Isolation and Characterization of Clostridioides difficile spores from contaminated “single-use” surgical gowns.

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Introduction

Clostridium difficile is a Gram-positive, spore-forming anaerobe that comprise either toxigenic or non-toxigenic strains. Toxigenic C. difficile usually possess Toxin A (TcdA) and Toxin B (TcdB); although some strains can be variant [1]. C. difficile can exist in vegetative bacterial form or as metabolically dormant, highly disinfectant resistant spores.

Sporules can attach to clinical surfaces for months via structures such as the exosporium and are implicated in organism transmission [2]. C. difficile infection (CDI) is the leading cause of antibiotic-and healthcare-associated diarrhoea globally [3]. One reason for high incidence rates is due to the adherence of spores to surgical gowns which can ‘trap’ the spores and transfer them to stainless steel surfaces and hospital floor vinyl [4].

Aim of Study

To determine whether C. difficile can be isolated from “used” hospital gowns. Any presumptive C. difficile will also be identified.

Suspected C. difficile NO2 growth Growth on CCFA Odour Chartreuse under UV Gram Stain Produces spores
6 + + + + + +
7 + + + + + +
8 + + + + + +
9 + + + + + +
12 + + + + + +
18 + + + + + +
19 + + + + + +
20 + + + + + +

Table 1: Phenotypic identification of the suspected C. difficile colonies extracted from contaminated gowns.

Methods

- “Used” hospital gowns were suspended in 15ml degassed Brain Heart Infusion broth + 0.1% Sodium Taurocholate (BHIST) and vortexed to dislodge bacteria. Broth was incubated for 3 days anaerobically (37°C) and then 4°C o/n to encourage sporulation. C. difficile strains DS1813, DS1748, R2021 were positive controls
- Broth was centrifuged at x500g and resuspended in sterile BHIST. 100ul was spotted onto C. difficile selective agar and incubated anaerobically 37°C, 48h. Resulting colonies were analysed for characteristic C. difficile colony morphology (Fig 1). Presumptive colonies were streaked onto degassed BHIST agar at 37°C, 48h.
- CDIFF QUIK CHECK COMPLETE® (TECHLAB) was used to identify C. difficile from presumptive colonies. Each sample was examined for the production of glutamate dehydrogenase (GDH) and toxins A/B (n=2).
- DNA Extraction: 0.2g Chelex 100 Resin was added to 4ml sterile deionized water and vortexed. 100µl was added to a loop of C. difficile in an Eppendorf, and heated to >90°C/12 min. Samples were centrifuged at x8000g/12min and supernatant was used as DNA extract. All samples were analysed using 16S-235 and toxin PCR

Results

- 23 colonies were isolated from the gowns. After phenotypic analysis (Table 1) only 8 isolates showed characteristic C. difficile growth. CDIFF QUIK CHECK COMPLETE® (TECHLAB) analysis confirmed these results (Figure ).
- After PCR analysis via 16S-235rRNA inter-spacer region [6] and toxin A and toxin B PCR [7], this was reduced to 5 final samples which are presumed to be C. difficile.
- These samples are currently undergoing final confirmatory testing at the National Anaerobic Reference unit, Cardiff, UK.

Conclusions and Future Work

- It was concluded that the surgical gowns from Rideout Hospital, USA, were contaminated with C. difficile.
- Future work will include conformational testing of all presumptive C. difficile strains at the National Anaerobic reference Unit, Cardiff, UK.
- Implications include gowns acting as fomites and the need to dispose of gowns immediately after use to prevent spore transfer
- Biocide testing will establish if current infection control measures and disinfectants (sporicides) are working.

References