Carriage of *Clostridium difficile* by wild animal species related to pig farms in Spain

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**INTRODUCTION**

The epidemiology of *Clostridium difficile* has changed. Reports of severe community-acquired *C. difficile* infections are becoming more numerous and thus it is now considered a potential emerging community-associated pathogen. Many of these epidemiological changes have been attributed to the emergence of the hypervirulent 027 and 078 ribotypes.

Several potential reservoirs were reported, i.e. companion, food and wild animals. In addition, contaminated meat, raw vegetables and water may also play an important role as sources for human infection. Thus, concerns about its zoonotic and foodborne potential are rising since there are indirect evidences of between-species transmission of some *C. difficile* types, particularly the 078 ribotype strains.

Little is known about the potential role of pets as reservoirs for *C. difficile*. A recent study showed the isolation of virulent *C. difficile* ribotypes from vermin species from a pig farm in the Netherlands and urban rats in Canada.

The main aim of this study was to determine whether some pet species commonly present in pig farms (rats, mice, voles and pigeons) and fattening pigs from the same farms were carriers of toxigenic *C. difficile* strains. In addition, the possible role of pet species as potential sources of infection for humans, pigs and other animal species and environments will be evaluated through the characterization of the isolated strains.

**MATERIAL AND METHODS**

**North East Spain Convenience Sampling**

29 fattening pig farms

**Intestinal content and/or environmental faces from:**

- Rats
- Mice
- Pigeons
- Voles
- Pigs

**Bacterial isolation**

- Pre-enriched in broth
- Ethyl alcohol
- Plated on blood agar and *C. difficile* agar

**DNA extraction**

- Boiling

**Conventional PCR**

- *tpi* gene
- *tcdA* gene
- *tcdB* gene
- *cdtA/ cdB* genes

**RESULTS**

**A total of 211 samples from 29 different farms were collected and tested for the presence of *C. difficile*** (Table 1)

- In 27 (12.8%) samples the bacterium was isolated. Most of the positive samples belonged to pig species or their droppings (81.5%)
- Of the 27 isolates, 25 (92.6%) had *tcdA* (repeating and non-repeating portions) and *tcdB* genes. From these strains 18 (72%) had also both *cdtA* and *cdtB* genes.
- Two of the isolates (from PHRT) presented high similarity with strain M129 (ribotype 078) and another one (RT) with strain 63P42 (ribotype 005) (oral presentation OPI. Martin-Uriell, I.)

**REFERENCES**


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**CONCLUSIONS**

- This study suggests that rodents may be an important source of toxigenic antimicrobial-resistant *C. difficile* farm contamination through their faeces, which could potentially contaminate other animals and people.
- Pigeons may also play a role in the maintenance of *C. difficile* within and among farm environments.
- The isolation of *C. difficile* from pest species from fattening pig farms may represent a risk factor for the further contamination of the food chain. Farm-to-fork studies should be implemented to establish all critical stages where meat could get contaminated with this bacterium. Prevention strategies could then be designed and applied to avoid contamination.
- To prove the relationship among isolates studied in this work, a detailed molecular comparison of the strains will be required.
- Given the isolation of similar *C. difficile* ribotypes to those isolated from humans, further studies on the genetic relationship between farm environment and humans *C. difficile* strains are required to help in the understanding of the epidemiology of this microorganism.

**Table 1.** Type and number of samples analysed for the presence of *C. difficile*.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>No. of farms</th>
<th>No. of positive farms (%)</th>
<th>No. of samples</th>
<th>No. of positive samples (%)</th>
<th>No. of MDR strains (%)</th>
<th>No. of toxigenic strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>7</td>
<td>1 (14.5)</td>
<td>26</td>
<td>5 (19.2)</td>
<td>0</td>
<td>5 (100)</td>
</tr>
<tr>
<td>PHRT</td>
<td>18</td>
<td>6 (33.3)</td>
<td>30</td>
<td>9 (30)</td>
<td>3 (33.3)</td>
<td>7 (77.8)</td>
</tr>
<tr>
<td>R</td>
<td>10</td>
<td>3 (30)</td>
<td>53</td>
<td>6 (11.2)</td>
<td>1 (16.7)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>PHR</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P</td>
<td>2</td>
<td>1 (50)</td>
<td>11</td>
<td>2 (18.2)</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td>PHCN</td>
<td>27</td>
<td>1 (3.7)</td>
<td>77</td>
<td>5 (6.5)</td>
<td>1 (20)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>29</td>
<td>10 (37)</td>
<td>211</td>
<td>27 (14.8)</td>
<td>4 (9.2)</td>
<td>25 (92.6)</td>
</tr>
</tbody>
</table>

¹RT: rat intestinal content; PHRT: rat environmental faeces; R: mouse intestinal content; PHR: mouse environmental faeces; P: pigeon intestinal content; PHCN: pool of pig faecal samples (per floor). ²MDR resistance (MDR): presence of antimicrobial resistance to 23 different classes of antimicrobial agents.

- All 27 isolates were fully susceptible to vancomycin and metronidazole. Slow growing metronidazole hetero-resistant subpopulations were not detected (Table 2).

- Four (14.8%) strains showed multidrug resistance (MDR) to clindamycin, tetracycline and erythromycin.

- Two out of 4 MDR isolates presented non-toxicogenic condition.

- Co-resistance to erythromycin and moxifloxacin was not observed.

**Table 2. In vitro activity of 6 antimicrobials against the *C. difficile* isolates**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Range (µg/mL)</th>
<th>Breakpoint (µg/mL)</th>
<th>No. resistant isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin</td>
<td>0.016-256</td>
<td>≥8*</td>
<td>12 (41.4)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.016-256</td>
<td>≥0.02*</td>
<td>16 (55.2)</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.016-256</td>
<td>≥2*</td>
<td>0</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.02-32</td>
<td>≥2*</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.016-256</td>
<td>≥16*</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.016-256</td>
<td>≥32</td>
<td>0</td>
</tr>
</tbody>
</table>

*The breakpoints for resistance established by the Clinical and Laboratory Standards Institute (CLSI) for anaerobic bacteria are those marked by an asterisk. The remaining breakpoints were based on the literature (Alvarez-Pérez et al., 2014).