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# Anti-streptococcal activity of AZD2563, a new oxazolidinone antimicrobial agent

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AZD 2563

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## Revised abstract

**Background:** Resistance emerging among several Gram-positive species has led to the development of several novel antimicrobial classes. Linezolid (LZD) was the initial clinically applied agent from the oxazolidinone class and AZD2563, a new agent, is described here.

**Methods:** A total of 525 streptococcal strains were tested and included: β-hemolytic (βhS; 266 strains) and viridans group (VgS; 256 strains) species. NCCLS broth microdilution and agar methods were utilized, testing eight comparison drugs including LZD, quinupristin-dalfopristin (Q-D), vancomycin (VAN), macrolides, clindamycin (CLI), penicillin (PEN), gentamicin (GEN) and levofloxacin (LEVX).

**Results:** PEN and macrolide resistance among VgS was 41 and 50%, respectively. $MIC_{50}$/$MIC_{90}$/% susceptible (≤ 2 µg/mL for AZD2563) results were: for βhS-AZD2563 (1/2/100%), LZD (1/2/100%), Q-D (0.25/0.25/100%), VAN (0.25/0.5/100%), and LEVX (0.5/1/99%); for VgS-AZD2563 (0.5/1/100%), LZD (1/1/100%), Q-D (0.5/1/99%), VAN (0.5/0.5/100%) and LEVX (1/1/98%). AZD2563 and LZD MIC modes were 0.5 or 1 µg/mL and 1 µg/mL, respectively. Killing curves showed modest concentration dependence, but static action. An enhanced bactericidal result was observed when AZD2563 was combined with GEN. LZD activity was also static. VAN combinations demonstrated indifference. No significant inter-method (agar versus broth) differences were noted for AZD2563 or LZD MIC results (100% ± one $\log_2$ dilution).

**Conclusions:** AZD2563 activity screening against these non-pneumococcal streptococci showed slightly greater potency for AZD2563 when compared to LZD. All AZD2563 MIC values were at ≤ 2 µg/mL, and this new oxazolidinone had features consistent with the oxazolidinone class. These results warrant further AZD2563 applications *in vivo* and studies against other Gram-positive species.

## Introduction

- Antimicrobial resistance among *Streptococcus* spp. has emerged regularly during the last 10–15 years, with resistance to penicillins and macrolides developing among all streptococcal species, including some β-hemolytic strains.
- As with the pneumococci, the viridans group species have acquired high levels of β-lactam resistance by altered PEN binding protein targets.
- These threats to contemporary infection chemotherapy have fostered the development of new classes of antimicrobial agents – everninomicins, oxazolidinones, streptogramins, lipopeptides, etc. – that have been focused toward Gram-positive pathogens, and modified structures of quinolones and cephems have expanded the potential range of use for these classes.
- This study examines the *in vitro* characteristics of AZD2563, a new oxazolidinone, using reference susceptibility test methods against *Streptococcus* spp. other than *Streptococcus pneumoniae*.

## Methods

### Antimicrobial agents

- AZD2563 was provided by AstraZeneca (Macclesfield, UK). Comparator drugs tested: LZD, Q-D, VAN, erythromycin (ERY), CLI, GEN, LEVX, and PEN, were obtained from the US manufacturers.

### Bacterial strains

- The 525 strains tested were utilized from various surveillance collections, amassed in the last 12 months by the JMI Laboratories, and included: viridans group streptococci (259 strains) and β-hemolytic streptococci (266 strains; five serogroups).
- Two or more laboratories identified each strain, all of which represented isolates derived from recent clinical cases.

### Susceptibility tests

- All strains were tested using the reference methods described for the broth microdilution technique by the NCCLS (2000).
- Broth microdilution trays were prepared in frozen form by TREK Diagnostics (Westlake, OH) and stored at -80°C until used. Antimicrobials were diluted in appropriate media for processing streptococci (Mueller–Hinton with 5% lysed horse blood). The inoculum used was 5 x $10^5$ cfu/mL, confirmed by regular colony counts.
- Quality control (QC) strains included fastidious (*S. pneumoniae* ATCC 49619) and rapid growing (*S. aureus* ATCC 29213) species. All QC results were within control ranges published by the NCCLS or product manufacturers.

## Results

**Table 1. *In vitro* activity of AZD2563 compared with that of eight other antimicrobial agents tested against 259 strains of viridans group streptococci.**

| Antimicrobial agent | $MIC_{50}$ (µg/mL) | $MIC_{90}$ (µg/mL) | Range (µg/mL) | % susceptible |
|---|---|---|---|---|
| AZD2563 | 0.5 | 1 | 0.06–2 | 100.0* |
| LZD | 1 | 1 | 0.12–2 | 100.0 |
| Q-D | 0.5 | 1 | 0.12–2 | 99.2 |
| VAN | 0.5 | 0.5 | 0.12–1 | 100.0 |
| ERY | 0.25 | 8 | ≤ 0.008–>16 | 50.0 |
| CLI | ≤ 0.5 | ≤ 0.5 | ≤ 0.5–>4 | 90.4 |
| GEN | 4 | > 8 | ≤ 0.25–>8 | 62.7 |
| LEVX | 1 | 1 | 0.06–>8 | 98.1 |
| PEN | 0.12 | 4 | ≤ 0.004–>8 | 59.2 |

*Value based on ≤ 2 µg/mL as no standard yet defined.

- Table 1 shows that the ($MIC_{90}$) activity of AZD2563 against the viridans group streptococci (1 µg/mL) was comparable to that for LZD (1 µg/mL), Q-D (1 µg/mL), VAN (0.5 µg/mL) and LEVX (1 µg/mL). These organisms had a resistance rate of 50.0% and 40.8% for ERY and PEN, respectively.

**Table 2. *In vitro* activity of AZD2563 compared with that of eight other antimicrobial agents tested against 266 strains of β-hemolytic streptococci.**

| Antimicrobial agent | $MIC_{50}$ (µg/mL) | $MIC_{90}$ (µg/mL) | Range (µg/mL) | % susceptible |
|---|---|---|---|---|
| AZD2563 | 1 | 2 | 0.25–2 | 100.0* |
| LZD | 1 | 2 | 0.5–2 | 100.0 |
| Q-D | 0.25 | 0.25 | 0.06–1 | 100.0 |
| VAN | 0.25 | 0.5 | 0.12–1 | 100.0 |
| ERY | 0.06 | 2 | ≤ 0.008–>16 | 83.1 |
| CLI | ≤ 0.5 | ≤ 0.5 | ≤ 0.5–>4 | 93.6 |
| GEN | > 8 | > 8 | ≤ 0.25–>8 | 11.3 |
| LEVX | 0.5 | 1 | 0.12–>8 | 99.2 |
| PEN | 0.03 | 0.06 | ≤ 0.004–0.5 | 98.5 |

*Value based on ≤ 2 µg/mL as no standard yet defined.

- Table 2 illustrates AZD2563 potency against five groups of β-hemolytic streptococci (266 strains). AZD2563 and LZD had equal activity at an $MIC_{90}$ of 2 µg/mL. The organism collection was modestly resistant to macrolides (16.9%) and CLI (6.4%).

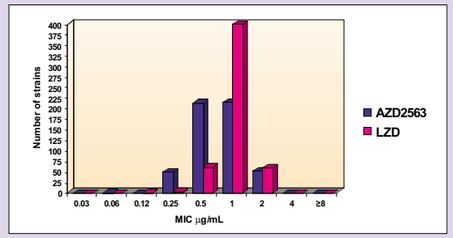
**Table 3. *In vitro* activity of AZD2563 compared with that of eight other antimicrobial agents tested against 525 strains of streptococci other than *S. pneumoniae*.**

| Antimicrobial agent | $MIC_{50}$ (µg/mL) | $MIC_{90}$ (µg/mL) | Range (µg/mL) | % susceptible |
|---|---|---|---|---|
| AZD2563 | 1 | 2 | 0.06–2 | 100.0* |
| LZD | 1 | 2 | 0.12–2 | 100.0 |
| Q-D | 0.25 | 2 | 0.06–2 | 99.6 |
| VAN | 0.5 | 0.5 | 0.12–1 | 100.0 |
| ERY | 0.06 | 4 | ≤ 0.008–>16 | 66.7 |
| CLI | ≤ 0.5 | ≤ 0.5 | ≤ 0.5–>4 | 92.0 |
| GEN | 8 | > 8 | ≤ 0.25–>8 | 36.7 |
| LEVX | 0.5 | 1 | 0.06–>8 | 98.7 |
| PEN | 0.06 | 0.5 | ≤ 0.004–8 | 79.1 |

*Value based on ≤ 2 µg/mL as no standard yet defined.

- The results for the entire collection of 525 strains (Table 3, Fig 1) illustrate the highly comparable endpoints ($MIC_{90}$) for both oxazolidinones (2 µg/mL), with the maximum MIC observed at 2 µg/mL.
- The widest spectra of activity were documented for the oxazolidinones (100.0%), VAN (100.0%), Q-D (99.6%) and LEVX (98.7%).

**Fig 1. MIC distributions for AZD2563 and LZD tested against 525 strains of non-pneumococcal *Streptococcus* spp.**




- Fig 1 demonstrates the potency advantage for AZD2563 versus LZD.

**Table 4. Comparisons of broth microdilution MICs to those of the agar dilution method (NCCLS)* for two oxazolidinones tested against 120 isolates of Gram-positive cocci.**

| Antimicrobial/organism (no. tested) | Broth/agar dilution MIC ratio | | | | |
|---|---|---|---|---|---|
| | ≤ 0.25 | 0.5 | 1 | 2 | ≥ 4 |
| **AZD2563** | | | | | |
| *S. pneumoniae* (30) | 0 | 0 | 30 | 0 | 0 |
| Other streptococci (30) | 0 | 13 | 17 | 0 | 0 |
| Staphylococci (30) | 0 | 0 | 30 | 0 | 0 |
| Enterococci (30) | 0 | 0 | 30 | 0 | 0 |
| Total (120)† | 0 | 13† | 107† | 0† | 0 |
| **LZD** | | | | | |
| *S. pneumoniae* (30) | 0 | 0 | 29 | 1 | 0 |
| Other streptococci (30) | 0 | 15 | 12 | 3 | 0 |
| Staphylococci (30) | 0 | 0 | 30 | 0 | 0 |
| Enterococci (30) | 0 | 0 | 30 | 0 | 0 |
| Total (120) | 0 | 15‡ | 101‡ | 4‡ | 0 |

*Testing was by reference NCCLS methods.
†All of the AZD2563 results were within the ± one $\log_2$ dilution range and 89.2% were at a ratio of 1.
‡All of the control LZD results were within the ± one $\log_2$ dilution range and 84.2% were at a ratio of 1.

- Table 4 shows the comparison of AZD2563 MICs determined by reference NCCLS agar and broth microdilution methods. A slightly lower MIC was observed with the broth-based methods for both oxazolidinones when testing non-pneumococcal streptococci, but all results were within ± one $\log_2$ dilution step.
- Killing curves showed modest concentration dependence, but static action (data not shown).
- An enhanced bactericidal result was observed when AZD2563 was combined with GEN, but VAN combinations demonstrated indifference (data not shown).

## Conclusions

- AZD2563 is a new oxazolidinone with activity ($MIC_{90}$) of 1–2 µg/mL against all non-pneumococcal streptococcal species.
- AZD2563 activity was at least equal to LZD.
- Reference methods used (agar and broth) gave comparable results, with broth MICs being slightly lower.
- AZD2563 *in vitro* characteristics against the streptococci tested were promising, and indicate that AZD2563 could be an alternative agent for the treatment of Gram-positive cocci including those resistant to other antimicrobial classes.

## Selected references

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