High prevalence of *Clostridium difficile* in soil, mulch and lawn samples from Western Australian (WA) hospitals

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**Background**

- *Clostridium difficile* is a Gram positive, anaerobic, spore forming enteropathogen that is responsible for causing a wide spectrum of diseases from mild diarrhoea to toxic megacolon and fulminant colitis.
- To prevent outbreaks, interventions commonly implemented include patient isolation, environmental cleaning and disinfection in hospitals.
- *Clostridium difficile* infections (CDI) have been encountered in increasing numbers in patients without previous healthcare contact, despite being a well established hospital pathogen.
- Community-acquired CDI (CA-CDI) represents a significant proportion of CDI diagnosed in WA hospitals. In 2011, CA-CDI cases comprised 26% of all hospital-acquired (HA) CDI cases in Australia and the rates have increased consistently since then.
- Non-human reservoirs of CDI include animals, food and the environment. Examples of common environmental sources are contaminated soil, lawn, mulch, sand, compost and water.

**Objectives**

This study reports the prevalence of *C. difficile* in the immediate outdoor environment of different hospitals, providing further insight into potential sources of CA-CDI.

**Materials and methods**

- In 2018, a total of 145 samples consisting of soil, mulch, lawn and sand were collected from the outdoor surroundings of four different old and new hospitals in Perth, WA.
- All samples were incubated in *C. difficile* selective enrichment broth (BHIB supplemented with cycloserine and cefoxitin) for at least 5 days, followed by alcohol shock and on culture on *C. difficile* selective media (ChromID, bioMérieux).
- Any putative *C. difficile* colonies were sub-cultured onto pre-reduced blood agar to be identified based on the distinctive horse dung odour, ground-glass colony morphology and the characteristic chartreuse fluorescence under long-wave UV light (360nm).
- PCR toxin gene profiling and ribotyping was performed, and PCR ribotypes (RTs) identified by comparing banding patterns to our reference library.

**Results**

- *C. difficile* was isolated from 90 out of 145 (62.0%) samples (Table 1).
- Overall, 19.8% (20/101) of the isolates were toxigenic (A+B+CDT--), n=18; A+B+CDT-, n=1; A+B+CDT, n=1 (as a novel strain).
- A total of 24 RTs were novel non-toxigenic strains followed by UK 010 (A-B+CDT--), UK 014/020 (A+B+CDT--), QX189 (A-B+CDT--), QX298 (A-B+CDT--), QX284 (A-B+CDT--) and UK 051 (A+B+CDT--) (Figure 1).
- 24 different previously identified PCR ribotypes were found (Table 1).
- UK 017 (A-B+CDT-), a strain that is endemic to the Asia-Pacific region, was also found from a newly laid lawn around one of the older hospitals.
- UK 027 and UK 078 (hypervarient strains) were not isolated.

**Discussion and conclusions**

- This is the first study to identify *C. difficile* in outdoor environment of various hospitals.
- The presence of highly diverse strains in hospitalised patients suggests the possibility of patients acquiring infections from sources/reservoirs external to the hospital (Table 1).
- Even though *C. difficile* is commonly found ubiquitously in soil, the presence of toxigenic strains especially UK 010/020, UK 013 and the Asian strain of much interest, UK 017, is of concern.
- It is possible that individuals from nearby Asian countries such as Thailand could have imported the strain on the soles of their shoes. Another plausible means of transmission might be vegetables imported from Asia.
- Despite this, the actual risk of disease remains unclear.
- Prominent ribotypes identified were UK 014/020 and 010 (Figure 1).
- UK 014/020 is a common strain identified among both CA and HA cases and the high prevalence of this particular strain in this study (especially from the entrances of the hospitals) further suggests the movement of *C. difficile* from community sources into the hospital setting (Figure 1).
- Figure 2 illustrates the common sources of CA-CDI in WA.
- CDI is fast becoming a One Health issue that requires a comprehensive approach to improving and safeguarding the health of human beings, animals and the environment (external and internal to hospitals).
- It is prudent for healthcare professionals, policy makers, veterinarians and researchers to be aware of the changing epidemiology of CDI.

**Future studies**

- Checking for antimicrobial resistance in the *C. difficile* isolates and conducting comparative genomic studies (such as WGS, MLSST, SNV analysis) to determine strain relatedness, between patients with CDI admitted in the respective hospitals included in this study.
- Examining the prevalence of *C. difficile* on shoes of healthcare staff in old and new WA hospitals.

**Table 1. Prevalence and toxin profile of *C. difficile* isolates**

<table>
<thead>
<tr>
<th>Main collection sites</th>
<th>No. of samples and prevalence (%)</th>
<th>Toxin profiles of known RTs isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Front of hospital (main and emergency entrances)</td>
<td>48 (33.2%)</td>
<td>A-B+CDT-</td>
</tr>
<tr>
<td>Back of hospital</td>
<td>35 (24.1%)</td>
<td>7</td>
</tr>
<tr>
<td>Sides of hospital</td>
<td>23 (15.9%)</td>
<td>6</td>
</tr>
<tr>
<td>Parks and patient sitting areas</td>
<td>30 (20.7%)</td>
<td>13</td>
</tr>
<tr>
<td>Carparks</td>
<td>9 (6.2%)</td>
<td>5</td>
</tr>
</tbody>
</table>

**Figure 1. PCR ribotypes of *C. difficile* in external surroundings of four WA hospitals**

**Figure 2. Common sources of CA-CDI transmission**