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Abstract (poster session)

Decreased *Clostridium difficile* spore viability following oritavancin exposure

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Objectives: We previously noted enhanced activity of oritavancin (ORI) compared to vancomycin (V) against *Clostridium difficile* (CD) in vitro. We determined whether ORI and V affected CD spore germination directly, or subsequent outgrowth. **Methods:** (i) CD PCR ribotype 027, 001 and 078 spores (~10⁶ cfu/ml) were incubated in Braziers broth containing no antibiotic, or 10mg/L ORI or V. Phase contrast microscopy (PCM) was used to monitor germination and outgrowth over 48 h, and cell suspensions were washed and plated onto Brazier's cycloserine-cefoxitin egg yolk agar with lysozyme (CCEYL) agar for total viable counts and spore viability testing. (ii) CD germination and outgrowth were monitored by PCM for 24 h after addition of 100mg/L ORI or V at different stages of CD PCR ribotype 027, 001 and 106 growth (0, 1, 2 & 4h). **Results:** (i) Spores incubated with ORI were able to convert from phase bright (PB; quiescent) to phase dark (PD; germinated), but were unable to outgrow into vegetative cells (VCs) unlike unchallenged controls. Germination of spores incubated with V was also reduced compared to controls (49-58% vs 63-73% VCs respectively), but to a lesser extent than with ORI (19-23%). Fewer (~2-6 log₁₀ cfu/ml decrease) VCs and spores were recovered after washing from ORI exposed samples than from controls or V-exposed samples, although there was inter-strain variation. For PCR ribotype 078, no spores or VCs could be recovered after ORI exposure. (ii) Effects of V and ORI addition at different stages of growth were similar. Controls showed conversion from PB to PD spores within 2h for all strains, and from PD to VCs within 2-4h incubation. CD cells exposed to ORI and V at T=0 or 1h converted from PB to PD spores, but not to VCs. CD exposed to ORI and V at 2 & 4h following outgrowth showed reduced VCs counts thereafter. Differences were noted between control germination curves of different CD strains in both experiments. **Conclusions:** ORI and V treated spores were able to convert from phase bright to phase dark, but were unable to form VCs. This indicates that ORI, like V, acts post-spore germination. Poor recovery of ORI-exposed spores vs V-exposed spores, despite washing provides further supportive evidence that ORI adheres to *C. difficile* spores, preventing effective outgrowth. Inter-strain variation in recovery of ORI-exposed CD spores on CCEYL may indicate that differences in spore surface properties between strains affects ORI binding.