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# Relatedness of human, animal and food *Clostridium difficile* strains

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## INTRODUCTION

*Clostridium difficile* is an important cause of infectious diarrhea in hospitals. The major risk factors for the development of nosocomial *C. difficile* infection include antibiotic therapy and increasing age. In animals, *C. difficile* also seems to be an important cause of enteric disease. Recent isolation of *C. difficile* in a variety of meat products reinforces the hypothesis about a potential risk of foodborne transmission.

## OBJECTIVES

The main objective of this study was to characterize and compare animal and human *C. difficile* strains with respect to the PCR-ribotype and the antibiotic resistance. Multilocus sequence typing (MLST) was performed in order to study clonal relationships of the isolates.

## MATERIAL AND METHODS

### Samples

Human *C. difficile* isolates were obtained from hospitalized patients. Animal isolates were collected from horses, pigs and cattle stools and from carcasses of pigs and cattle at slaughter.

### Methods

#### *C. difficile* typing

Isolates were characterized by PCR ribotyping and tested for the presence of toxin genes encoding the 3 toxins (tcdA, tcdB and cdtA) using Genotype Cdiff Test System. This test made possible the detection of deletions in the regulator gene *tcdC* (39bp deletion) and *gyrA* gen mutation associated with moxifloxacin resistance.

#### MLST analysis

Seven housekeeping loci were to determine genetic relatedness of the isolates. For PCR-ribotype 014 and 078, the band patterns of animal and human strains were compared.

#### *Antimicrobial resistance*

Isolates were tested for susceptibilities to a total of 7 antimicrobial agents (rifampin, tetracycline, erythromycin, clindamycin, vancomycin, metronidazole and moxifloxacin) by disc diffusion (n=4) and E-test (n=3) respectively.

## RESULTS

Twenty different PCR ribotypes were identified, including PCR-ribotype 078, 014 and 015. Only 7 PCR-ribotypes were negative for all toxin genes: UCL270 (n=1), UCL103 (n=1), UCL36 (n=2), UCL9 (n=2) and UCL 261 (n=1).

Only PCR-ribotypes 078, 023, UCL5a and UCL11 presented all the types of toxin genes while all of the other types were positive for both of toxin genes *tcdA* and *tcdB,* but negative for *cdtA* gene encoding for the binary toxin. A 39 bp deletion in the regulator gene *tcdC*, which is associated with an increased production of toxins in hyper-virulent *C. difficile* strains, was found in all PCR-ribotypes 078, 023, UCL5a and UCL11.

Most of these PCR-ribotypes had a mutation in the *GyrA* gene. This mutation is related with moxifloxacin resistance. Phylogenetic analysis showed that human and animal isolates with the same PCR-ribotype cluster in the same lineage.

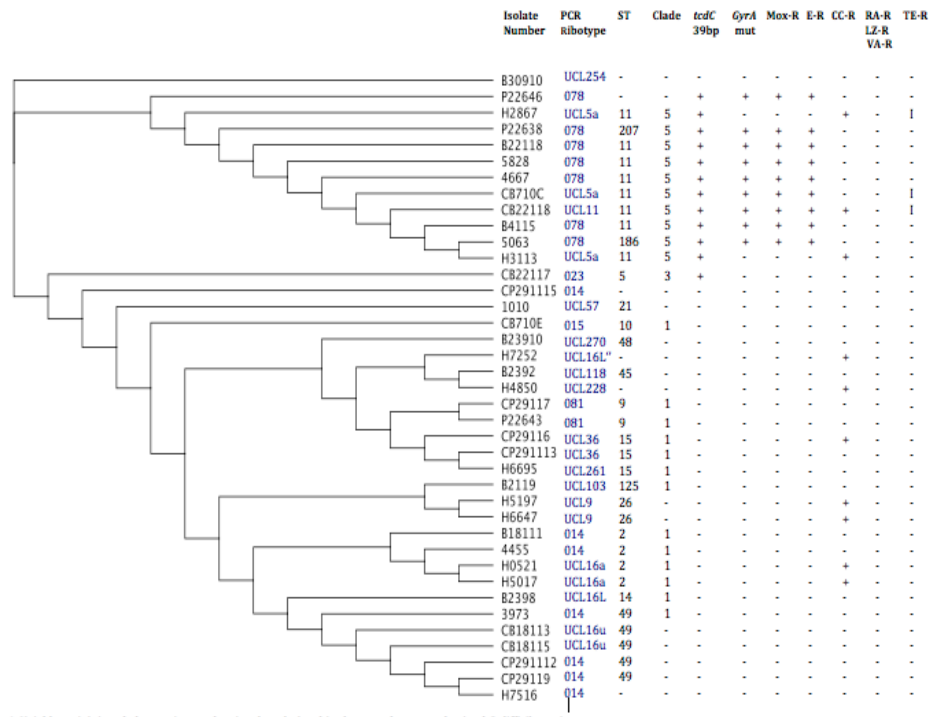


**Figure 1. Neighbour-joining phylogenetic tree showing the relationships between human and animal *C. difficile* strains**
**ST**: sequence type; ***tcdC 39bp:*** presence of deletions in the regulator gene *tcdC*; ***gyrA mut:*** presence of mutation in the *gyrA* gene; **MXF-R:** moxifloxacin resistance; **E-R:** erythromycin resistance; **CC-R:** clindamycin resistance; **RA-R:** rifampin resistance; **LZ-R:** metronidazole resistance; **VA-R:** vancomycin resistance. **TE-R:** tetracycline resistance.
**B :** cattle stools : **P** : pig stools : **H** : horse stool : **CB** : cattle carcasses: **CP**: pig carcasses: **isolate number:** human stools

## CONCLUSIONS

The multi-locus sequence typing analysis showed that animal and carcass *C. difficile* isolates largely overlap with human PCR-ribotypes. Furthermore, strains that are prevalent in humans are also present in different animals and in carcasses at slaughter, suggesting a potential risk of foodborne infections linked to *C. difficile.* The overlap between strains from animal and human host suggest a potential risk of interspecies transmission.