Determination of Disk Diffusion and MIC Quality Control Ranges for BC-3781 using CLSI Multi-Laboratory M23-A3 Study Design

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ABSTRACT

We developed disk diffusion (DD) and MIC CLSI quality control (QC) guidelines for an investigational pleuromutilin, BC-3781, against four G.1 pathogens. Methods: These studies followed the CLSI M23-A3 (2008), M02-A10 (2009), M100-S20 (2010), and M114-A1 (2009). S. aureus (SA) ATCC 29213 (DD), ATCC 25923 (MIC), H. influenzae (HI) ATCC 49247 and S. pneumoniae (SPN) ATCC 49619 were tested with 3 or more media lots and 3 control methods (DD, broth microdilution (BMD), and agar dilution) in three laboratories. For DD, sites used 3 agar lots, generating 3 DD diameters. BMD methods included pleuromutilins, macrolides, and fluoroquinolones. Results: The table lists proposed DD and MIC QC ranges for BC-3781. BMD QC values (% of total) observed were: SA ATCC 29213 at 0.12 µg/mL (98.6%), HI ATCC 49247 at 1 µg/mL (98.1%), and SPN ATCC 49619 at 0.5 µg/mL (98.9%). Inter-laboratory variability occurred with HI resulting in exclusion of one laboratory from the final analysis. Testing MIC endpoints were also noted for HI. In one laboratory, a test was executed, but the BC-3781 activity was not observed. Conclusions: Proposed BMD and DD QC ranges established here will assist clinical or reference laboratories in the testing of clinical isolates and facilitate the regulatory review process for BC-3781. Inter-laboratory discord should be very unusual events.

MATERIALS & METHODS

An eight-laboratory study was used to develop the guidelines for disk diffusion and broth microdilution MIC methods and used study design criteria as published in the Clinical and Laboratory Standards Institute (CLSI) M23-A3 document.

INTRODUCTION

The pleuromutilin class was discovered from an edible mushroom, Pleurotus mutilus, which has a unique mode of action that inhibits bacterial protein synthesis by binding to the peptidyl-transferase center of the 50S ribosomal subunit. Pleuromutilins have no cross-resistance to trimethoprim, but trimethoprim, a semi-synthetic pleuromutilin, has shown some reduced susceptibility due to gene mutations that encode the 235 FRNA.

BC-3781, a pleuromutilin from Nabriva Therapeutics AG (Vienna, Austria), is in clinical development for intravenous and/or oral treatment of acute bacterial skin and skin structure infections (ABSSSI) and community-acquired bacterial pneumonia (CABP).

RESULTS

• S. pneumoniae ATCC 49619 has a proposed MIC QC range of 0.06-0.5 µg/mL for BC-3781 (Figure 3) which includes 98.6% of results excluding one outlier laboratory (H).
• Table 1 reports DD QC ranges for BC-3781 against S. aureus ATCC 29213 showing a seven mm range (26-32 mm; 97.9%). The DD QC ranges results for BC-3781 and H. influenzae ATCC 49247 are shown in Table 2 where a range of 22-28 mm (96.0%) is proposed. The MIC portion of the study utilized frozen-form, reference broth microdilution panels provided by TREK Diagnostics (Cleveland, OH). The panels contained three medium lots of commercially-prepared Mueller-Hinton broth (Difco, Detroit, MI). Three lots of Mueller-Hinton agar were used, one lot of H. influenzae ATCC 49247 (Difco). The range for BC-3781 of 0.5 to 2 µg/mL only included 94.3% of all results after excluding one laboratory (H).

• No significant medium or disk lot variations were noted. Control agents tested provided acceptable internal controls, with each antimicrobial having >99% of all values within the CLSI published ranges.

• Inter-laboratory discord should be very unusual events.

CONCLUSIONS

• The results from this collaborative study provide the initial BC-3781 broth microdilution QC ranges for 5. S. aureus ATCC 29213 or 29523, H. influenzae ATCC 49247 and S. pneumoniae ATCC 49619.

• As this novel pleuromutilin agent progresses through human clinical trials, the susceptibility testing results can be accurately validated by concurrent quality assurance procedures listed here.

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