A total of 93 clinical strains of enterococci were included in this study. Fifty strains were susceptible to vancomycin, i.e., MIC <4 µg/ml; of the 50 and Enterococcus faecalis. Forty-three strains were resistant to vancomycin, are recognized on the GAIN list (Generating Antibiotic Incentives Now) of pathogens for which drug candidates would obtain priority review for approval by the FDA. Intrinsically less susceptible to antibiotics and the combination of high-level resistance to ampicillin, vancomycin, and teicoplanin (VanA enterococci) are resistant to intermediate level vancomycin (MIC 64-256 µg/ml) and susceptible to teicoplanin (MIC <0.5 µg/ml). VanD enterococci (E. faecium) are resistant to intermediate level vancomycin (MIC 64-256 µg/ml) and teicoplanin (MIC <1 µg/ml). However, the MIC of solithromycin for 100% of the enterococci tested (fifty vancomycin-susceptible strains and forty-three vancomycin-resistant strains) was 0.5 µg/ml. However, the MIC of solithromycin for 100% of the enterococci tested (fifty vancomycin-susceptible strains and forty-three vancomycin-resistant strains) was 1 µg/ml.

Conclusion: New antibiotics are needed for the treatment of enterococcal infections. Solithromycin is being studied in Phase 3 studies in CAP and should be studied in other indications including enterococcal infections.

Solithromycin

**Materials and Methods**

**Drugs:** MICs of the following drugs were determined: azithromycin (Sigma, Lot# E446421/3v), ceftriaxone (Cerus, Lot# FMD-CET-028), deoxyn (Sigma, Lot#B035050), gentamicin (Sigma, Lot#K7501531/17v), linezolid (Sigma, Lot#028667/40), penicillin (Sigma, Lot#BCR75607W), cefoxitin (Sigma, Lot#E4511546), meropenem (Merck Sharp Dohme, Lot# MDL-503-20), and vancomycin (Sigma, Lot# 102801745). Drugs were diluted and studied for testing per recommendations in CLSI M100-S22 (5).

**Organisms:** Clinical strains were obtained from blood cultures of patients submitted to the Clinical Microbiology Laboratories at the University of Rochester Medical Center, Rochester, NY, in 2011, 2012 and 2013.

**MIC Determinations:** Prior to testing, clinical strains were sub-cultured onto Tryptic Soy Agar with 5% sheep blood for 18-20 hours at 35 °C in ambient air. MICs of SOLI (Solithromycin) and comparator drugs for clinical strains were determined by broth microdilution methodology in carbon-adjusted Mueller Hinton Broth as recommended by CLSI M7-A8 (6). Organisms suspended from fresh agar cultures were adjusted to yield a final test inoculum of 5 x 10^8 CFU/ml. Inoculated broth microdilution trays were incubated for 24 hours at 35 °C in ambient air.

The MIC endpoints for drugs were read as the concentrations at which no growth, or a significant reduction of growth, was observed by visual inspection after incubation. The performance of test reagents (including drug potency) and equipment, and test personnel was monitored using accurate quality control organisms as recommended by CLSI M100-S22 (5). MICs of all drugs for quality control organisms tested in parallel with test organisms were within acceptable ranges as recommended by CLSI M100-S22 (5).

**Characterization of Vancomycin Resistant in Clinical Strains:** Vancomycin-resistant enterococci can be characterized on the basis of phenotype which includes resistance to vancomycin and presence or absence of resistance to teicoplanin (7). VanA enterococci are resistant to high level vancomycin (MIC ≥100 µg/ml) and resistant to high level teicoplanin (MIC >8 µg/ml). VanB enterococci are resistant to vancomycin over a wide range (MIC ≥4-1000 µg/ml) and are susceptible to teicoplanin (MIC ≤2 µg/ml). VanC enterococci (E. faecium and E. casseliflavus) are intrinsically resistant to vancomycin (MIC ≥32 µg/ml) and susceptible to teicoplanin (MIC <2 µg/ml). VanD enterococci (E. faecium) are resistant to intermediate level vancomycin (MIC 64-256 µg/ml) and teicoplanin (MIC ≤2 µg/ml).

**Results**

A total of 93 clinical strains of enterococci were included in this study. Fifty strains were susceptible to vancomycin, i.e., MIC ≤2 µg/ml of the 50 vancomycin-susceptible strains. 41 were E. faecalis, 9 were E. faecium and 1 was E. gallinarum. Forty-three strains were resistant to vancomycin, i.e., MIC ≥32 µg/ml of the 43 vancomycin-resistant strains, 38 were E. faecium, 3 were E. faecalis, 1 was E. casseliflavus and 1 was E. raffinosus. Phenotypic resistance to vancomycin in the 43 vancomycin-resistant strains was categorized according to levels of vancomycin resistance and teicoplanin resistance or susceptibility revealed the following: 37 VanA, E. faecium and 1 VanA, E. faecalis, 2 VanB, E. faecalis and 1 VanB, E. faecium, 1 VanC, E. casseliflavus and 1 VanD, E. raffinosus.

For purposes of data analysis, results for vancomycin-susceptible strains are presented separately from results for vancomycin-resistant strains. The range of MICs for 50% of strains and MICs for 90% of strains for all drugs against vancomycin-susceptible strains (41 strains of E. faecalis) were 0.004-1 µg/ml and 1 µg/ml, respectively.

The range of MICs and MIC 90 of solithromycin for vancomycin-susceptible strains were 0.002-1 µg/ml and 0.5 µg/ml, respectively. Solithromycin was the most active compound tested against vancomycin-susceptible Enterococcus.

**Conclusion:** Solithromycin was more active against vancomycin-susceptible enterococci (MIC_50 0.03 µg/ml) than it was against vancomycin-resistant strains (MIC_50 0.5 µg/ml). However, the MIC of solithromycin for 100% of the enterococci tested (fifty vancomycin-susceptible strains and forty-three vancomycin-resistant strains) was 1 µg/ml. In addition, solithromycin was more active than penicillin, vancomycin, cefazolin, azithromycin, and teicoplanin against these strains and was the most active compound tested. This work confirms a previous study (4).

Solithromycin could be bacteriostatic or bactericidal sometimes depending on the growth conditions. However, it is more potent in vitro than linezolid, which is also a protein synthesis inhibitor and is also bacteriostatic. Solithromycin could also be an alternative for combination against enterococci, especially for long-term use as it is being developed in oral and intravenous formulations. Further studies are planned to be conducted in animal models.