

# Correlation of *In vitro* Susceptibility Testing Results for an Investigational Pleuromutilin (BC-3781) Using MIC and Disk Diffusion Methods Against Gram-Positive Pathogens

D.J. Biedenbach<sup>1</sup>, H.S. Sader<sup>1</sup>, S.D. Putnam<sup>1</sup>, S. Paukner<sup>2</sup>, R. Novak<sup>2</sup>, Z. Ivezic-Schoenfeld<sup>2</sup>, R.N. Jones<sup>1</sup>

<sup>1</sup> JMI Laboratories, North Liberty, Iowa, USA

<sup>2</sup> Nabriva Therapeutics AG, Vienna, Austria

Nabriva Therapeutics AG

Leberstrasse 20

1110 Vienna, Austria

www.nabriva.com

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## Amended Abstract

**Objectives:** To establish the intermethod agreements for testing BC-3781 using the CLSI reference broth microdilution (BMD) MIC and disk diffusion (DD) tests for staphylococci, streptococci and enterococci. This pleuromutilin agent has a unique mode of action which interferes with bacterial protein synthesis without cross-resistance (R) to other antimicrobial classes.

**Methods:** Strains with pleuromutilin-R phenotypes were included to define the epidemiologic cutoff value (ECV)/susceptibility (S) breakpoints for this agent. These strains included 11 non-wildtype (WT) staphylococcal isolates that carried either *cf* or *vga(A)*. The enterococcal population also included strains with non-WT BC-3781 MICs and vancomycin-R isolates. The overall collection was dominated by recent (2008-2009) clinical isolates of staphylococci (316), streptococci (302) and *E. faecium* (EFM; 112). Isolates were tested by BMD and DD, using a 20- $\mu$ g disk concentration and CLSI methods (M07-A8, 2009; M02-A10, 2009). Media used were Mueller-Hinton broth or agar supplemented with blood products (horse or sheep blood). Comparator agents included azithromycin, linezolid and clindamycin.

**Results:** Using an ECV MIC of  $\leq 1$  mg/l and a correlate zone diameter at  $\geq 20$  mm for the BC-3781 DD, resulted in no intermethod discords for the staphylococci. One organism was noted at a BC-3781 MIC of 1 mg/l (methicillin-S CoNS; zone diameter at 24 mm) and one strain with a MIC of 2 mg/l (methicillin-R *S. aureus*; zone diameter at 15 mm). All non-WT strains had zone diameters around the 20- $\mu$ g BC-3781 disk of 6-17 mm. The WT population of EFM contained MIC values at  $\leq 1$  mg/l and zone diameters at  $\geq 21$  mm and excellent separation between WT and non-WT enterococci was observed. All streptococci would be considered S or WT at MICs of  $\leq 1$  mg/l and zone diameters of  $\geq 20$  mm, except for two isolates in the *S. bovis* group (2 of 10 strains tested; 20%). Cross-R or -S with other agents (macrolides, oxazolidinones, lincosamides) was not demonstrated.

**Conclusions:** The proposed/tentative ECV breakpoints ( $\leq 1$  mg/l and  $\geq 20$  mm) proposed were without intermethod error for any organism group. If an intermediate category ( $\leq 2$  mg/l, 17-19 mm) was applied, intermethod correlations would also be very acceptable with extremely rare (0.4 %) minor error. This study demonstrates that accurate breakpoints can be selected and should be considered for use in the early clinical trials for BC-3781, a promising agent for the cutaneous infections.

Organism/MIC Interpretation (no.)	No. at DD Interpretation:	
	S or WT ( $\geq 20$ mm)	R or non-WT ( $\leq 19$ mm)
<i>Staphylococcus</i> spp. (316)		
S or WT ( $\leq 1$ mg/l)	305	0
R or non-WT ( $\geq 2$ mg/l)	0	11
<i>Enterococcus faecium</i> (112)		
S or WT ( $\leq 1$ mg/l)	80	0
R or non-WT ( $\geq 2$ mg/l)	0	32
<i>Streptococcus</i> spp. (302)		
S or WT ( $\leq 1$ mg/l)	300	0
R or non-WT ( $\geq 2$ mg/l)	0	2

## Introduction

BC-3781 belongs to the pleuromutilin class of antimicrobial agents which have a novel mode of action overcoming common resistance mechanisms to other antimicrobial classes. In bacteria, pleuromutilins inhibit protein synthesis by binding to the peptidyl transferase component of the 50S subunit of ribosomes. BC-3781 is highly active against Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), and Gram-negative pathogens associated with community-acquired bacterial pneumonia. After the conclusive findings of ongoing clinical trials, it is anticipated that both an intravenous and an oral dosing formulation will be available making this the first agent in its class with these properties.

A preliminary disk content study was performed to determine the appropriate disk concentration to be applied for BC-3781 against target pathogens. That pilot investigation established the 20- $\mu$ g disk as most appropriate for providing the best correlation with MIC values and to differentiate the wildtype susceptible isolates from the resistant organism population. The purpose of this investigation was to determine the intermethod correlation between the reference broth microdilution MIC values and 20- $\mu$ g disk diffusion zone diameter results for BC-3781, when tested against a large collection of target pathogens including, staphylococci, streptococci, and *Enterococcus faecium*.

## Materials and Methods

**Bacterial isolates:** A large sample of 730 recent (2008-2009) clinical isolates was tested, dominantly from the United States (USA; 52.2 %) and Europe (39.2 %). Species/genus groups included 214 strains of *S. aureus* including methicillin-susceptible *S. aureus* (MSSA; 102) and methicillin-resistant *S. aureus* (MRSA; 112 strains). MRSA isolates represented strains having various SCCmecA types and USA typed clones including USA300 community-acquired (CA) MRSA. The non-wildtype sub-population (eight strains, MIC values of 2 to  $>16$  mg/l) was selected based on elevated MIC values for other pleuromutilin compounds (retapamulin and/or tiamulin) and was included in the study to facilitate the evaluation of the epidemiologic MIC and disk diffusion categorical breakpoints for BC-3781. Coagulase-negative *Staphylococcus* spp. (CoNS; 102 strains), included methicillin-susceptible CoNS (51 strains), methicillin-resistant CoNS (51 strains) and non-wildtype isolates with regard to pleuromutilin susceptibility. *E. faecium* (112 strains) included vancomycin-susceptible (78 strains), and vancomycin-resistant (34 strains; VanA and VanB phenotypes) populations. Streptococci (302 strains) included  $\beta$ -haemolytic species (BHS; groups A, B, C, F and G; 202 strains) and viridans group streptococci ( $\geq 6$  species including *S. bovis*-group; 100 strains). Isolates were mainly derived from documented bacteremias (72.6 %) and skin and skin structure infections (21.8 %).

**Susceptibility testing:** MIC values for pathogens were determined using the reference Clinical and Laboratory Standards Institute (CLSI) broth microdilution method as described in M07-A8 (2009). Ninety-six-well frozen-form assay panels were produced by JMI Laboratories (North Liberty, Iowa, USA) and consisted of two media types, cation-adjusted Mueller-Hinton broth and cation-adjusted Mueller-Hinton broth with 2-5 % lysed horse blood (for testing of streptococci). Disk diffusion tests per the CLSI M02-A10 (2009) method using commercially prepared (Remel, Lenexa, Kansas, USA) 150 mm agar plates containing, Mueller-Hinton agar or Mueller-Hinton agar with 5 % sheep blood for streptococci. 20- $\mu$ g BC-3781 disks were provided by MAST Group (Merseyside, United Kingdom). Comparison susceptibility disks were provided by Becton-Dickinson ( Sparks, Maryland, USA) and included linezolid, azithromycin and clindamycin. Quality control (QC) ranges and interpretive criteria for both MIC and zone diameters for comparator compounds were as published in the CLSI M100-S20 (2010) document. Tested QC strains included *S. aureus* ATCC 29213, *S. aureus* ATCC 25923 (disk diffusion only), and *Streptococcus pneumoniae* ATCC 49619. The MIC and zone diameter results were compared using analysis found in CLSI M23-A3 (2008).

## Results

- BC-3781 demonstrated activity against staphylococci (MIC<sub>90</sub>, 0.12-0.25 mg/l) including methicillin-resistant strains, as well as vancomycin-susceptible and -resistant *E. faecium* (MIC<sub>90</sub>, 16 mg/l),  $\beta$ -haemolytic streptococci (MIC<sub>90</sub>, 0.06 mg/l) and viridans group streptococci (MIC<sub>90</sub>, 0.5 mg/l), as shown in Table 1.
- Among the *S. aureus* isolates that exhibited non-wildtype MIC values for the BC-3781, those that had MIC values  $>16$  mg/l harboured *cf* and those with MIC values between 2 and 8 mg/l carried the *vga(A)* gene. CoNS isolates showing elevated MIC results ( $>16$  mg/l) for BC-3781 also carried either *cf* or *vga(A)*. Staphylococci isolates recovered from human clinical species carrying these resistance determinants remain extremely rare among clinical isolates submitted to the SENTRY program.
- Figure 1 shows the BC-3781 MIC distribution that clearly illustrates a bimodal distribution of wildtype isolates (MIC range,  $\leq 0.008$ -1 mg/l) and non-wildtype organisms with MIC results at  $\geq 2$  mg/l.
- For all 730 strains (Figure 2), a clear separation of wildtype and non-wildtype organisms can be achieved. No intermethod categorical discords were observed when using a susceptible breakpoint at  $\leq 1$  mg/l and a correlate zone diameter at  $\geq 20$  mm for the 20- $\mu$ g BC-3781 disk. All non-wildtype strains had zone diameters around the BC-3781 disk of 6-17 mm.

Figure 1. Frequency of MIC distribution for BC-3781 (all isolates; 730)

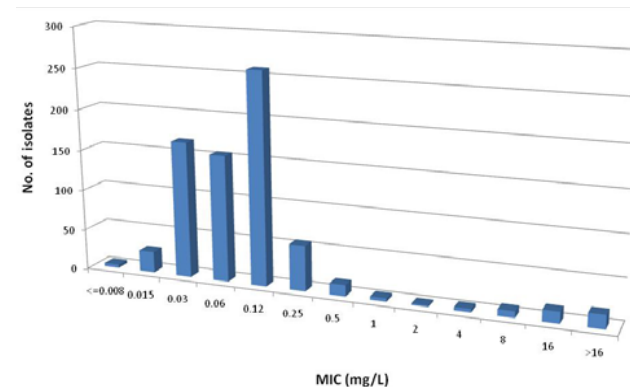


Table 1. MIC frequency distributions of BC-3781 tested against 730 strains of Gram-positive bacterial pathogens

Organism (no. tested)	no. (%) of strains inhibited at each MIC (mg/l):												
	$\leq 0.008$	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	$>16$
<i>S. aureus</i> (214)		2 (0.9)	13 (6.1)	171 (79.9)	20 (9.4) <sup>a</sup>	-	<sup>b</sup>	1 (0.5)	2 (0.9)	2 (0.9)	-	-	3 (1.4)
Oxacillin-susceptible (102)		2 (2.0)	10 (9.8)	79 (77.5)	11 (10.8)	-	<sup>b</sup>	-	-	-	-	-	-
Oxacillin-resistant (112)			3 (2.7)	92 (82.1)	9 (8.0)	-	<sup>b</sup>	1 (0.9)	2 (1.8)	2 (1.8)	-	-	3 (2.7)
Coagulase-negative staphylococci <sup>c</sup> (102)	1 (1.0)	12 (11.8)	69 (67.7)	12 (11.8)	2 (2.0)	2 (2.0)	1 (1.0) <sup>b</sup>	-	-	-	-	-	3 (2.9)
Oxacillin-susceptible (51)		7 (13.7)	37 (72.6)	4 (7.8)	-	1 (2.0)	1 (2.0) <sup>b</sup>	-	-	-	-	-	1 (2.0)
Oxacillin-resistant (51)	1 (2.0)	5 (9.8)	32 (62.8)	8 (15.7)	2 (3.9)	1 (2.0)	<sup>b</sup>	-	-	-	-	-	2 (3.9)
<i>E. faecium</i> (112)		2 (1.8)	16 (14.3)	46 (41.1)	10 (9.0)	5 (4.5)	1 (0.9)	1 (0.9)	-	6 (5.4)	14 (12.5)	-	11 (9.8)
Vancomycin-susceptible (78)		1 (1.3)	9 (11.5)	30 (38.5)	7 (9.0)	2 (2.6)	1 (1.3)	1 (1.3)	-	5 (6.4)	13 (16.7)	-	9 (11.5)
Vancomycin-resistant (34)		1 (2.9)	7 (20.6)	16 (47.1)	3 (8.8)	3 (8.8)	-	-	-	2 (2.9)	1 (2.9)	-	2 (5.9)
$\beta$ -hemolytic streptococci <sup>d</sup> (202)	1 (0.5)	21 (10.4)	140 (69.3)	37 (18.3)	3 (1.5)	-	-	-	-	-	-	-	-
Viridans streptococci group <sup>e</sup> (100)	3 (3.0)	4 (4.0)	11 (11.0)	20 (20.0)	27 (27.0)	24 (24.0)	7 (7.0)	2 (2.0)	2 (2.0)	-	-	-	-

<sup>a</sup> Bolded results represent the MIC<sub>90</sub> value.

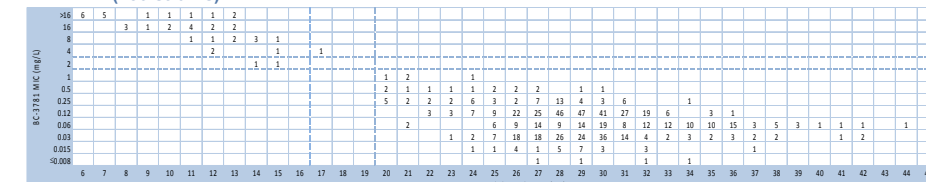
<sup>b</sup> Epidemiologic cutoff value (ECV) representing the highest MIC for the wildtype population. BC-3781 MIC results at  $\geq 2$  mg/l (*S. aureus* and CoNS) are non-wildtype values.

<sup>c</sup> Includes: *Staphylococcus auricularis* (one strain), *S. capitis* (eight strains), *S. caprae* (one strain), *S. carnosus* (one strain), *S. chromogenes* (one strain), *S. epidermidis* (53 strains), *S. haemolyticus* (five strains), *S. hominis* (14 strains), *S. lugdunensis* (eight strains), *S. schleiferi* (one strain), *S. simulans* (three strains), *S. succinus* (one strain) and *S. warneri* (four strains).

<sup>d</sup> Includes: Group A *Streptococcus* (105 strains), Group B *Streptococcus* (67 strains), Group C *Streptococcus* (nine strains), Group F *Streptococcus* (two strains), and Group G *Streptococcus* (19 strains).

<sup>e</sup> Includes: *Streptococcus anginosus* (11 strains), *S. bovis* (10 strains), *S. constellatus* (five strains), *S. gordonii* (2 two strains), *S. intermedius* (one strain), *S. mitis* (27 strains), *S. oralis* (six strains), *S. parasanguinis* (nine strains), *S. salivarius* (19 strains), *S. sanguinis* (10 strains), and *S. vestibularis* (one strain).

Figure 2. BC-3781 scattergram comparing MIC and zone diameter results for all bacterial strains<sup>a</sup> tested (730 strains)



<sup>a</sup> Includes: *Enterococcus faecium* (112 strains), *Staphylococcus aureus* (214 strains), *S. auricularis* (one strain), *S. capitis* (eight strains), *S. caprae* (one strain), *S. carnosus* (one strain), *S. chromogenes* (one strain), *S. epidermidis* (53 strains), *S. haemolyticus* (five strains), *S. hominis* (14 strains), *S. lugdunensis* (eight strains), *S. schleiferi* (one strain), *S. simulans* (three strains), *S. warneri* (four strains), *S. succinus* (one strain), *Streptococcus anginosus* (10 strains), *S. bovis* (10 strains), *S. constellatus* (five strains), *S. gordonii* (two strains), *S. intermedius* (one strain), *S. mitis* (27 strains), *S. oralis* (six strains), *S. parasanguinis* (nine strains), *S. salivarius* (19 strains), *S. sanguinis* (10 strains), *S. vestibularis* (one strain), Group A *Streptococcus* (105 strains), Group B *Streptococcus* (67 strains), Group C *Streptococcus* (nine strains), Group F *Streptococcus* (two strains), and unspiculated coagulase-negative staphylococci (one strain).

- If an intermediate category was defined at 2 mg/l and correlate zones of 17-19 mm, the calculated error rates would be: very major error (false-susceptible by disk test) = 0.0 %, major error (false-resistant by disk test) = 0.0 %, minor error (intermediate by one of the compared tests) = 3 occurrences or 0.4 % and an absolute categorical agreement of 99.6 %.

- Utilizing these breakpoints, the strains contributing to the minor intermethod errors included two MRSA and one *E. faecium*. These results are highly acceptable.

## Conclusions

- BC-3781 was very active against *S. aureus* and CoNS, including oxacillin-resistant strains, but demonstrated little potency against isolates harboring *cf* or *vga(A)*. Staphylococci isolates recovered from human clinical species carrying these resistance determinants are extremely rare.
- Correlation of the 20- $\mu$ g BC-3781 zone diameters with CLSI reference MIC values was excellent with extremely rare (0.4 %) minor intermethod error when using a susceptible breakpoint of  $\leq 1$  mg/l ( $\geq 20$  mm). These tentative criteria should be considered for the application in clinical trials.

- The investigational agent BC-3781 shows promising activity against the vast majority of prevalent Gram-positive pathogens producing skin and skin structure infections. Pending appropriate clinical trial studies, BC-3781 remains a promising adjunct for management of acute cutaneous infections and has accurate MIC and disk diffusion *in vitro* susceptibility test methods with tentative breakpoint criteria.

## Selected References

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