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Original Article

Ceftobiprole activity against Gram-positive and Gram-negative pathogens causing bone and joint infections in the United States from 2016 to 2020

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ABSTRACT

Bone and joint infections (BJIs) present significant treatment challenges. Ceftobiprole, a broad-spectrum cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*, is approved in many European and other countries for the treatment of adults with community- and hospital-acquired pneumonia, excluding ventilator-associated pneumonia. In this study, the *in vitro* activity of ceftobiprole and comparators was evaluated against clinical isolates collected from BJIs in the USA from 2016 to 2020. Gram-positive pathogens made up 70.6% of all BJI isolates and included *S. aureus* (47.4% of all isolates), β-hemolytic streptococci, coagulase-negative staphylococci, and *Enterococcus faecalis*. Ceftobiprole was highly active against *S. aureus* ($MIC_{50/90}$ values, 0.5/1 mg/L; 99.6% susceptible using the European Committee on Antimicrobial Susceptibility Testing susceptibility breakpoint of ≤2 mg/L for the treatment of pneumonia patients) and exhibited potent activity against the other Gram-positive cocci and the predominant BJI Gram-negative groups. These results support the further evaluation of ceftobiprole for this potential indication.

1. Introduction

Bone and joint infections (BJIs) pose clinical challenges and often require prolonged antimicrobial therapy and surgical debridement [1–3]. BJIs comprise native/prosthetic joint infections, osteomyelitis, and spinal infections (discitis, vertebral osteomyelitis, and epidural abscess) and appear to be increasing in incidence [1,2,4].

Most BJIs are caused by Gram-positive cocci (GPC; predominantly *Staphylococcus* spp.) although other pathogens like the *Enterobacterales, Pseudomonas aeruginosa*, and anaerobes are also isolated at lower frequencies [1,4,5]. For example, Kremers et al. (2015) [4] reported that *Enterobacterales* and *P. aeruginosa* made up 18% of the isolates from patients with diabetes mellitus-related osteomyelitis, and Aggarwal et al. (2014) [6] reported that Gram-negative species comprised 6.6% of the isolates obtained at a single US site from patients with periprosthetic joint infections.

Current initial treatment options for BJIs involving GPC are varied but generally include penicillinase-resistant β-lactams like oxacillin, cephalosporins, or clindamycin [1,5,7]. Methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) often make up a significant percentage of the *Staphylococcus* spp. isolates [5,8]. For instance, Sarkissian et al. (2016) [9] reported an increase of MRSA from 11.8% in 2001-2002 to 34.8% in 2009-2010 among community-acquired musculoskeletal *S. aureus* infections in children, and Aggarwal et al. (2014) [6] reported that MRSA (48.1%) and MRCoNS (51.9%) constituted a large portion of their respective staphylococcal species isolated from periprosthetic joint infections in a US medical center from 2000-2011. In such cases, administration of a glycopeptide like vancomycin is recommended [1,5,7,10]. For the treatment of prosthetic joint infections caused by staphylococci, the Infectious Diseases Society of America also recommends treatment with rifampin as part of an antimicrobial cocktail [10]. Much remains to be learned about the optimal treatment of BJIs, including the best route of antimicrobial administration and the duration of therapy [2,11,12].

Ceftobiprole is a broad-spectrum parenteral cephalosporin that exhibits potent activity against many GPC (including MRSA) and *Enterobacterales* that do not produce extended-spectrum β-lactamases or carbapenemases [13–18]. For example, ceftobiprole exhibited $MIC_{50/90}$ values of 1/2 mg/L against MRSA and 0.03/0.06 mg/L

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against *Klebsiella pneumoniae* isolates that did not display an ESBL phenotype [18].

Ceftobiprole obtained regulatory approval in Europe and several other countries for the treatment of hospital-acquired pneumonia (excluding ventilator-associated pneumonia) and community-acquired bacterial pneumonia in adults [19–23]. Ceftobiprole also showed efficacy in a clinical study of patients with acute bacterial skin and skin structure infections [24] which is part of a phase 3 clinical development program to support a New Drug Application in the US for acute bacterial skin and skin structure infections and *S. aureus* bacteremia.

Although not indicated for the treatment of BJIs, ceftobiprole's unique spectrum, safety profile, and robust bactericidal activity against many important pathogens [17,20-22,25,26] suggest that it may also be an attractive therapeutic candidate for the treatment of BJIs.

Several studies have documented the *in vitro* activity of ceftobiprole against BJI pathogens and pathogens from prosthetic joint infections [27–30]. Moreover, preclinical studies in animal models of osteomyelitis [31,32] and foreign body infections [33] have shown good penetration of ceftobiprole into bone matrix and marrow as well as eradication of methicillin-resistant and -susceptible staphylococci. Importantly, ceftobiprole has also been shown to be active in penetrating and eradicating biofilms produced by staphylococci, including MRSA and MRCoNS [34].

There have been 2 reports on the efficacy of ceftobiprole to treat BJIs in patients. The first was a case report of polymicrobial osteomyelitis and septic joint infection involving MRSA, *Enterobacter cloacae*, and *Peptostreptococcus* (*Anaerococcus*) *prevotii* in a diabetic foot infection that was effectively managed with the administration of ceftobiprole monotherapy coupled with surgical debridement [35]. Second, the Canadian usage registry database reported that ceftobiprole was used in combination with daptomycin or vancomycin to treat 8 patients with BJIs caused by MRSA; the clinical outcome was improvement or cure for 7 of the 8 patients [36].

The present study examines the activity of ceftobiprole and comparators against documented BJI isolates collected from United States (US) medical centers during 2016-2020 as part of the SENTRY Antimicrobial Surveillance Program.

2. Materials and methods

2.1. Bacterial isolates tested

As part of the ceftobiprole SENTRY Antimicrobial Surveillance Program in the US during 2016-2020, a total of 565 non-duplicate clinical isolates from BJIs were submitted from 30 medical centers across all 9 US Census Bureau divisions. Most isolates (399) were Gram-positive, while 166 isolates were from Gram-negative species (Table 1). The most prevalent Gram-negative species are shown in Table 2.

All organisms were isolated from documented BJIs, 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 using standard microbiological methods and matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker, Billerica, MA).

2.2. Susceptibility testing methods

Susceptibility to ceftobiprole and comparator agents was tested using current Clinical and Laboratory Standards Institute (CLSI) methods [37,38]. CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) interpretive criteria were applied according to current guidelines [38,39]. Since there are no approved CLSI or FDA breakpoints for ceftobiprole, the EUCAST susceptibility breakpoints for *S. aureus* (2 mg/L) and *Enterobacterales* (0.25 mg/L) were used. Importantly, these susceptibility breakpoints are only applicable for parenteral dosing of ceftobiprole in patients with pneumonia. Further work is required to understand the relevance of these breakpoints to the potential treatment of BJIs. US FDA product package insert criteria for tigecycline were used as an alternative breakpoint source as indicated [40]. *S. aureus* isolates were classified as MRSA according to their level of oxacillin resistance (MIC ≥4 mg/L).

JMI Laboratories followed current CLSI quality assurance practices when performing the susceptibility tests. MIC values were validated by concurrently testing CLSI-recommended [38] American Type Culture Collection quality control reference strains. Quality control ranges for tested reference strains were those criteria published by CLSI [38]. The inoculum density during susceptibility testing was monitored by bacterial colony counts.

3. Results

3.1. Organisms isolated

The most common Gram-positive species isolated from BJIs were *S. aureus* (47.4% overall [268/565 isolates]; 34.3% of which were MRSA [92/268 isolates]) followed by β-hemolytic streptococci (BHS; 9.7% [55/565 isolates]), CoNS (6.2% overall [35/565 isolates]; 65.7% of which were MRCoNS [23/35 isolates]), and *E. faecalis* (5.1%; [29/565 isolates]) (Table 1). Twelve additional isolates from lower-prevalence Gram-positive species were also collected but are not considered here.

Although the majority of BJI isolates were Gram-positive, we also investigated the antimicrobial susceptibilities of Gram-negative isolates that were obtained from patients with BJIs. Of the 565 total BJI isolates, 166 (29.4%) were from Gram-negative species. Unlike the Gram-positive BJI isolates, which were dominated by a single species (*S. aureus*), the Gram-negative BJI isolates spanned many species. The 4 most frequently isolated species were *P. aeruginosa* (33 isolates; 5.8% [33/565] of all BJI isolates), *Enterobacter cloacae* species complex (25 isolates; 4.4% [25/565]), *E. coli* (24 isolates; 4.2% [24/565]), and *Proteus mirabilis* (20 isolates; 3.5% [20/565]).

3.2. Activity of ceftobiprole against Gram-positive BJI pathogens

The ceftobiprole MIC distributions for each tested Gram-positive species or organism group are shown in Table 1, and a summary table of antimicrobial activities for ceftobiprole and comparators is shown in Table 3. Ceftobiprole was potent when tested against all 268 *S. aureus* isolates ($MIC_{50/90}$, 0.5/1 mg/L; 99.6% susceptible), methicillin-susceptible *S. aureus* (MSSA; $MIC_{50/90}$, 0.5/0.5 mg/L; 100.0% susceptible), and methicillin-resistant *S. aureus* (MRSA; $MIC_{50/90}$, 1/2 mg/L; 98.9% susceptible) (Table 3). Against the MSSA isolate subset, ceftobiprole and ceftaroline were the most potent cephalosporins (MIC_{90}, 0.5 mg/L and 0.25 mg/L, respectively) tested and were 16- to 32-fold more potent than ceftriaxone (MIC_{90}, 8 mg/L). All MSSA isolates were susceptible to ceftobiprole as well as ceftaroline, ceftriaxone, daptomycin, linezolid, oxacillin, tigecycline, trimethoprim-sulfamethoxazole, and vancomycin at their respective breakpoints (Table 3).

As expected, all β-lactams displayed higher MIC values when tested against the MRSA subset, but ceftobiprole (MIC_{90}, 2 mg/L) and ceftaroline (MIC_{90}, 1 mg/L) maintained good activity, as expected (Table 3). The MRSA isolates also exhibited high levels of resistance against levofloxacin (68.5% resistant, CLSI), clindamycin (28.3%, CLSI), and erythromycin (85.9%, CLSI). Greater than 90.0% of the MRSA isolates were susceptible to ceftobiprole as well as ceftaroline, daptomycin, gentamicin, linezolid, tigecycline, trimethoprim-sulfamethoxazole, and vancomycin at their respective breakpoints (Table 3).

Against the full set of 35 CoNS isolates (Table 1), the ceftobiprole $MIC_{50/90}$ values were 1/1 mg/L, and this activity was largely

Table 1
Antimicrobial activity of ceftobiprole tested against the main Gram-positive species and organism groups from bone and joint infections.

Species/organism group (no. of isolates)	No. 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	>[a]		
Staphylococcus aureus (268)						0	1	1	45	132	67	21	1		0.5	1
						0.0	0.4	0.7	17.5	66.8	91.8	99.6	100.0			
Methicillin-susceptible (176)						0	1	1	45	127	2				0.5	0.5
						0.0	0.6	1.1	26.7	98.9	100.0					
Methicillin-resistant (92)									0	5	65	21	1		1	2
									0.0	5.4	76.1	98.9	100.0			
Coagulase-negative staphylococci (35)[b]							0	5	2	10	15	0	3		1	1
							0.0	14.3	20.0	48.6	91.4	91.4	100.0			
Methicillin-susceptible (12)							0	4	2	4	2				0.25	1
							0.0	33.3	50.0	83.3	100.0					
Methicillin-resistant (23)							0	1	0	6	13	0	3		1	4
							0.0	4.3	4.3	30.4	87.0	87.0	100.0			
Enterococcus faecalis (29)						0	1	3	5	14	2	2	2		0.5	2
						0.0	3.4	13.8	31.0	79.3	86.2	93.1	100.0			
β-hemolytic streptococci (55)[c]	0	1	3	12	13	25	1								0.015	0.03
	0.0	1.8	7.3	29.1	52.7	98.2	100.0									
Streptococcus agalactiae (31)				0	5	25	1								0.03	0.03
				0.0	16.1	96.8	100.0									
Streptococcus dysgalactiae (7)		0	1	0	6										0.015	
		0.0	14.3	14.3	100.0											
Streptococcus pyogenes (16)	0	1	1	12	2										0.008	0.015
	0.0	6.2	12.5	87.5	100.0											

[a] Greater than the highest concentration tested.
[b] Organisms included *Staphylococcus caprae* (1), *Staphylococcus epidermidis* (18), *Staphylococcus haemolyticus* (4), *Staphylococcus hominis* (2), *Staphylococcus lugdunensis* (6), *Staphylococcus pseudintermedius* (1), and *Staphylococcus simulans* (3).
[c] Organisms also included *Streptococcus canis* (1) (data not shown).

Table 2
Antimicrobial activity of ceftobiprole and comparators tested against the main Gram-negative species and organism groups from bone and joint infections.

Species/organism group (no. of isolates)	No. and cumulative % of isolates inhibited at MIC (mg/L) of:														MIC_{50}	MIC_{90}
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>[a]		
Pseudomonas aeruginosa																
Ceftobiprole (33)							0	6	11	4	4	4		4	2	>16
							0.0	18.2	51.5	63.6	75.8	87.9		100.0		
Cefepime (33)							0	4	10	6	3	6		4	4	>16
							0.0	12.1	42.4	60.6	69.7	87.9		100.0		
Ceftaroline (33)									0	2	4			27	>8	>8
									0.0	6.1	18.2			100.0		
Enterobacter cloacae species complex																
Ceftobiprole (25)		0	10	8	2	0	1	1	0	1	1	0		1	0.06	4
		0.0	40.0	72.0	80.0	80.0	84.0	88.0	88.0	92.0	96.0	96.0		100.0		
Cefepime (24)					18	1	0	1	1	2	0	0	0	1	≤0.12	4
					75.0	79.2	79.2	83.3	87.5	95.8	95.8	95.8	95.8	100.0		
Ceftaroline (24)			0	1	6	6	6	1	0	0	0			4	0.25	>8
			0.0	4.2	29.2	54.2	79.2	83.3	83.3	83.3	83.3			100.0		
Escherichia coli																
Ceftobiprole (24)		0	10	10	0	0	1	0	0	0	0	0		3	0.06	>16
		0.0	41.7	83.3	83.3	83.3	87.5	87.5	87.5	87.5	87.5	87.5		100.0		
Cefepime (24)					18	2	0	1	0	0	0	1		2	≤0.12	16
					75.0	83.3	83.3	87.5	87.5	87.5	87.5	91.7		100.0		
Ceftaroline (24)				9	4	3	3	1	0	0	1	0		3	0.12	>16
				37.5	54.2	66.7	79.2	83.3	83.3	83.3	87.5	87.5		100.0		
Proteus mirabilis																
Ceftobiprole (20)	0	2	15	2	0	0	0	0	0	0	0	0		1	0.03	0.06
	0.0	10.0	85.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0		100.0		
Cefepime (20)					19	0	0	1							≤0.12	≤0.12
					95.0	95.0	95.0	100.0								
Ceftaroline (20)				12	7	0	0	0	0	0	0			1	≤0.06	0.12
				60.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0			100.0		

[a] Greater than the highest concentration tested.

Table 3
Activity of ceftobiprole and comparator antimicrobial agents against Gram-positive cocci from bone and joint infections.

Species or organism group (no. of isolates) Antimicrobial agent	mg/L			CLSI[a]			EUCAST[a]		
	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R	%S	%I	%R
Staphylococcus aureus (268)									
Ceftobiprole	0.5	1	0.06-4				99.6		0.4
Ceftaroline	0.25	1	≤0.06-2	98.1	1.9[b]	0.0	98.1[c]	1.9	0.0
							98.1[d]		1.9
Ceftriaxone	4	>8	≤0.25->8	65.7		34.3			
Clindamycin	≤0.25	>2	≤0.25->2	86.9	0.0	13.1	86.6	0.4	13.1
Daptomycin	0.25	0.5	≤0.12-1	100.0			100.0		0.0
Erythromycin	0.25	>8	≤0.06->8	51.1	5.6	43.3	51.9	1.9	46.3
Gentamicin	≤1	≤1	≤1->8	98.1	0.0	1.9	98.1[e]		1.9
Levofloxacin	0.25	>4	0.06->4	70.1	1.1	28.7	[f]	70.1	29.9
Linezolid	1	2	≤0.12-4	100.0		0.0	100.0		0.0
Oxacillin	0.5	>2	≤0.25->2	65.7		34.3	65.7		34.3
Tetracycline	≤0.5	≤0.5	≤0.5->8	91.8	1.5	6.7	91.0	0.7	8.2
Tigecycline	0.06	0.12	0.03-0.5	100.0[g]			100.0		0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5->4	97.4		2.6	97.4	0.0	2.6
Vancomycin	1	1	0.5-2	100.0	0.0	0.0	100.0		0.0
MSSA (176)									
Ceftobiprole	0.5	0.5	0.06-1				100.0		0.0
Ceftaroline	0.25	0.25	≤0.06-0.5	100.0	0.0[b]	0.0	100.0[c]	0.0	0.0
							100.0[d]		0.0
Ceftriaxone	4	8	≤0.25-8	100.0		0.0			
Clindamycin	≤0.25	≤0.25	≤0.25->2	94.9	0.0	5.1	94.3	0.6	5.1
Daptomycin	0.25	0.5	≤0.12-0.5	100.0			100.0		0.0
Erythromycin	0.25	>8	≤0.06->8	71.0	8.0	21.0	72.2	2.8	25.0
Gentamicin	≤1	≤1	≤1->8	99.4	0.0	0.6	99.4[e]		0.6
Levofloxacin	0.25	0.5	0.06->4	91.5	0.6	8.0	[f]	91.5	8.5
Linezolid	1	2	≤0.12-4	100.0		0.0	100.0		0.0
Oxacillin	0.5	1	≤0.25-2	100.0		0.0	100.0		0.0
Tetracycline	≤0.5	≤0.5	≤0.5->8	94.3	0.6	5.1	93.2	1.1	5.7
Tigecycline	0.12	0.12	0.03-0.5	100.0[g]			100.0		0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5-2	100.0		0.0	100.0	0.0	0.0
Vancomycin	1	1	0.5-2	100.0	0.0	0.0	100.0		0.0
MRSA (92)									
Ceftobiprole	1	2	0.5-4				98.9		1.1
Ceftaroline	1	1	0.25-2	94.6	5.4[b]	0.0	94.6[c]	5.4	0.0
							94.6[d]		5.4
Ceftriaxone	>8	>8	>8->8	0.0		100.0			
Clindamycin	≤0.25	>2	≤0.25->2	71.7	0.0	28.3	71.7	0.0	28.3
Daptomycin	0.25	0.5	≤0.12-1	100.0			100.0		0.0
Erythromycin	>8	>8	0.12->8	13.0	1.1	85.9	13.0	0.0	87.0
Gentamicin	≤1	≤1	≤1->8	95.7	0.0	4.3	95.7[e]		4.3
Levofloxacin	4	>4	0.06->4	29.3	2.2	68.5	[f]	29.3	70.7
Linezolid	1	2	0.5-4	100.0		0.0	100.0		0.0
Oxacillin	>2	>2	>2->2	0.0		100.0	0.0		100.0
Tetracycline	≤0.5	8	≤0.5->8	87.0	3.3	9.8	87.0	0.0	13.0
Tigecycline	0.06	0.12	0.03-0.5	100.0[g]			100.0		0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5->4	92.4		7.6	92.4	0.0	7.6
Vancomycin	1	1	0.5-2	100.0	0.0	0.0	100.0		0.0
Coagulase-negative staphylococci (CoNS; 35)[h]									
Ceftobiprole	1	1	0.12-4						
Ceftaroline	0.25	0.5	≤0.06-2						
Ceftriaxone	>8	>8	1->8	34.3		65.7			
Clindamycin	≤0.25	>2	≤0.25->2	71.4	5.7	22.9	71.4	0.0	28.6
Daptomycin	0.25	0.5	≤0.12-1	100.0			100.0		0.0
Erythromycin	>8	>8	≤0.06->8	31.4	0.0	68.6	31.4	0.0	68.6
Gentamicin	≤1	>8	≤1->8	80.0	0.0	20.0	80.0[i]		20.0
Levofloxacin	0.25	>4	0.12->4	51.4	2.9	45.7	[f]	51.4	48.6
Linezolid	0.5	1	0.25-2	100.0		0.0	100.0		0.0
Oxacillin	>2	>2	≤0.25->2	34.3		65.7	28.6		71.4
Tetracycline	≤0.5	1	≤0.5->8	94.3	0.0	5.7	91.4	2.9	5.7
Tigecycline	0.12	0.12	0.03-0.25				100.0		0.0
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5->4	68.6		31.4	68.6	14.3	17.1
Vancomycin	1	2	0.5-2	100.0	0.0	0.0	100.0		0.0
MSCoNS (12)[j]									
Ceftobiprole	0.25	1	0.12-1						
Ceftaroline	0.12	0.25	≤0.06-0.25						
Ceftriaxone	4	4	1-4	100.0		0.0			
Clindamycin	≤0.25	>2	≤0.25->2	83.3	0.0	16.7	83.3	0.0	16.7
Daptomycin	≤0.12	0.5	≤0.12-1	100.0			100.0		0.0
Erythromycin	0.25	>8	≤0.06->8	58.3	0.0	41.7	58.3	0.0	41.7
Gentamicin	≤1	≤1	≤1-≤1	100.0	0.0	0.0	100.0[i]		0.0
Levofloxacin	0.25	0.25	0.12-0.25	100.0	0.0	0.0	[f]	100.0	0.0

(*continued*)

Table 3 (*Continued*)

Species or organism group (no. of isolates) Antimicrobial agent	mg/L			CLSI[a]			EUCAST[a]		
	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R	%S	%I	%R
Linezolid	0.5	1	0.5-1	100.0		0.0	100.0		0.0
Oxacillin	0.5	1	≤0.25-1	100.0		0.0	83.3		16.7
Tetracycline	≤0.5	≤0.5	≤0.5-1	100.0	0.0	0.0	100.0	0.0	0.0
Tigecycline	0.06	0.12	0.03-0.12				100.0		0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5-4	91.7		8.3	91.7	8.3	0.0
Vancomycin	0.5	1	0.5- 2	100.0	0.0	0.0	100.0		0.0
MRCoNS (23)[k]									
Ceftobiprole	1	4	0.12-4						
Ceftaroline	0.25	2	0.12-2						
Ceftriaxone	>8	>8	4->8	0.0		100.0			
Clindamycin	≤0.25	>2	≤0.25->2	65.2	8.7	26.1	65.2	0.0	34.8
Daptomycin	0.25	0.5	≤0.12-0.5	100.0			100.0		0.0
Erythromycin	>8	>8	≤0.06->8	17.4	0.0	82.6	17.4	0.0	82.6
Gentamicin	≤1	>8	≤1->8	69.6	0.0	30.4	69.6[i]		30.4
Levofloxacin	4	>4	0.12->4	26.1	4.3	69.6	[f]	26.1	73.9
Linezolid	0.5	1	0.25-2	100.0		0.0	100.0		0.0
Oxacillin	>2	>2	1->2	0.0		100.0	0.0		100.0
Tetracycline	≤0.5	2	≤0.5->8	91.3	0.0	8.7	87.0	4.3	8.7
Tigecycline	0.12	0.12	0.06-0.25				100.0		0.0
Trimethoprim-sulfamethoxazole	2	>4	≤0.5->4	56.5		43.5	56.5	17.4	26.1
Vancomycin	1	2	0.5-2	100.0	0.0	0.0	100.0		0.0
Enterococcus faecalis (29)									
Ceftobiprole	0.5	2	0.06-4						
Ampicillin	1	1	0.5-2	100.0		0.0	100.0	0.0	0.0
Ceftaroline	2	8	1->8						
Daptomycin	1	1	≤0.25-2	100.0	0.0	0.0			
Levofloxacin	1	>4	0.25->4	75.9	0.0	24.1	75.9[l]		24.1
Linezolid	1	2	0.5-2	100.0	0.0	0.0	100.0		0.0
Teicoplanin	≤0.5	≤0.5	≤0.5- >16	96.6	0.0	3.4	96.6		3.4
Tigecycline	0.06	0.12	0.03-0.12	100.0[m]			100.0		0.0
Vancomycin	1	2	0.5->16	96.6	0.0	3.4	96.6		3.4
β-hemolytic streptococci (55)[n]									
Ceftobiprole	0.015	0.03	0.002-0.06						
Ceftaroline	0.015	0.015	≤0.008-0.03	100.0			100.0		0.0
Ceftriaxone	0.06	0.06	≤0.015-0.12	100.0			100.0		0.0
Clindamycin	≤0.25	>2	≤0.25->2	65.5	1.8	32.7	67.3		32.7
Daptomycin	0.12	0.25	≤0.06-1	100.0			100.0		0.0
Erythromycin	0.12	>16	≤0.015->16	50.9	0.0	49.1	50.9	0.0	49.1
Levofloxacin	0.5	1	0.12-2	100.0	0.0	0.0	0.0	100.0	0.0
Linezolid	1	1	0.5-2	100.0			100.0		0.0
Meropenem	0.03	0.06	≤0.008-0.12	100.0			100.0		0.0
Penicillin	0.03	0.06	≤0.008-0.06	100.0			100.0		0.0
Tetracycline	>4	>4	≤0.25->4	34.5	1.8	63.6	34.5	0.0	65.5
Vancomycin	0.5	0.5	0.25-0.5	100.0			100.0		0.0

CLSI = Clinical and Laboratory Standards Institute; EUCAST = European Committee on Antimicrobial Susceptibility Testing; I = intermediate; MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-susceptible *Staphylococcus aureus*; MRCoNS = methicillin-resistant coagulase-negative staphylococci; MSCoNS = methicillin-susceptible coagulase-negative staphylococci; R = resistant; S = susceptible.

[a] Criteria as published by [38] and [39]; the EUCAST ceftobiprole *S. aureus* susceptibility breakpoint value, which is valid for parenteral dosing of pneumonia patients, was used as indicated. Further work is required to understand the relevance of this breakpoint to the potential treatment of BJIs.

[b] Intermediate is interpreted as susceptible-dose dependent.

[c] Using other than pneumonia breakpoints.

[d] Using pneumonia breakpoints.

[e] For systemic infections, aminoglycosides must be used in combination with other active therapy.

[f] An arbitrary susceptible breakpoint of ≤0.001 mg/L and/or >50 mm has been published by EUCAST indicating that susceptible should not be reported for this organism-agent combination and intermediate should be interpreted as susceptible increased exposure.

[g] US FDA breakpoints were applied.

[h] Organisms included *Staphylococcus caprae* (1), *Staphylococcus epidermidis* (18), *Staphylococcus haemolyticus* (4), *Staphylococcus hominis* (2), *Staphylococcus lugdunensis* (6), *Staphylococcus pseudintermedius* (1), and *Staphylococcus simulans* (3).

[i] For systemic infections, aminoglycosides must be used in combination with other active therapy.

[j] Organisms included *Staphylococcus caprae* (1), *Staphylococcus epidermidis* (2), *Staphylococcus lugdunensis* (6), *Staphylococcus pseudintermedius* (1), and *Staphylococcus simulans* (2).

[k] Organisms included *Staphylococcus epidermidis* (16), *Staphylococcus haemolyticus* (4), *Staphylococcus hominis* (2), and *Staphylococcus simulans* (1).

[l] Uncomplicated UTI only.

[m] US FDA breakpoints published for vancomycin-susceptible isolates were applied to all isolates.

[n] Organisms included *Streptococcus agalactiae* (31), *Streptococcus canis* (1), *Streptococcus dysgalactiae* (7), and *Streptococcus pyogenes* (16).

unaffected by oxacillin resistance ($MIC_{50/90}$, 1/4 mg/L against MRCoNS; 65.7% of the CoNS isolates tested were resistant to oxacillin [Table 3]). Ceftaroline (MIC_{90}, 0.5 mg/L) was the most potent cephalosporin tested against CoNS and was 2-fold more potent than ceftobiprole (MIC_{90}, 1 mg/L) (Table 3). The CoNS isolates showed high resistance rates for all tested comparators except daptomycin, linezolid, tetracycline, tigecycline, and vancomycin (all >90.0% susceptible).

Ceftobiprole demonstrated potent activity against 29 *E. faecalis* isolates ($MIC_{50/90}$, 0.5/2 mg/L; Table 1) but was inactive against *Enterococcus faecium* isolates (MIC_{50}, >4 mg/L; data not shown), as expected. The *E. faecalis* isolates were all susceptible to ampicillin,

daptomycin, linezolid, and tigecycline (Table 3). A total of 3.4% of the *E. faecalis* isolates were resistant to vancomycin.

Ceftobiprole was very potent against the BHS, with all isolates inhibited at ≤0.06 mg/L ($MIC_{50/90}$, 0.015/0.03 mg/L; Table 1). *Streptococcus agalactiae* was the most prevalent species observed (56.4% of the BHS subset). All BHS isolates were susceptible to ceftaroline, ceftriaxone, daptomycin, levofloxacin, linezolid, meropenem, penicillin, and vancomycin. Resistance to clindamycin and erythromycin was 32.7% and 49.1%, respectively (Table 3).

3.3. Activity of ceftobiprole against Gram-negative BJI pathogens

Table 2 displays the *in vitro* activity of ceftobiprole, ceftaroline, and cefepime against the 4 most frequently isolated Gram-negative groups. The $MIC_{50/90}$ values for ceftobiprole were similar to or more potent than those of ceftaroline and cefepime against the Gram-negative isolate subsets. Using the EUCAST ceftobiprole pneumonia susceptibility breakpoint value of 0.25 mg/L, 81.9% of the 116 *Enterobacterales* isolates from this study were susceptible to ceftobiprole (data not shown); the relevance of the EUCAST ceftobiprole susceptibility criteria to BJIs is not known.

4. Discussion

The distribution of BJI isolates collected at US medical centers during 2016-2020 was similar to previously published data: most isolates were GPC and predominantly *S. aureus*. Aerobic Gram-negative rods were also isolated but at lower frequencies [4,5]. Importantly, the *in vitro* activity of ceftobiprole that was measured in this study against the BJI isolate set was nearly identical to previously reported activity against combined US isolates obtained from various infection types [16,18].

Ceftobiprole was highly active against clinical BJI isolates from the major Gram-positive pathogen groups. The *S. aureus* isolate set (47.4% of all BJI isolates), including MRSA, was 99.6% susceptible to ceftobiprole using the EUCAST susceptibility breakpoint for the treatment of pneumonia. Although no susceptibility breakpoints have been established for the other Gram-positive isolate sets considered in this study, ceftobiprole exhibited potent *in vitro* activity against the BHS ($MIC_{50/90}$, 0.015/0.03 mg/L), *E. faecalis* ($MIC_{50/90}$, 0.5/2 mg/L), and CoNS ($MIC_{50/90}$, 1/1 mg/L) isolate sets from patients with BJIs.

Further study of ceftobiprole for empiric and targeted therapy of BJIs should be considered based on its: (1) broad spectrum of activity that includes prevalent Gram-positive (especially MRSA) and non-ESBL *Enterobacterales* causative pathogens [18]; (2) penetration into bone and efficacy in rabbit *S. aureus* osteomyelitis models [31,32] and rat foreign-body infection models [33]; (3) activity against biofilm-producing organisms [34]; (4) good safety profile [21]; and (5) encouraging preliminary results from 2 small clinical studies in which ceftobiprole was used to treat patients with BJIs [35,36].

5. Conclusions

Ceftobiprole exhibited potent *in vitro* antibacterial activity against the prevalent Gram-positive (including MRSA) and Gram-negative *Enterobacterales* species associated with BJIs. This observation, efficacy in animal osteomyelitis and foreign-body infection models [31−33], and 2 reports in which ceftobiprole exhibited efficacy against patients with BJIs [35,36] support its further evaluation for this potential indication.

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Authors' contributions

Leonard R. Duncan: writing - original draft, writing - review and editing, project administration, and visualization. Kamal A. Hamed: writing - review and editing, and funding acquisition. Jennifer I. Smart: writing - review and editing, and funding acquisition. Michael A. Pfaller: writing - original draft. Robert K. Flamm: conceptualization. Rodrigo E. Mendes: supervision, writing - review and editing.

Declaration of competing interest

JIS is an employee of Basilea Pharmaceutica International Ltd. KAH was an employee of Basilea Pharmaceutica International Ltd. while this work was conducted. LRD, MAP, RKF, and REM have no conflicts to disclose. This study was funded in part by Basilea Pharmaceutica International Ltd., which was involved in the design and decision to present these results, and JMI Laboratories received compensation fees for services in relation to preparing the manuscript. 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., Astellas 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, Diamond V, 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, U.S. Food and Drug Administration, Venatorx Pharmaceuticals, Inc., Weill Cornell Medicine, and Wockhardt Ltd.

Supplementary materials

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