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# Isolation and characterization of Clostridioides difficile spores from contaminated single-used surgical gowns

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# 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.

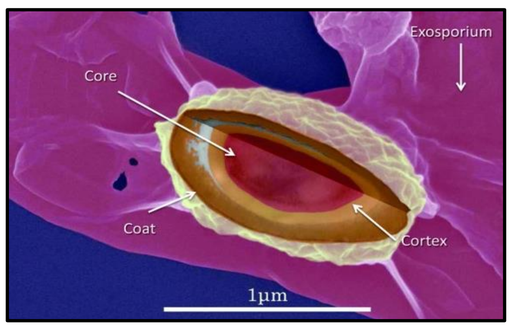


Figure 1: Spore surface of a *C. difficile* clinical isolate. SEM image [1]

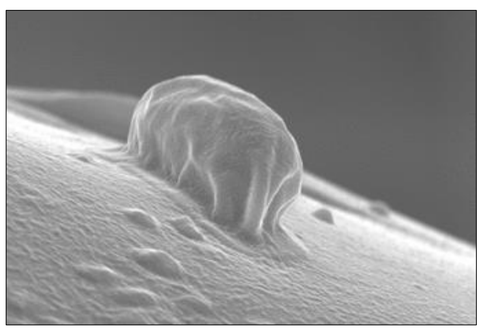

Figure 2: Spore surface of a *C. difficile* clinical isolate. SEM image [4]

Spores 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* number | 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.

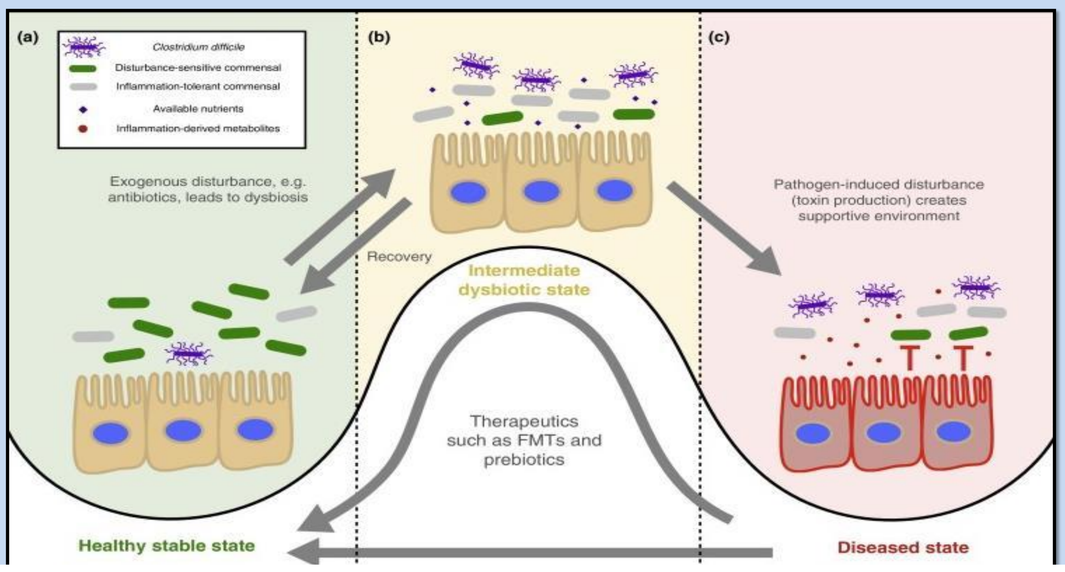


Figure 3: Life cycle of *C. difficile* in the gut. (A) toxigenic *C. difficile* may be present in healthy human microbiota.(B) dysbiosis occurs (e.g. due to antibiotics) which allows for colonisation of *C. difficile*. (C) *C. difficile* toxins lead to inflammation of the distal gut and thus *C. difficile* mediated disease [5]

## 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.
- This work aims to limit the prevalence of spores and spread of infection in hospitals.

## Methods

- "Used" hospital gowns were suspended in 15ml degassed Brain Heart Infusion broth + 0.1% Sodium Taurocholate (BHIST) and vortex mixed to dislodge bacteria. Broth was incubated for 3d 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 x5000g and resuspended in sterile BHIST. 100ul was spread onto *C. difficile* selective agar and incubated anaerobically 37°C, 48h. Resulting colonies were analysed for characteristic *C. difficile* colony morphology (Fig ). Presumptive colonies were streaked onto degassed BHIST agar at 37°C, 48h.

Figure 4: Colonies of *C. difficile* CD630 on degassed BHI agar.

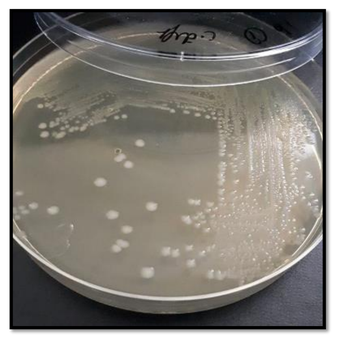

Figure 5 : Suspected *C. difficile* on BHIST agar.

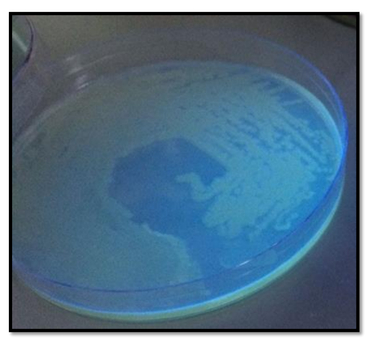

Figure 6 : Suspected *C. difficile* under UV light. *Chartreuse*

- CDIFF QUIK CHEK 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-23S 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 CHEK COMPLETE® (TECHLAB) analysis confirmed these results (Figure ).
- After PCR analysis via 16S-23SrRNA 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.


Figures A, B and C: A shows a full positive result on the C.DIFF QUIK CHEK; B shows a negative result and C shows a result positive for the antigen but negative for toxin A/B.

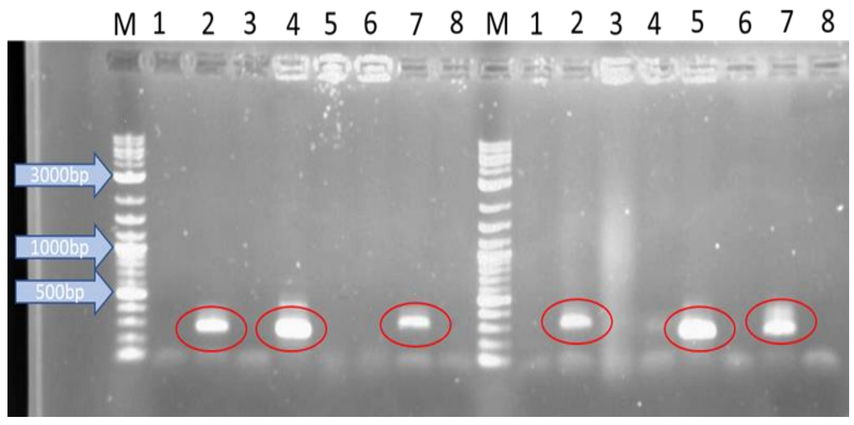


Figure: Detection of 16s-23s rRNA intergenic spacer region. M represents 1Kb Plus DNA Ladder marker, Lane 1 = control strain DS1813, lane 2 = control strain R20291, lane 3 = control strain DS1748, lane 4 = sample 7, lane 5 = sample 8, lane 6 = sample 9, lane 7 = Non toxigenic control DS1684 and lane 8 = negative control. Samples are shown in Table 1.

## References

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