# *Clostridium difficile in the Community: Food for Thought*
# *Prof. Tom Riley,*
# *A Webber Training Teleclass*

## *Clostridium difficile* in the Community: Food for Thought!


Tom Riley

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Hosted by Jane Barnett
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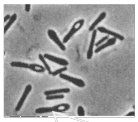
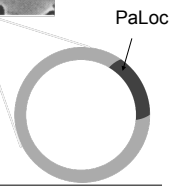
## *C. difficile* infection



- Most common cause of infectious diarrhoea in hospital patients
- 2 major virulence factors:
  - toxin A (an enterotoxin)
  - toxin B (a cytotoxin)
- 3rd "binary" toxin



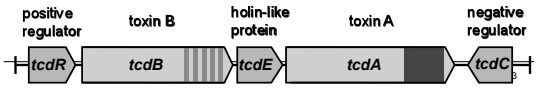



Bartlett, JG, *Clin Infect Dis* 1994,18:S265-72

## Toxin A & toxin B

- Large structurally and functionally related proteins
- Genes are contained on a 19.6-kB Pathogenicity Locus (PaLoc) which is absent in non-toxigenic strains
- Majority of pathogenic strains produce both toxins which affect actin cytoskeleton
- Polymorphisms in the PaLoc can affect toxin production - toxin A-negative, toxin B-positive strains

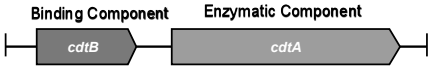
Toxin phenotypes
- $A^+B^+$
- $A^-B^+$
- $A^-B^-$






## Binary toxin

- Additional toxin produce by 2-5% of isolates
- Consists of two component proteins, the genes for which are contained within the CDT locus on the chromosome
- Actin-specific ADP-ribosyltransferase
- Unknown significance in disease, but associated with increased severity of diarrhoea





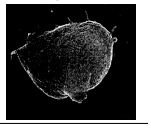

## Cytopathic effects

Effect on Chinese hamster ovary cells

Infect Immun 2001; 69:5487-93.

**CONTROL**

**Toxin B**



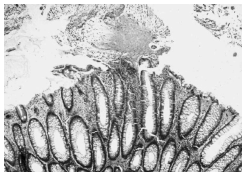
Cellular Morphology
- Cell-rounding
- Detachment from extracellular matrix

Cellular Processes
- Activation of caspases → apoptosis
- Decrease in integrity of tight-cell junctions
- Inflammatory response
  - Release of cytokines & chemokines
  - Production of reactive oxygen intermediates



## Histological effects

- Massive inflammatory response
- Recruitment of polymorphonuclear neutrophils to area
- Increase in epithelial permeability



Kelly *et al*. N Engl J Med 1994;330:257-262.



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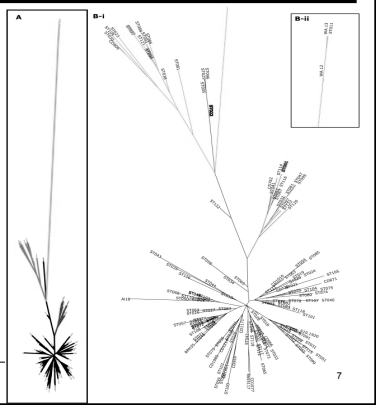
Relative evolutionary relatedness of five main subgroups and demonstration of microdiversity of subgroups.

Stabler R.A. et al. Macro- and micro diversity of *Clostridium difficile* isolates from diverse sources and geographical locations. *PLoS One* 2012;7:e31559




Branch colouring: black = clade 1, red = clade 2 (inc ST-1/RT027), blue = clade 3 (inc ST-22/ RT023), orange = clade 4 (inc ST-37/RT017), green = clade 5 (inc ST-11/RT078).

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## Risk factors for getting *C.difficile* ?

- Exposure to the organism – how much?
- Exposure to antibiotics – clindamycin, then cephalosporins, now fluoroquinolones
- Maybe others now?

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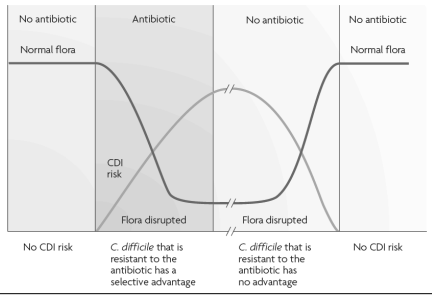
## Effect of antibiotics on normal flora




Rupnik *et al*. Nat Rev Microbiol 2009;7:526-36.

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The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## A Predominantly Clonal Multi-Institutional Outbreak of *Clostridium difficile*–Associated Diarrhea with High Morbidity and Mortality

Vivian G. Loo, M.D., Louise Poirier, M.D., Mark A. Miller, M.D., Matthew Oughton, M.D., Michael D. Libman, M.D., Sophie Michaud, M.D., M.P.H., Anne-Marie Bourgault, M.D., Tuyen Nguyen, M.D., Charles Frenette, M.D., Mirabelle Kelly, M.D., Anne Vibien, M.D., Paul Brassard, M.D., Susan Fenn, M.L.T., Ken Dewar, Ph.D., Thomas J. Hudson, M.D., Ruth Horn, M.D., Pierre René, M.D., Yury Monczak, Ph.D., and André Dascal, M.D.

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Investigation

## Investigation into outbreaks of *Clostridium difficile* at Stoke Mandeville Hospital, Buckinghamshire Hospitals NHS Trust

July 2006

Healthcare Commission

http://www.healthcarecommission.org.uk/_db/_documents/Stoke_Mandeville.pdf

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Daily Mail, Saturday, September 1, 2007 — Page 37

**Superbug kills war hero who survived three years as a PoW**

By Luke Salkeld

THE family of a distinguished war veteran have criticised the hospital where he was infected by a killer bug.

Major Sam Weller – who survived three years as a prisoner of war – died after catching Clostridium Difficile following an operation on his hip. Yesterday, he had lived until he had been released by the courts in the Philippines.

Major Weller, 88, had surgery at Gloucestershire Royal Hospital but was given a course of antibiotics.

Weeks later he died and an inquest was told the medicine had left him vulnerable to catching the superbug.

Yesterday, his family criticised the hospital treatment he received and standards of ...

Fearless officer: Major Sam Weller, left, who was decorated for his bravery, is pictured with his brother Tony in 1947

ALMOST 56,000 vulnerable and elderly patients have been infected with C. Diff in the past year.

Between January and March alone, 15,592 caught the bug – an astonishing 22 per cent rise on the previous three months.

C.Diff, which is spread by doctors and nurses, is a bigger killer than MRSA. It claimed 2,247 lives in 2005 – a 60 per cent rise on the previous year.

It exists naturally in the stomachs of many healthy adults, where it is kept under control by 'friendly' bacteria.

Problems start if the balance of bacteria is disturbed, perhaps as a result of taking antibiotics for another infection. Then the 'friendly' bacteria are killed off, the C. Diff is able to multiply and produce the toxins which cause diarrhoea and, in the worst cases, a fatal infection of the abdomen.

2,247

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Comparison of the Burdens of Hospital-Onset, Healthcare Facility–Associated *Clostridium difficile* Infection and of Healthcare-Associated Infection due to Methicillin-Resistant *Staphylococcus aureus* in Community Hospitals

Becky A. Miller, MD;[1] Luke F. Chen, MD, MPH;[1]
Daniel J. Sexton, MD;[1] Deverick J. Anderson, MD, MPH[1]

We sought to determine the burden of nosocomial *Clostridium difficile* infection in comparison to other healthcare-associated infections (HAIs) in community hospitals participating in an infection control network. Our data suggest that *C. difficile* has replaced MRSA as the most common etiology of HAI in community hospitals in the southeastern United States.

*Infect Control Hosp Epidemiol* 2011;32(4):387-390

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## *C. difficile* PCR ribotype 027

- More severe disease
- Produces more toxins A and B
- Produces binary toxin
- Fluoroquinolone resistant
- Epidemic spread across North America and UK/Europe from early 2000s
- Numbers dropping in UK/Europe
- Still major issue in USA
- Three clusters in Australia since 2009

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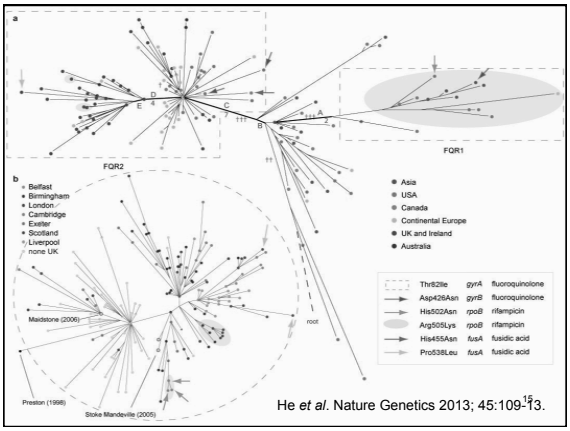


He *et al*. Nature Genetics 2013; 45:109-13.

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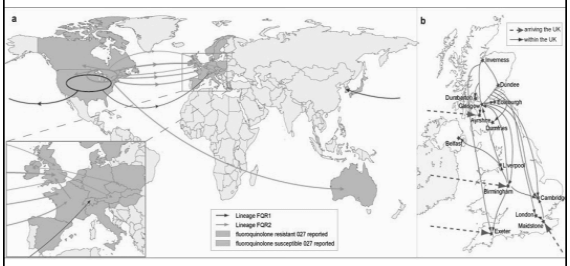
He *et al*. Nature Genetics 2013; 45:109-13.

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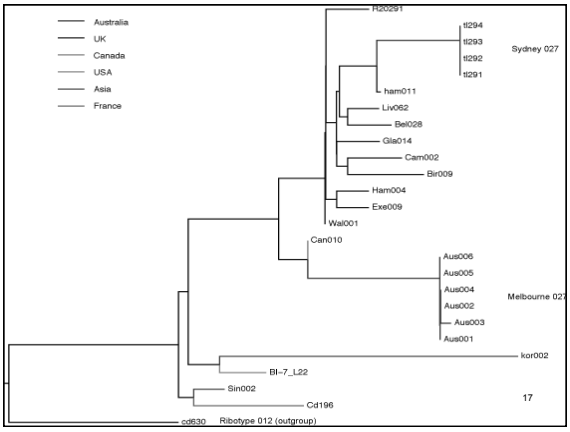


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## CDI in Australia

- Not a notifiable infection
- But mandatory reporting by hospitals since 2010
- Reporting of "hospital identified" cases of CDI

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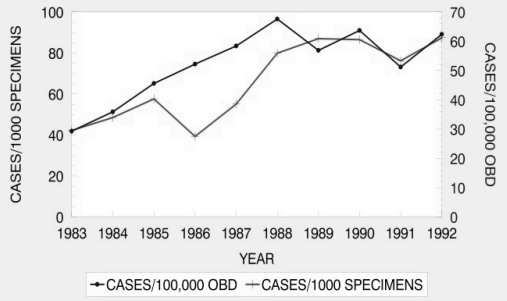
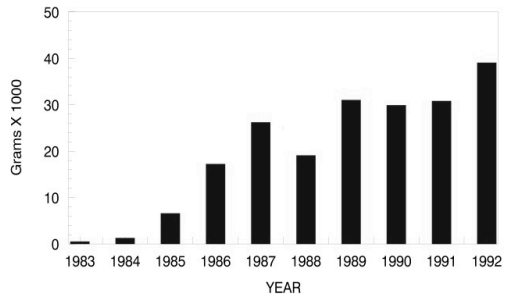
Fig.2 Incidence of *Clostridium difficile*-associated diarrhoea at SCGH, 1983-92




Cephalosporin use SCGH, 1983-92

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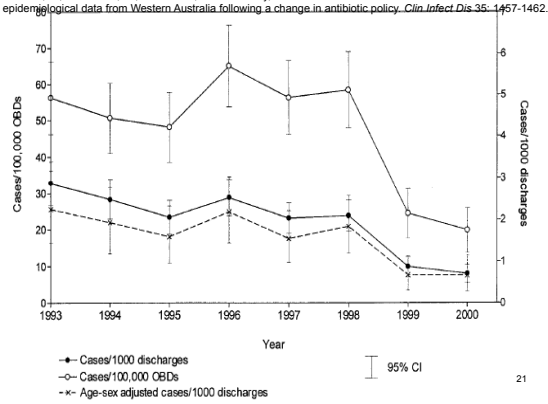
Thomas, C., Stevenson, M., Williamson, D.J. and Riley, T.V. 2002. *Clostridium difficile*-associated diarrhoea: epidemiological data from Western Australia following a change in antibiotic policy. *Clin Infect Dis* 35: 1457-1462.

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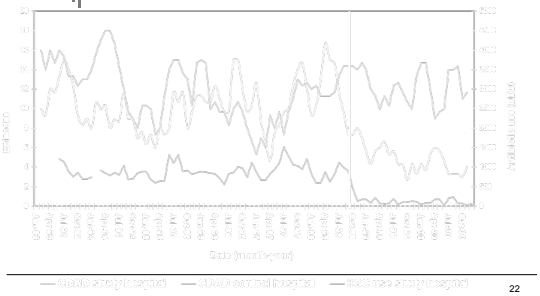
**C.difficile: monthly episodes 1993-2000**



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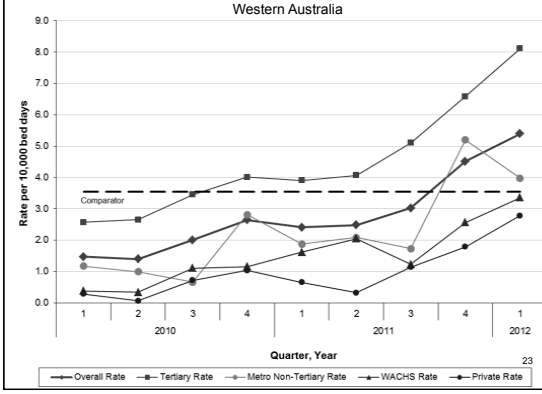
Aggregate *Clostridium difficile* Infection Rates

Western Australia

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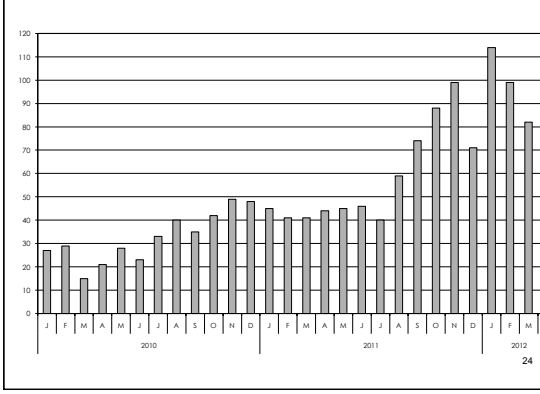
CDI CASES 2010 - 2012

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## Reasons for increase

- Changes in test numbers
  - Some evidence of this
  - Greater awareness
- Changes in testing methods
  - Yes – when and what impact?
- If a real increase then why?
  - Healthcare associated vs community-associated
  - Changes in risk factors??????

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The Epidemiology of Community-Acquired *Clostridium difficile* Infection: A Population-Based Study

Sahil Khanna, MBBS[1], Darrell S. Pardi, MD, MS, FACG[1], Scott L. Aronson, MD[1,2], Patricia P. Kammer, CCRP[1], Robert Orenstein, DO[3], Jennifer L. St Sauver, PhD[4], W. Scott Harmsen, MS[5] and Alan R. Zinsmeister, PhD[5]

**Study highlights**

**What is current knowledge?**

- *Clostridium difficile infection* is increasing worldwide with hospitalization and antibiotic exposure as the most common risk factors.
- The epidemiology and characteristics of community-acquired *Clostridium difficile* infection are not well defined.

**What is new here?**

- A major proportion of *Clostridium difficile* infection patients is community-acquired.
- These patients are younger, often lack traditional risk factors, and have less severe disease than patients with hospital-acquired infection.

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*Am J Gastroenterol.* 2012 January ; 107(1): 89–95. doi:10.1038/ajg.2011.398.

## Community acquired CDI

- *C. difficile* was the most common enteric pathogen detected.
- Most patients had only mild to moderate diarrhoea.
- In the majority of patients the diarrhoea was protracted.
- 80-85% of patients had received antimicrobial agents in the 3 months preceding onset of diarrhoea.
- Most treated successfully with metronidazole.

Riley et al. Diarrhoeal disease due to *Clostridium difficile* in general practice. *Pathology* 1991: 23: 346-349.
Riley et al. Community-acquired *Clostridium difficile*-associated diarrhea. *Clin Infect Dis* 1995: 20 (Suppl 2): S263-S265.

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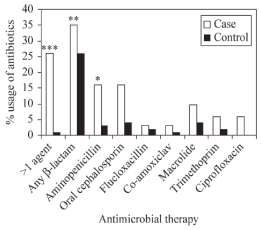


**Figure 1.** Comparative antibiotic usage in randomly selected community-associated CDI cases and controls. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Contact with infants <2 years old significantly associated with CDI

Wilcox *et al. J Antimicrob Chemother* 2008; 62: 388-396.

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## Community acquired CDI

- This is not new!
- Very much under-diagnosed for years
- *C. difficile* is ubiquitous
- Many sources in the community
- All animals get colonised at birth incl. humans
- But – generally requires exposure to an infectious dose AND prior gut insult
- Risk factors need further investigation

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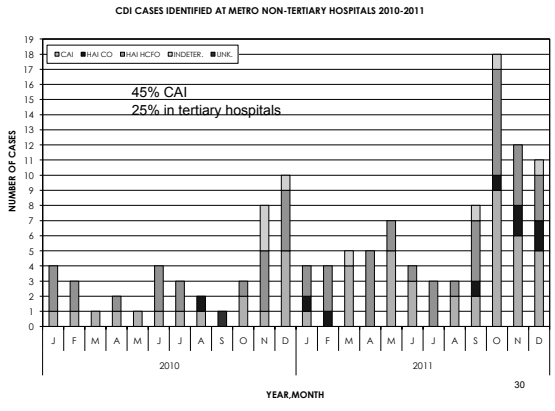


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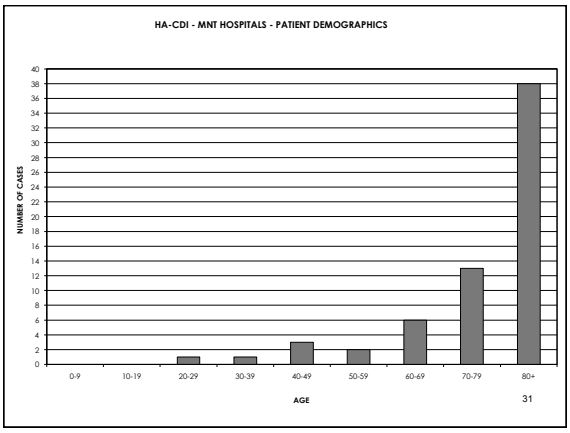


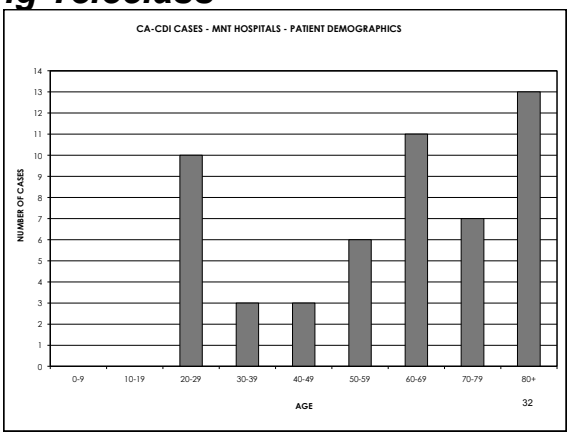


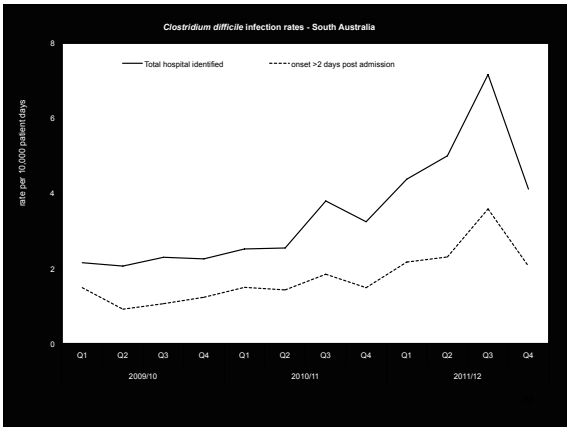


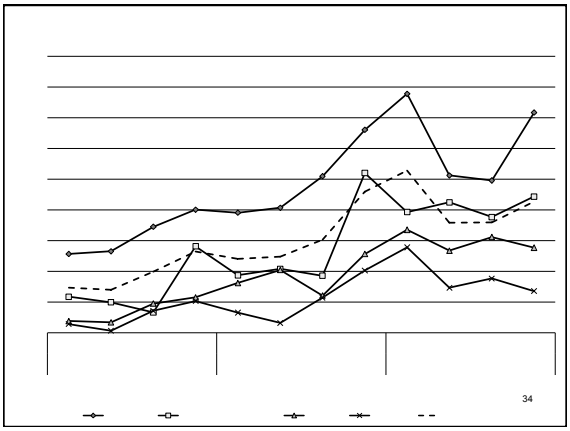


## *C. difficile* PCR ribotype 244

- More severe disease – attributable mortality 30% (Dr Rhonda Stuart)
- Currently community acquired
- Produces more toxins A and B
- Produces binary toxin, *tcdC* mutation at pos.117
- Fluoroquinolone susceptible
- Putative 027 with GeneXpert
- Sept-Oct 2010 ACSQHC snapshot – one isolate
- 2011/12 3rd most common ribotype ~5%

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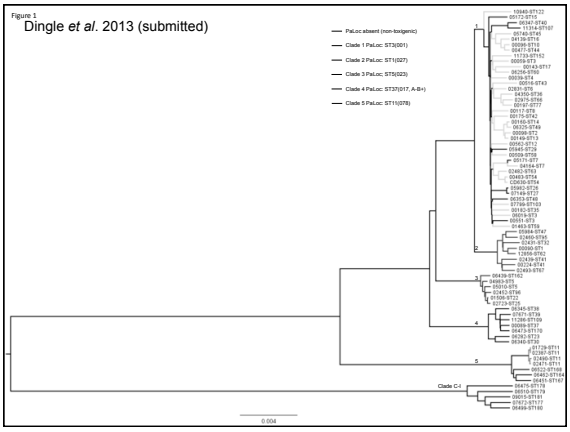


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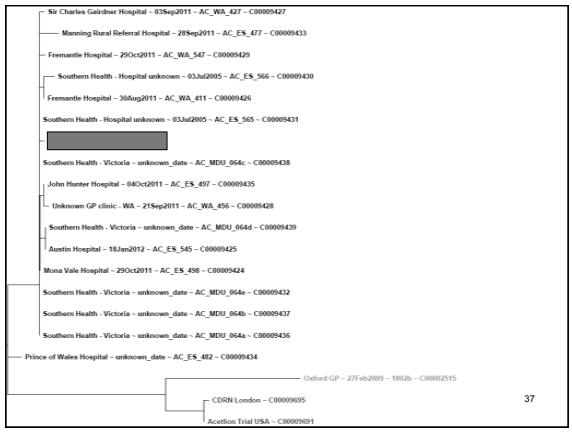


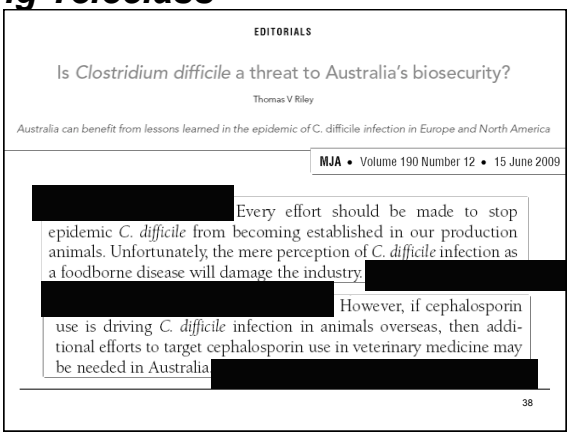


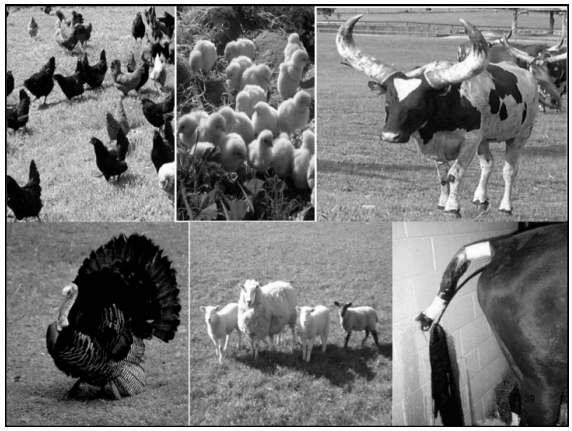


Table. Source and characteristics of *Clostridium difficile* isolates obtained from retail meats sold in Tuscon, Arizona, USA, 2007*

| Meat product | No. samples cultured | Total no. (%) positive | Ribotype | Toxinotype | Δ*tcdC*, bp† | PFGE type | No. (%) pos |
|---|---|---|---|---|---|---|---|
| Ground beef (uncooked) | 26 | 13 (50) | 027 | III | 18 | NAP1 | 1 (3.8) |
| | | | | | | NAP1-related | 2 (7.7) |
| | | | 078 | V | 39 | NAP7 | 8 (30.8) |
| | | | | | | NAP8 | 2 (7.7) |
| Summer sausage (ready to eat) | 7 | 1 (14.3) | 027 | III | 18 | NAP1 | 1 (14.3) |
| Ground pork (uncooked) | 7 | 3 (42.9) | 027 | III | 18 | NAP1-related | 1 (14.3) |
| | | | 078 | V | 39 | NAP7 | 2 (28.6) |
| Braunschweiger (ready to eat) | 16 | 10 (62.5) | 027 | III | 18 | NAP1 | 2 (12.5) |
| | | | | | | NAP1-related | 1 (6.2) |
| | | | 078 | V | 39 | NAP7 | 7 (43.8) |
| Chorizo (uncooked) | 10 | 3 (30.0) | 027 | III | 18 | NAP1-related | 1 (10.0) |
| | | | 078 | V | 39 | NAP7 | 2 (20.0) |
| Pork sausage (uncooked) | 13 | 3 (23.1) | 027 | III | 18 | NAP1-related | 1 (7.7) |
| | | | 078 | V | 39 | NAP7 | 2 (15.4) |
| Ground turkey (uncooked) | 9 | 4 (44.4) | 078 | V | 39 | NAP7 | 4 (44.4) |
| Totals | 88 | 37 (42.0) | 027 | III | 18 | NAP1 | 4 (4.4) |
| | | | | | | NAP1-related | 6 (6.7) |
| | | | 078 | V | 39 | NAP7 | 25 (27.8 |
| | | | | | | NAP8 | 2 (2.2) |

*All samples were positive for *cdtB*, which encodes the binding component of binary toxin. PFGE, pulsed-field gel electrophoresis.
†Deletions in *tcdC* regulatory gene.

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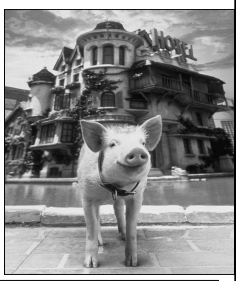
**Songer *et al. Emerg Infect Dis* 2009; 15: 819-821**

## *C. difficile* in pigs

- Early this century outbreaks of CDI in 5d old piglets in USA - high mortality (16%)
- Since 2000, *C. difficile* the major & most common cause of enteritis in neonatal piglets in USA
- Economic losses
- Pig ribotype 078
- 078 now infecting people in Europe and USA, 3rd most common
- ? Food source or environment



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## *Clostridium difficile* infection in Europe: a hospital-based survey

Martijn P Bauer, Daan W Notermans, Birgit H B van Benthem, Jon S Brazier, Mark H Wilcox, Maja Rupnik, Dominique L Monnet, Ioan T van Dissel, Ed J Kuijper, for the ECDIS Study Group*

Lancet 2011; 377: 63–73

Methods We set up a network of 106 laboratories in 34 European countries.

| Microbiological characteristics | |
|---|---|
| Most frequent PCR-ribotypes of toxigenic isolates | |
| 014/020 | 61/389 (16%) |
| 001 | 37/389 (10%) |
| 078 | 31/389 (8%) |
| 018 | 23/389 (6%) |
| 106 | 20/389 (5%) |
| 027 | 19/389 (5%) |
| 002 | 18/389 (5%) |
| 012 | 17/389 (4%) |
| 017 | 14/389 (4%) |

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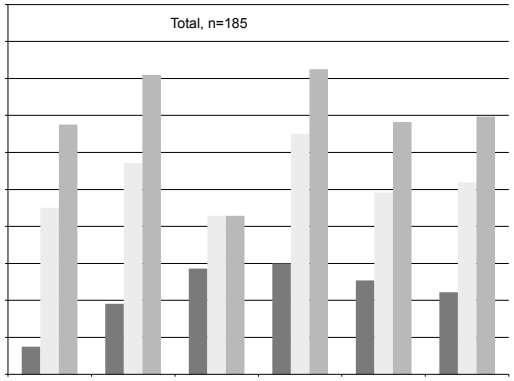


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## *C. difficile* in cattle in Australia

- 2008/9: adult cattle, 151 carcass washings and 151 gut contents from WA
  - No *C. difficile*
- 2009/10: 280 faecal samples from adults E Australia
  - 5 positives (1.8%)
- 2012: 360 <7 day old veal calves, several abattoirs in Vic and Queensland (4% in 2-6 month old calves)
  - 56% positive

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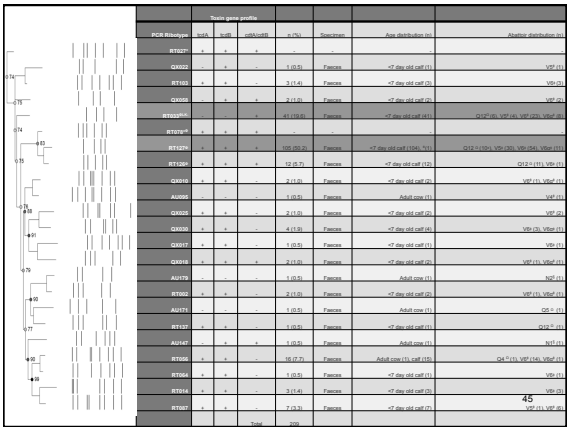

| PCR Ribotype | Toxin gene profile | | | n (%) | Specimen | Age distribution (n) | Abattoir distribution (n) |
|---|---|---|---|---|---|---|---|
| RT027[1] | + | + | + | - | - | - | - |
| QX022 | - | + | - | 1 (0.5) | Faeces | <7 day old calf (1) | V3[F] (1) |
| RT103 | + | + | - | 3 (1.4) | Faeces | <7 day old calf (3) | V9[-] (3) |
| QX023 | - | + | + | 2 (1.0) | Faeces | <7 day old calf (2) | V9[F] (2) |
| RT033[2,3] | - | - | + | 41 (19.0) | Faeces | <7 day old calf (41) | Q12[F-] (6), V3[F-] (4), V9[F-] (23), V9bf[F-] (8) |
| RT078[4] | + | + | + | - | - | - | - |
| RT127[5] | + | + | + | 105 (50.2) | Faeces | <7 day old calf (104), ?(1) | Q12[F-] (101), V9[-] (3), V9bf[-] (1) |
| RT126[6] | + | + | + | 12 (5.7) | Faeces | <7 day old calf (12) | Q12[F-] (11), V9[-] (1) |
| QX015 | + | + | - | 2 (1.0) | Faeces | <7 day old calf (2) | V9[F] (1), V9bf[-] (1) |
| AU099 | - | - | - | 1 (0.5) | Faeces | Adult cow (1) | V4[F] (1) |
| QX024 | + | + | - | 2 (1.0) | Faeces | <7 day old calf (2) | V9[F] (2) |
| QX039 | + | + | - | 4 (1.9) | Faeces | <7 day old calf (4) | V9[-] (3), V9bf[-] (1) |
| QX017 | + | + | - | 1 (0.5) | Faeces | <7 day old calf (1) | V9[-] (1) |
| QX019 | + | + | + | 2 (1.0) | Faeces | <7 day old calf (2) | V9[F-] (1), V9bf[F-] (1) |
| AU179 | - | - | - | 1 (0.5) | Faeces | Adult cow (1) | N3[F-] (1) |
| RT002 | + | + | - | 2 (1.0) | Faeces | <7 day old calf (2) | V9[F-] (1), V9bf[F-] (1) |
| AU171 | - | - | - | 1 (0.5) | Faeces | Adult cow (1) | Q5[F-] (1) |
| RT137 | + | + | - | 1 (0.5) | Faeces | <7 day old calf (1) | Q12[F-] (1) |
| AU147 | - | + | + | 1 (0.5) | Faeces | Adult cow (1) | N1[F-] (1) |
| RT056 | + | + | - | 16 (7.7) | Faeces | Adult cow (1), calf (15) | Q4[F-] (1), V9[F-] (14), V9bf[F-] (1) |
| RT084 | + | + | - | 1 (0.5) | Faeces | <7 day old calf (1) | V9[-] (1) |
| RT014 | + | + | - | 3 (1.4) | Faeces | <7 day old calf (3) | V9[-] (3) |
| RT087 | + | + | - | 7 (3.3) | Faeces | <7 day old calf (7) | V3[F-] (1), V9[F-] (6) |
| | | | Total | 209 | | | |

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## Contaminated vegetables

- Bakri *et al*. *Clostridium difficile* in ready-to-eat salads, Scotland. Emerg Infect Dis 2009;15: 817-8. (3/40 [7.5%] positive)
- Metcalf *et al*. *Clostridium difficile* in vegetables, Canada. Letts Appl Microbiol 2010; 51: 600-2. (5/111 [4.5%] positive)
- Al Saif and Brazier. The distribution of *Clostridium difficile* in the environment of South Wales. J Med Microbiol 1996; 45: 133-7.(7/300 [2.3%] positive)
- MUSHROOMS!

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## Our 1st theory

- Contaminated Australian meat or vegetables
- Driven by flu season plus antibiotics
- But can't find RT 244 in any animals!
- Doesn't account for all the increase
- RT 244 comes from North America

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## Ribotype 251

- 2nd major new RT emerged in Australia
- Similar to RT 244
- Community acquired, severe disease
- Binary toxin positive but no *tcdC* deletion or mutation
- There no putative 027 with GeneXpert
- Groups with 027 by PFGE
- Cluster found in USA along with a cluster of 244

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## Our 2nd theory

- Contaminated food imported from North America
- 96% of Australian food locally produced
- A seasonality of RT 244 infections
- Exactly the same problem occurring in NZ with the same food importation patterns as Australia
- But probably endemic local food-borne disease also

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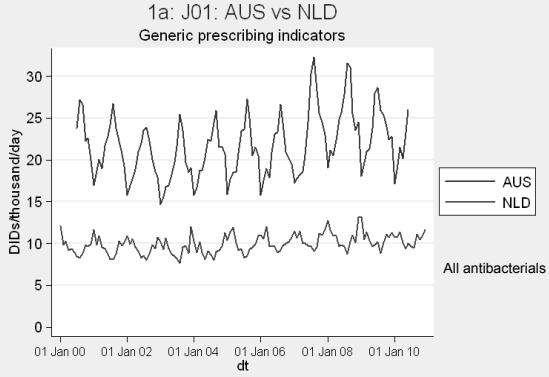


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Courtesy of John Turnidge

## Animal/human connections

- Ribotype 126
- Ribotype 127
- Ribotype 033

cattle

- Ribotype 237 - pigs
- Ribotype ??? – horses
- Many other new ribotypes from animals: usually binary toxin positive, that are starting to appear in humans

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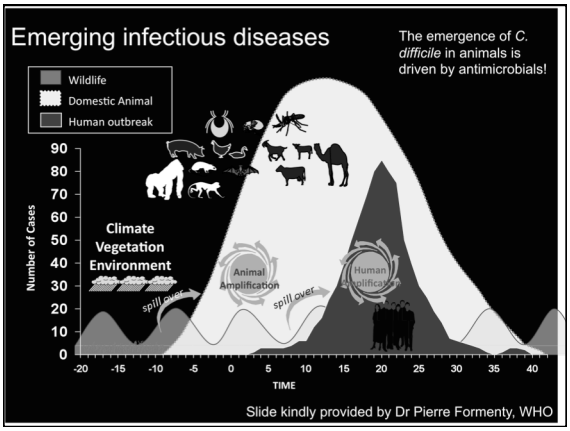


## To summarise the issues

- Major new human health problem in Australia (and NZ) – community CDI
- Need to find the source/reservoir
- Need to prevent establishment of RTs 244/251 in hospitals
- Now a major animal health problem (pigs/horses)
- Gross contamination of the environment OUTSIDE hospitals - probable contamination of food
- CDI is a zoonosis
- Will require a One Health approach to resolve

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University of Leeds (Mark Wilcox)

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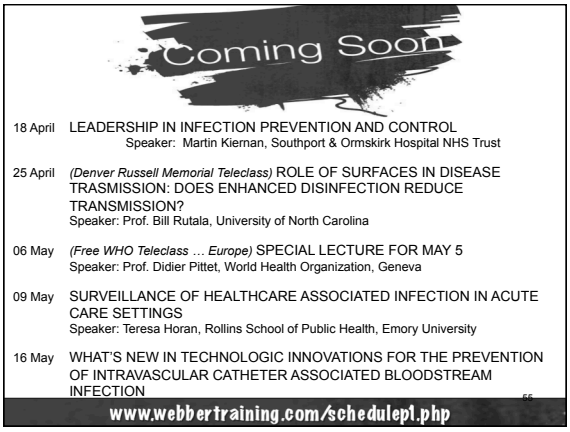
***Clostridium difficile in the Community: Food for Thought***
***Prof. Tom Riley,***
***A Webber Training Teleclass***





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