Bacterial Strains & Inoculum Preparation
50 unique strains of M. pneumoniae were included in 14 macrolide-susceptible clinical isolates obtained between 1980 and 2013 from several states in the USA and from Shanghai, China. Acquired macrolide-resistant isolates were obtained between 2009 and 2013 from several states in the USA and from Shanghai, China. All 5 drugs were tested.

MIC Determination
MIC values were obtained in a powder form of known purity from their respective manufacturers or from commercial sources. Drugs were dissolved according to each manufacturer’s instructions and were in accordance with CLSI guidelines. An appropriate amount of each powered drug was weighed to 10 mg of a stock solution allowing for the color change of each agent. If not used immediately, stock solutions were frozen at -4°C.

Broth Minimum Inhibition Assay (CLSI Method)
Each well was tested in two-fold dilutions of medium, vol, media, and growth controls in accordance with CLSI guidelines. Microtiter trays were incubated aerobically at 37°C in a rotating incubator for 24 hours. Color change from pink to yellow indicative of glucose metabolism. A final determination of antibiotic activity of lefamulin and comparators was confirmed by preparing 6 serial dilutions of the inoculum (0.1 ml inoculum in 0.9 ml of SP4 broth) antibiotic beyond the MIC (1:100 dilution). A subculture onto SP4 agar was prepared by preparing 6 serial dilutions of the inoculum (0.1 ml inoculum in 0.9 ml of SP4 broth) antibiotic beyond the MIC (1:100 dilution). A subculture onto SP4 agar was prepared to verify organism viability. Subcultures were incubated at 37°C and agar plates were incubated at 37°C in air plus 5% CO₂ until the growth control showed change from pink to yellow. A subculture onto SP4 agar was prepared to verify organism viability. Subcultures were incubated at 37°C and agar plates were incubated in an anaerobic chamber for 24 hours.

MIC Breakpoints
MICs were recorded as the lowest concentration of antimicrobial inhibiting color change in SP4 broth. Results were considered valid if the control agar plate for organism tested regardless of resistance phenotype, inhibiting all 50 strains at concentrations of ≤ 0.004 µg/mL. Lefamulin demonstrated bactericidal activity against all 8 strains tested regardless of resistance phenotype. In conclusion, lefamulin is a promising agent for infections caused by M. pneumoniae in the respiratory tract.

RESULTS
• Lefamulin had potent activity against all M. pneumoniae isolates (n=50) with all MIC values ≤ 0.004 µg/mL. MIC50 and MIC90 subgroups were ≤ 0.002 mg/L and 0.008 mg/L, respectively (Table 1).
• This activity was comparable to the activity of azithromycin when tested against the MS subset of organisms (MICrange: 0.0005-0.001 mg/L). Results were considered valid if the control agar plate for organism tested regardless of resistance phenotype, inhibiting all 50 strains at concentrations of ≤ 0.004 µg/mL. Lefamulin demonstrated bactericidal activity against all 8 strains tested regardless of resistance phenotype. In conclusion, lefamulin is a promising agent for infections caused by M. pneumoniae in the respiratory tract.

REFERENCES