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Ceftobiprole activity when tested against contemporary bacteria causing bloodstream infections in the United States (2016–2017)

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ABSTRACT

Ceftobiprole medocaril, the prodrug of ceftobiprole, is an advanced-generation cephalosporin that is approved in many European and non-European countries for the treatment of adults with hospital-acquired pneumonia (excluding ventilator-associated pneumonia) and community-acquired pneumonia and is currently being evaluated in a global phase 3 clinical trial of patients with *Staphylococcus aureus* bacteremia. This study investigated the in vitro activity of ceftobiprole and comparators against a total of 5466 gram-positive and -negative isolates from bloodstream infections (BSIs) that were collected in the United States during 2016 and 2017 as part of the SENTRY Antimicrobial Surveillance Program. Ceftobiprole was highly active (isolates were >99% susceptible) against *S. aureus* (including methicillin-resistant *S. aureus*), coagulase-negative staphylococci, *Enterococcus faecalis*, streptococci, and non–extended-spectrum β-lactamase (non-ESBL) phenotype *Enterobacteriaceae*. As expected, lower activities were observed against *Enterococcus faecium*, ESBL-phenotype *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. These results support further clinical evaluation of ceftobiprole for the treatment of BSIs caused by susceptible organisms.

1. Introduction

Bloodstream infections (BSIs) are associated with considerable morbidity, short-term mortality rates of 13–24% (Goto and Al-Hasan, 2013; Laupland and Church, 2014; Nielsen et al., 2014; Weiner et al., 2016), long-term excess mortality (Edmond et al., 1996; Goto and Al-Hasan, 2013; Leibovici, 2013; Martin et al., 1989), and increased healthcare costs (Goto and Al-Hasan, 2013; Vrijens et al., 2010). A recent review estimated that 575,000–677,000 episodes of BSI occur annually in North America (536,000–628,000 in the United States [USA] and 40,000–49,000 in Canada) and over 1.2 million episodes occur each year in Europe (Goto and Al-Hasan, 2013). These estimates are likely conservative given reports of increasing trends in the occurrence of BSIs in both hospital and community settings (Goto and Al-Hasan, 2013; Laupland and Church, 2014; Timbrook et al., 2017; Uslan et al., 2007; Weiner et al., 2016).

Early detection and appropriate antimicrobial therapy reduce mortality associated with BSIs (Timbrook et al., 2017); however, rates of BSI caused by antimicrobial-resistant bacteria are increasing worldwide, further complicating patient management (Ammerlaan et al., 2013; Friedman et al., 2016). The problem of antimicrobial resistance requires a coordinated global response to safeguard human health against current and emerging threats (Queenan et al., 2016). Antimicrobial resistance surveillance is an essential component of this response because it helps to define the scope of the resistance problem, guide the appropriate use of antibiotics, and inform the new drug development process (Perez and Villegas, 2015; Queenan et al., 2016). Adopting molecular rapid diagnostic testing to detect and identify resistant pathogens and to enhance our understanding of the resistance mechanisms whereby the bacteria avoid the effects of antibiotics is also important. This information would be used to modify older agents or to develop new agents such that potent activity is maintained against key target pathogens (Brogan and Mossialos, 2013; Spellberg et al., 2008; Timbrook et al., 2017).

Ceftobiprole is an attractive expanded-spectrum parenteral cephalosporin that exhibits potent antimicrobial activity against many gram-positive and gram-negative pathogens, including methicillin (oxacillin)-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci (MRCoNS), *Enterobacteriaceae*, and *Pseudomonas aeruginosa* (Davies et al., 2007; Farrell et al., 2014; Fritsche et al., 2008; Walkty et al., 2011; Zhanel et al., 2008).

Currently, ceftobiprole is approved for the treatment of adults with hospital-acquired pneumonia (excluding ventilator-associated

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pneumonia [VAP]) and community-acquired bacterial pneumonia in Europe (17 countries), Argentina, Canada, Jordan, Peru, and Saudi Arabia (Awad et al., 2014; Liapikou et al., 2015; Syed, 2014; Zevtera, 2013).

Ceftobiprole was designated a qualified infectious disease product by the United States Food and Drug Administration (US FDA) and is being evaluated in separate phase 3 clinical trials for 1) the treatment of adult patients with acute bacterial skin and skin structure infections and 2) the treatment of adult patients with *S. aureus* bacteremia (including infective endocarditis). The purpose of these clinical trials is to expand ceftobiprole's potential indications and support its registration in the United States. This study concerns ceftobiprole's in vitro antimicrobial activity against BSI pathogens.

Since 1997, the SENTRY Antimicrobial Surveillance Program has collected and tested BSI pathogens from around the world (Biedenbach et al., 2004). Data on pathogen prevalence and antimicrobial resistance rates from this program have been reported, and these results can be compared to the results from other studies that monitor BSI pathogens (Ammerlaan et al., 2013; Biedenbach et al., 2004; Laupland and Church, 2014). In general, for example, *S. aureus* and *Escherichia coli* are the most commonly isolated species associated with BSIs (Ammerlaan et al., 2013; Biedenbach et al., 2004; Laupland and Church, 2014; Weiner et al., 2016). The present study updates the prevalence and trends of gram-positive and gram-negative organisms causing BSIs in the United States and examines the activity of ceftobiprole and comparator antimicrobial agents tested against 5466 BSI isolates collected from US medical centers during 2016 and 2017 as part of the SENTRY Antimicrobial Surveillance Program.

2. Materials and methods

2.1. Bacterial strains tested

A total of 5466 nonduplicate, consecutive clinical isolates from the ceftobiprole SENTRY Antimicrobial Surveillance Program in the United States were submitted from 32 medical centers across all 9 US Census Bureau divisions during 2016–2017 (Table 1). All organisms were isolated from documented BSIs, and only 1 isolate per patient infection episode was included in the surveillance collection. Species identification was performed at the participating medical centers and confirmed at the monitoring laboratory (JMI Laboratories, North Liberty, IA) using the Vitek 2 System (bioMérieux, Hazelwood, MO) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker, Billerica, MA) when necessary.

2.2. Susceptibility testing methods

All isolates were tested by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (CLSI, 2018a) in cation-adjusted Mueller–Hinton broth (with 5% lysed horse blood added for testing fastidious streptococci and Haemophilus test medium used for testing *Haemophilus influenzae*). Minimal inhibitory concentration (MIC) values were generated using frozen-form panels manufactured by JMI Laboratories. All tested agents were provided by their respective manufacturers as reagent-grade formulations of the active compounds. Susceptibility interpretations were based upon the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints recently established for ceftobiprole: *S. aureus* (susceptible ≤2 mg/L, resistant >2 mg/L), *Streptococcus pneumoniae* (susceptible ≤0.5 mg/L, resistant >0.5 mg/L), *Enterobacteriaceae* (susceptible ≤0.25 mg/L, resistant >0.25 mg/L), and non–species-specific (susceptible ≤4 mg/L, resistant >4 mg/L) (EUCAST, 2018; Zevtera, 2013). In cases where ceftobiprole susceptibilities are reported based on the non–species-specific breakpoint (e.g., *Enterococcus faecalis*), we note that further studies are required to evaluate the full clinical utility of ceftobiprole against such organisms. MIC results for tested comparators obtained against clinical isolates were interpreted using CLSI M100 breakpoint criteria, where published (CLSI, 2018b). US FDA product package insert criteria (tigecycline) or EUCAST criteria (colistin and tigecycline) were used as alternative breakpoint sources, as necessary (EUCAST, 2018). *S. aureus* strains were classified as MRSA according to their oxacillin MIC values (resistant MIC, ≥4 mg/L). The CLSI screening criterion for potential extended-spectrum β-lactamase (ESBL) production (ceftazidime, ceftriaxone, and/or aztreonam MIC value ≥2 mg/L) was used to categorize *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* isolates as exhibiting an ESBL phenotype (CLSI, 2018b). Multidrug-resistant (MDR; nonsusceptible to at least 3 antimicrobial classes) *P. aeruginosa* isolates were classified according to recently recommended guidelines (Magiorakos et al., 2012) using the following antimicrobial class representatives: cephalosporins (ceftazidime and cefepime), penicillins plus β-lactamase inhibitors (piperacillin-tazobactam), fluoroquinolones (levofloxacin), aminoglycosides (amikacin and gentamicin), carbapenems (imipenem), and polymyxins (colistin). Results are reported as MIC distributions (ceftobiprole only), $MIC_{50/90}$ values (MIC values encompassing 50% and 90% of isolates tested, respectively), and percent of susceptible and resistant isolates according to CLSI (as well as FDA and EUCAST, when necessary) interpretive criteria.

Concurrently tested American Type Culture Collection (ATCC) quality control (QC) strains included *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and ATCC 35218, *P. aeruginosa* ATCC 27853, *S. pneumoniae* ATCC 49619, and *H. influenzae* ATCC 49247. All QC results were within published limits (Anderegg et al., 2004; CLSI, 2018b).

Table 1
Top 10 bloodstream infection pathogen species/groups from the US SENTRY Program (2016–2017).

Organism/organism group	Number (% of all isolates)
Staphylococcus aureus	1371 (25.1)
Escherichia coli	1102 (20.2)
Klebsiella pneumoniae	527 (9.6)
Enterococcus spp.	481 (8.8)
Coagulase-negative staphylococci	374 (6.8)
Pseudomonas aeruginosa	258 (4.7)
β-hemolytic streptococci	254 (4.6)
Enterobacter spp.	225 (4.1)
Viridans group streptococci	146 (2.7)
Serratia marcescens	127 (2.3)

Additional isolates from less prevalent species or groups were also tested (data not shown).

3. Results

3.1. Rank order of BSI pathogens and distribution of resistant phenotypes by census division

Overall, the top 10 pathogen species/groups encompassed 89.0% (4865/5466) of all BSI isolates (Table 1). The most common 3 species were *S. aureus* (25.1%), *E. coli* (20.2%), and *K. pneumoniae* (9.6%) (Table 1). Among the remaining 7 pathogen species/groups listed, 4 were gram-positive cocci, 77.5% of which were classified as community-acquired (where data were available; not shown).

The overall frequencies of key resistant phenotypes per species among the 2016–2017 BSI isolates were: MRSA (40.7%), vancomycin-resistant (MIC, >32 mg/L) *Enterococcus faecium* (VR-EFM) isolates (65.1%), ESBL-phenotype *E. coli* isolates (18.1%), ESBL-phenotype *K. pneumoniae* isolates (15.4%), and MDR *P. aeruginosa* isolates (13.6%) (Table 2). The prevalence of BSIs caused by these resistant phenotypes varied according to the respective US census division (Table 2). At least 50% of BSI *S. aureus* isolates from the South Atlantic (division 5; 50.0%) and East South Central (division 6; 66.1%) divisions were MRSA versus a low of 28.1% MRSA in the Pacific division (division 9). Likewise, 80% or greater of *E. faecium* BSI isolates from divisions 5 and 6 exhibited

Table 2
Select resistant phenotypes of bloodstream pathogens collected from the United States stratified by census division (% resistant phenotype in each census division).

	US census divisions (% of isolates within tested species)[a,b]									
Organism/organism group	1	2	3	4	5	6	7	8	9	All
MRSA	33.8	41.3	37.6	31.3	50.0	**66.1**	41.5	33.3	28.1	40.7
Vancomycin-resistant *E. faecium*	70.0	59.2	73.7	30.0	**88.2**	80.0	62.5	73.3	46.2	65.1
ESBL-phenotype *E. coli*	16.1	22.0	8.5	9.2	9.7	15.0	**27.5**	23.6	27.4	18.1
ESBL-phenotype *K. pneumoniae*	12.2	**22.9**	6.9	13.2	18.9	0.0	19.6	14.8	13.3	15.4
MDR *P. aeruginosa*	**28.6**	6.7	3.6	4.5	3.6	**28.6**	19.7	0.0	19.2	13.6

MRSA = methicillin-resistant *S. aureus*; ESBL = extended-spectrum β-lactamase; MDR = multidrug-resistant.
[a] US census divisions: 1, New England; 2, Middle Atlantic; 3, East North Central; 4, West North Central; 5, South Atlantic; 6, East South Central; 7, West South Central; 8, Mountain; 9, Pacific.
[b] Highest value in bold.

a vancomycin-resistant (VRE) phenotype (88.2% and 80.0%, respectively), whereas less than 50% of *E. faecium* isolates from the West North Central division (division 4) and division 9 expressed this phenotype (30.0% and 46.2%, respectively) (Table 2). BSI *E. coli* isolates with an ESBL phenotype were frequently observed (>20%) in the following census divisions: Middle Atlantic (division 2; 22.0%), West South Central (division 7; 27.5%), Mountain (division 8; 23.6%), and division 9 (27.4%) but comprised less than 10% in the East North Central (division 3; 8.5%), division 4 (9.2%), and division 5 (9.7%) (Table 2). Although the ESBL-phenotype frequency among BSI *K. pneumoniae* isolates was quite low in divisions 3 (6.9%) and 6 (0.0% [0/32 isolates]), high rates of ESBL-phenotype *K. pneumoniae* isolates were detected in divisions 2 (22.9%), 5 (18.9%), and 7 (19.6%). The MDR phenotype was common among BSI *P. aeruginosa* isolates from New England (division 1; 28.6%) and divisions 6 (28.6%), 7 (19.7%), and 9 (19.2%) but accounted for less than 10% (range 0.0–6.7%) of isolates from the other divisions (Table 2).

3.2. Activity of ceftobiprole against US BSI pathogens from 2016 to 2017

MIC distributions for ceftobiprole against each tested species or organism group are shown in Table 3. Ceftobiprole was very potent when tested against all 1371 *S. aureus* isolates ($MIC_{50/90}$, 0.5/2 mg/L; 99.7% susceptible), methicillin-susceptible *S. aureus* (MSSA; $MIC_{50/90}$, 0.5/0.5 mg/L; 100.0% susceptible), and MRSA ($MIC_{50/90}$, 1/2 mg/L; 99.3% susceptible) (Table 3).

The 374 CoNS isolates were 100% susceptible to ceftobiprole at the EUCAST non–species-related susceptibility breakpoint of ≤4 mg/L ($MIC_{50/90}$, 0.5/1 mg/L), and this activity was largely unaffected by oxacillin resistance ($MIC_{50/90}$, 1/1 mg/L against MRCoNS).

Ceftobiprole demonstrated potent activity against 299 *E. faecalis* isolates ($MIC_{50/90}$, 0.5/2 mg/L; 99.7% inhibited at the EUCAST non–species-related susceptibility breakpoint of ≤4 mg/L) but was largely inactive against *E. faecium* isolates ($MIC_{50/90}$, >4/>4 mg/L; 19.8% inhibited at an MIC value of ≤4 mg/L) (Table 3).

Ceftobiprole was very potent against the tested groups of streptococci: the $MIC_{50/90}$ values against 116 *S. pneumoniae* isolates were 0.015/0.12 mg/L (99.1% susceptible), and 96.6% of viridans group streptococci (VGS; $MIC_{50/90}$, 0.03/0.25 mg/L) and all (100.0%) β-hemolytic streptococci (BHS; $MIC_{50/90}$, 0.015/0.03 mg/L) were inhibited by ≤0.5 mg/L of ceftobiprole (Table 3).

The activity of ceftobiprole against the *Enterobacteriaceae* (2253 isolates) exhibited a bimodal distribution, with 84.9% of isolates being inhibited at an MIC value of ≤0.25 mg/L (the EUCAST susceptibility breakpoint) and 12.0% having MIC values of ≥8 mg/L (Table 3). Against *E. coli,* the ceftobiprole $MIC_{50/90}$ values were 0.03/>16 mg/L (83.9% susceptible). Ceftobiprole exhibited excellent activity against non–ESBL-phenotype *E. coli* (99.6% susceptible), but lower activity was observed against isolates with an ESBL phenotype (13.5% susceptible) (Table 3).

Similar to *E. coli*, bimodal ceftobiprole activity was observed against *K. pneumoniae* (Table 3; $MIC_{50/90}$ values of 0.03/>16 mg/L; 86.0% susceptible). Ceftobiprole was highly active against non-ESBL *K. pneumoniae* (446 isolates, 100.0% susceptible), but ESBL-phenotype isolates (81 isolates, 15.4%) displayed higher resistance rates for ceftobiprole (91.4% resistant) (Table 3).

Compared to *K. pneumoniae,* ceftobiprole susceptibility was lower among *K. oxytoca* isolates (80.5% susceptible) (Table 3). Notably, *K. oxytoca* isolates that exhibited either an ESBL phenotype or non-ESBL phenotype were less susceptible to ceftobiprole (0.0% and 89.9% susceptible, respectively) than the corresponding subsets of *K. pneumoniae* isolates (8.6% and 100.0% susceptible, respectively) (Table 3).

Ceftobiprole activity against isolates of other *Enterobacteriaceae* species, including *Enterobacter* spp. (80.0% susceptible), *Citrobacter* spp. (85.3% susceptible), *P. mirabilis* (94.4% susceptible), indole-positive Proteeae (93.3% susceptible), and *Serratia* spp. (90.2% susceptible), was similar to the activity that was observed against *E. coli* and *K. pneumoniae* (Table 3).

The activity of ceftobiprole against 258 *P. aeruginosa* isolates ($MIC_{50/90}$, 2/16 mg/L; 76.7% susceptible at ≤4 mg/L [EUCAST non–species-related breakpoint]) was lower than that seen for the *Enterobacteriaceae* (Table 3). As expected, ceftobiprole exhibited little activity against *Acinetobacter* spp. (MIC_{90}, >16 mg/L) and *Stenotrophomonas maltophilia* (MIC_{50}, >16 mg/L) (Table 3).

Ceftobiprole was equally active against β-lactamase–negative ($MIC_{50/90}$, 0.06/0.12 mg/L) and β-lactamase–positive (MIC_{50}, 0.06 mg/L) *H. influenzae* isolates (Table 3). Ceftobiprole also exhibited potent activity against *Moraxella catarrhalis*, although only 2 isolates were tested (Table 3).

3.3. Activities of ceftobiprole and comparators against gram-positive cocci

Table 4 summarizes the activities of ceftobiprole and comparators against *S. aureus* isolates. Ceftobiprole and ceftaroline exhibited potent activity (MIC_{90}, 0.5 mg/L and 0.25 mg/L, respectively) against the MSSA isolate subset and were 16- to 32-fold more potent than ceftriaxone (MIC_{90}, 8 mg/L). All MSSA isolates were susceptible to ceftobiprole, ceftaroline, ceftriaxone, daptomycin, linezolid, tigecycline, and vancomycin at their respective breakpoints (Table 4).

The overall MRSA rate was 40.7% (Table 2). All tested β-lactams exhibited higher MIC values against the MRSA subset compared to the MSSA subset. The MIC_{90} values for ceftobiprole and ceftaroline were 2 mg/L and 1 mg/L, respectively (Table 4). Ceftobiprole was 2-fold less potent than ceftaroline, linezolid, or vancomycin and 4-fold less potent than daptomycin by MIC_{90} value. These MRSA isolates exhibited high levels of resistance to levofloxacin (72.8% resistant). Greater than 92.3% of MRSA isolates were susceptible to ceftobiprole, ceftaroline, daptomycin, gentamicin, linezolid, tetracycline, tigecycline, trimethoprim-sulfamethoxazole, and vancomycin at their respective breakpoints (Table 4).

Susceptibility results for all CoNS, including MR isolates, are presented in Table 4. A total of 64.7% of the CoNS isolates tested were resistant to oxacillin (Table 4). Against the full CoNS isolate set, ceftaroline (MIC_{90}, 0.5 mg/L) was 2-fold more potent than ceftobiprole (MIC_{90}, 1 mg/L), and ceftriaxone activity was poor (MIC_{90}, >8 mg/L) (Table 4). At the EUCAST non–species-related breakpoint of ≤4 mg/L, all CoNS isolates were susceptible to ceftobiprole (Table 3). The antibiogram results

Table 3
Antimicrobial activity of ceftobiprole tested against the main organisms and organism groups.

Organism/organism group (number of isolates)	Number and cumulative % of isolates inhibited at MIC (mg/L) of:																MIC_{50}	MIC_{90}
	≤0.001	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>[a]		
Staphylococcus aureus (1371)							0	4	241	605	358	159	4				0.5	2
							0.0	0.3	17.9	62.0	88.1	99.7	100.0					
MSSA (813)							0	4	240	568	1						0.5	0.5
							0.0	0.5	30.0	99.9	100.0							
MRSA (558)								0	1	37	357	159	4				1	2
								0.0	0.2	6.8	70.8	99.3	100.0					
Coagulase-negative staphylococci						13	12	50	49	90	143	11	6				0.5	1
(374)						3.5	6.7	20.1	33.2	57.2	95.5	98.4	100.0					
MSCoNS (132)						13	12	49	40	16	2						0.12	0.5
						9.8	18.9	56.1	86.4	98.5	100.0							
MRCoNS (242)							0	1	9	74	141	11	6				1	1
							0.0	0.4	4.1	34.7	93.0	97.5	100.0					
Enterococcus spp. (481)						5	4	18	46	131	65	59	14			139	1	>4
						1.0	1.9	5.6	15.2	42.4	55.9	68.2	71.1			100.0		
Enterococcus faecalis (299)						3	4	17	45	128	53	39	9			1	0.5	2
						1.0	2.3	8.0	23.1	65.9	83.6	96.7	99.7			100.0		
Vancomycin-susceptible						3	4	17	42	126	50	35	8			1	0.5	2
(≤4 mg/L) (286)						1.0	2.4	8.4	23.1	67.1	84.6	96.9	99.7			100.0		
Vancomycin-nonsusceptible								0	3	2	3	4	1				1	2
(>4 mg/L) (13)								0.0	23.1	38.5	61.5	92.3	100.0					
Enterococcus faecium (172)									0	3	8	18	5			138	>4	>4
									0.0	1.7	6.4	16.9	19.8			100.0		
Vancomycin-susceptible									0	2	8	18	4			28	4	>4
(≤4 mg/L) (60)									0.0	3.3	16.7	46.7	53.3			100.0		
Vancomycin-nonsusceptible									0	1	0	0	1			110	>4	>4
(>4 mg/L) (112)									0.0	0.9	0.9	0.9	1.8			100.0		
Streptococcus pneumoniae (116)	0	1	1	32	54	10	4	3	3	7	1						0.015	0.12
	0.0	0.9	1.7	29.3	75.9	84.5	87.9	90.5	93.1	99.1	100.0							
Penicillin-susceptible meningitis	0	1	1	31	50	4	3										0.015	0.015
(≤0.06 mg/L) (90)	0.0	1.1	2.2	36.7	92.2	96.7	100.0											
Penicillin-resistant meningitis			0	1	4	6	1	3	3	7	1						0.12	0.5
(≥0.12 mg/L) (26)			0.0	3.8	19.2	42.3	46.2	57.7	69.2	96.2	100.0							
Penicillin-susceptible	0	1	1	32	54	10	4	3	3	6							0.015	0.12
non-meningitis (≤2 mg/L) (114)	0.0	0.9	1.8	29.8	77.2	86.0	89.5	92.1	94.7	100.0								
Penicillin-intermediate									0	1	1						0.5	
nonmeningitis (=4 mg/L) (2)									0.0	50.0	100.0							
Ceftriaxone-nonsusceptible										0	1							
nonmeningitis (≥2 mg/L) (1)										0.0	100.0							
Viridans group streptococci (146)		0	3	15	39	27	27	16	8	6	2	2				1	0.03	0.25
		0.0	2.1	12.3	39.0	57.5	76.0	87.0	92.5	96.6	97.9	99.3				100.0		
β-hemolytic streptococci (254)	0	1	5	73	77	97	1										0.015	0.03
	0.0	0.4	2.4	31.1	61.4	99.6	100.0											
Streptococcus pyogenes (80)	0	1	4	56	18	1											0.008	0.015
	0.0	1.2	6.2	76.2	98.8	100.0												
Streptococcus agalactiae (122)			0	2	24	95	1										0.03	0.03
			0.0	1.6	21.3	99.2	100.0											
Other (52)		0	1	15	35	1											0.015	0.015
		0.0	1.9	30.8	98.1	100.0												
Enterobacteriaceae (2253)				3	58	1248	451	107	46	23	20	14	12	10	6	255	0.03	>16
				0.1	2.7	58.1	78.1	82.9	84.9	85.9	86.8	87.4	88.0	88.4	88.7	100.0		
Escherichia coli (1102)				1	24	690	176	19	15	6	3	1	1	0	2	164	0.03	>16
				0.1	2.3	64.9	80.9	82.6	83.9	84.5	84.8	84.8	84.9	84.9	85.1	100.0		
ESBL phenotype (200)					0	8	5	6	8	3	2	1	1	0	2	164	>16	>16
					0.0	4.0	6.5	9.5	13.5	15.0	16.0	16.5	17.0	17.0	18.0	100.0		
Non-ESBL phenotype (902)				1	24	682	171	13	7	3	1						0.03	0.06
				0.1	2.8	78.4	97.3	98.8	99.6	99.9	100.0							
Klebsiella pneumoniae (527)				0	22	338	77	13	3	2	5	5	1	1	0	60	0.03	>16
				0.0	4.2	68.3	82.9	85.4	86.0	86.3	87.3	88.2	88.4	88.6	88.6	100.0		
ESBL phenotype (81)					0	4	0	2	1	2	5	5	1	1	0	60	>16	>16
					0.0	4.9	4.9	7.4	8.6	11.1	17.3	23.5	24.7	25.9	25.9	100.0		
Non-ESBL phenotype (446)				0	22	334	77	11	2								0.03	0.06
				0.0	4.9	79.8	97.1	99.6	100.0									
Klebsiella oxytoca (77)					0	8	11	24	19	5	2	1	0	1	0	6	0.12	2
					0.0	10.4	24.7	55.8	80.5	87.0	89.6	90.9	90.9	92.2	92.2	100.0		
ESBL phenotype (8)										0	1	0	0	1	0	6	>16	
										0.0	12.5	12.5	12.5	25.0	25.0	100.0		
Non-ESBL phenotype (69)					0	8	11	24	19	5	1	1					0.12	0.5
					0.0	11.6	27.5	62.3	89.9	97.1	98.6	100.0						
Enterobacter spp. (225)				0	3	94	65	13	5	3	3	5	9	8	3	14	0.06	8
				0.0	1.3	43.1	72.0	77.8	80.0	81.3	82.7	84.9	88.9	92.4	93.8	100.0		
Citrobacter spp. (34)					0	21	7	0	1	1	2	0	1	0	0	1	0.03	1
					0.0	61.8	82.4	82.4	85.3	88.2	94.1	94.1	97.1	97.1	97.1	100.0		
Proteus mirabilis (90)				0	7	63	15	0	0	0	0	0	0	0	0	5	0.03	0.06

(continued on next page)

Table 3 *(continued)*

Organism/organism group (number of isolates)	Number and cumulative % of isolates inhibited at MIC (mg/L) of:																MIC_{50}	MIC_{90}
	≤0.001	0.002	0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>[a]		
				0.0	7.8	77.8	94.4	94.4	94.4	94.4	94.4	94.4	94.4	94.4	94.4	100.0		
Indole-positive Proteeae (30)				2	2	18	5	1	0	0	0	0	0	0	0	2	0.03	0.06
				6.7	13.3	73.3	90.0	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	100.0		
Serratia spp. (133)					0	4	80	33	3	5	5	2	0	0	1		0.06	0.25
					0.0	3.0	63.2	88.0	90.2	94.0	97.7	99.2	99.2	99.2	100.0			
Pseudomonas aeruginosa (258)									0	4	55	101	38	24	30	6	2	16
									0.0	1.6	22.9	62.0	76.7	86.0	97.7	100.0		
Acinetobacter spp. (66)				2	4	6	5	6	5	14	8	2	1	2	0	11	0.5	>16
				3.0	9.1	18.2	25.8	34.8	42.4	63.6	75.8	78.8	80.3	83.3	83.3	100.0		
Stenotrophomonas maltophilia (59)															0	59	>16	>16
															0.0	100.0		
Haemophilus influenzae (31)					0	13	9	6	3								0.06	0.12
					0.0	41.9	71.0	90.3	100.0									
β-lactamase positive (7)					0	2	2	2	1								0.06	
					0.0	28.6	57.1	85.7	100.0									
β-lactamase negative (24)					0	11	7	4	2								0.06	0.12
					0.0	45.8	75.0	91.7	100.0									
Haemophilus parainfluenzae (1)					0	1												
					0.0	100.0												
Moraxella catarrhalis (2)						0	1	0	1								0.06	
						0.0	50.0	50.0	100.0									

MSSA = methicillin-susceptible *S. aureus*; MRSA = methicillin-resistant *S. aureus*; MSCoNS = methicillin-susceptible coagulase-negative staphylococci; MRCoNS = methicillin-resistant coagulase-negative staphylococci; ESBL = extended-spectrum β-lactamase.

[a] Greater than the highest concentration tested.

for comparators against CoNS isolates showed high resistance rates for all tested drugs except for daptomycin, linezolid, tigecycline, and vancomycin (96.0% to 100.0% susceptible).

All *E. faecalis* isolates were susceptible to ampicillin, daptomycin, linezolid, and tigecycline, and almost all isolates were susceptible to ceftobiprole (99.7%; using the EUCAST non–species-related breakpoint; see Materials and Methods) and vancomycin (95.7%) (Tables 3 and 4).

E. faecium isolate testing revealed 65.1% overall resistance to vancomycin (Table 4). Like ampicillin, ceftobiprole displayed limited activity against *E. faecium* isolates (MIC_{50}, >4 mg/L), regardless of vancomycin susceptibility patterns (Table 3). Daptomycin, linezolid, and tigecycline (98.8%, 98.8%, and 99.4% susceptible, respectively) provided the best coverage against *E. faecium* (Table 4).

Susceptibility testing results for *S. pneumoniae* isolates ($n = 116$; 1.7% penicillin-NS [MIC, ≥4 mg/L]) are shown in Tables 3 and 4. Ceftobiprole and ceftaroline (99.1% and 100.0% susceptible, respectively, by EUCAST criteria) were the most active β-lactam agents tested against *S. pneumoniae*. All isolates were susceptible to levofloxacin and linezolid. Resistance was higher for erythromycin (32.8%), tetracycline (12.9%), and trimethoprim-sulfamethoxazole (6.0%) (Table 4).

By MIC_{90} value, the most potent β-lactam agents tested against the VGS isolates were ceftaroline and ceftobiprole (MIC_{90}, 0.06 mg/L for ceftaroline and 0.25 mg/L for ceftobiprole), and 96.6% of VGS were susceptible to ceftobiprole at the *S. pneumoniae* susceptible breakpoint of 0.5 mg/L (Tables 3 and 4). Penicillin resistance was 3.4%, and levofloxacin resistance was 12.3%. Clindamycin and erythromycin resistance rates were 13.0% and 49.3%, respectively. Daptomycin and linezolid (both 100.0% susceptible) showed the greatest coverage against the VGS isolate group (Table 4).

Ceftobiprole was very potent (MIC_{90}, 0.03 mg/L) against BHS ($n = 254$), and all MIC values were ≤ 0.06 mg/L (Table 3). The BHS isolates were 100% susceptible to ceftaroline, ceftriaxone, daptomycin, linezolid, penicillin, and vancomycin. Levofloxacin resistance was 0.4%. Resistance to clindamycin and erythromycin was 17.7% and 34.8%, respectively (Table 4).

3.4. Activities of ceftobiprole and comparators against Enterobacteriaceae

The activities of ceftobiprole and comparison agents tested against *Enterobacteriaceae* isolates ($n = 2253$) are summarized in Table 5. Against all *Enterobacteriaceae*, 84.9% of isolates were susceptible to ceftobiprole (Tables 3 and 5). Susceptibility rates were 99.4% for meropenem, 96.9% for tigecycline, 95.9% for imipenem, 93.3% for piperacillin-tazobactam, 89.3% for gentamicin, 88.8% for cefepime, 87.4% for ceftazidime, 86.8% for aztreonam, and 83.7% for ceftriaxone. Ceftaroline susceptibility was 77.6% (Table 5).

Against all 1102 *E. coli* isolates, tigecycline (99.9% susceptible), meropenem (99.7% susceptible), imipenem (99.7% susceptible), colistin (99.6% susceptible), and piperacillin-tazobactam (95.4% susceptible) were the most active agents tested (Table 5). Like the expanded-spectrum cephalosporins, ceftobiprole activity against *E. coli* was bimodal (Tables 3 and 5) with $MIC_{50/90}$ values of 0.03/>16 mg/L (83.9% of isolates susceptible at the EUCAST breakpoint of ≤0.25 mg/L). Resistance rates against *E. coli* for ampicillin-sulbactam, levofloxacin, and trimethoprim-sulfamethoxazole were 33.8%, 30.1%, and 37.9%, respectively (Table 5). More than 11% (range 11.7% [ceftazidime] to 18.5% [ceftaroline]) of *E. coli* isolates were resistant to each of the remaining agents (Table 5).

Among 902 non–ESBL-phenotype *E. coli* (81.9% of all isolates), ceftobiprole ($MIC_{50/90}$, 0.03/0.06 mg/L), cefepime ($MIC_{50/90}$, ≤0.12/≤0.12 mg/L), ceftriaxone ($MIC_{50/90}$, ≤0.06/0.12 mg/L), imipenem ($MIC_{50/90}$, ≤0.12/≤0.12 mg/L), and meropenem ($MIC_{50/90}$, ≤0.015/0.03 mg/L) were the most potent (Table 5). Resistance was high for ampicillin-sulbactam (24.5%), levofloxacin (19.5%), and trimethoprim-sulfamethoxazole (32.9%) against non-ESBL *E. coli* (Tables 3 and 5).

Tables 3 and 5 display the activity of ceftobiprole against *K. pneumoniae* isolates ($n = 527$). Colistin (99.2% susceptible), the carbapenems (99.1–99.4% susceptible), and tigecycline (97.9% susceptible) provided the best overall susceptibility rates.

Non–ESBL-phenotype *K. pneumoniae* isolates (84.6%, 446 isolates) were susceptible to most antimicrobial agents tested (range 92.8% [trimethoprim-sulfamethoxazole] to 100.0% [aztreonam, cefepime, ceftazidime, ceftriaxone, imipenem, and meropenem]) (Table 5). Susceptibility to ampicillin-sulbactam was lower at 85.4%. Ceftobiprole was very active against the non–ESBL-phenotype subset of *K. pneumoniae* (100.0% susceptible) (Tables 3 and 5).

3.5. Activities of ceftobiprole and comparators against nonfermentative gram-negative bacilli

Colistin (MIC_{90}, 1 mg/L; 100.0% susceptible) was the most potent agent tested against *P. aeruginosa* isolates (Table 5). Ceftobiprole inhibited 76.7% of *P. aeruginosa* at ≤4 mg/L (Table 3), while amikacin,

Table 4
Activity of ceftobiprole and comparator agents when tested against selected gram-positive bacterial species from bloodstream infection isolates collected in the United States (2016–2017).

Organism/organism group (number of isolates) Antimicrobial agent	Antimicrobial activity (mg/L)			CLSI[a]			EUCAST[a]		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%I	%R
Methicillin-resistant *Staphylococcus aureus* (558)									
Ceftobiprole	1	2	0.25 to 4				99.3		0.7
Ceftaroline	0.5	1	0.12 to 2	94.4	5.6	0.0	94.4	5.6	0.0[b]
Ceftriaxone	>8	>8	8 to >8	0.0		100.0			
Clindamycin	≤0.25	>2	≤0.25 to >2	69.0	0.4	30.6	69.0	0.0	31.0
Daptomycin	0.25	0.5	≤0.12 to 2	99.8			99.8		0.2
Erythromycin	>8	>8	≤0.06 to >8	11.3	4.5	84.2	11.8	1.4	86.7
Gentamicin	≤1	≤1	≤1 to >8	95.7	0.2	4.1	95.7		4.3
Levofloxacin	4	>4	0.12 to >4	25.4	1.8	72.8	25.4		74.6
Linezolid	1	1	0.25 to 4	100.0		0.0	100.0		0.0
Tetracycline	≤0.5	1	≤0.5 to >8	95.5	0.9	3.6	92.3	2.7	5.0
Tigecycline	0.06	0.12	≤0.015 to 0.5	100.0[c]			100.0		0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >4	96.1		3.9	96.1	0.4	3.6
Vancomycin	1	1	0.25 to 2	100.0	0.0	0.0	100.0		0.0
Methicillin-susceptible *Staphylococcus aureus* (813)									
Ceftobiprole	0.5	0.5	0.12 to 1				100.0		0.0
Ceftaroline	0.25	0.25	≤0.06 to 0.5	100.0	0.0	0.0	100.0	0.0	0.0[b]
Ceftriaxone	4	8	1 to 8	100.0		0.0			
Clindamycin	≤0.25	≤0.25	≤0.25 to >2	96.4	0.2	3.3	96.2	0.2	3.6
Daptomycin	0.25	0.5	≤0.12 to 1	100.0			100.0		0.0
Erythromycin	0.25	>8	≤0.06 to >8	66.8	8.2	25.0	68.3	3.4	28.3
Gentamicin	≤1	≤1	≤1 to >8	99.0	0.2	0.7	98.6		1.4
Levofloxacin	0.25	4	≤0.03 to >4	87.1	0.2	12.7	87.1		12.9
Linezolid	1	2	0.25 to 4	100.0		0.0	100.0		0.0
Tetracycline	≤0.5	≤0.5	≤0.5 to >8	96.8	0.5	2.7	95.3	0.4	4.3
Tigecycline	0.06	0.12	≤0.015 to 0.25	100.0[c]			100.0		0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5 to >4	98.8		1.2	98.8	0.2	1.0
Vancomycin	1	1	0.25 to 2	100.0	0.0	0.0	100.0		0.0
Coagulase-negative staphylococci (374)									
Ceftobiprole	0.5	1	≤0.03 to 4				100.0[d]		0.0
Ceftaroline	0.25	0.5	≤0.06 to 2						
Ceftriaxone	8	>8	≤0.25 to >8	35.3		64.7			
Clindamycin	≤0.25	>2	≤0.25 to >2	67.6	1.6	30.7	67.4	0.3	32.4
Daptomycin	0.25	0.5	≤0.12 to 1	100.0			100.0		0.0
Erythromycin	>8	>8	≤0.06 to >8	35.0	5.6	59.4	36.6	2.7	60.7
Gentamicin	≤1	>8	≤1 to >8	69.8	2.7	27.5	67.6		32.4
Levofloxacin	0.25	>4	0.06 to >4	53.7	1.1	45.2	53.7		46.3
Linezolid	0.5	1	≤0.12 to >8	96.0		4.0	96.0		4.0
Oxacillin	>2	>2	≤0.25 to >2	35.3		64.7	35.6		64.4
Tetracycline	≤0.5	>8	≤0.5 to >8	84.5	0.0	15.5	74.8	9.4	15.8
Tigecycline	0.12	0.25	0.03 to 0.5				100.0		0.0
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5 to >4	64.4		35.6	64.4	20.9	14.7
Vancomycin	1	2	0.25 to 4	100.0	0.0	0.0	100.0		0.0
β hemolytic streptococci (254)									
Ceftobiprole	0.015	0.03	0.002 to 0.06				100.0[d]		0.0
Ceftaroline	≤0.008	0.015	≤0.008 to 0.03	100.0			100.0		0.0
Ceftriaxone	0.03	0.06	≤0.015 to 0.12	100.0			100.0		0.0
Clindamycin	≤0.25	>2	≤0.25 to >2	80.7	1.6	17.7	82.3		17.7
Daptomycin	0.12	0.25	≤0.06 to 1	100.0			100.0		0.0
Erythromycin	0.06	>16	≤0.015 to >16	64.4	0.8	34.8	64.4	0.8	34.8
Levofloxacin	0.5	1	0.25 to >4	99.2	0.4	0.4	99.2		0.8
Linezolid	1	2	0.5 to 2	100.0			100.0	0.0	0.0
Penicillin	0.015	0.06	≤0.008 to 0.12	100.0			100.0		0.0
Tetracycline	>4	>4	≤0.25 to >4	47.2	2.4	50.4	46.1	1.2	52.8
Vancomycin	0.5	0.5	0.25 to 0.5	100.0			100.0		0.0
***Enterococcus faecalis* (299)**									
Ceftobiprole	0.5	2	≤0.03 to >4				99.7[d]		0.3
Ampicillin	1	2	≤0.5 to 4	100.0		0.0	100.0	0.0	0.0
Ceftaroline	2	8	≤0.06 to >8						
Daptomycin	0.5	1	≤0.25 to 4	100.0					
Levofloxacin	1	>4	0.06 to >4	76.6	0.0	23.4	76.6		23.4[e]
Linezolid	1	2	0.25 to 2	100.0	0.0	0.0	100.0		0.0
Teicoplanin	≤0.5	≤0.5	≤0.5 to >16	95.7	0.0	4.3	95.7		4.3
Tigecycline	0.06	0.12	≤0.015 to 0.12	100.0[c]			100.0	0.0	0.0
Vancomycin	1	2	0.25 to >16	95.7	0.0	4.3	95.7		4.3
***Enterococcus faecium* (172)**									
Ceftobiprole	>4	>4	0.5 to >4				19.8[d]		80.2
Ampicillin	>16	>16	≤0.5 to >16	19.8		80.2	18.6	1.2	80.2
Ceftaroline	>8	>8	0.12 to >8						
Daptomycin	1	2	≤0.25 to >8	98.8					
Levofloxacin	>4	>4	0.25 to >4	12.2	7.0	80.8	19.2		80.8[e]
Linezolid	1	2	0.25 to 8	98.8	0.6	0.6	99.4		0.6

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Table 4 *(continued)*

Organism/organism group (number of isolates) Antimicrobial agent	Antimicrobial activity (mg/L)			CLSI[a]			EUCAST[a]		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%I	%R
Teicoplanin	>16	>16	≤0.5 to >16	36.6	4.7	58.7	34.9		65.1
Tigecycline	0.06	0.12	≤0.015 to 0.5				99.4	0.6	0.0
Vancomycin	>16	>16	0.25 to >16	34.9	0.0	65.1	34.9		65.1
Viridans group streptococci (146)									
Ceftobiprole	0.03	0.25	0.004 to >2						
Ceftaroline	0.015	0.06	≤0.008 to 0.5						
Ceftriaxone	0.12	0.5	≤0.015 to >2	94.5	2.1	3.4	90.4		9.6
Clindamycin	≤0.25	>2	≤0.25 to >2	86.3	0.7	13.0	87.0		13.0
Daptomycin	0.25	0.5	≤0.06 to 1	100.0					
Erythromycin	0.5	>16	≤0.015 to >16	48.6	2.1	49.3			
Levofloxacin	1	>4	0.25 to >4	87.7	0.0	12.3			
Linezolid	1	1	0.12 to 2	100.0					
Penicillin	0.06	1	≤0.008 to >4	69.9	26.7	3.4	79.5	17.1	3.4
Tetracycline	1	>4	≤0.25 to >4	61.6	4.1	34.2			
***Streptococcus pneumoniae* (116)**									
Ceftobiprole	0.015	0.12	0.002 to 1				99.1		0.9
Ceftaroline	≤0.008	0.06	≤0.008 to 0.25	100.0[f]			100.0		0.0
Ceftriaxone	0.03	0.5	≤0.015 to 2	93.1	6.0	0.9[f]	93.1	6.9	0.0
				99.1	0.9	0.0[g]			
Clindamycin	≤0.25	≤0.25	≤0.25 to >2	90.4	0.0	9.6	90.4		9.6
Erythromycin	0.03	>16	≤0.015 to >16	64.7	2.6	32.8	64.7	2.6	32.8
Levofloxacin	1	1	0.5 to 2	100.0	0.0	0.0	100.0		0.0
Linezolid	1	2	0.5 to 2	100.0			100.0	0.0	0.0
Penicillin	0.015	0.5	≤0.008 to 4	77.6	16.4	6.0[h]	77.6		22.4[f]
				77.6		22.4[i]	77.6	20.7	1.7[g]
				98.3	1.7	0.0[j]			
Tetracycline	≤0.25	>4	≤0.25 to >4	87.1	0.0	12.9	87.1	0.0	12.9
Trimethoprim-sulfamethoxazole	0.25	1	≤0.12 to >4	83.6	10.3	6.0	91.4	2.6	6.0
Vancomycin	0.25	0.5	0.12 to 0.5	100.0			100.0		0.0

S = susceptible; I = intermediate; R = resistant.
[a] Criteria as published by CLSI (CLSI, 2018b) and EUCAST (EUCAST, 2018).
[b] Using other than pneumonia breakpoints.
[c] FDA breakpoints published 2017-DEC-13 (applied to all *E. faecalis* but approved for vancomycin-susceptible isolates only).
[d] Using the pharmacokinetic/pharmacodynamic (non–species-related) breakpoint based on the standard dose (EUCAST, 2018).
[e] Uncomplicated urinary tract infection only.
[f] Using meningitis breakpoints.
[g] Using nonmeningitis breakpoints.
[h] Using oral breakpoints.
[i] Using parenteral meningitis breakpoints.
[j] Using parenteral nonmeningitis breakpoints.

cefepime, ceftazidime, gentamicin, and piperacillin-tazobactam inhibited 98.1%, 86.0%, 88.4%, 87.6%, and 85.7% of the isolates at their respective breakpoints (Table 5). For most isolates, ceftobiprole (MIC_{50}, 2 mg/L) was 8-fold more potent than ceftaroline (MIC_{50}, 16 mg/L). The greatest resistance among comparators was observed for levofloxacin (16.7%), aztreonam (15.9%), and imipenem (15.9%) (Table 5).

Ceftobiprole inhibited 80.3% of the *Acinetobacter* spp. isolates (Table 3) at ≤4 mg/L, and its activity was greater than that of ampicillin-sulbactam (77.3% susceptible), cefepime (74.2% susceptible), and ceftazidime (74.2% susceptible). Among other agents tested, colistin (MIC_{90}, 2 mg/L; 90.9% susceptible) and tigecycline (MIC_{90}, 2 mg/L) were the most active, while amikacin, gentamicin, imipenem, and levofloxacin exhibited susceptibility rates of 81.8 to 84.8% (Table 5).

4. Discussion

The SENTRY Antimicrobial Surveillance Program has collected and tested clinical isolates from various infection types since 1997 (Pfaller et al., 2001). Biedenbach et al. (2004) described the rank order and frequencies of the top 10 bacterial BSI pathogen species/groups that were isolated in North and Latin America and Europe during the first 6 years (1997–2002) of the SENTRY Program. Overall, the top 10 pathogen species/groups in North America for the years 1997–2002 accounted for 92.1% of all BSI isolates and were similar to the top 10 pathogen groups shown in Table 1 for the 2016/2017 SENTRY Program in the United States (89.0% of total; *S. pneumoniae* was 11th in prevalence in 2016–2017). Whereas *S. aureus* (26.0% of total), *E. coli* (17.7%), and CoNS (11.5%) were the 3 most frequently isolated pathogens in 1997–2002, CoNS (6.8% of total) fell to fifth in rank order in 2016–2017 following *S. aureus* (25.1%), *E. coli* (20.2%), *K. pneumoniae* (9.6%), and *Enterococcus* spp. (8.8%) (Table 1). The decrease in CoNS as a cause of BSIs was likely the result of greater diligence by microbiology laboratories in preventing and identifying skin contaminants coupled with greater attention to preventing catheter-associated BSIs and emerging antimicrobial-resistant *K. pneumoniae* and enterococci in recent years (Ammerlaan et al., 2013; Biedenbach et al., 2004; Weiner et al., 2016). Among key resistant phenotypes causing BSIs in North America during 1997–2002, the frequency of MRSA (32.4% overall, range 22.4% [1997] to 39.1% [2002]) and VRE (15.1% overall, range 13.0% [1997] to 17.7% [2002]) increased over time, while ESBL *Klebsiella* spp. (5.8% overall, range 7.9% [1998] to 4.9% [2002]) and MDR *P. aeruginosa* (2.2% overall, range 1.6% [1998] to 3.0% [2002]) remained relatively stable (Biedenbach et al., 2004). In comparison, the frequency of each of these resistant phenotypes was higher for US BSI isolates during 2016–2017 (MRSA, 40.7%; VRE, 26.0% overall [4.3% and 65.1% for *E. faecalis* and *E. faecium*, respectively]; ESBL *K. pneumoniae*, 15.4%; and MDR *P. aeruginosa*, 13.6%), underscoring the increasing problem with antimicrobial-resistant bacteria in the United States (Table 2).

The SENTRY Program results from 2016 to 2017 that are reported here confirm that ceftobiprole is highly active against many gram-positive and gram-negative pathogens that cause serious infections. Importantly, ceftobiprole exhibited potent antimicrobial activity against MRSA, MRCoNS, and penicillin-resistant *S. pneumoniae* and exhibited

Table 5
Activity of ceftobiprole and comparator agents when tested against selected gram-negative bacterial species from bloodstream infection isolates collected in the United States (2016 to 2017).

Organism/organism group (number of isolates) Antimicrobial agent	Antimicrobial activity (mg/L)			CLSI[a]			EUCAST[a]		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%I	%R
***Enterobacteriaceae* (2253)**									
Ceftobiprole	0.03	>16	≤0.008 to >16				84.9		15.1
Ampicillin-sulbactam	16	>32	≤0.5 to >32	48.6	16.9	34.5	48.6		51.4
Aztreonam	0.12	16	≤0.03 to >16	86.8	1.6	11.7	84.3	2.5	13.2
Cefepime	≤0.12	8	≤0.12 to >16	88.8	1.8	9.4[b]	87.3	2.4	10.3
Ceftaroline	0.12	>32	≤0.015 to >32	77.6	4.2	18.2	77.6		22.4
Ceftazidime	0.25	16	0.03 to >32	87.4	1.3	11.3	84.7	2.7	12.6
Ceftriaxone	≤0.06	>8	≤0.06 to >8	83.7	0.8	15.5	83.7	0.8	15.5
Colistin	0.12	>8	≤0.06 to >8				86.3		13.7
Gentamicin	0.5	>8	≤0.12 to >8	89.3	0.6	10.1	88.9	0.4	10.7
Imipenem	≤0.12	0.5	≤0.12 to >8	95.9	3.2	1.0	99.0	0.8	0.1
Levofloxacin	0.06	>4	≤0.03 to >4	80.4	1.7	17.9	77.2	2.2	20.6
Meropenem	0.03	0.06	≤0.015 to 16	99.4	0.1	0.5	99.5	0.4	0.1
Piperacillin-tazobactam	2	8	≤0.5 to >64	93.3	3.3	3.4	90.1	3.2	6.7
Tigecycline	0.25	1	≤0.06 to 8	96.9	2.8	0.2[c]	94.0	2.9	3.1
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5 to >4	74.3		25.7	74.3	0.6	25.1
***Escherichia coli* (1102)**									
Ceftobiprole	0.03	>16	≤0.008 to >16				83.9		16.1
Ampicillin-sulbactam	16	>32	≤0.5 to >32	44.9	21.3	33.8	44.9		55.1
Aztreonam	0.12	>16	≤0.03 to >16	84.9	2.4	12.7	82.2	2.7	15.1
Cefepime	≤0.12	>16	≤0.12 to >16	85.5	1.7	12.8[b]	84.5	1.7	13.8
Ceftaroline	0.12	>32	≤0.015 to >32	79.8	1.7	18.5	79.8		20.2
Ceftazidime	0.25	16	0.03 to >32	86.5	1.8	11.7	83.5	3.0	13.5
Ceftriaxone	≤0.06	>8	≤0.06 to >8	82.9	0.2	16.9	82.9	0.2	16.9
Colistin	0.12	0.25	≤0.06 to >8	99.6[d]			99.6		0.4
Gentamicin	1	>8	≤0.12 to >8	85.1	0.1	14.8	84.7	0.5	14.9
Imipenem	≤0.12	≤0.12	≤0.12 to 4	99.7	0.1	0.2	99.8	0.2	0.0
Levofloxacin	0.06	>4	≤0.03 to >4	67.8	2.1	30.1	66.2	1.0	32.8
Meropenem	≤0.015	0.03	≤0.015 to 8	99.7	0.0	0.3	99.7	0.3	0.0
Piperacillin-tazobactam	2	8	≤0.5 to >64	95.4	2.3	2.4	93.0	2.4	4.6
Tigecycline	0.12	0.25	≤0.06 to 4	99.9	0.1	0.0[c]	99.8	0.1	0.1
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5 to >4	62.1		37.9	62.1	0.8	37.1
Non–ESBL-phenotype *Escherichia coli* (902)									
Ceftobiprole	0.03	0.06	≤0.008 to 1				99.6		0.4
Ampicillin-sulbactam	8	>32	≤0.5 to >32	52.3	23.2	24.5	52.3		47.7
Aztreonam	0.06	0.25	≤0.03 to 1	100.0	0.0	0.0	100.0	0.0	0.0
Cefepime	≤0.12	≤0.12	≤0.12 to 2	100.0	0.0	0.0[b]	99.8	0.2	0.0
Ceftaroline	0.06	0.25	≤0.015 to 16	96.2	2.0	1.8	96.2		3.8
Ceftazidime	0.12	0.5	0.03 to 1	100.0	0.0	0.0	100.0	0.0	0.0
Ceftriaxone	≤0.06	0.12	≤0.06 to 1	100.0	0.0	0.0	100.0	0.0	0.0
Colistin	0.12	0.25	≤0.06 to >8	99.8[d]			99.8		0.2
Gentamicin	0.5	4	≤0.12 to >8	90.2	0.0	9.8	89.8	0.4	9.8
Imipenem	≤0.12	≤0.12	≤0.12 to 0.5	100.0	0.0	0.0	100.0	0.0	0.0
Levofloxacin	≤0.03	>4	≤0.03 to >4	78.4	2.1	19.5	77.1	0.9	22.1
Meropenem	≤0.015	0.03	≤0.015 to 0.12	100.0	0.0	0.0	100.0	0.0	0.0
Piperacillin-tazobactam	2	4	≤0.5 to >64	98.2	0.8	1.0	97.5	0.8	1.8
Tigecycline	0.12	0.25	≤0.06 to 1	100.0	0.0	0.0[c]	100.0	0.0	0.0
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5 to >4	67.1		32.9	67.1	0.4	32.4
***Klebsiella pneumoniae* (527)**									
Ceftobiprole	0.03	>16	0.015 to >16				86.0		14.0
Ampicillin-sulbactam	8	>32	1 to >32	73.1	7.2	19.7	73.1		26.9
Aztreonam	0.06	16	≤0.03 to >16	88.4	0.6	11.0	86.3	2.1	11.6
Cefepime	≤0.12	8	≤0.12 to >16	88.8	1.3	9.9[b]	88.2	1.1	10.6
Ceftaroline	0.12	>32	≤0.015 to >32	82.4	2.5	15.2	82.4		17.6
Ceftazidime	0.25	16	0.03 to >32	87.7	1.3	11.0	85.4	2.3	12.3
Ceftriaxone	≤0.06	>8	≤0.06 to >8	86.5	0.6	12.9	86.5	0.6	12.9
Colistin	0.12	0.25	≤0.06 to 8	99.2[d]			99.2		0.8
Gentamicin	0.25	1	≤0.12 to >8	91.1	0.9	8.0	91.1	0.0	8.9
Imipenem	≤0.12	0.25	≤0.12 to >8	99.4	0.2	0.4	99.6	0.2	0.2
Levofloxacin	0.06	1	≤0.03 to >4	92.0	1.9	6.1	87.3	4.0	8.7
Meropenem	0.03	0.03	≤0.015 to 16	99.1	0.2	0.8	99.2	0.4	0.4
Piperacillin-tazobactam	4	16	≤0.5 to >64	93.2	2.8	4.0	87.1	6.1	6.8
Tigecycline	0.5	1	≤0.06 to 4	97.9	2.1	0.0[c]	95.4	2.5	2.1
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5 to >4	82.7		17.3	82.7	0.2	17.1
Non–ESBL-phenotype *Klebsiella pneumoniae* (446)									
Ceftobiprole	0.03	0.06	0.015 to 0.25				100.0		0.0
Ampicillin-sulbactam	8	16	1 to >32	85.4	7.0	7.6	85.4		14.6
Aztreonam	0.06	0.12	≤0.03 to 0.5	100.0	0.0	0.0	100.0	0.0	0.0
Cefepime	≤0.12	≤0.12	≤0.12 to 1	100.0	0.0	0.0[b]	100.0	0.0	0.0
Ceftaroline	0.12	0.25	≤0.015 to 2	97.3	2.5	0.2	97.3		2.7
Ceftazidime	0.12	0.5	0.03 to 1	100.0	0.0	0.0	100.0	0.0	0.0
Ceftriaxone	≤0.06	0.12	≤0.06 to 1	100.0	0.0	0.0	100.0	0.0	0.0

(continued on next page)

Table 5 *(continued)*

Organism/organism group (number of isolates) Antimicrobial agent	Antimicrobial activity (mg/L)			CLSI[a]			EUCAST[a]		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%I	%R
Colistin	0.12	0.25	≤0.06 to 8	99.5[d]			99.5		0.5
Gentamicin	0.25	0.5	≤0.12 to >8	98.9	0.0	1.1	98.9	0.0	1.1
Imipenem	≤0.12	0.25	≤0.12 to 1	100.0	0.0	0.0	100.0	0.0	0.0
Levofloxacin	0.06	0.25	≤0.03 to >4	98.0	0.4	1.6	95.5	1.8	2.7
Meropenem	0.03	0.03	≤0.015 to 0.25	100.0	0.0	0.0	100.0	0.0	0.0
Piperacillin-tazobactam	2	8	≤0.5 to >64	98.0	1.6	0.4	94.8	3.1	2.0
Tigecycline	0.25	0.5	≤0.06 to 4	98.9	1.1	0.0[c]	98.0	0.9	1.1
Trimethoprim-sulfamethoxazole	≤0.5	1	≤0.5 to >4	92.8		7.2	92.8	0.0	7.2
***Pseudomonas aeruginosa* (258)**									
Ceftobiprole	2	16	0.5 to >16				76.7[e]		23.3
Amikacin	4	8	0.5 to >32	98.1	0.4	1.6	92.6	5.4	1.9
Ampicillin-sulbactam	>32	>32	32 to >32						
Aztreonam	8	>16	0.5 to >16	77.1	7.0	15.9	3.1	81.0	15.9
Cefepime	2	16	0.5 to >16	86.0	10.5	3.5	86.0		14.0
Ceftaroline	16	>32	1 to >32						
Ceftazidime	2	32	0.5 to >32	88.4	1.6	10.1	88.4		11.6
Colistin	1	1	0.12 to 2	100.0		0.0	100.0		0.0
Gentamicin	2	8	0.25 to >8	87.6	6.2	6.2	87.6		12.4
Imipenem	1	8	≤0.12 to >8	79.5	4.7	15.9	84.1	10.1	5.8
Levofloxacin	0.5	>4	0.12 to >4	79.1	4.3	16.7	69.8		30.2
Piperacillin-tazobactam	4	64	0.5 to >64	85.7	4.7	9.7	85.7		14.3
Tigecycline	8	>8	1 to >8						
***Acinetobacter* spp. (66)**									
Ceftobiprole	0.5	>16	≤0.008 to >16				80.3[e]		19.7
Amikacin	2	>32	≤0.25 to >32	84.8	0.0	15.2	84.8	0.0	15.2
Ampicillin-sulbactam	2	32	≤0.5 to >32	77.3	6.1	16.7			
Aztreonam	>16	>16	4 to >16						
Cefepime	4	>16	≤0.12 to >16	74.2	4.5	21.2			
Ceftaroline	2	>32	0.12 to >32						
Ceftazidime	4	>32	0.5 to >32	74.2	4.5	21.2			
Colistin	0.25	2	≤0.06 to >8	90.9		9.1	90.9		9.1
Gentamicin	0.5	>8	≤0.12 to >8	81.8	1.5	16.7	81.8		18.2
Imipenem	0.25	>8	≤0.12 to >8	84.8	0.0	15.2	84.8	0.0	15.2
Levofloxacin	0.25	>4	≤0.03 to >4	83.3	1.5	15.2	81.8	0.0	18.2
Piperacillin-tazobactam	2	>64	≤0.5 to >64	70.8	4.6	24.6			
Tigecycline	0.25	2	≤0.06 to 8						
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5 to >4	78.8		21.2	78.8	3.0	18.2

ESBL = extended-spectrum β-lactamase; S = susceptible; I = intermediate; R = resistant.

[a] Criteria as published by CLSI (CLSI, 2018b) and EUCAST (EUCAST, 2018).

[b] Intermediate interpreted as susceptible-dose dependent.

[c] FDA breakpoints published 2017-DEC-13.

[d] Percentage of wild type based on epidemiological cutoff value. CLSI M100 (CLSI, 2018b).

[e] Using the pharmacokinetic/pharmacodynamic (non-species related) breakpoint based on the standard dose (EUCAST, 2018).

activity similar to cefepime against gram-negative bacilli. Ceftobiprole also exhibited good in vitro activity against the *E. faecalis* isolate set, although its clinical utility as monotherapy against this species requires additional investigation. When compared to earlier reports (Farrell et al., 2014; Fritsche et al., 2008; Pfaller et al., 2018; Walkty et al., 2011), the data reported here for 2016–2017 isolates do not reveal any unexpected shifts in the MIC distributions for ceftobiprole with respect to potential target pathogens and indicate that ceftobiprole merits further study in the treatment of serious infections like BSI with etiologies that involve gram-positive cocci (particularly MRSA), gram-negative bacilli, or mixed infections.

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References

Ammerlaan HS, Harbarth S, Buiting AG, Crook DW, Fitzpatrick F, Hanberger H, et al. Secular trends in nosocomial bloodstream infections: antibiotic-resistant bacteria increase the total burden of infection. Clin Infect Dis 2013;56:798–805.

Anderegg TR, Jones RN, Sader HS. Quality control guidelines for BAL9141 (RO 63-9141), an investigational cephalosporin, when reference MIC and standardized disk diffusion susceptibility test methods are used. J Clin Microbiol 2004;42:3356–8.

Awad SS, Rodriguez AH, Chuang YC, Marjanek Z, Pareigis AJ, Reis G, et al. A phase 3 randomized double-blind comparison of ceftobiprole medocaril versus ceftazidime plus

linezolid for the treatment of hospital-acquired pneumonia. Clin Infect Dis 2014;59:51–61.
Biedenbach DJ, Moet GJ, Jones RN. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002). Diagn Microbiol Infect Dis 2004;50:59–69.
Brogan DM, Mossialos E. Incentives for new antibiotics: the options market for antibiotics (OMA) model. Glob Health 2013;9:58.
Clinical and Laboratory Standards Institute (CLSI). M07Ed11E. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: Eleventh edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2018a.
Clinical and Laboratory Standards Institute (CLSI). M100Ed28E. Performance standards for antimicrobial susceptibility testing: 28th informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2018b.
Davies TA, Page MG, Shang W, Andrew T, Kania M, Bush K. Binding of ceftobiprole and comparators to the penicillin-binding proteins of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Antimicrob Agents Chemother 2007;51:2621–4.
Edmond MB, Ober JF, Dawson JD, Weinbaum DL, Wenzel RP. Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. Clin Infect Dis 1996;23:1234–9.
EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.0, January 2018. Available at http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.0_Breakpoint_Tables.pdf, 2018.
Farrell DJ, Flamm RK, Sader HS, Jones RN. Ceftobiprole activity against over 60,000 clinical bacterial pathogens isolated in Europe, Turkey, and Israel from 2005 to 2010. Antimicrob Agents Chemother 2014;58:3882–8.
Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clin Microbiol Infect 2016;22:416–22.
Fritsche TR, Sader HS, Jones RN. Antimicrobial activity of ceftobiprole, a novel antimethicillin-resistant *Staphylococcus aureus* cephalosporin, tested against contemporary pathogens: results from the SENTRY Antimicrobial Surveillance Program (2005–2006). Diagn Microbiol Infect Dis 2008;61:86–95.
Goto M, Al-Hasan MN. Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin Microbiol Infect 2013;19:501–9.
Laupland KB, Church DL. Population-based epidemiology and microbiology of community-onset bloodstream infections. Clin Microbiol Rev 2014;27:647–64.
Leibovici L. Long-term consequences of severe infections. Clin Microbiol Infect 2013;19:510–2.
Liapikou A, Cilloniz C, Torres A. Ceftobiprole for the treatment of pneumonia: a European perspective. Drug Des Devel Ther 2015;9:4565–72.
Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.
Martin MA, Pfaller MA, Wenzel RP. Coagulase-negative staphylococcal bacteremia. Mortality and hospital stay. Ann Intern Med 1989;110:9–16.
Nielsen SL, Pedersen C, Jensen TG, Gradel KO, Kolmos HJ, Lassen AT. Decreasing incidence rates of bacteremia: a 9-year population-based study. J Infect 2014;69:51–9.
Perez F, Villegas MV. The role of surveillance systems in confronting the global crisis of antibiotic-resistant bacteria. Curr Opin Infect Dis 2015;28:375–83.
Pfaller MA, Acar J, Jones RN, Verhoef J, Turnidge J, Sader HS. Integration of molecular characterization of microorganisms in a global antimicrobial resistance surveillance program. Clin Infect Dis 2001;32:S156–67.
Pfaller MA, Flamm RK, Duncan LR, Streit JM, Castanheira M, Sader HS. Antimicrobial activity of ceftobiprole and comparator agents when tested against contemporary gram-positive and -negative organisms collected from Europe (2015). Diagn Microbiol Infect Dis 2018;91:77–84.
Queenan K, Hasler B, Rushton J. A one health approach to antimicrobial resistance surveillance: is there a business case for it? Int J Antimicrob Agents 2016;48:422–7.
Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, et al. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis 2008;46:155–64.
Syed YY. Ceftobiprole medocaril: a review of its use in patients with hospital- or community-acquired pneumonia. Drugs 2014;74:1523–42.
Timbrook TT, Morton JB, McConeghy KW, Caffrey AR, Mylonakis E, LaPlante KL. The effect of molecular rapid diagnostic testing on clinical outcomes in bloodstream infections: a systematic review and meta-analysis. Clin Infect Dis 2017;64:15–23.
Uslan DZ, Crane SJ, Steckelberg JM, Cockerill III FR, St Sauver JL, Wilson WR, et al. Age- and sex-associated trends in bloodstream infection: a population-based study in Olmsted County, Minnesota. Arch Intern Med 2007;167:834–9.
Vrijens F, Hulstaert F, Van de Sande S, Devriese S, Morales I, Parmentier Y. Hospital-acquired, laboratory-confirmed bloodstream infections: linking national surveillance data to clinical and financial hospital data to estimate increased length of stay and healthcare costs. J Hosp Infect 2010;75:158–62.
Walkty A, Adam HJ, Laverdiere M, Karlowsky JA, Hoban DJ, Zhanel GG, et al. In vitro activity of ceftobiprole against frequently encountered aerobic and facultative gram-positive and gram-negative bacterial pathogens: results of the CANWARD 2007–2009 study. Diagn Microbiol Infect Dis 2011;69:348–55.
Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. Infect Control Hosp Epidemiol 2016;37:1288–301.
Zevtera. Summary of product characteristics. Rickmansworth, United Kingdom: Basilea Medical Ltd.; 2013.
Zhanel GG, Lam A, Schweizer F, Thomson K, Walkty A, Rubinstein E, et al. Ceftobiprole: a review of a broad-spectrum and anti-MRSA cephalosporin. Am J Clin Dermatol 2008;9:245–54.