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

Rodriguez C1, Taminiau B2, Avesani V2, Van Broeck J1, Delmée M2 and Daube G1

1. University of Liège, Faculty of Veterinary Medicine, Food Science Department - Food Microbiology, Liège, Belgium
2. Catholic University of Louvain, Faculty of Medicine, Microbiology Unit, Brussels, Belgium

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 gene mutation associated with moxifloxacin resistance.

**MLST analysis**

Seven housekeeping loci were used 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. Phyllogenetic analysis showed that human and animal isolates with the same PCR-ribotype cluster in the same lineage.

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.