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Proposed susceptibility testing criteria for AZD2563, a novel long-acting oxazolidinone

T Anderegg, D Biedenbach, J Kirby, and RN Jones. The JONES Group/JMI Laboratories, North Liberty, Iowa, USA

Contact details: Dr Ronald N Jones
The Jones Group/JMI Laboratories
345 Beaver Kreek Center, Suite A
North Liberty, IA, 52317, USA
Tel: +1 319 665 3370
Fax: +1 319 665 3371
E-mail: ronald-jones@jmilabs.com

Introduction

- During the last several decades, there has been an increase in the number of Gram-positive pathogens responsible for clinical infections and consequently has led to a rise in the number of types of antimicrobial resistances found among these pathogens.
- Depending on the geographical location, it is not uncommon to see oxacillin-resistant or glycopeptide-intermediate staphylococci, vancomycin-resistant enterococci, and penicillin- and macrolide-resistant pneumococci clinical isolates.[1-3] With the continuing increase in multi-drug resistances there has been a push to develop new and novel antimicrobial agents such as the oxazolidinones.
- The oxazolidinones, which include the already licensed linezolid, work by inhibiting bacterial protein synthesis by stopping the formation of the 70S initiation complex. Time-kill studies show that the oxazolidinones are most likely to be bacteriostatic.
- AZD2563 is a novel oxazolidinone that has been shown to have activity against staphylococci, enterococci and streptococci as well as rarer Gram-positive pathogens like *Listeria* spp., *Bacillus* spp., and *Corynebacterium* spp.. Preclinical in-vivo studies confirm that AZD2563 has a favorable PK/PD profile that might support once-daily dosing.[4]
- This study summarizes the results of 1572 Gram-positive isolates tested by National Committee for Clinical Laboratory Standards (NCCLS) reference methods for broth microdilution and disk diffusion.[5-8] Agar dilutions were also performed on 120 of the Gram-positive isolates and compared with the broth microdilution results. Quality control ranges for AZD2563 were also suggested by a multi-laboratory study.

Methods

Bacterial isolates

- A total of 1572 Gram-positive bacterial isolates were tested from a collection of recent clinical and surveillance isolates.
- The staphylococci isolates included 169 oxacillin-susceptible and 144 oxacillin-resistant *Staphylococcus aureus* and 65 oxacillin-susceptible and 234 oxacillin-resistant coagulase-negative staphylococci (CoNS; represents 14 different species).
- The 305 enterococci isolates tested comprised 196 vancomycin-susceptible (VAN-S) *Enterococcus faecalis*, 11 vancomycin-resistant (VAN-R) *E. faecalis*, 43 VAN-S *Enterococcus faecium*, 39 VAN-R *E. faecium*, 4 linezolid-resistant and 2 linezolid-intermediate *E. faecium*, 2 *Enterococcus avium*, 2 *Enterococcus raffinosus*, 3 *Enterococcus casseliflavus* and 3 *Enterococcus gallinarum*.
- The streptococci isolates consisted of 305 *Streptococcus pneumoniae* (about 40% were penicillin-resistant or -intermediate), 150 streptococci viridans group and 150 β-hemolytic streptococci (includes 50 group A, 83 group B, 8 group C, 1 group F, 8 group G).
- A total of 50 other rare Gram-positive species (11 *Bacillus* spp., 12 *Listeria* spp., 13 *Corynebacterium* spp., 10 *Micrococcus* spp., 3 *Stomatococcus* spp., and 1 *Aerococcus* spp.) were also tested. QC strains obtained from the American Type Culture and Collection (ATCC; Rockville, MD), included *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 25923 and 29213, and *E. faecalis* ATCC 29212.

Susceptibility test methods

- MIC and disk diffusion test methods followed the guidelines in the NCCLS.[5] A 0.5% McFarland was made for each organism tested and was plated on Mueller-Hinton agar for staphylococci, enterococci and *Bacillus* spp. or Mueller-Hinton agar supplemented with 3-5% sheep blood for streptococcal and other rare Gram-positive species.
- For the broth microdilution panels, the 0.5% McFarland was diluted in either Mueller-Hinton broth for staphylococci, enterococci, and *Bacillus* spp. or Mueller-Hinton broth supplemented with 3-5% defibrinated horse blood for streptococcal and other rare Gram-positive species. The dilution was then plated with a semi-automatic inoculator into the dry-form microdilution panel prepared by TREK Diagnostics (Westlake, OH).
- Zone diameters for the disk diffusion tests were read with digital calipers and recorded to the nearest whole millimeter while the broth microdilution panels were read at 80% inhibition of the antibiotic in the panel.

Results

- Results of testing 1572 Gram-positive pathogens against AZD2563 by MIC and disk diffusion methods are summarized in Figures 1-6.
- Figure 1 shows complete AZD2563 activity versus *S. aureus* and Figure 2 against CoNS. All MICs were ≤2 µg/ml (proposed breakpoint) and had zones of inhibition at ≥20 mm. Only one intermethod, minor error was noted for *S. aureus* (0.3%).

Figure 1. Scattergram comparing the AZD2563 MIC and zone diameters for *S. aureus*

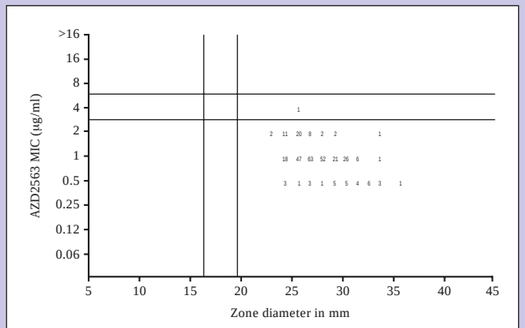
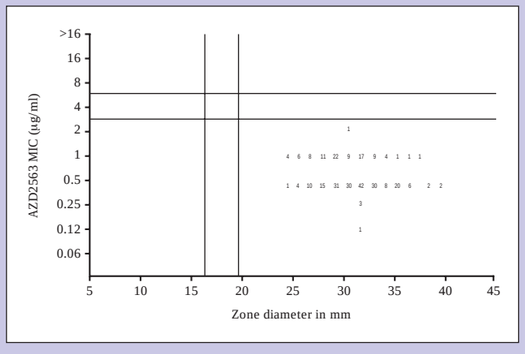
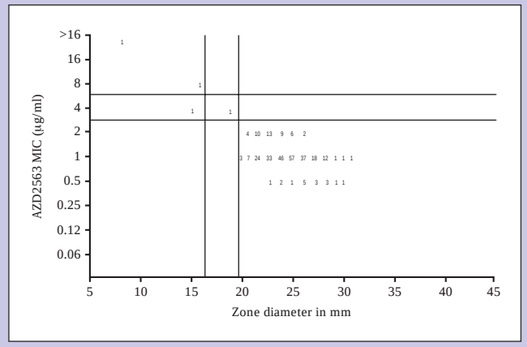


Figure 2. Scattergram comparing the AZD2563 MIC and zone diameters for CoNS



- Figure 3 shows the scattergram for enterococcal AZD2563 MIC and disk diffusion comparisons. This collection included linezolid-resistant strains that also were resistant (MIC, ≥8 µg/ml) to AZD2563. Applying the proposed susceptible interpretive criteria, again only one minor error (0.3%) occurred.

Figure 3. Scattergram comparing the AZD2563 MIC and zone diameters for enterococci



- Figures 4 and 5 illustrate the AZD2563 scattergrams for the streptococcal collections of strains. AZD2563 was very active against these isolates and at proposed breakpoints, no intermethod errors were detected.

Figure 4. Scattergram comparing the AZD2563 MIC and zone diameters for *S. pneumoniae*

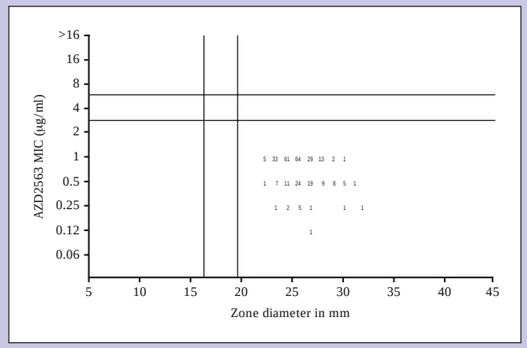
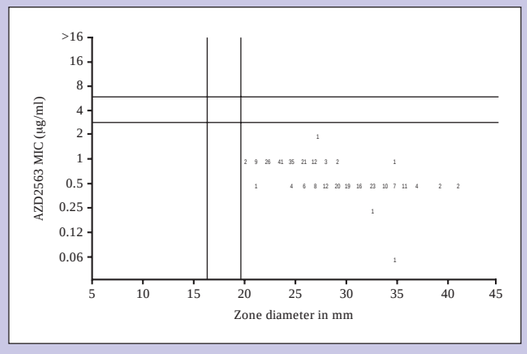
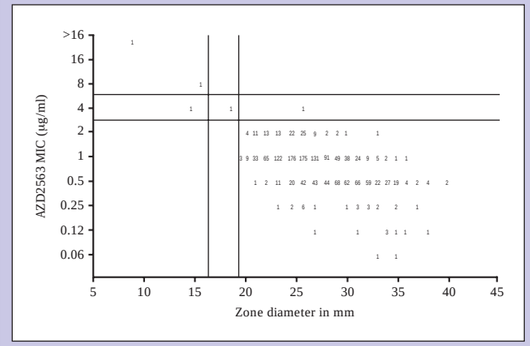


Figure 5. Scattergram comparing the AZD2563 MIC and zone diameters for other streptococci



- Figure 6 shows the entire experiment (1572 strains) for the AZD2563 in-vitro susceptibility tests. The tentative MIC breakpoints justified by PK/PD testing (≤2 µg/ml) clearly separates susceptible and resistant populations (G2576U mutations) of organisms.

Figure 6. Scattergram summarizing all 1572 Gram-positive organisms tested against AZD2563 by NCCLS reference (M7-A5) and standardized (M2-A7) methods



- The overall intermethod error rates were:
  - very major - 0.0%
  - major - 0.0%
  - minor - 0.1% (1 *S. aureus* and 1 *E. faecium*)
  - absolute categorical agreement at 99.9%.

Conclusions

- The testing of this new oxazolidinone, AZD2563, by NCCLS methods yields reliable and accurate results for the following tentative breakpoints when tested against all Gram-positive species:
  – susceptible at ≤2 µg/ml (≥20 mm)
  – resistant at ≥8 µg/ml (≤16 mm).

References

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