Molecular Epidemiology of Clostridium difficile in Hong Kong

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**Clostridium difficile**

- Anaerobic, Gram-positive, spore-forming bacillus
- Antibiotic associated diarrhea (AAC) and pseudomembranous colitis
- Global dissemination of a hypertoxigenic strain of PCR ribotype 027, toxinotype III, and North American Pulse-field type 1 (R-027 NAP1)
  - more severe colitis
  - higher mortality rate
- A sporadic case of R-027 was reported in Hong Kong in 2008
  - tcdA+, tcdB+, binary toxins+, tcdC(deletion)
Trend of isolation of toxigenic *Clostridium difficile* in a healthcare region in Hong Kong

* Period 1 data were acquired from laboratory information and hospital record system.
Study design

- One year study in 2009
- A healthcare region of 5 hospitals:
  - 1 acute care university teaching hospital (1,400 beds)
  - 4 chronic care hospitals (110-524 beds)
- Hospitalized patients suffering from GI tract infection:
  - stool specimens collected for culture and cytotoxin assay for *C. difficile*
Methods

Isolation of *C. difficile*:
- Cycloserine -Cefoxitin-Fructose Agar (CCFA)
- ID by VITEK ANI card
- E-test (metronidazole, vancomycin, ciprofloxacin)

Cytotoxin detection:
- Hela Cell (Qualitative) + Antitoxin neutralization
  - Stool filtrate
  - *C. difficile* culture filtrate
- ELISA for *C. difficile* Toxin A and B (Quantitative)
  - TOX A/B IITM kit (TechLab, Netherlands)
  - PCR ribotype 002
Patients
(2,310)

Patients isolated with toxigenic C. difficile
(307)

CCCNA
Stool filtrate: +ve
Culture filtrate: +ve
(145)

CCCNA
Stool filtrate: -ve
Culture filtrate: +ve
(162)
Molecular typing

PCR ribotyping (Philip Bidet *et al.* 2000)
- 23 control strains with known ribotypes (gift from Dr E. J. Kuijper)
- phylogenetic analysis by Bionumerics ver 6.0

Surface layer protein A (SlpA)-typing (Joost *et al.* 2009)
Distribution of ribotype of toxin producing strain of *C. difficile* in 2009

- ribotype 002: 70.07%
- ribotype 001: 11.61%
- ribotype og39: 10.11%
- ribotype 012: 3.80%
- ribotype 014: 2.30%
- ribotype 017: 1.20%
- Other pattern: 0.60%
- Non-typable: 0.30%
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Sporulation ability of R-002

Miles and Misra Method (Hedges et al, 1978):

Sub-culture bacterial suspension to 2 sets of plates -
  • (A) incubated for 3 days anaerobic 37°C
  • (B) incubated for 2 days aerobic 37°C, followed by incubation of 3 days anaerobic 37°C

Sporulation frequency (%) : B / A
Symptomatic infection / asymptomatic colonization

Symptomatic infection defined as patients suffering from diarrhea at the time of specimen collection.

Scoring system for the severity of symptomatic infection:

- One point: Age > 60; temperature > 38.3°C; Albumin level < 2.5mg/dL; peripheral WBC count >15,000 cell/mm3 within 48h of symptoms onset.

- Two points (severe *C. difficile* associated diarrhea [CDAD]):
  - Endoscopic evidence of pseudomembranous colitis
  - Treatment in ICU
Sporulation and toxin production of 35 strains of *Clostridium difficile* ribotype 002 isolated in Hong Kong (2009)
<table>
<thead>
<tr>
<th></th>
<th>Symptomatic infection</th>
<th>Asymptomatic colonization</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>17#</td>
<td>12#</td>
<td>--</td>
</tr>
<tr>
<td>• CDAD: 10</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>• Mild infection: 7</td>
<td></td>
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<tr>
<td>Toxin level of C. difficile R-002</td>
<td>123.85 U/ml</td>
<td>96.13U/ml</td>
<td>0.29</td>
</tr>
<tr>
<td>Sporulation frequencies of C. difficile R-002</td>
<td>28.9%</td>
<td>3.5%</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

# All 17 patients with symptomatic infection were defined as healthcare-associated infection
PCR 002 in Hong Kong

- Resistant to ciprofloxain (MIC >32μg/ml)
- tcdA and tcdB toxin genes +
- Intact tcdC genes (no deletion)
- No binary toxin gene
- Mean level of toxin production = 112.38 U/ml
- Mean sporulation frequency of 28.9%
  - non-002 ribotypes (3.5% ; p<0.001).
Summary

- R-002 is the predominant *C. difficile* ribotype in Hong Kong.
- Mean sporulation frequency of R-002 strains was significantly higher than other ribotypes.
- Symptomatic infection correlates significantly with higher rate of sporulation, but not with toxin production level in R002.
  - Epidemic strains reported with an increased inherent sporulation (Akerlund *et al.*, 2008).
  - Transmission of *C. difficile* in healthcare was primarily mediated by spores (Alfa *et al.*, 2008).
- R-002 in Hong Kong becomes an epidemic strain and contributes to the major *C. difficile* nosocomial transmission in Hong Kong?
Acknowledgement

The University of Hong Kong
• Gilman Siu
• Jacqueline Tsang
• Oscar Lam
• KY Yuen
• Vincent Cheng

Leiden University, The Netherlands:
• Dr E. J. Kuijper

Research Fund for Control of Infectious Diseases, Hong Kong