Update on *Clostridium difficile* pathogenesis and epidemiology

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Clostridium difficile infection (CDI)

- a toxin-mediated intestinal disease

- clinical outcomes
  - asymptomatic colonization
  - mild diarrhea
  - colitis (including abdominal pain, fever, and leukocytosis)
  - inflammatory lesions and pseudomembranes in the colon (typical for PMC)
  - toxic megacolon or bowel perforation, sepsis, shock, and death.
**Clostridium difficile** infection – risk factors

- **disturbance of intestinal flora**
  - neonate
  - other
  - hospital
  - food
  - endogenous
  - exogenous

- **colonization by CD**
  - toxins A/B
  - no toxin
  - asympt.

- **diarrhoea**
  - age
  - IC
  - IgG
  - IgA
  - ....

- **PMC**
  - death

M. Delmee, UCL, Brussels
Virulence and pathogenesis

1. Transmissibility
entry into host
release and spread
host range and reservoirs

2. Survival and colonization of the host
overcoming the host barriers
adherence

3. Factors to harm the host
enzymes
toxins
Transmissibility

• Reservoirs

  gut microorganism, soil and environment
  previously most infections from hospital reservoir
  importance of new reservoirs

• Survival in environment

  spores and sporulation properties
Nosocomial vs. community-acquired

Hospital

introduction of new types RARE

introduction of new types FREQUENT
Clostridium difficile – other reservoirs?

Humans

Animals

Environment

transmission (no, yes?)
overlap of genotypes
how?
contact, food
Clostridium difficile – other reservoirs?
C. difficile in animals

• C. difficile is described in several animal species
camels, seals, deer, elephant, tiger
laboratory rodents (hamster, guinea pigs, rabbits, mice)
cats, dogs, horses, pigs, calves, poultry

• disturbed normal gut flora (!)
antibiotics
young age
diet (inhibiting growth of intestinal microorganisms; Bojesen et al., Vet. Microbiol., 2006)

• diseased or healthy animals (multiplying hosts)
no clear link between presence of C. difficile and disease
piglets (Alvarez-Perez et. al., 2009)
calves (Rodriguez-Palacios et al., 2006; Hammitt et al., 2007)
Analysis of gut microbiota - poultry

DHPLC analysis of total gut eubacterial 16S RNA genes

C. difficile negative
C. difficile positive

Lactobacillus  Enterococcus  bifidobacteria

7FP Hyperdiff project (www.clostridia.net/hyperdiff)
J. Skraban, M. Rupnik et al, unpublished
## C. difficile types in humans and animals

<table>
<thead>
<tr>
<th>host</th>
<th>number of ribotypes found</th>
<th>most prevalent ribotypes or toxinotypes</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>humans</td>
<td>app. 300</td>
<td>0/014, 0/001;; III/027; 0/20; VIII/017 (V/078)</td>
<td>Barbut et al., 2007 Bauer et al., 2010</td>
</tr>
<tr>
<td>horses</td>
<td>10 to 12</td>
<td>(V/078 (up to 35%))</td>
<td>Keel et al., 2007; Arroyo et al., 2005</td>
</tr>
<tr>
<td>calves</td>
<td>3 to 8</td>
<td>V/078 (up to 94%)</td>
<td>Rodriguez-Palacios et al., 2006; Hammitt et al., 2008 Keel et al., 2007</td>
</tr>
<tr>
<td>piglets</td>
<td>2 to 4</td>
<td>V/078 (up to 83%) USA</td>
<td>Keel et al., 2007 Avbersek et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V/066 (up to 67%) Slovenia</td>
<td></td>
</tr>
</tbody>
</table>
Types in humans and animals are indistinguishable

- **calves (Canada)** (Rodriguez-Palacios et al., Emerg.Infect.Dis., 2006)
  8 ribotypes
  7 of them also in human isolates (same time/geogr. area)
  078 (V), 017 (VIII), 027 (III), 033 (XI), 077 (0), 014 (0)

- **calves and pigs (USA)** (Keel et al., 2007)
  4 ribotypes
  all of them known in human isolates
  078 (V), 017 (VIII), 033 (XI), 002 (0), 126 (ND)

- **pigs (The Netherlands)** (Goorhuis et al., 2008)
  ribotype 078
  also emerging highly virulent type in human infections
  pig and human strains can be identical with MLVA
### C. difficile genotypes found in food

<table>
<thead>
<tr>
<th>food</th>
<th>country</th>
<th>% of positive samples</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ground meat (beef and/or pork)</td>
<td>Canada</td>
<td>20%</td>
<td>M26/tox-, 077, 014,M31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6,7 %</td>
<td>M26/tox-, 077, 014,M31,J, C, F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 %</td>
<td>078, 027</td>
</tr>
<tr>
<td>ground meat (chicken)</td>
<td>Canada</td>
<td>12,8 %</td>
<td>078</td>
</tr>
<tr>
<td>ground meat (beef and/or pork)</td>
<td>USA</td>
<td>50 %</td>
<td>027, 078</td>
</tr>
<tr>
<td>ground meat (beef and pork)</td>
<td>Austria</td>
<td>3 %</td>
<td>AI-57, 053</td>
</tr>
<tr>
<td>ground beef</td>
<td>France</td>
<td>3 %</td>
<td>(not 078, not 027)</td>
</tr>
<tr>
<td>ground beef</td>
<td>Sweden</td>
<td>2,4 %</td>
<td>nd</td>
</tr>
</tbody>
</table>

Rupnik and Songer, Advan Food Nutrit Res, 2010
C. difficile in food other than meat

- **Salads**
  ready-to-eat salads 7.5% (Bakri et al., 2009)
  Scotland, but none of the products originated in the UK

- **Milk**
  raw milk samples, Austria, 0% (Jobstl et al., 2010)

- **Raw vegetables**
  cucumber, onion, potato, mushroom, carrot, radish (Al Saif and Brazier, 1996)
**C. difficile** as foodborne infection?

- number of spores
  - from 20 to 240 spores / g of meat (Weese et al., 2009)
  - infection dose not known

- temperature stability of spores at recommended cooking temperature for meat (Rodriguez-Palacios et al., 2007)

- ready to eat products
  - meat products (sausages…) positive in 36,9 % (Songer et al., 2009)
  - salads, raw vegetables
Clostridium difficile – new reservoirs?

- increase of community-associated CDI
- increasingly important as animal pathogen

new potential reservoir for human infections

- overlap of genotypes
- cases of direct animal↔human transmissions

possible transmission routes

- contact
- environment
- food
Transmission - sporulation related properties

- kinetics of sporulation/germination
  epidemic strains \( \uparrow \) sporulation capacity
  (increase in sub-inhibitory concentrations of non-chlorine-based cleaning agents) (Fawley et al., 2007)

- resistance of spores to the environmental conditions

- attachment of spores
027 isolates sporulate earlier and produce more spores than non-027 strains

Mean Spore Accumulation over Time

\[ *p = 0.001 \]

\[ **p = 0.0001 \]

G. Vedantam, University of Arizona, Tucson, Arizona
Survival in the host and colonization

- Antibiotic resistance
- Adhesion
C. difficile antibiotic resistance as a virulence factor

C. difficile and antibiotic resistance

• low (but emerging?) resistance to antibiotic used for CDI treatment

• high virulence associated with antibiotic resistance to different antibiotic classes
  (clindamycin, cephalosporins, fluoroquinolones)
  first wave of CDI – clindamycin resistant strains
  second wave of CDI – fluoroquinolone resistant strains

• routine testing
  not needed
  useful in the outbreak situation or during increased infection rates
Adhesion

• in humans – colon
  is adhesion important?

• adhesion properties of vegetative cells and spores

• molecules involved in adhesion
Pre-incubation of Caco-2 BBE cells with **BI-17** SLP decreases strain **BI-17** adherence

SLP (surface layer proteins) of *C. difficile* are responsible for app. 50% of adhesion

G. Vedantam, University of Arizona, Tuscon Arizona
**C. difficile** toxins – genes and proteins

**Large clostridial cytotoxins**
- **TcdA** (toxin A, enterotoxin)
- **TcdB** (toxin B, cytotoxin)

**Clostridial binary toxins**
- **binary toxin CDT**
Role of the toxins in the pathogenesis

Main disease signs and symptoms
secretory diarrhea
inflammation of colonic mucosa

Toxin A (enterotoxin)   TcdA
Toxin B (cytotoxin)    TcdB

- TcdB – essential virulence factor
- Amount of toxin produced
- Variability in toxin genes
- Importance of binary toxin
TcdA and TcdB – local enterotoxicity

synergistic action of TcdA and TcdB

cytotoxicity

immunomodulatory effects

neuronal component
TcdB – systemic effects

- Lung damage
  hamster model
  (Lyerly et al., Infect. Immun, 1985)

- Damage and edema in cardiac tissue
  Zebrafish embryos
  (Hamm EE, et al., PNAS, 2006)
TcdB – essential virulence factor

- new tools for genetic modification of *C. difficile*
- toxin deficient mutants (Lyras et al., Nature, 2009;)

<table>
<thead>
<tr>
<th>strain</th>
<th>virulence in hamster model</th>
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<tbody>
<tr>
<td>wt</td>
<td>+++ (9/10 animals died)</td>
</tr>
<tr>
<td>TcdA-only</td>
<td>--- (4/19 animals died; TcdB+ revertants)</td>
</tr>
<tr>
<td>TcdB-only</td>
<td>+++ (16/17 animals died)</td>
</tr>
</tbody>
</table>
TcdB as an essential virulence factor

pro:
- toxin production in clinical isolates
  - A+B+ yes disease +++
  - A−B+ yes disease +++
  - A+B− not known

- TcdB more potent than TcdA on human intestinal tissue
  (Riegler et al., 1995)
  - mucosal necrosis
  - decreasing barrier function

contra:
- early hamster experiments (pure toxins)
  - TcdA diarrhea/death +++
  - TcdB ---
  - TcdB/TcdA↓ death +++

- clinical A-B+ strains not virulent in hamster model (Depitre et al., 1993)
TcdB and TcdA are important virulence factors

<table>
<thead>
<tr>
<th>Strain</th>
<th>wt</th>
<th>TcdA-only</th>
<th>TcdB-only</th>
<th>TcdA and B K/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virulence in hamster model</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>nd</td>
</tr>
<tr>
<td>Lyras et al, 2009</td>
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<td>Kuehne et al., 2010</td>
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Variability of *C. difficile* toxin loci

**PaLoc** (toxins A and B)

- 27 variants - toxinotypes

**CdtLoc** (binary toxin CDT)

- 2 main variant forms
Variability in *tcd* genes - mutations

- differences among *tcd* genes
  mainly found in *tcdB* gene and *tcdC* gene
  *tcdA* gene more conserved

- differences within a *tcd* gene
  clustering within positions 1-868
Differences in *tcdb* – nucleotide level

- Minimal identity
  - 87% (types with deletions)
  - 90% (types w/o deletion)
Is binary toxin a virulence factor?

- A-B-CDT+ strains isolated from severe cases in horses

- A-B-CDT+ strains and animal models (Geric et al., JID, 2006)
  
  - hamster colonization ++, disease –
  - rabbit ileal loop assay enterotoxic effect +++

- Binary toxin positive strains (A+B+CDT+) more likely associated with severe disease (Barbut et al., JMM, 2005; Terhes et al., JCM, 2004)
Summary

Virulence potential of *C. difficile* strains is a combination of different properties (toxins, adhesion, sporulation, resistance)

Ribotype can be informative but is not always a marker for increased virulence

Other emerging reservoirs for CDI (animals, food, environment)