Whole-genome analysis of *Clostridioides difficile* strains isolated from horses in Japan

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INTRODUCTION

*Clostridioides difficile* has been recognized as an important cause of acute enterocolitis in horses (1). At the 9th ICDS, we reported the observation of various PCR ribotypes (RTs) in cases of *C. difficile* infection (CDI) of thoroughbred racehorses in Japan (8). Some of the RTs (CDI) of these cases were the same RT as those in humans, such as the hypervirulent RT 027 and 078. This raises the possibility of *C. difficile* as a potential zoonotic agent. We conducted whole-genome sequencing (WGS) analysis of *C. difficile* isolates in Japan and compared the sequences with a number of other *C. difficile* genome sequences.

MATERIALS AND METHODS

**Bacterial strains**

We tested 34 *C. difficile* isolates obtained from 32 horses. The isolates were obtained from feces or intestinal contents collected between May 2010 and July 2016. All isolates were toxin-producing strains as confirmed by PCR (4-5, 10).

**PCR ribotyping**

PCR ribotyping was performed according to the modified Stubbs method described by Kato et al. (6, 8).

**Whole-genome analysis and computer-based molecular epidemiological analysis**

The isolates were sequenced on an Illumina NextSeq 500 sequencer, with 612 publicly available *C. difficile* genome sequences as references. Computer-based multi-locus sequence typing (MLST) and antimicrobial resistance (AMR)-based genome analysis were conducted on a whole-genome based genome analyzer (GenEpid-J, 11). The core genome single nucleotide polymorphism (SNP)-based phylogenetic tree was constructed by the approximately-maximum-likelihood phylogenetic method in FastTree (7). Quinolone-resistant-determining regions (QRDRs) gyrA and gyrB of moxifloxacin-resistant isolates were sequenced (9).

RESULTS AND DISCUSSION

**Table 1**. Molecular genotyping, toxins produced, antimicrobial resistance genes or mutations detected, and susceptibility to 5 antimicrobials of 34 equine isolates of *C. difficile*.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>PCR ribotype</th>
<th>MLST</th>
<th>Serotype</th>
<th>Pathogenicity</th>
<th>Toxins</th>
<th>Amr1</th>
<th>Amr2</th>
<th>Amr3</th>
<th>Amr4</th>
<th>Amr5</th>
<th>AMR</th>
<th>MIC (µg/ml)</th>
<th>S/R</th>
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<tr>
<td>horse 1</td>
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**Relation between PCR ribotype and MLST sequence type (Table 1)**

The isolates were classified into 11 RTs and 13 MLST types. Ribotype corresponded to MLST type in all isolates except RT 014 and RT 056.

**Core genome SNP-based phylogenetic analysis (Figure 1)**

- The RT 078 isolates were divided into 3 sublineages which corresponded to their geographical and temporal differences.
- One lineage was associated with a nosocomial outbreak in a clinic.
- Isolates in the 2 RTs (especially RT 078, RT 027, and RT 017) in horses were indistinguishable from isolates from humans and bovines despite host and geographical differences among them.
- High genetic relatedness between equine and clinical isolates indicates the possibility of transmission of *C. difficile* between horses and humans.
- RT n13124 (A/B*C") was closely related to RT 027.

**Antimicrobial susceptibility and resistance gene detected by GenEpid-J (Table 1)**

- All equine isolates were susceptible to metronidazole (MIC: <1 µg/ml) and vancomycin (MIC: 0.5–1 µg/ml).
- tet(M) was detected in all RT 078 and RT 017 isolates and one RT 014 isolate. These isolates had reduced susceptibility to moxifloxin. In contrast, the presence of tet(49) and tet(5) did not seem to influence susceptibility. erm(B) was detected in all RT 017 isolates with reduced susceptibility to azithromycin.
- Two RT 078 and three RT 017 isolates were resistant to moxifloxacin. The same nucleotide mutation (T245→C) corresponding to an amino acid substitution (Thr82→Val) was observed in QRDR gyrA. Another amino acid substitution (Ile146→Ala) was detected in the two RT 078 isolates.
- Aminoglycoside-resistance genes (aac(6')-aph(2')-Iex, aac(6')-aph(2'-Iex), and aph(2'-Iex)-k) were detected in some isolates despite *C. difficile* natural resistance to aminoglycosides.
- No isolate with low susceptibility to metronidazole and vancomycin were observed.
- The presence of the resistance genes and mutations seemed to be related to more RT than to the consumption of these agents in equine practice in Japan.

CONCLUSIONS

- WGS analysis suggested the presence of a particular sublineage of RT 078 in Japan, which was associated with a nosocomial outbreak in an equine clinic.
- High genetic relatedness between equine and human isolates suggests the possibility of transmission of *C. difficile* between horses and humans.
- Some antimicrobial resistance genes detected by WGS, corresponding to phenotypy, were detected in some RTs.
- The sources of *C. difficile* in the Japanese equine population are still unknown. Therefore, continuous observation will be important from the perspective of the "One Health" concept.

REFERENCES

6. Yoshinari Katayama, Equine Research Institute, Japan Racing Association, Japan.
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