P0799
Poster Session III
C. difficile: antimicrobial susceptibility and treatment

PERSISTENCE AND REMOVAL OF FIDAXOMICIN FROM C. DIFFICILE SPORES, AND IMPACT ON SPORE RECOVERY

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Objectives:

We have previously shown that fidaxomicin activity persists on *C. difficile* spores, and may prevent subsequent spore recovery. The nature of the interaction between fidaxomicin and the spore coat is poorly understood. Here we investigate the effect of different methods to remove fidaxomicin on spore persistence and the down-stream detection of spores.

Methods:

C. difficile ribotype 001 spores were exposed to solutions containing 200 mg/L fidaxomicin or no antimicrobial for 30 mins. 1 mL aliquots were centrifuged and the resultant spore pellet washed 3 times in 500 µl of PBS, CTAB, DMSO or EtOH. After the final w ash, all samples were re-suspended in PBS, transferred to fresh tubes and rinsed twice in PBS to remove any solvent effects. Fidaxomicin persistence on washed spores was determined by large-plate bioassay using *K. rhizophila* indicator organism. Zones of inhibition of washed spore preps were compared with a fidaxomicin calibration series. Recovery of washed spores was determined by culture on Brazier's CCEYL agar following serial dilution to 10^{-7}.

Results:

Fidaxomicin activity persisted on PBS (~4 mg/L) and CTAB (~16 mg/L) washed spores. However, no residual activity was detected on DMSO or EtOH w ashed spores (Figure 1), indicating that these solvents dissociated bound fidaxomicin. Inhibition of spore recovery correlated with fidaxomicin persistence as measured by bioassay. No inhibition of spore recovery (compared with non-fidaxomicin-exposed control) was observed following DMSO or EtOH wash, but recovery was inhibited following PBS and CTAB washes. The inhibition of spore recovery was affected by dilution. Inhibition of PBS washed spore recovery w as only evident in neat samples. Inhibition of CTAB washed spore recovery was evident in neat, 1:10 and 1:100 dilutions (comparable to non-washed, fidaxomicin-exposed-spores) but not in higher dilutions.

Conclusion:

Extent of fidaxomcin persistence on washed *C. difficile* spores is dependent on the choice of wash solution, which may relate to solvent polarity. DMSO and EtOH removed spore-associated fidaxomicin activity, whereas PBS and CTAB did not. This work provides further evidence of the existence and nature of fidaxomicin persistence on *C. difficile* spores.

Figure 1 - Persistance of fidaxomicin acitivity on washed C. difficile spores