**CAN SHOE SOLES CONTRIBUTE TO C. DIFFICILE DISSEMINATION IN HOSPITAL ENVIRONMENT?**

Sandra Janezic¹,², Ines Blazevic², David Eyre³,⁴, Aleksander Kocuvan², Maja Rupnik¹,²

¹National Laboratory for Health, Environment and Food, Maribor, Slovenia, ²University of Maribor, Faculty of Medicine, Maribor, Slovenia, ³Nuffield Department of Medicine, University of Oxford, Oxford, UK, ⁴Big Data Institute, University of Oxford, Oxford, UK

**INTRODUCTION AND AIM**

Shoe soles are shown to have a dense pathogenic load and shoes can be a reservoir and a vector that spreads bacteria through different environments, posing threat to human infections (Rashid et al., 2016). Two previous community studies demonstrated high rate of *C. difficile* contamination of shoe soles (up to 43 %) and high prevalence of PCR ribotypes that are commonly isolated from humans, animals and different natural environments (Janezic et al., 2018, Alam et al., 2014).

The aim of this study was to evaluate the presence of *C. difficile* on shoe soles to explore their possible contribution to the dissemination of *C. difficile* spores in hospital environment.

**METHODS**

**SAMPLING**

Single large teaching hospital - different departments. Shoe soles swabs were taken from health-care staff (physicians, nurses, cleaning staff and paramedics), medical students and a visitor. Sampling was performed in May, July and August in 2014.

**ISOLATION OF C. difficile**

Shoe soles: surfaces were swabbed with pre-moistened Polystyrene™ sponges (MWE) and placed into 50 ml sterile centrifuge tubes. Sponges were incubated in enrichment medium BHI supplemented with 0.1 % L-cysteine, 0.5% yeast extract, 0.1% taurocholic acid sodium salt and *C. difficile* selective supplement (SR0096E, Oxoid). After enrichment and ethanol shock *C. difficile* was isolated on selective plates chromID™ *C. difficile* (BioMerieux).

Clinical samples: *C. difficile* isolated from stool samples from the same hospital in routine diagnostic laboratory were included in the comparison.

All isolates were identified with MALDI-TOF MS (Bruker).

**TYPING**

**WHOLE GENOME SEQUENCING AND SNV ANALYSIS**

For whole genome sequencing, bacterial DNA was isolated with QIAamp DNA Mini Kit (Qiagen) following protocol for isolation of DNA from Gram-positive bacteria. Paired-end libraries were generated with Nextera XT Library preparation kit (Illumina) following manufacturer’s protocol. Libraries were sequenced on MiSeq (Illumina) with 600-cycle MiSeq Reagent Kit v3, aiming for the theoretical coverage of 100x. SNV analysis was performed as described previously (De Silva et al., 2016).

**RESULTS**

- **Almost two-thirds (62 %, 34 out of 55)** of shoe swabs were **positive** for *C. difficile*. (Table 1)
- **Eleven different PCR ribotypes** were identified. Most common were PCR ribotypes 010 (n=8), 027 (n=8), 014 (n=7), 018 (n=5) which are also PCR ribotypes that are commonly isolated from patients.

<table>
<thead>
<tr>
<th>Department</th>
<th># of swabs tested</th>
<th># of C. difficile positive swabs</th>
<th># of PCR ribotypes</th>
<th>PCR ribotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dept. of Gastroenterology</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>010, 012, 027, 025</td>
</tr>
<tr>
<td>Dept. of Infectious Disease and Febrile Conditions</td>
<td>27</td>
<td>19</td>
<td>7</td>
<td>010, 012, 014, 027, 018, 012, 022</td>
</tr>
<tr>
<td>Dept. of Internal Intensive Med.</td>
<td>11</td>
<td>6</td>
<td>4</td>
<td>010, 014, 027, 176</td>
</tr>
<tr>
<td>Dept. of Cardiology</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>002, 014, 023, 027</td>
</tr>
</tbody>
</table>

- **Fourteen shoe isolates and 39 matching (temporal and spatial) clinical isolates of PCR ribotypes 027 (n=31), 014/020 (n=15) and 002 (n=7)** were selected for whole genome sequencing and high-resolution single nucleotide variant (SNV) analysis to determine their relatedness on a whole genome level.

- **With core genome SNV analysis 12 out of 14 (86 %) of shoe sole isolates had ≤2 SNVs difference from one or more human clinical isolates which is consistent with recent transmission** (Figure 1).

- Genetically related pairs of clinical and shoe strains were isolated from 3 days to 20 weeks apart and were found both within the same departments as well as in different departments.

**CONCLUSION**

We demonstrated a high rate of *C. difficile* contamination of shoe soles in hospital environment and high prevalence of PCR ribotypes that are commonly isolated from humans. This study also shows that shoe soles could play an important part in the dissemination of *C. difficile* spores in hospital environment.

**REFERENCES**


