# *Clostridium difficile* in a South African Hospital Setting: Incidence and Antibiotic Resistance

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## Introduction

• Little is known with regard to the epidemiology of *Clostridium difficile* infections in South Africa and there is no published data concerning the antibiotic resistance profiles of local strains

• Testing for *C. difficile* is carried out in the majority of South African healthcare laboratories using standard enzyme immunoassay-based (EI-based) procedures that detect the presence of *C. difficile* toxins A and B in a stool sample

• EI-based tests are suspected to lack both sensitivity and specificity and are only done on request by the attending physician

## Aim

• The goal of this pilot study was to evaluate various test procedures for the detection of *C. difficile* in stool samples compared to the reference standard of selective culture and to obtain epidemiological data that can be used to begin to track the infectious spread of *C. difficile* in the South African hospital environment

## Methods

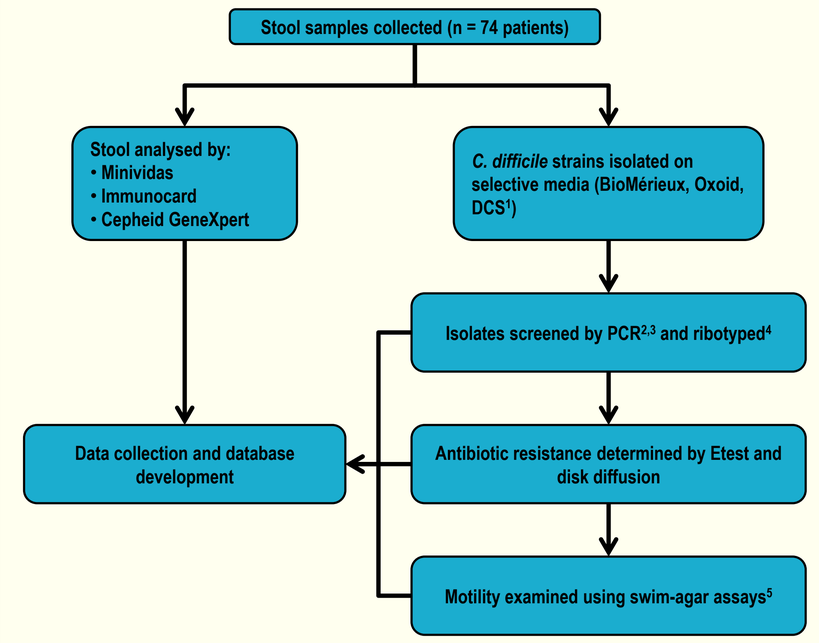


Figure 1: Schematic flow diagram of sample treatment

## Results

### *C. difficile* clinical isolates

• There was no significant relationship between *C. difficile* infection and patient age ($p > 0.05$)
• None of the isolates possessed the binary toxin genes, *cdtA* and *cdtB,* and no ribotype 027 strains were recorded
• Almost half (7/18) isolates belonged to the ribotype 017 group, many of which showed resistance to several of the antibiotics tested
• No isolates were resistant to vancomycin or metronidazole, although some ribotype 017 strains displayed a tendency toward decreased susceptibility to metronidazole (MIC = 2-6 µg/ml)

Table 2 – Summary of PCR results and antibiotic resistance profiles for *C. difficile* clinical isolates

| | | | | | ETest[1] | Disk Diffusion[1] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Specimen No | Age | PCR | Ribotype | Motility | Met MIC (µg/ml) | Van (5µg) | Ery (15µg) | Clin (2µg) | Cip (5µg) | Mox (5µg) | Ceft (30µg) | Cefo (5ug) |
| SCH5806553 | 33 | A+B+Bi- | | - | 0.125 | S | R | R | R | S | I | R |
| SCH5806999 | 58 | A-B+Bi- | 017 | - | 2 | S | R | R | R | R | R | R |
| SCH5819316 | 53 | A+B+Bi- | 014/020 | + | 0.19 | S | S | R | I | S | I | R |
| SCH5824693 | 62 | A-B+Bi- | 017 | + | 0.5 | S | R | R | R | R | R | R |
| SCH5824696 | 40 | A+B+Bi- | 015 | + | 0.25 | S | S | R | R | S | R | R |
| SCH5842529 | 58 | A-B+Bi- | 017 | + | 0.125 | S | R | R | R | R | R | R |
| SCH5842753 | 11 | A+B+Bi- | | - | 0.38 | S | S | R | R | S | R | R |
| SCH5845567 | 77 | A-B+Bi- | 017 | + | 0.38 | S | R | R | R | R | R | R |
| SCH5845556 | 44 | A-B+Bi- | 017 | + | 0.19 | S | S | R | R | R | R | R |
| SCH5858033 | 77 | A+B+Bi- | | + | 0.19 | S | S | R | R | S | I | R |
| SCH5865760 | 87 | A-B+Bi- | 017 | + | 6 | S | R | R | R | S | I | R |
| SCH5868162 | 40 | A+B+Bi- | 014/020 | + | 0.125 | S | S | R | R | S | I | R |
| SCH5864722 | 79 | A-B+Bi- | 017 | + | 4 | S | R | R | R | R | I | R |
| SCH5910574 | 24 | A-B-Bi- | | + | 0.19 | S | S | R | R | S | S | R |
| SCH5909173 | 33 | A+B+Bi- | 015 | + | 0.125 | S | S | R | R | S | I | R |
| SCH5941858 | 45 | A+B+Bi- | | + | 0.25 | S | R | R | R | S | I | R |
| SCH5972702 | 15 | A+B+Bi- | | - | 0.19 | S | R | R | R | S | I | R |
| SCH5901795 | 33 | A+B+Bi- | 015 | + | 0.19 | S | S | R | R | S | I | R |

[1]Key: (Met) Metronidazole, (Van) Vancomycin, (Ery) Erythromycin, (Clin) Clindomycin, (Cip) Ciprofloxacin, (Mox) Moxifloxacin, (Ceft) Ceftriaxone, (Cefo) Cefotaxime

### Evaluation of diagnostic tests

Table 1: Diagnostic sensitivity and specificity of testing procedures

| | Immunocard | | MiniVidas | | GeneXpert | |
|---|---|---|---|---|---|---|
| | pos | neg | pos | neg | pos | neg |
| Culture +ve† | 5 | 12 | 8 | 9 | 14 | 3 |
| Culture -ve‡ | 2 | 55 | 3 | 54 | 4 | 53 |
| Sensitivity (%)* | 29.4 | | 47.1 | | 82.4 | |
| Specificity (%)** | 96.5 | | 94.7 | | 93.0 | |

†Number of culture positive samples that tested positive and negative for *C.difficile* using each of the clinical diagnostic tests
‡Number of culture negative samples that tested positive and negative for *C. difficile* using each of the clinical diagnostic tests
*Sensitivity of each test calculated by dividing the total number of culture positive, test positive samples by the total number of culture positive samples
**Specificity of each test calculated by dividing the total number of culture negative, test negative samples by the total number of culture negative samples

### Motility assays

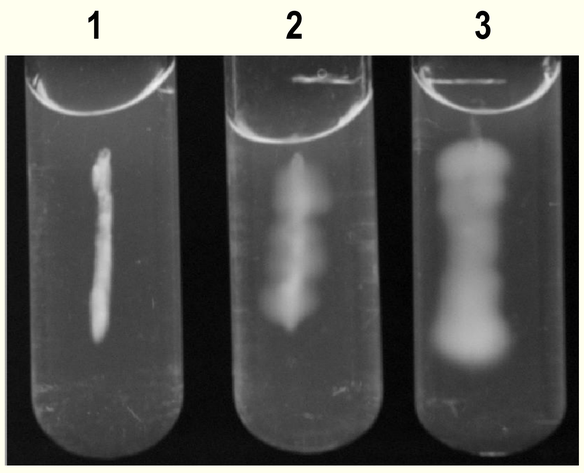


Figure 2: Determination of strain motility. (1) SCH5806553 (non-motile), (2) SCH5845567 (motile), (3) SU1149 (highly motile)

## Conclusions

• The EI-based tests currently used in the clinical diagnosis of *C. difficile* are not sensitive enough to be effective in diagnosing *C. difficile* infection in the South African context
• GeneXpert showed good correlation with culture and good specificity
• Ribotype 017 strains predominated and displayed resistance to multiple antibiotics, many of which are used currently at Groote Schuur Hospital
• There was no relationship between strain ribotype and motility suggesting that motility is a strain specific phenotype

### References

1. Darkoh, C., H.L. DuPont, and H.B. Kaplan. 2012. J Clin Microbiol **49**:4219-4224.
2. Lemee, L., A. Dhalluin, S. Testelin, *et al.* 2004. J Clin Microbiol **42**:5710-5714.
3. Stubbs, S., M. Rupnik, M. Gibert, *et al.* 2000. FEMS Microbiol Lett **186**:307-312.
4. Stubbs, S., J. Brazier, G.L. O'Neill, *et al.* 1999. J Clin Microbiol **37**:461-463.
5. Tasteyre, A., M.C. Barc, T. Karjalainen, *et al.* 2000. Microbiology **146**(part 4):957-966.
6. Reynolds, C.B., J.E. Emerson, L. de la Riva, *et al.* 2011. PLoS Pathog **7**:e1002024.

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