ciprofloxacin, ceftriaxone, amdinocillin, and trimethoprim-sulfamethoxazole) or when breakpoints exist for only one agency (cefadroxil and nitroxoline [EUCAST]). A single value (CLSI) was also reported for amoxicillin-clavulanate tested at a 2:1 ratio. When breakpoints differ between CLSI and EUCAST guidelines (fosfomycin and nitrofurantoin), the percentages of isolates considered susceptible with each breakpoint are labeled accordingly.

Resistant subsets. CLSI breakpoints were applied to define isolates with a phenotype of resistance to the following standard-of-care agents: amoxicillin-clavulanate, ciprofloxacin, fosfomycin, mecillinam, nitrofurantoin, and trimethoprim-sulfamethoxazole. The ESBL-producing phenotype was defined for *E. coli* as MIC values of $\geq$2 μg/mL for aztreonam, ceftazidime, or ceftriaxone (29). Isolates meeting these criteria can produce ESBL, have plasmid AmpC, and/or overexpress the intrinsic AmpC gene but are described here as presumptive ESBL producers. All *E. coli* strains were susceptible to meropenem. The MDR designation for isolates was similar to the criteria published by Magiorakos et al. (32), who define MDR as not susceptible to $\geq$1 agent in $\geq$3 antimicrobial classes. The antimicrobial classes and representative drugs used in the *E. coli* MDR analysis included broad-spectrum cephalosporins (ceftriaxone and ceftazidime), carbapenems (meropenem), a broad-spectrum penicillin combined with a β-lactamase inhibitor (piperacillin-tazobactam), fluoroquinolones (ciprofloxacin and levofloxacin), and aminoglycosides (gentamicin and amikacin).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.03 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.01 MB.

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