

## D-134. Assessment of Intrinsic Antibacterial Activity Using Fluorescence Microscopy.

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**Background:** Conventional MIC methods use fixed time (typically 24 h) incubation of an antimicrobial agent and target organism in culture medium, after which visible bacterial growth is assessed. An MIC does not reveal time-dependent antimicrobial effects: these can be assessed by quantitative recovery of bacteria from the test mixture at intervals during incubation. The present study augmented this approach, using fluorescent nucleic acid stains to directly visualize bacterial cell viability and morphology at each time point.

**Methods:** Antibacterial activities of cefotaxime, ciprofloxacin and gentamicin were assessed against *Escherichia coli* and *Staphylococcus aureus* strains. MICs were determined, then strains were exposed to 1x and 10xMIC of each compound in Mueller Hinton Broth with shaking incubation at 37°C. At t=0, 1, 2, 6 and 24 h, colony counts were performed on each mixture and cell viability was studied using differential live/dead fluorescent staining. **Results:** *E. coli*: Cefotaxime at 10xMIC reduced colony count by  $10^3$  after 6 h and by  $10^4$  after 24 h. This was reflected by direct viable cell counts, which also revealed cell elongation after 2 h exposure. At the MIC, cefotaxime produced a temporary decrease in both colony count and cell viability after 6 h, both of which increased again overnight. Ciprofloxacin at 1x and 10xMIC produced comparable reductions in colony count of  $>10^3$  over 24 h, but cell viability remained at ~ 50%. *S. aureus*: Gentamicin at 10xMIC reduced colony count by  $10^3$  after 6 h, but direct viable cell count was only reduced by <50% at this point. Subsequently, colony counts recovered 100-fold after 24 h.

**Conclusions:** Direct viable cell counts using fluorescence microscopy did not always provide equivalent data to colony counts for characterization of antimicrobial activity. Evidence of “viable but non-culturable” bacterial cells was found after gentamicin and ciprofloxacin exposure, but gentamicin-exposed *S. aureus* cells regained cultivability after overnight incubation.