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Antimicrobial Activity of the Pleuromutilin Antibiotic BC-3781 against Bacterial Pathogens Isolated in the SENTRY Antimicrobial Surveillance Program in 2010

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BC-3781 is a novel semisynthetic pleuromutilin antibiotic inhibiting bacterial protein synthesis. BC-3781 has completed a phase 2 clinical trial in acute bacterial skin and skin structure infections (ABSSSI). Its antibacterial spectrum additionally covers the predominant pathogens causing community-acquired bacterial pneumonia (CABP). In this study, the antibacterial activity of BC-3781 was evaluated against a contemporary collection of 10,035 bacterial isolates predominately causing ABSSSI and CABP, among other infections, collected within the SENTRY Antimicrobial Surveillance Program worldwide in 2010. BC-3781 exhibited potent activity against organisms commonly isolated from ABSSSI such as *Staphylococcus aureus* ($MIC_{50/90}$, 0.12/0.12 μg/ml; 99.8% inhibited at ≤0.5 μg/ml), beta-hemolytic streptococci ($MIC_{50/90}$, 0.03/0.03 μg/ml; 99.3% inhibited at ≤0.5 μg/ml), and coagulase-negative staphylococci (CoNS; $MIC_{50/90}$, 0.06/0.12 μg/ml; 97.8% inhibited at ≤1 μg/ml). BC-3781 displayed similar MIC distributions among methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *S. aureus* strains. BC-3781 was also active against *Enterococcus faecium*, with 76.3% of vancomycin-susceptible and 97.0% of vancomycin-resistant isolates being inhibited at BC-3781 concentrations of ≤1 μg/ml. Beta-hemolytic and viridans group streptococci were highly susceptible to BC-3781, with 99.3% and 96.7% of isolates inhibited at ≤0.5 μg/ml, respectively. Further, activity of BC-3781 against *Streptococcus pneumoniae* ($MIC_{50/90}$, 0.12/0.25 μg/ml), *Haemophilus influenzae* ($MIC_{50/90}$, 1/2 μg/ml), and *Moraxella catarrhalis* ($MIC_{50/90}$, 0.12/0.25 μg/ml) was not negatively influenced by β-lactamase production or resistance to other antimicrobial classes tested. In all, BC-3781 displayed a very potent antibacterial profile including the most prevalent bacterial pathogens causing ABSSSI and CABP, thus warranting further clinical development of this antibiotic in these and possibly other indications.

Acute bacterial skin and skin structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP) pose a significant problem to public health despite a rather large armamentarium of antibiotics available to fight these infections. Cellulitis, abscess, and other skin and soft tissue infections are among the most common infections treated in hospitals. Of increasing concern is the rapidly rising frequency of ABSSSI caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Particularly, the incidence of community-acquired MRSA (CA-MRSA) infections has risen dramatically since the beginning of this millennium (1, 2). CA-MRSA is now the most common pathogen cultured from patients with ABSSSI in emergency departments in most U.S. cities (3). Among respiratory tract infections, CABP and nosocomial pneumonia, including ventilator-associated bacterial pneumonia (VABP) and hospital-acquired bacterial pneumonia (HABP), remain, despite advances in antimicrobial therapy, supportive care methods, and the implementation of a broad range of preventive measures, leading causes of morbidity and mortality worldwide (4–6). Treatment of CABP often requires systemic antibiotic therapy and thus is associated with substantial health care costs (7).

Furthermore, epidemic antibiotic resistance in combination with the global pandemic of highly virulent pathogens like MRSA has underscored the urgent need for novel antibacterial agents suitable to combat serious bacterial infections caused by resistant organisms (8). Particularly, the resistance development of *S. aureus* and beta-hemolytic streptococci, being the most common organisms isolated among ABSSSI, as well as *Streptococcus pneumoniae* and *Haemophilus influenzae*, being commonly associated with CABP, against established classes of antimicrobials such as macrolides, tetracyclines, and β-lactam antibiotics constantly strengthens the need for new antibiotic compounds overcoming existing resistance problems (7, 9, 10).

BC-3781 is a novel compound belonging to the pleuromutilin class of antibiotics. Pleuromutilins inhibit bacterial protein synthesis by selectively binding to the peptidyl transferase center of the bacterial ribosome and preventing the correct positioning of the CCA ends of tRNAs for peptide transfer (11, 12). Although the first pleuromutilins were described already 6 decades ago, synthetic modifications led to only three approved compounds, tiamulin and valnemulin for the treatment of bacterial infections in swine and poultry and retapamulin for the topical treatment of impetigo and uncomplicated skin and skin structure infections (due to methicillin-susceptible *S. aureus* [MSSA] only) in humans (13–17). BC-3781 is the first systemic pleuromutilin derivative in development for intravenous and oral administration to combat serious infections in humans. It has completed clinical phase 2 in ABSSSI (18–23). The spectrum of activity of this new antibacterial comprises of potent activity against organisms commonly isolated from ABSSSI, as well as those causing CABP, including multidrug-resistant Gram-positive cocci and those causing atypical pneumonia, such as *Mycoplasma pneumoniae* ($MIC_{50/90}$, 0.006/0.006 μg/

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TABLE 1 Number of organisms tested against BC-3781, stratified by site or type of infection

Organism(s)	No. of isolates tested (%)			
	ABSSI	Respiratory tract	Bacteremia	Others
S. aureus	2,347 (42.5)	832 (15.1)	1,887 (34.1)	461 (8.3)
CoNS	154 (17.5)	7 (0.8)	576 (65.6)	141 (16.1)
E. faecium	95 (17.7)	5 (0.9)	358 (66.8)	78 (14.5)
S. pneumoniae	7 (0.5)	1,261 (85.6)	195 (13.2)	10 (0.7)
Viridans group *Streptococcus* spp.	43 (17.6)	2 (0.8)	147 (60.0)	53 (21.6)
Beta-hemolytic *Streptococcus* spp.	401 (52.6)	24 (3.1)	162 (21.2)	176 (23.1)
H. influenzae	0 (0.0)	360 (100.0)	0 (0.0)	0 (0.0)
M. catarrhalis	0 (0.0)	252 (99.6)	1 (0.4)	0 (0.0)
Total	3,047 (30.4)	2,743 (27.3)	3,326 (33.1)	919 (9.2)

ml), *Chlamydophila pneumoniae* ($MIC_{50/90}$, 0.02/0.04 μg/ml), and *Legionella pneumophila* ($MIC_{50/90}$, 0.06/0.5 μg/ml) (22, 23). BC-3781 has also been shown to be active against *Enterococcus faecium* (particularly vancomycin-resistant strains [VRE]), which has become an important nosocomial pathogen, whereas it is not active against *Enterococcus faecalis* (23).

In this study, the antibacterial *in vitro* activity of BC-3781 was assessed against a worldwide contemporary collection of Gram-positive and fastidious Gram-negative bacterial isolates, collected within the SENTRY Antimicrobial Surveillance Program platform in 2010, which commonly, but not exclusively, cause ABSSSI and CABP, among other bacterial infections.

MATERIALS AND METHODS

Organism collection. The activity of BC-3781 was determined against bacterial pathogens mainly collected from bacterial skin and soft tissue infections (30.4%), respiratory tract infections (27.3%), and bloodstream infections (bacteremia; 33.1%) from patients aged 0 to 98 years old as listed in Tables 1 and 2. A total of 10,035 unique patient isolates (only one strain per patient) were collected within the SENTRY Program during 2010. The strains were consecutively collected (prevalence mode) from medical centers in the United States (37 centers in all nine census regions) and multiple countries in Europe and Asia (25 medical centers in the United Kingdom, Sweden, Germany, Spain, Italy, France, Turkey, Ireland, Poland, Belgium, Israel, and Portugal), Latin America (10 medical

TABLE 2 Frequency of occurrence of BC-3781 MICs for all pathogens (10,035) tested

Organism(s) (no.)	Cumulative % of isolates inhibited at BC-3781 MIC (μg/ml) of:												
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16
Staphylococcus aureus (5,527)	0.0	0.1	1.0	43.1	94.6	99.5	99.8	99.8	99.9	99.9	99.9	>99.9	100.0
MSSA (3,157)	0.0	0.2	1.0	46.9	98.2	99.7	99.8	99.8	99.9	99.9	99.9	99.9	100.0
MRSA (2,370)	0.0	0.1	0.9	38.1	89.7	99.2	99.8	99.8	100.0	100.0			
Coagulase-negative *Staphylococcus* spp. (878)	0.6	2.7	46.1	85.4	93.7	94.6	96.6	97.8	98.5	99.0	99.3	99.4	100.0
MSCoNS (241)	1.2	3.7	49.8	92.9	97.9	97.9	97.9	99.6	99.6	100.0			
MRCoNS (637)	0.3	2.4	44.7	82.6	92.2	93.4	96.1	97.2	98.1	98.6	99.1	99.2	100.0
Viridans group streptococci (245)	4.1	15.1	27.3	47.8	70.6	84.1	96.7	98.4	100.0				
Beta-hemolytic streptococci (763)	0.5	19.0	93.2	98.4	99.0	99.3	99.3	99.3	99.3	99.6	99.6	100.0	
Group A *Streptococcus* (267)	0.4	37.8	98.9	100.0									
Group B *Streptococcus* (334)	0.6	9.6	94.3	98.2	98.5	98.5	98.5	98.5	98.5	99.1	99.1	100.0	
Enterococcus faecium (536)	0.0	0.2	2.4	42.9	76.5	81.5	86.0	88.1	89.6	91.8	92.5	94.6	100.0
Vancomycin susceptible (232)	0.0	0.0	1.3	31.5	62.5	69.0	75.4	76.3	78.0	81.9	83.2	87.9	100.0
Vancomycin nonsusceptible (304)	0.0	0.3	3.3	51.6	87.2	91.1	94.1	97.0	98.4	99.3	99.7	99.7	100.0
Streptococcus pneumoniae (1,473)	0.2	1.0	4.3	18.0	62.3	95.0	99.8	100.0					
Penicillin susceptible (903)	0.3	1.0	4.0	17.6	61.2	94.0	99.8	100.0					
Penicillin intermediate (258)	0.0	1.2	7.8	18.2	55.0	93.4	99.6	100.0					
Penicillin resistant (312)	0.0	0.6	2.6	18.9	71.2	99.0	100.0						
Haemophilus influenzae (360)	0.0	0.3	0.3	0.3	0.3	3.3	38.3	86.1	98.3	99.4	100.0		
β-Lactamase positive (85)	0.0	0.0	0.0	0.0	0.0	4.7	30.6	80.0	97.6	98.8	100.0		
β-Lactamase negative (275)	0.0	0.4	0.4	0.4	0.4	2.9	40.7	88.0	98.5	99.6	100.0		
Moraxella catarrhalis (253)	2.8	3.6	4.3	6.7	69.2	98.8	100.0						

centers in Argentina, Brazil, Chile, and Mexico), and the Asia-Pacific region (12 medical centers in Australia, New Zealand, China, Taiwan, Hong Kong, Korea, Malaysia, and Singapore). A total of 51.2% of the isolates were collected from the United States, 30.2% from Europe, 10.4% from Latin America, and 8.2% from the Asia-Pacific region. Of the isolates, 2,069 (30.6%) were of nosocomial origin, 4,769 (47.5%) were community acquired, and 3,197 (31.9%) were unclassified.

Susceptibility test methods. MICs for BC-3781 and the comparator agents were determined according to CLSI guidelines (24) using validated microdilution panels manufactured by TREK Diagnostics (Cleveland, OH). Cation-adjusted Mueller-Hinton broth was used for testing of nonfastidious organisms and with 2.5 to 5% lysed horse blood added for testing of streptococci. *Haemophilus* test medium was used for testing of *H. influenzae*. Interpretive criteria for comparator agents were used as published by the CLSI and EUCAST (25, 26). For tigecycline, U.S. FDA breakpoints were applied (27). Quality control was performed as recommended by the CLSI using the following strains: *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, and *E. faecalis* ATCC 29212 (24).

RESULTS AND DISCUSSION

BC-3781 demonstrated potent activity against a large collection of *S. aureus* isolates ($n = 5,527$), with a $MIC_{50/90}$ of 0.12/0.12 μg/ml. Overall, 99.8% of *S. aureus* strains were inhibited at ≤0.5 μg/ml of BC-3781, with similar MIC distributions among MSSA and MRSA (Table 2). Only 6 MSSA and 4 MRSA strains out of 5,527 *S. aureus* isolates (0.18%) had BC-3781 MICs of ≥2 μg/ml, elevated relative to that of the wild-type population (MIC range, 0.015 to 0.5 μg/ml [Table 2]) and when considering a provisional susceptibility cutoff value of ≤1 μg/ml established for Gram-positive cocci (28). All *S. aureus* isolates with elevated BC-3781 MICs are currently being characterized genetically to elucidate potential resistance mechanisms. Compared with other antibiotics tested, BC-3781 was among the compounds displaying the lowest MICs against *S. aureus* isolates. Linezolid ($MIC_{50/90}$, 1/1 μg/ml), vancomycin ($MIC_{50/90}$, 1/1 μg/ml), and tigecycline ($MIC_{50/90}$, 0.12/0.25 μg/ml) showed complete activity (100.0% susceptibility) against *S. aureus*, and very few isolates were nonsusceptible to daptomycin ($MIC_{50/90}$, 0.25/0.5 μg/ml; >99.9% susceptible) and quinupristin-dalfopristin ($MIC_{50/90}$, ≤0.5/≤0.5 μg/ml; 99.9% susceptible [Table 3). MSSA strains showed high rates of susceptibility (≥95%) to all comparator agents tested, except levofloxacin (8.3% resistant) and erythromycin (22.7 to 23.9% resistant). Particularly against MRSA (overall worldwide MRSA rate of 42.9%), which displayed high rates of resistance to various comparator agents, including erythromycin (84.1 to 84.6%), clindamycin (36.4 to 36.5%) and levofloxacin (71.2%), BC-3781 was, with a $MIC_{50/90}$ of 0.12/0.25 μg/ml (Table 2), one of the most active antibiotics among the tested compounds (Table 3).

BC-3781 was also one of the most active compounds against coagulase-negative *Staphylococcus* spp. (CoNS; $MIC_{50/90}$, 0.06/0.12 μg/ml; 97.8% inhibited at ≤1 μg/ml), which displayed slightly lower BC-3781 MICs than did *S. aureus* (Table 2). Vancomycin ($MIC_{50/90}$, 1/2 μg/ml), linezolid ($MIC_{50/90}$, 0.5/1 μg/ml), daptomycin ($MIC_{50/90}$, 0.25/0.5 μg/ml), quinupristin-dalfopristin ($MIC_{50/90}$, ≤0.5/≤0.5 μg/ml), and tigecycline ($MIC_{50/90}$, 0.12/0.25 μg/ml) exhibited near complete activity (>99% susceptibility) against these organisms, whereas the majority of CoNS strains in this study were resistant to oxacillin (MRCoNS; 72.6%), erythromycin (64.3 to 65.0%), or levofloxacin (49.2% [data not shown]; CLSI and EUCAST susceptibility or resistance criteria applied). Nineteen CoNS strains (2.2%), with 94.7% of those being MRCoNS, showed elevated BC-3781 MICs (≥2 μg/ml [Table 2]). Further characterization of these organisms is warranted to understand the mechanisms responsible for the elevated MICs.

Beta-hemolytic *Streptococcus* spp., the second most common causative organisms of ABSSSI, were very susceptible to BC-3781, with a $MIC_{50/90}$ of 0.03/0.03 μg/ml. Overall, 99.0% of beta-hemolytic streptococci were inhibited at BC-3781 concentrations of ≤0.12 μg/ml. Group A streptococci (*S. pyogenes*) were slightly more susceptible to BC-3781 (37.8% and 98.9% inhibited at ≤0.015 and ≤0.03 μg/ml, respectively) than group B streptococci (*S. agalactiae*; 9.6% and 94.3% inhibited at ≤0.015 and ≤0.03 μg/ml, respectively) (Table 2). Only 5 strains of 763 beta-hemolytic *Streptococcus* isolates exhibited elevated BC-3781 MICs (4 and 16 μg/ml) and were identified as *S. agalactiae* (Table 2). Overall, beta-hemolytic streptococci demonstrated high rates of susceptibility to most antimicrobial agents tested, except erythromycin, doxycycline, and clindamycin. Against these antibacterials, group A streptococci showed higher susceptibility rates (90.6%, 85.4%, and 95.9%, respectively [Table 3]) than group B (63.8%, 16.2%, and 78.4% to 78.7%, respectively [Table 3]) or other beta-hemolytic streptococci (79.0%, 91.4%, and 66.7%, respectively [data not shown]).

BC-3781 also showed potent activity against viridans group streptococci ($MIC_{50/90}$, 0.12/0.5 μg/ml), with all isolates being inhibited at BC-3781 concentrations of ≤2 μg/ml (Table 2). Among viridans group streptococci, 75.1% and 86.5% of strains were susceptible to penicillin according to CLSI and EUCAST breakpoint criteria, respectively (Table 3). Linezolid, vancomycin, quinupristin-dalfopristin, tigecycline, and daptomycin showed complete activity (100.0% susceptibility) against these species (Table 3).

BC-3781 also demonstrated *in vitro* activity against *E. faecium*, with 88.1% of isolates being inhibited at ≤1 μg/ml ($MIC_{50/90}$, 0.12/4 μg/ml [Tables 2 and 3]). BC-3781 MICs were lower among vancomycin-nonsusceptible strains (MIC_{50}, 0.06 μg/ml, and MIC_{90}, 0.25 μg/ml) than among vancomycin-susceptible strains (MIC_{50}, 0.12 μg/ml, and MIC_{90}, >16 μg/ml [Table 2]), as already observed in earlier studies (23). Elevated BC-3781 MICs of ≥2 μg/ml were shown against 23.7% of vancomycin-susceptible isolates, but only with 3.0% of vancomycin-nonsusceptible strains. Further investigations to clarify the mechanism responsible for these distributions are needed. Tigecycline, daptomycin, and linezolid were highly active against *E. faecium*, with susceptibility rates of ≥98.7%, while the susceptibility rates were lower for quinupristin-dalfopristin (88.2%) and vancomycin (43.3%). Similar to BC-3781, quinupristin-dalfopristin was slightly more active against vancomycin-nonsusceptible *E. faecium* ($MIC_{50/90}$, ≤0.5/1 μg/ml; 97.0% susceptible) than against vancomycin-susceptible *E. faecium* ($MIC_{50/90}$, ≤0.5/2 μg/ml; 76.7% susceptible). BC-3781 was not evaluated against *E. faecalis* since previous investigations showed no significant activity against this species (23).

S. pneumoniae was very susceptible to BC-3781 ($MIC_{50/90}$, 0.12/0.25 μg/ml), with 100.0% of strains inhibited at BC-3781 concentrations of ≤1 μg/ml (Tables 2 and 4). BC-3781 activity was not adversely affected by resistance to penicillin, since *S. pneumoniae* strains that were susceptible (61.3% according to breakpoints established by the CLSI for oral penicillin V), intermediate (17.5%), and resistant (MIC ≥ 2 μg/ml; 21.2%) to penicillin displayed identical BC-3781 $MIC_{50/90}$s (0.12/0.25 μg/ml; Table 2). Penicillin-resistant *S. pneumoniae* strains exhibited high rates of resistance to macrolides (84.6 to 85.1%), doxycycline (66.7%;

TABLE 3 Activities of BC-3781 and comparator antimicrobial agents against bacterial pathogens causing predominantly ABSSSI and other infections

Species (no. tested) and antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	CLSI %S/%R[a]	EUCAST %S/%R[a]
S. aureus (5,527)					
BC-3781	0.12	0.12	0.015–>16		
Clindamycin	≤0.25	>2	≤0.25–>2	82.0/17.8	81.4/18.0
Daptomycin	0.25	0.5	≤0.06–2	>99.9/−	>99.9/<0.1
Doxycycline	0.12	0.25	≤0.06–>8	97.9/0.4	95.3/3.1
Erythromycin	2	>4	≤0.25–>4	49.2/49.1	49.2/50.0
Levofloxacin	≤0.5	>4	≤0.5–>4	63.4/35.3	63.4/35.3
Linezolid	1	1	≤0.12–4	100.0/0.0	100.0/0.0
Oxacillin	0.5	>2	≤0.25–>2	57.1/42.9	57.1/42.9
Quinupristin-dalfopristin	≤0.5	≤0.5	≤0.5–>4	99.9/0.1	99.9/0.1
Tigecycline[b]	0.12	0.25	≤0.03–0.5	100.0/−	100.0/0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5–>4	97.7/2.3	97.7/2.0
Vancomycin	1	1	0.25–2	100.0/0.0	100.0/0.0
MRSA (2,370)					
BC-3781	0.12	0.25	0.015–4		
Clindamycin	≤0.25	>2	≤0.25–>2	63.5/36.4	63.1/36.5
Daptomycin	0.25	0.5	≤0.06–2	99.9/−	99.9/0.1
Doxycycline	0.12	1	≤0.06–>8	96.2/0.8	93.0/5.4
Erythromycin	>4	>4	≤0.25–>4	15.0/84.1	15.0/84.6
Levofloxacin	>4	>4	≤0.5–>4	26.8/71.2	26.8/71.2
Linezolid	1	1	≤0.12–2	100.0/0.0	100.0/0.0
Oxacillin	>2	>2	>2	0.0/100.0	0.0/100.0
Quinupristin-dalfopristin	≤0.5	≤0.5	≤0.5–>4	99.9/0.1	99.9/0.1
Tigecycline[b]	0.12	0.25	≤0.03–0.5	100.0/−	100.0/0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5–>4	95.8/4.2	95.8/3.8
Vancomycin	1	1	0.25–2	100.0/0.0	100.0/0.0
Beta-hemolytic *Streptococcus* spp. (763)					
BC-3781	0.03	0.03	≤0.008–16		
Clindamycin	≤0.25	>2	≤0.25–>2	87.3/12.6	87.4/12.6
Daptomycin	≤0.06	0.25	≤0.06–0.5	100.0/−	100.0/0.0
Doxycycline	0.25	8	≤0.06–>8		51.1/48.1
Erythromycin	≤0.25	>4	≤0.25–>4	76.4/22.7	76.4/22.7
Levofloxacin	≤0.5	1	≤0.5–>4	99.2/0.4	95.8/0.8
Linezolid	1	1	0.5–2	100.0/−	100.0/0.0
Penicillin	≤0.03	0.06	≤0.03–0.12	100.0/−	100.0/0.0
Quinupristin-dalfopristin	≤0.5	≤0.5	≤0.5–1	100.0/0.0	
Tigecycline[b]	≤0.03	0.06	≤0.03–0.25	100.0/−	100.0/0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5–>4		98.4/1.4
Vancomycin	0.25	0.5	≤0.12–1	100.0/−	100.0/0.0
S. pyogenes, group A streptococci (267)					
BC-3781	0.03	0.03	≤0.008–0.06		
Clindamycin	≤0.25	≤0.25	≤0.25–>2	95.9/4.1	95.9/4.1
Daptomycin	≤0.06	≤0.06	≤0.06–0.25	100.0/−	100.0/0.0
Doxycycline	0.12	8	≤0.06–>8		85.4/13.5
Erythromycin	≤0.25	≤0.25	≤0.25–>4	90.6/9.4	90.6/9.4
Levofloxacin	≤0.5	1	≤0.5–>4	98.5/0.7	91.8/1.5
Linezolid	1	1	0.5–1	100.0/−	100.0/0.0
Penicillin	≤0.03	≤0.03	≤0.03–0.12	100.0/−	100.0/0.0
Quinupristin-dalfopristin	≤0.5	≤0.5	≤0.5	100.0/0.0	
Tigecycline[b]	≤0.03	0.06	≤0.03–0.12	100.0/−	100.0/0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5–>4		97.4/2.2
Vancomycin	0.25	0.5	≤0.12–0.5	100.0/−	100.0/0.0
S. agalactiae, group B streptococci (334)					
BC-3781	0.03	0.03	≤0.008–16		
Clindamycin	≤0.25	>2	≤0.25–>2	78.4/21.3	78.7/21.3
Daptomycin	0.12	0.25	≤0.06–0.5	100.0/−	100.0/0.0
Doxycycline	8	8	≤0.06–>8		16.2/83.2

(Continued on following page)

TABLE 3 (Continued)

Species (no. tested) and antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	CLSI %S/%R[a]	EUCAST %S/%R[a]
Erythromycin	≤0.25	>4	≤0.25–>4	63.8/34.4	63.8/34.4
Levofloxacin	≤0.5	1	≤0.5–>4	99.7/0.3	97.6/0.3
Linezolid	1	1	0.5–1	100.0/–	100.0/0.0
Penicillin	≤0.03	0.06	≤0.03–0.06	100.0/–	100.0/0.0
Quinupristin-dalfopristin	≤0.5	≤0.5	≤0.5–1	100.0/0.0	
Tigecycline[b]	≤0.03	0.06	≤0.03–0.25	100.0/–	100.0/0.0
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5–>4		99.7/0.3
Vancomycin	0.5	0.5	0.25–1	100.0/–	100.0/0.0
Viridans group *Streptococcus* spp.[c] (245)					
BC-3781	0.12	0.5	≤0.008–2		
Clindamycin	≤0.25	≤0.25	≤0.25–>2	90.2/8.6	91.4/8.6
Daptomycin	0.25	0.5	≤0.06–1	100.0/–	
Doxycycline	0.25	>8	≤0.06–>8		
Erythromycin	≤0.25	>4	≤0.25–>4	51.8/46.1	
Linezolid	1	1	0.25–2	100.0/–	
Levofloxacin	1	2	≤0.5–>4	94.7/4.5	
Penicillin	0.06	0.5	≤0.03–>4	75.1/3.7	86.5/3.7
Quinupristin-dalfopristin	≤0.5	1	≤0.5–1	100.0/0.0	
Tigecycline[b]	≤0.03	0.06	≤0.03–0.12	100.0/–	
Trimethoprim-sulfamethoxazole	≤0.5	2	≤0.5–>4		
Vancomycin	0.5	0.5	≤0.12–1	100.0/–	100.0/0.0
E. faecium (536)					
BC-3781	0.12	4	0.015–>16		
Ampicillin	>8	>8	≤1–>8	5.8/94.2	5.8/94.2
Daptomycin	2	2	≤0.06–4	100.0/–	
Doxycycline	4	>8	≤0.06–>8	55.6/32.8	
Erythromycin	>4	>4	≤0.25–>4	3.4/87.1	
Levofloxacin	>4	>4	≤0.5–>4	4.7/93.7	
Linezolid	1	1	0.5–>8	98.7/0.9	99.1/0.9
Quinupristin-dalfopristin	≤0.5	2	≤0.5–>4	88.2/4.7	88.2/0.2
Tigecycline[b]	0.12	0.25	≤0.03–>4	99.1/–	99.1/0.2
Vancomycin	>16	>16	0.25–>16	43.3/56.3	43.3/56.7

[a] %S, percentage of susceptible organisms; %R, percentage of resistant organisms. Criteria were as published by the CLSI (2012) and EUCAST (2011) (25, 26).
[b] U.S. FDA breakpoints were applied (Tygacil label [27]).
[c] Includes the following species (isolate numbers): *S. anginosus* (22), *S. bovis* (9), *S. canis* (2), *S. constellatus* (6), *S. gallolyticus* (11), *S. gordonii* (5), *S. intermedius* (2), *S. milleri* (5), *S. mitis* (64), *S. mutans* (4), *S. oralis* (16), *S. parasanguinis* (7), *S. salivarius* (20), *S. sanguinis* (13), *S. vestibularis* (2), and unspecified viridans group streptococci (57).

EUCAST criteria), cefuroxime (99.7 to 100.0%), and trimethoprim-sulfamethoxazole (69.2%) but remained susceptible to the fluoroquinolones tested (levofloxacin; $MIC_{50/90}$, 1/1 μg/ml; 98.1% susceptible). Further, *S. pneumoniae* isolates resistant to macrolides (36.2 to 37.4%) or to levofloxacin (1.0 to 1.1%) were fully susceptible to BC-3781, with $MIC_{50/90}$s of 0.12/0.25 μg/ml for both subsets of organisms (data from *S. pneumoniae* subsets not shown).

BC-3781 displayed good activity against the Gram-negative respiratory pathogen *H. influenzae* ($MIC_{50/90}$, 1/2 μg/ml), and its activity was not adversely affected by β-lactamase production (23.6% of strains produced β-lactamases [Table 2]). Both β-lactamase-positive and -negative subsets exhibited high rates of susceptibility to cefuroxime (74.4 to 98.6% according to CLSI and EUCAST criteria), imipenem (99.7 to 100.0%), and moxifloxacin (99.7 to 100.0%). High susceptibility rates were also observed for azithromycin (98.3%) and clarithromycin (81.3%) according to CLSI susceptibility criteria; however, susceptibility rates were much lower (0.8% and 0.3%, respectively) when EUCAST breakpoints were applied for these compounds (Table 4).

Moraxella catarrhalis strains exhibited lower BC-3781 MICs ($MIC_{50/90}$, 0.12/0.25 μg/ml) than did *H. influenzae*. The highest BC-3781 MIC among *M. catarrhalis* strains was 0.5 μg/ml (Table 2). *M. catarrhalis* isolates displayed high rates of susceptibility to all compounds tested (93.5 to 100.0%) except for cefuroxime (72.3 to 99.6% susceptible).

When the antimicrobial activity of BC-3781 was evaluated in the geographic context (data not shown), BC-3781 was found to display excellent activity against all *S. aureus* isolates, with no apparent differences between geographic regions among MSSA isolates ($MIC_{50/90}$ for all regions except Latin America, 0.12/0.12 μg/ml; $MIC_{50/90}$ for Latin America, 0.06/0.12 μg/ml). The rate of MRSA among *S. aureus* isolates from the United States was the highest, at 51.4%. BC-3781 displayed slightly higher activity against MRSA isolates from the United States ($MIC_{50/90}$, 0.12/0.12 μg/ml) than from the rest of the world ($MIC_{50/90}$ from all other regions, 0.12/0.25 μg/ml). Among CoNS overall, and in particular methicillin-resistant CoNS (MRCoNS), isolates from the United States were again slightly more susceptible to BC-3781 ($MIC_{50/90}$ for both subsets, 0.03/0.06 μg/ml) than were isolates from the

TABLE 4 Activities of BC-3781 and comparator antimicrobial agents against bacterial pathogens causing predominantly respiratory tract infections

Organism (no. tested) and antimicrobial agent	MIC^{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	CLSI %S/%R[a]	EUCAST %S/%R[a]
S. pneumoniae (1,473)					
BC-3781	0.12	0.25	≤0.008–1		
Azithromycin	≤0.25	>4	≤0.25–>4	62.6/36.6	61.7/37.4
Ceftriaxone	≤0.06	1	≤0.06–8	91.3/1.2	78.0/1.2
Cefuroxime	≤0.12	8	≤0.12–>16	73.3/24.0	72.0/26.7
Clarithromycin	≤0.25	>32	≤0.25–>32	63.2/36.6	63.2/36.6
Doxycycline	0.25	8	≤0.06–>8		73.9/25.2
Erythromycin	≤0.25	>4	≤0.25–>4	62.8/36.2	62.8/36.2
Imipenem	≤0.12	0.5	≤0.12–1	79.6/4.4	100.0/0.0
Levofloxacin	1	1	≤0.5–>4	98.9/1.0	98.9/1.1
Linezolid	1	1	≤0.12–4	99.9/−	100.0/0.0
Moxifloxacin	≤0.5	≤0.5	≤0.5–>4	99.0/0.7	98.7/1.3
Penicillin[b]	≤0.03	4	≤0.03–>4	88.5/0.5	
Penicillin[c]	≤0.03	4	≤0.03–>4	61.3/21.2	61.3/11.5
Tigecycline[d]	≤0.03	0.06	≤0.03–0.5	99.7/−	
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5–>4	68.3/23.2	74.0/23.2
Vancomycin	0.25	0.5	≤0.12–1	100.0/−	100.0/0.0
H. influenzae (360)					
BC-3781	1	2	0.015–8		
Ampicillin	≤1	>8	≤1–>8	74.4/23.3	74.4/25.6
Azithromycin	1	2	≤0.25–>4	98.3/−	0.8[e]/1.7
Ceftriaxone	≤0.06	≤0.06	≤0.06–0.5	100.0/−	99.2/0.8
Cefuroxime	1	2	≤0.12–>16	98.6/0.6	74.4/7.5
Ciprofloxacin	≤0.03	≤0.03	≤0.03–1	100.0/−	99.7/0.3
Clarithromycin	8	16	≤0.25–>32	81.3/2.5	0.3/1.1
Doxycycline	0.5	0.5	0.12–2		98.9/0.0
Erythromycin	4	8	0.25–>8		0.3/2.8
Imipenem	0.5	1	≤0.12–4	100.0/−	99.7/0.3
Moxifloxacin	≤0.5	≤0.5	≤0.5–1	100.0/−	99.7/0.3
Tigecycline[d]	0.25	0.25	0.06–1	90.6/−	
Trimethoprim-sulfamethoxazole	≤0.5	>4	≤0.5–>4	68.1/29.4	68.1/31.7
M. catarrhalis (253)					
BC-3781	0.12	0.25	≤0.008–0.5		
Azithromycin	≤0.25	≤0.25	≤0.25–2	99.6/−	99.6/0.4
Ceftriaxone	0.25	0.5	≤0.06–1	100.0/−	100.0/0.0
Cefuroxime	1	2	≤0.12–8	99.6/0.0	72.3/1.6
Ciprofloxacin	≤0.03	≤0.03	≤0.03–0.5	100.0/−	100.0/0.0
Clarithromycin	≤0.25	≤0.25	≤0.25–4	99.6/−	98.7/0.4
Doxycycline	0.12	0.25	≤0.06–4		99.6/0.4
Erythromycin	0.25	0.25	≤0.06–4	99.6/−	93.5/0.4
Imipenem	≤0.12	≤0.12	≤0.12–0.25		100.0/0.0
Moxifloxacin	≤0.5	≤0.5	≤0.5		100.0/0.0
Penicillin	>4	>4	≤0.03–>4		
Tigecycline[d]	0.06	0.25	≤0.03–0.5		
Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5–>4	94.5/2.0	94.5/3.2

[a] %S, percentage of susceptible organisms; %R, percentage of resistant organisms. Criteria were as published by the CLSI (2012) and EUCAST (2011) (25, 26).
[b] Criteria as published by the CLSI (2012) for "Penicillin parenteral (non-meningitis)" (25).
[c] Criteria as published by the CLSI (2012) for "Penicillin (oral penicillin V)" (25).
[d] U.S. FDA breakpoints were applied (Tygacil drug information) (27).
[e] Percentage inhibited at ≤0.25 μg/ml (26).

other regions ($MIC_{50/90}$, 0.06/0.12 μg/ml). BC-3781 displayed similar good activity against vancomycin-susceptible *E. faecium* from all geographic regions (MIC_{50}, 0.12 μg/ml). The vancomycin resistance rate was significantly higher among *E. faecium* isolates from the United States (80.2%) and Latin America (64.3%) than from Europe (17.6%) and the Asia-Pacific region (19.4%). Against *H. influenzae*, BC-3781 displayed slightly greater activity against European isolates ($MIC_{50/90}$, 1/1 μg/ml) than against U.S. isolates ($MIC_{50/90}$, 1/2 μg/ml). No apparent difference between geographic regions was noted for the BC-3781 activity against *S. pneumoniae*, viridans group streptococci, or *M. catarrhalis* isolates.

In conclusion, BC-3781 demonstrated potent antibacterial *in vitro* activity against a large contemporary collection (10,035 iso-

lates) of the most prevalent bacterial pathogens causing ABSSSI and CABP, among other infections, such as staphylococci, beta-hemolytic and viridans group streptococci, *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*, including multidrug-resistant isolates. Activity was also shown against *E. faecium* (including VRE), which became an important cause of nosocomial infections such as bloodstream infections (29). In this study, BC-3781 was shown to be one of the most active antibiotics *in vitro*, and its activity was not negatively influenced by resistance to other antimicrobial classes tested, such as macrolides, lincosamides, tetracyclines, fluoroquinolones, or β-lactam antibiotics. Further, no major difference was noted for the BC-3781 activity across various geographic regions. According to the recently completed clinical phase 2 study involving patients with ABSSSI, there is good evidence that the excellent *in vitro* activity is translated into potent clinical efficacy comparable to those of already marketed antibiotics, notably vancomycin, which was used as a comparator in this clinical study (18–21). Further clinical phase 3 trials will define the role of BC-3781 for the treatment of CABP and ABSSSI as well as other types of infections.

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