C. difficile Type 027: Virulent new Superbug?

Dr. Jon Brazier
Anaerobe Reference Lab
NPHS Microbiology
Cardiff
University Hospital of Wales
Introduction

• *Clostridium difficile* disease (antibiotic associated diarrhoea/colitis/pseudomembranous colitis/toxic megacolon) is a well recognised healthcare-associated infection problem.

• Figures for England and Wales in 2004 reached >44,000 diagnoses of CDD recorded as *C. difficile* toxin positive stools.

• Despite the medical profession being aware of this pathogen since the mid 1970’s, the popular press have only recently become aware of this “new superbug” which causes more actual disease than MRSA of which they have been aware for years.
History of *C. difficile* typing in Cardiff

- *C. difficile* typing for the UK was set up in Cardiff in the early 1990’s. First method used was PyMS, but limitations of this method meant a molecular scheme was needed.

- PCR amplification of the 16S-23S intergenic spacer region was selected as a likely target.

- O’Neil *et al* (1996) modified a described method and the ARL developed a library of types using a numeric nomenclature with a new three-figure number being assigned every time a new banding pattern was encountered. Eg. Type 001 was the first. To date we have >160 different types.

- Type 027 was first assigned in 1998 to a new banding pattern noted in an isolate from a culture collection from Prof. Popoff in Paris that originated from a 28yr old woman with PMC. It was later studied by the toxinotyping method as belonging to toxinotype type III and was also positive for binary toxin production.

- Until 2004 it was considered an unimportant strain.
Target for *C. difficile* PCR ribotyping

- Multiple copies of the rRNA gene complex. Size of the intergenic spacer region ranges from 250-600bp with the O’Neill primers. (Anaerobe 1996;2:205-9)

Library of >160 different types constructed in GelCompar
Images are captured digitally and compared to our library of types using the GelCompar software package.
PCR ribotyping patterns of Types 001 and 027
**Clostridium difficile** PCR ribotypes in hospital patients in England and Wales 1995-2003
**C. difficile outbreak in North America**

- In 2003, in Southern Quebec and the Montreal district of Canada an epidemic of *C. difficile* disease (CDD) was recorded. By January 2005, 30 hospitals in Quebec were reporting rates of *C. diff* infections at five times their normal level of 3 per 10,000 p.b.d. At the Centre Hospitalier Universitaire de Sherbrooke (CHUS) the percentage of patients dying within 30 days of a diagnosis of CDD rose from 4.7% in 1991/2 to 8.6% in 2002 and 13.8% in 2003. During the same interval the incidence per 100,000 individuals aged >65 increased from 102 (1991-2) to 866 (2003).

- This strain has now been identified in several US states including Maine, Georgia, Pennsylvania and New Jersey.
- It is PCR ribotype 027 and has been assigned as PFGE Type NAP1 to describe the clonal international strain (NAP1/027).
- The Quebec government sponsored an International meeting in October 2005 to review their outbreak experiences and learn of *C. diff* problems in UK and USA.
History of Type 027 in the UK

- By random, Type 027 was found in isolation as a single isolate in a small batch from hospital patients from Preston in Jan. 1999.
- Isolates submitted as part of an MSc project of a BMS in Birmingham revealed another single Type 027 isolate in April 2002.
- It was not seen again until March 2004 when Rose Gallagher, an ICN from Stoke Mandeville hospital contacted the ARL about a severe ongoing outbreak of *C. difficile*. Between Feb-June 2004 they had 150 cases with 12 deaths.
The First Recognised UK Hospital Outbreak with Type 027.
(Government Enquiry pending)

Stoke Mandeville Hospital, Aylesbury
Jeremy Laurance – Health Editor, The Independent
Infection expert resigned over hospital’s failure to control new superbug

Protests mount after 12 die and 300 infected at Stoke Mandeville
Lethal bug is costing NHS £160m a year - 

Switch your healthcare

£50 a month average saving

Why Are You Shamed
By Your Mistakes
In English?
“International *C. difficile* Grapevine”

- Contacts were made between ARL, CDC Atlanta and Montreal in Canada resulting in an exchange of isolates to compare outbreak strains.
- Canadian and American isolates sent to ARL all belonged to PCR ribotype 027 and UK USA and Canadian isolates were all >94% identical by PFGE and some were 100%.
PFGE Typing of UK, USA and Canadian isolates.

- A = Type 017
- C = Type 002
- B = Type 106
- D = Type 001
- E = Type 027 (Popoff's reference)
- F = Type 027 (Preston UK)
- H = Type 027 (Stoke Mandeville)
- I = Type 027 (Newcastle UK)
- G = Type 027 (Canada)

Gram stain of *C. difficile* Type 027
The Royal Devon and Exeter Hospital submitted 18 isolates of *C. difficile* to ARL with a history of an outbreak that coincided with a change of antibiotic regimen for treatment of CAP patients to moxifloxacin.

- 15 of 18 isolates belonged to Type 027.
The patients who died at the hospital had an average age of 85.

A hospital bug which has killed hundreds of patients nationwide was a factor in the deaths of 13 people at the Royal Devon and Exeter Hospital.
Outbreak of *Clostridium difficile* infection in a hospital in south east England.

June 23\textsuperscript{rd} 2005; 25:15

There has been an outbreak of *Clostridium difficile* associated disease in an acute hospital in south east England, which is currently under investigation. Laboratory tests have confirmed that many isolates from this outbreak are of type 027, which is unusual in the United Kingdom..... contact Dr. Jon Brazier at the Anaerobe Reference Laboratory, tel: 02920 742378 or 742171 to discuss further typing investigations.
The Third Hospital: “Superbug kills one at hospital”

One person has died and nine other patients have been infected with superbug *Clostridium difficile* at Oldchurch Hospital in Romford, Essex.

Seven of nine isolates submitted belonged to Type 027.
Three English 027 Outbreaks as of June 23rd 2005
The Conservative MP for Aylesbury tabled many questions to the Minister for Health on the outbreak in his constituency - Stoke Mandeville Hospital. Some of these could only be answered by the Anaerobe Reference Lab. Eg. Where else has this strain been detected?

As PQ’s are in the public domain (Hansard), in locations where 027 has been identified the local press seized on the story.

We had complaints about breaching confidentiality!!
Type 027 and toxin production

- A report from CDC Atlanta in conjunction with Acambis Inc. at a conference stated that the North American outbreak strain produced between 8 to 16 fold greater amounts of toxins A and B \textit{in vitro} compared to other strains.
- This would tie in with clinical information of more severe disease and increased death rates seen in outbreaks with this strain.
- Hyper-toxin production was believed to be due to an 18bp deletion in the \textit{TcdC} gene that regulates toxin production.
- Paper published in The Lancet 24\textsuperscript{th} Sept 2005 (Warny, Pepin, Fang, Killgore, Thompson, Brazier, Frost and McDonald) “Toxin production by an emerging strain of \textit{C. difficile} associated with outbreaks of severe disease in North America and Europe”
PCR for *TcdC* 18bp gene deletion in Type 027

All 331bp products were Type 027
Typical results for *TidC* partial gene deletions using the Beckman Coulter CEQ 8000 sequencer*

Absent
349bp Product (Type 001)

Present
331bp Product (Type 027)

* Dr. Lewis White
TcdC gene sequence deletions
Comparative *in vitro* toxin A production of PCR ribotypes*.

![Bar chart showing toxin production over time for different ribotypes.]

- **A** = Type 001
- **C** = Type 027
- **D** = Type 027

* Dr. Simon Jackson’s group UWCM
The Dutch Connection

- In late June 2005, Dr. Ed Kuijper from Leiden University Medical College gets in touch informing me of a serious outbreak of *C. difficile* infections in a Dutch hospital.
- We exchanged isolates and found Type 027 was the cause.
- Shortly afterwards, a second Dutch hospital was involved due to a known 027 patient being transferred.
- A few weeks later another hospital in Amsterdam was also identified.
- In October, a Belgian hospital became affected.
Factors allegedly associated with 027 infections

- In Canada and USA the increased use of proton pump inhibitors (PPI’s) was identified as a potential risk factor for *C. diff* infections.
- Pepin et al (CID Nov. 2005) reported that fluoroquinolone antibiotics (gatifloxacin, levoflox, moxiflox*) were the most important risk factor for CDD during an epidemic with Type 027.
- The majority of affected patients are >75yrs old with reported higher rates of severe disease requiring colectomy and increased mortality.
- *UK Type 027’s are resistant to moxi, levo, erythro, and imipenem."
Metronidazole results from Department of Health Study data

<table>
<thead>
<tr>
<th>MIC results</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.047</td>
<td>0.001</td>
</tr>
<tr>
<td>0.064</td>
<td>0.027</td>
</tr>
<tr>
<td>0.094</td>
<td>other</td>
</tr>
<tr>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>&gt;1.5</td>
<td></td>
</tr>
</tbody>
</table>
Erythromycin results from Department of Health Study data

EM MIC results

- 0.19
- 0.5
- 0.75
- 1
- 1.5
- 2
- 3
- 4
- 6
- >256

Percentage

- 001
- 106
- 027
- Other
Levofloxacin results from Department of Health Study data

Levofloxacin MIC results

Percentage

LE

MIC results

0.32 6 8 12 24 32 >32

0% 5% 10% 15% 20% 25% 30% 35% 40%
Imipenem results from Department of Health Study data

Percentage

IP MIC results

0% 5% 10% 15% 20% 25% 30% 35%

0.32 3 4 6 8 12 16 24 32 >32

001 106 027 other
DoH/HPA *C. difficile* Surveillance Scheme

- Due to begin in Jan 2004, this scheme finally started in early 2005.
- Nine HPA Regional labs request *C. diff* toxin positive stools from hospitals in their region in a given week (max. 10), culture for *C. diff* and send the isolates to ARL for typing and susceptibility testing.
- 1,000 isolates a year sampled on a rotational basis.
- In Wales, the NPHS planned 60 isolates sampled in the month of October to be obtained from around the country as a snapshot of what strains are in circulation in Welsh hospitals.
- So far we have received 20 isolates from Bangor, Rhyl and Wrexham, none of which were Type 027.
The true extent of Type 027 emerges!

- As the DoH surveillance scheme takes effect we unearth Type 027 in other English hospitals.
- On being told, some Consultant Microbiologists said:
  - “Oh $***! ”
  - “I’m not surprised, we’ve been having a terrible time”
- However, not all indicated that they had an outbreak or noted anything unusual to their level of *C. difficile* disease.
- As the word spread, numerous other hospitals requested investigations in the absence of any change in rates or severity of disease – the “Have we got it??” syndrome created a lot of work for the ARL.
Known Locations with PCR ribotype 027 as of Nov. 29th
PCR ribotypes of *C. difficile* in UK referrals from Jan. 2004 – June 2005 (n=338)
Is “Superbug” publicity Good or Bad?

- One hospital in the Midlands was found to have 027 as part of the DoH/HPA surveillance scheme.
- After being told of this finding, the Consultant Microbiologist informed his local Trust Management team. They were so concerned about bad publicity they:
  - Converted a medical ward into a new isolation ward,
  - Changed to chlorine based disinfectants for ward cleaning,
  - Emphasised soap and water hand washing prior to alcohol hand rubs,
  - Changed antibiotic policy, instigated multi-disciplinary ward rounds.
  - He phoned ARL to say how helpful our typing results were!
Summary:

C. difficile Type 027: Virulent new superbug?

- **Virulent?** Evidence for = in vitro toxin assay, $TcdC$ gene deletion, general increased incidence of disease where it is present, anecdotal evidence of increased deaths and severity of disease, spreading to other countries. Against = some cases not severe, some cases are community acquired, other types have $TcdC$ gene deletions.

- **New?** For = has only recently been detected causing outbreaks, Against = first UK isolate was in 1999,

- **Superbug?** ........??? Definition – ask the Editor of The Sun!