International Clostridium difficile Animal Strain Collection


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INTERNATIONAL Clostridium difficile ANIMAL STRAIN COLLECTION


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Background

Animals have been recognized as an important potential reservoir of Clostridium difficile and according to recent studies the overlap between PCR ribotypes of human and animal isolates seems to be increasing (Bakker et al., 2010; Gould and Limbagho, 2010; Janezic et al., 2012; Keel et al., 2007; Koene et al., 2011).

Here we report on an International Clostridium difficile animal strain collection that was established to enhance comparative studies on animal-associated strains and contribute to interlaboratory exchange of the strains.

The goal of the collection is to include one PCR ribotype per species per country/laboratory.

Results and Discussion

All 100 included strains were distributed into 39 different PCR ribotypes. Up to 17 different PCR ribotypes can be found within a single animal species and up to 15 different PCR ribotypes per country.

Within one standard (agarose based) PCR ribotype several PCR ribotypes could be distinguished by capillary gel based PCR ribotyping (Table 1; PCR ribotypes 014/020, 078, 002, 045, 056, 033).

Five strains are nontoxigenic while toxigenic strains account for 95.0% and belong to 10 different toxigenotypes: 0, I, II, III, IV, V, VI, VIII, XII and XIX. PCR ribotypes 078, 126, 014/020, 012 and 002 that are frequently associated with animals (Keel et al., 2007; Janezic et al., 2012) represent 40.0% of all strains.

Materials and methods

C. difficile strains:

Altogether 100 strains from 12 different countries were contributed. Collected strains originate from 11 different animal species, including pets, horses and food animals. Approximately half (58.0%) of the strains are from cattle and pigs (Table 1).

For every strain additional available information on animal host and strain was obtained.

Molecular characterization of C. difficile strains:

All collected strains were characterized by toxinotyping (Rupnik et al., 1998; http://www.mf.uni-mb.si/usx/). In addition, binary toxin genes were detected by PCR as described in Stubbs et al. (2000).

Standard agarose gel-based PCR ribotyping was used as described by Bidet et al. (1999) and results analyzed by BioNumerics software 5.10 (Applied Maths).

Strains were also typed by capillary gel electrophoresis-based ribotyping using primers for standard agarase gel-based PCR ribotyping with flourescein labelled 16S primer (Indra et al., 2008). PCR ribotype patterns were analyzed and identified on a web-based database WebRib (http://webrib.ags.at).

Table 1. Distribution of C. difficile genotypes by species and country given as toxinotype/standard PCR ribotype [capillary gel electrophoresis-based PCR ribotype].

<table>
<thead>
<tr>
<th>Country</th>
<th>Animal host</th>
<th>Total number of strains per animal species</th>
<th>Number of different PCR ribotypes per animal species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>Dog</td>
<td>29</td>
<td>17</td>
</tr>
<tr>
<td>Belgium</td>
<td>Cattle</td>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>Canada</td>
<td>Horse</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>Rabbit/Hare</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Denmark</td>
<td>Partridge</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Germany</td>
<td>Poultry</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Italy</td>
<td>Goose</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Scotland</td>
<td>Crow</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Slovenia</td>
<td>Raccoon</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

mb typing not done; new RT-PCR ribotype has on webrib database not yet been determined; subtype/ribbon has on webrib not yet been determined