Clostridium difficile: an emerging zoonotic pathogen

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Diagnostic et surveillance des maladies infectieuses
21nd Novembre 2013
Centre culturel de Woluwé-St-Pierre
Background

• Since toxigenic *C. difficile* was recognized as the major cause of antibiotic-associated diarrhea and pseudomembranous colitis in 1978, many outbreaks have been documented

• In the last years, an enhanced virulence and increased antibiotic resistance of *C. difficile* strains (PCR-ribotype 078/NAP-1/B1) has been observed

• There are emerging data on the occurrence of *C. difficile* infection in the community: non-hospitalized and younger patients with absence of other traditional risk factors

**Hypothesis about a potential risk of foodborne infections linked to *C. difficile***
Background

- Patients with serious illnesses and prolonged hospitalizations are at particular risk, as people above 65 years of age

- The increased risk of acquiring *C. difficile* in the elderly may be due to age-related changes in intestinal flora, immune senescence or the presence of underlying diseases

- There is not much data describing the prevalence and molecular epidemiology of *C. difficile* in nursing homes in absence of an epidemic situation

Arvand, M., et al., 2012. High prevalence of *Clostridium* difficile colonization among nursing home residents in Hesse, Germany. Plosone, 7, e30183.
Clostridium difficile Infection in Nursing homes

**Clostridium difficile in animals and food**

- In animals, *C. difficile* appears to be an important cause of enteric disease.

- Asymptomatic carriage of *C. difficile* in animals has been also described.

- *C. difficile* has been recently isolated from many types of meat products.

- *C. difficile* meat isolates are correlated with the types implicated in human disease.
The possibility that *C. difficile* infection has a food or animal origin has recently been evoked due to the presence of *C. difficile* strains in food animals and meat. These strains are similar to those found in humans.
Objectives

• Determine the presence of *C. difficile* in young animals on farms

• Determine the presence of *C. difficile* in intestinal contents and on carcasses in animals at the slaughterhouse

• Evaluate the presence of *C. difficile* in retail meat

• Characterize the isolates by PCR-ribotype, presence of toxin genes and toxigenic activity in order to compare the strains with the main PCR-ribotypes found in humans in Belgium
Study design

Farm animals
*From January to July 2011*

- **Piglets faecal samples**
  - Samples from 23 piglets were collected from 3 different breeding farms
  - Piglets were stimulated to make them defecate in individual tubes
  - The piglets don’t have diarrhea and were still suckling

- **Calves faecal samples**
  - Faecal samples of 18 calves were collected from 5 different local farms
  - Calves were less than 3 months of age at the time of sampling
Study design

Slaughter animals

*From January to July 2011*

200 intestinal samples from cattle and pigs were collected over 9 different visits to a local slaughterhouse.

All intestinal contents were collected from the slaughter line, directly from the large intestine in the viscera processing area.
Study design

Intestinal contents, carcass samples and meat samples

From September to December 2011

A total of 100 intestinal samples and 100 carcass samples from cattle and pigs were collected 2 h after slaughter in the chilling room.

A total of 133 beef samples and 107 pork samples were collected.
Methodology

- **Direct and enrichment culture**
  Home-made cycloserine cefoxitin fructose taurocholate
  

- ***C. difficile* latex agglutination rapid test Kit DR 1107A Oxoid**
- **Detection of a species-specific internal fragment of *tpi*, detection of genes for toxin B, toxin A and binary toxin (*cdtA*) by PCR et Genotype Cdiff test system**
  
  (Lemée et al., 2004. Multiplex PCR targeting *tpi* (triose phosphate isomerase), *tcdA* (toxin A), and *tcdB* (toxin B) genes for toxigenic culture of *Clostridium difficile*. *J Clin Microbiol*, 42, 5710-14)
  

- **Cytotoxicity assay using confluent monolayer MRC-5 cells**
  Cytotoxic activity was confirmed using a specific *C. difficile* antitoxin kit (T500, TechLab, USA)
  
  (Rodriguez et al., 2012. *Clostridium difficile* in young farm animals and slaughter animals in Belgium. *Anaerobe*, 18, 621-625)

- **PCR-ribotyping**
  
Results and discussion:

C. difficile in farm and slaughter animals

Farm

Prevalence 78.3%
Main PCR-ribotypes
078/002

Prevalence 0-1%
Main PCR-ribotypes
078/UCL46

Prevalence 6.9-9.9%
Main PCR-ribotypes
078/ Great variety of types (UCL5, 014, 002)

Slaughterhouse

Similar prevalences and types were previously reported in other countries as Canada, The Netherlands, Slovenia or Spain

Avbersek et al., 2009. Diversity of Clostridium difficile and other animals in Slovenia. Anaerobe, 15, 252-5.
Results and discussion: 

C. difficile on pigs and cattle carcasses

There are similar studies describing C. difficile on pig and cattle carcasses at the slaughterhouse in North America and Canada

First isolation in Europe

Prevalence 7%
Main PCR-ribotypes 014/081/UCL36

Prevalence 7.9%
Great variety of types (UCL5a/UCL16u)

Susick et al., 2012. Longitudinal study comparing the dynamics of Clostridium difficile in conventional and antimicrobial free pigs at farm and slaughter. Vet Microbiol 25, 172-78.


Results and discussion: 
*C. difficile* in retail meat

Prevalence 2.3%
Main PCR-ribotypes 078/014

Prevalence 4.7%
Main PCR-ribotypes 078/014/UCL57

Prevalence of *C. difficile* previously reported in meat
- **America**: 1.8 - 20% of positives
- **Europe**: 3% of positives
- Main PCR-ribotypes in America 078 and 027

First isolation of PCR-Ribotypes 078 and 014 in retail meats in Europe

Discussion: Ribotypes distribution in Belgian hospitals

• In 2011 in Belgium, the most prevalent PCR ribotypes in hospitals were
  • 014***, 002*, 027, 078***, 020, UCL46*, UCL16l*, UCL26, 001, 023*, UCL23f, 012, UCL16b, 015*, UCL5a**, UCL20a*, and UCL49 sorted by decreasing values in number of isolates.

Intestinal contents    Carcasses    Meat

• Overlap of PCR-ribotypes isolated from meat and human samples (MLST)
Conclusions

• This study documented that animals are carriers of *C. difficile* at slaughter, and carcass contamination occurs inside the slaughterhouse

• PCR-ribotypes 014 and 078 were the most frequently identified. These ribotypes were also the most common isolates from patients in Belgium

• The results obtained prove that toxigenic *C. difficile* is present in meat in Belgium. However, the clinical relevance of ingesting spores with food needs further investigation
ASSOCIATION OF CLASSICAL MICROBIOLOGY AND TARGETED METAGENOMIC ANALYSIS TO EVALUATE THE PRESENCE OF CLOSTRIDIUM DIFFICILE IN A BELGIAN NURSING HOME
Objectives

• To evaluate and follow the prevalence of *C. difficile* among older people in a nursing home

• To establish a relationship between other intestinal bacterial populations and *C. difficile* colonization

• To evaluate the global evolutions of the total microflora and the relation with the *C. difficile* presence
Nursing home study

Capacity: 110 beds
- 34 nursing home
- 61 nursing home and long-term care
- 15 day centers

Employees
- 73
Study design

STOOL SAMPLES
From March to June 2013

• During a 4-month period, stool samples from a group of 23 elderly care home residents were collected weekly.

• Two samples per person were collected: the first sample was cultivated and examined for *C. difficile* by classical microbiological methods and the second one was used to study the microbial biodiversity of the faeces content by amplicon sequencing coupled to microbial metagenomic analysis.
Metagenomics

High throughput sequencing

Bioinformatics
Results: Prevalence of *C. difficile* in nursing home residents

*C. difficile* recovery:

- 7/23 (30.4%) residents were (at least one week) positive for *C. difficile*
- *C. difficile* was detected in 13/30 (43.3%) episodes of diarrhea and we found 25/47 (53.2%) samples positive but without diarrhea
- 4/13 (30.7%) residents positive for *C. difficile* had previously received an antibiotic therapy. 10/13 (77%) positive residents didn’t get any antibiotic therapy
The results so far:

80 samples sequenced and analyzed: 6300 OTUs

Positive detection of *Clostridium difficile*:

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Relative proportions of the different bacterial families
There is a bacterial composition that appears stable along the weeks
The story so far

• *C. difficile* prevalence of 30.4% in a Belgian nursing home

• The most common PCR-ribotype identified was 027

• Residents have all their microbiota print

• Metagenomics analysis can’t substitute targeted protocols

• But it offers a global picture of the microbiota context:
  – With correlations
  – Identifications
  – Follow up
ACKNOWLEDGEMENTS

Public slaughterhouse of Liège

Nursing Home Sainte-Joséphine (Theux-Belgium)

Prof. Georges Daube
Dr. Bernard Taminiau
Dr. Nicolas Korsak

Prof. Michel Delmée
Véronique Avesani
Johan Van Broeck
Eléonore Lyeza