**INTRODUCTION**

Clostridioides difficile is the most common cause of healthcare-associated diarrhea, in 2011 the burden was estimated at 453,000 infections and 29,000 deaths with 80.8% of the infections being considered healthcare-associated and 24.2% of outbreak occurring during hospitalization (Lessa et al., 2015). Most patients remain asymptomatic after infection but often continue to shed C. difficile in their stool. In the healthcare environment this can pose a concern since C. difficile spores can persist in the environment for long periods of time. Contamination of the environment, especially high-touch surfaces in patient bathrooms and rooms can be sources of infection.

In a recent study to determine bioburden on high-touch healthcare environmental surfaces, C. difficile was recovered culture from 1.7% of total samples (Dunn 2014). While the range was too low G. et al (2014) the infection rate of C. difficile is unknown and the presence of spores in a patient’s environment after clearing is a concern. Current methods to quickly identify C. difficile involve use of PCR: however, PCR only identify the presence of C. difficile and is not capable of distinguishing if the DNA is from live or dead cells. This distinction is critical when assessing the threat to a patient’s health. Since healthcare is not capable of distinguishing if the DNA is from live or dead cells.

**METHODS**

1. A known spore stock (10^8/10^7 spores/mL) was serial diluted down to 10^1 spores/mL.
2. Three different inoculum matrices were evaluated at three different inoculated spore concentrations (~10^4, 10^2, and 10^1 spores/mL):
   - Spores only in Phosphate buffered saline with 0.02% Tween 80® (PBST)
   - Spores mixed with 20% ATS
   - Spores mixed with 20% ATS and 10% C. difficile spores
3. Two different inoculation methods (1. direct addition of the spore suspension and 2. spiking of a flocked swab) and 3 different volumes (10µL, 100µL, and 1mL) were evaluated.
4. There was one negative control (PBST) for each media, inoculation type, and inoculum level.
5. C. difficile spores + ATS was added to broth, the inoculum suspension was a log10 lower than expected and thus only 10^1 spores/mL inoculum is directly comparable to the other sample matrices.
6. Sample results were recorded at 24, 48, and 72 hours.

**RESULTS**

- **Positive Broth Tubes at 24 h for 10 µL Inoculation Volume (n = 10)**
  - CDBB-TC: 3 positives
  - CCFB: 2 positives
  - CCMB-TAL: 3 positives

- **Positive Broth Tubes at 24 h for 100 µL Inoculation Volume (n = 10)**
  - CDBB-TC: 3 positives
  - CCFB: 2 positives
  - CCMB-TAL: 3 positives

**CONCLUSIONS**

- **At 24 h:**
  - Spores only: positive results varied but overall CCFB and CCMB-TAL had more positive broth tubes than CDBB-TC.
  - Spores + ATS+E_fecalis: CDFB-TC was equivalent to or higher than CDFB and CCMB-TAL when a spiked swab was used to inoculate the broth.
  - When 1 mL eluent was placed in broth, the anaerobically incubated media were more often positive.
  - By 48 h almost all samples were positive for all three broths, inoculation methods, volumes and concentrations.
  - At 72 h all samples (except possible true negatives) were positive with a LOD of 10^5 spores/mL.
  - Media, inoculation method, and volume of sample are equal within 72 h no matter what the sample matrix was and thus which method to use to test environmental samples may be more dependent on lab capacity.
  - The option to do environmental sampling and broth culture is available to labs with and without an anaerobe chamber.

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