Prevalent *Clostridium difficile* genotypes among human and animal isolates other than ribotype 078

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INTRODUCTION

*Clostridium difficile* is one of the most important nosocomial pathogens (Barlett, 1994). However, in recent years the incidence of community-acquired *C. difficile* infection has increased and *C. difficile* has also emerged as a pathogen in animals (Chemenuil et al., 2005; Gould and Limbug, 2010; Senger and Anderson, 2007).

PCR ribotype 078 is currently the most frequently isolated strain from pigs and calves and is also increasingly reported in human population particularly in those with community associated infections (Kcel et al., 2007; Limbugo et al., 2009). In Slovenia strains other than ribotype 078 are prevalent in humans and animals.

The objective of this study was to compare prevalent PCR ribotypes isolated from humans and different animals with pulsed-field gel electrophoresis and antimicrobial susceptibility testing to determine their relatedness.

MATERIALS AND METHODS

**Strains:** Altogether 90 representative *C. difficile* isolates from 7 most frequently isolated PCR ribotypes (014/020, 012, 023, 029 and SLO 037) were analyzed (Table 1).

- 52 human strains isolated as a part of routine *C. difficile* testing from three different Slovenian hospitals.
- 18 animal strains isolated from pigs (n=3), poultry (n=8), cats (n=1), calves (n=2) and dogs (n=4) isolated from different Slovenian farms and households.
- Selected strains had been isolated between 2006 and 2009.

**Toxinotyping:** was performed as described by Janezic and Rupnik (2010), using two different

**PCR ribotyping:** Intergenic spacer regions were amplified with primers described by Bidet et al. (1999).

**Antimicrobial susceptibility testing:** Minimum inhibitory concentrations (MICs) for 6 antimicrobial agents (rifampin (RI), meropenem (ME), erythromycin (EM), piperacillin/tazobactam (PTC), tetracycline (TC) and clindamycin (CM)) were determined by E-test method.

**PFGE:** Pulsed field gel electrophoresis was performed as described by Janecic and Rupnik (2010) using two different

**RESULTS AND DISCUSSION**

- There is a considerable overlap between *C. difficile* ribotypes isolated from humans and animals (Table 1).
- Two most prevalent PCR ribotypes in humans and animals were 014/020 and 002 accounting for 22.9 % and 8.9 % (humans), and 14.2 % and 8.2 % (animals), respectively. These two ribotypes are also among the most prevalent ribotypes in Europe (Bauer et al., 2010).

<table>
<thead>
<tr>
<th>Source</th>
<th>% of strains</th>
<th>No. of different ribotypes</th>
<th>002 (n=4)</th>
<th>014/020 (n=4)</th>
<th>012 (n=1)</th>
<th>023 (n=3)</th>
<th>029 (n=2)</th>
<th>SLO 037 (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>humans</td>
<td>40</td>
<td>8</td>
<td>100</td>
<td>68.9</td>
<td>13.3</td>
<td>4.0</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>animals</td>
<td>39</td>
<td>5</td>
<td>100</td>
<td>14.2</td>
<td>13.3</td>
<td>8.2</td>
<td>2.6</td>
<td>4.0</td>
</tr>
</tbody>
</table>

- Majority of strains of a single PCR ribotype grouped together with PFGE regardless which restriction enzyme was used (SmaI or SacII) data not shown).

- Human and animal isolates of the same PCR ribotype cluster together with PFGE and had also similar MIC values for all antibiotics tested.

- In the first PFGE (clustern 1-5 on the figure 1) animal isolates had indistinguishable banding pattern (100 % similar) when restriction was performed with SmaI. However, when restriction was performed with SacII, only one pig isolate was 100 % similar to the human isolate (figure 1).

- In general, human isolates had higher MICs for EM and TC values than animal isolates for all antibiotics tested (Table 2).

- With a few exceptions all strains within a single ribotype also had similar MIC values for all antibiotics tested.

- Within ribotype 014/020 three strains had MICs for MX >256 mg/L and one had MICs for EM and CM >256 mg/l. All four strains grouped together with other 014/020 strains (Figure 1).

- Two out of seven Paloe-negative strains (SLO 010 and SLO 037) had MICs for EM and CM >256 mg/L. One had MICs for MX and CM <256 mg/L. In contrast to 014/020 strains this three strains grouped together but not with other Paloe-negative strains (Figure 2).

**REFERENCES**

Our results show that isolates of the same PCR ribotype also group together with PFGE and that animal and human isolates of the same PCR ribotype are genetically similar which is in agreement with previous studies on ribotype 078 strains (Baker et al., 2010; Jiang et al., 2008). Currently most laboratories uses SmaI restriction enzyme for PFGE typing of *C. difficile*, but our results show that use of two different enzymes simultaneously could improve discriminatory power of PFGE.