COMPARISON AND MOLECULAR CHARACTERIZATION OF ANIMAL AND HUMAN *CLOSTRIDIUM DIFFICILE* STRAINS

Rodriguez C[1], Taminiau[1] B, Korsak N[1], Avesani V[2], Van Broeck J[2], Delmée M[2] and Daube G[1]

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

INTRODUCTION

Clostridium difficile is the leading pathogen responsible for infectious antibiotic associated diarrhea in hospitals. In animals, as pigs, calves and horses, *C. difficile* also seems to be an important cause of enteric disease. Moreover, 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 analysis (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

Isolates were characterized by PCR ribotyping and tested for the presence of toxin genes encoding enterotoxin A (tcdA), cytotoxin B (tcdB) and binary toxin A (cdtA) using Genotype Cdiff Test System. This test made possible the detection of deletions in the regulator gene *tcdC* (18bp and 39bp deletions or single base deletion at position 117) and *gyrA* gen mutation associated with moxifloxacin resistance.

Seven housekeeping loci (*adk, atpA, dxr, glyA, recA, sodA* and *tpi*) were to determine genetic relatedness of the animal isolates. For PCR-ribotype 014 and 078, the band patterns of animal and human strains were compared.

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

RESULTS

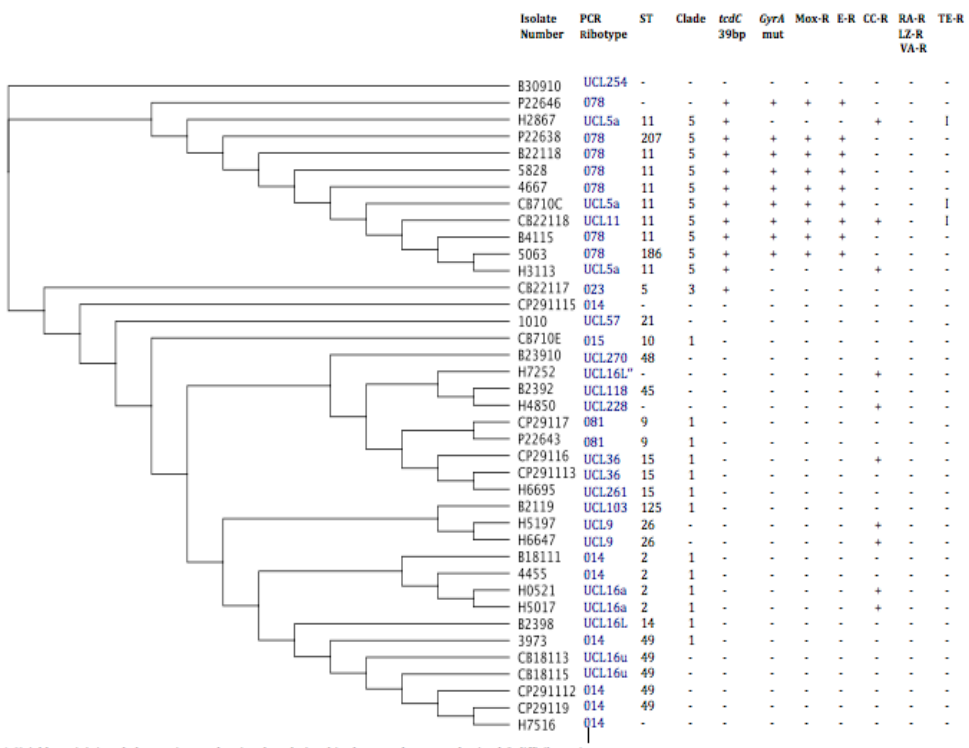

Figure 1. Neighbour-joining phylogenetic tree showing the relationships between human and animal *C. difficile* strains
ST: sequence type; ***tcdC 39bp:*** presence of deletions in the regulator gene *tcdC*; ***gyrA mut:*** presence of mutation in the *gyrA* gene; **MXF-R:** moxifloxacin resistance; **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

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.

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.*