Milestones in *Clostridium difficile* Research: Basic and Clinical

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Disclosures: DNG holds patents for the treatment and prevention of CDI licensed to ViroPharma/Shire, and is a consultant or advisory board member for Merck, Cubist, Actelion, Sanofi Pasteur, Rebiotix, Pfizer, Summit and ViroPharma/Shire.

Views expressed are those of the presenter and do not necessarily reflect the views of the U.S. Department of Veterans Affairs.

Choice of milestones is the prerogative of the presenter. Apologies to all whose excellent contributions I may have overlooked or forgotten. RED Boxes are my favorites.

Research Milestone Topic Areas

- CDI Discovery and Basic/Genetic Milestones
- Animal model Milestones
- Epidemiologic Milestones
  - Clinical
  - Molecular/Typing
- Treatment Milestones
- Diagnostic Milestones
- Infection Control and Prevention Milestones
- Immunologic Milestones
- Biotherapeutics and Microbiome Milestones

Milestones that Antedate CDI

- First description of pseudomembranous colitis (PMC)

  Finney JMT. *Bull Johns Hopkins Hosp* **1893**;4:53

- First description of *Clostridium difficile* (in infants)

  Hall IC, O'Toole E. *Am J Dis Child* **1935**;49:390

- First description of rodent model of antibiotic-associated colitis (AAC) in guinea pigs

  Hambre DM, et al. *Am J Med Sci* **1943**:206:642

- Report of 94 post-operative cases of pseudomembranous enterocolitis 1925-1952

  Pettet JD et al, SG&O **1954**;98:546-52

- Report of 14 cases of PMC, 5 in colonic obstruction, 9 in medical patients

  Goulston SJM, McGovern VJ, Gut **1965**;6:207-12

Milestones that Antedate CDI

- Lincomycin as a Cause of PMC, (8 patients, 3 deaths, 1 colectomy) Scott AJ et al *Lancet* **1973**:II:1232-34
- Clindamycin-associated Colitis. A prospective study. (200 Consecutive clindamycin recipients: 21% diarrhea, 10% PMC, 38% diarrhea onset after clindamycin discontinued, benign course if recognized early and clindamycin stopped)
  Tedesco FJ, et al. *Ann Int Med* **1974**;81:429

Solving the Mystery of Clindamycin Colitis: The Discovery of *C. difficile* and Toxins

- Cytopathic effect of stools from patients with PMC

  Larson HE, et al. *Br Med J* **1977**;1:1246
- Vancomycin prolongs survival in hamsters with antibiotic associated cecitis (AAC)

  Bartlett JG, et al. *Gastroenterol* **1977**;73:772
- Stools from patients with PMC contain a toxin neutralized by *C. sordellii* antitoxin

  Rifkin GD, et al. *Lancet* November **1977**;2:1103
- Toxigenic clostridia cause PMC in patients

  Bartlett JG, et al. *N Engl J Med* March **1978**;298:531
- *C. difficile* isolated from one patient with PMC, toxin neutralized by *C. sordellii* antitoxin, not others

  George WL et al *Lancet* April **1978**;1:802
- *C. difficile* isolated from multiple patients with PMC

  Larson HE et al. *Lancet* May **1978**;1:1063-6

CDI Milestone Development was Stifled for over a Decade: Why?

- Clinical complacency that the disease was a nuisance that was readily managed
- Lack of clinical awareness and tracking of CDI
- Discovery early on of very highly effective treatment agents (vancomycin and metronidazole) stifled new drug development
- An inappropriate sense that everything about CDI was known (see quote)
- Waning use of anaerobic culture for diagnosis
- Restrictions on use of the hamster model
- Reluctance by major funding agencies to support grants in *C. difficile* research, perhaps as a result of the above points

Chapter 1, Introduction: *Clostridium difficile*: Its Role in Intestinal Disease (1988)

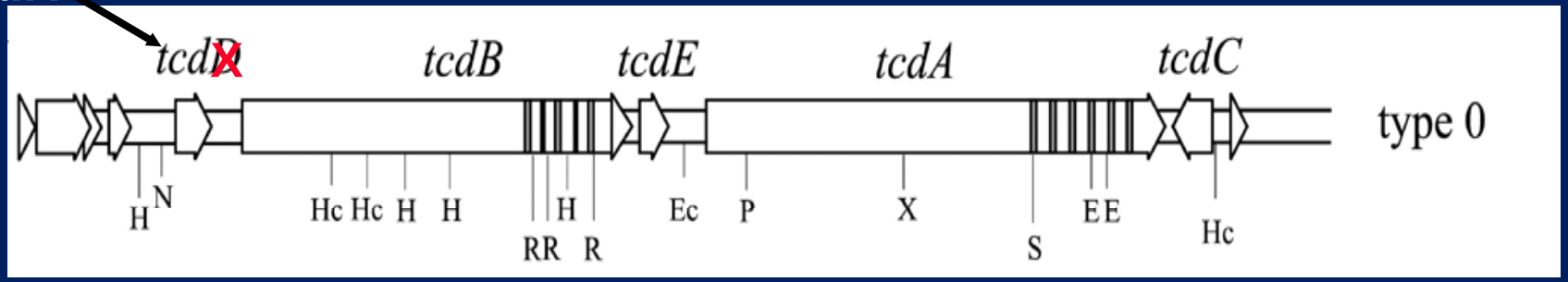
… Most of the important information regarding this microbe and the associated enteric infection is now known. …There remain nuances of this disease that are poorly understood, but there is no doubt that this potentially lethal pathogen is now largely controlled and patients who have a life threatening infection are now managed with diagnostic and therapeutic modalities that are extremely effective.

Clostridium difficile: Its Role in Intestinal Disease. Rolfe RD, Finegold SM [Eds]. Academic Press, San Diego, CA. 1988

Basic Milestone Studies of *C. difficile* Toxins

- Large Clostridial toxins (LCTs): 2 large (~300 kDa), single-unit proteins which share 49% aa homology
- Toxin A 'enterotoxin': Marked tissue damage and fluid response in experimental animals
  Lyerly DM, et al. Infect Immun **1982**;47:349
- Toxin A given orally to animals caused disease but toxin B did not Lyerly DM Infect Immun **1985**;47:349-52
- Toxin B 'cytotoxin': Potent cytotoxin, also active on human colonic strips in vitro
  Riegler M, et al. J Clin Invest **1995**;95:2004
- Mechanism of action: Glycosylation of small GTP-binding proteins (Rho subfamily) involved in cell cytoskeleton organization Just I..Aktories K, et al. Nature **1995**:375:500

Genetic Basis for Toxin Production:

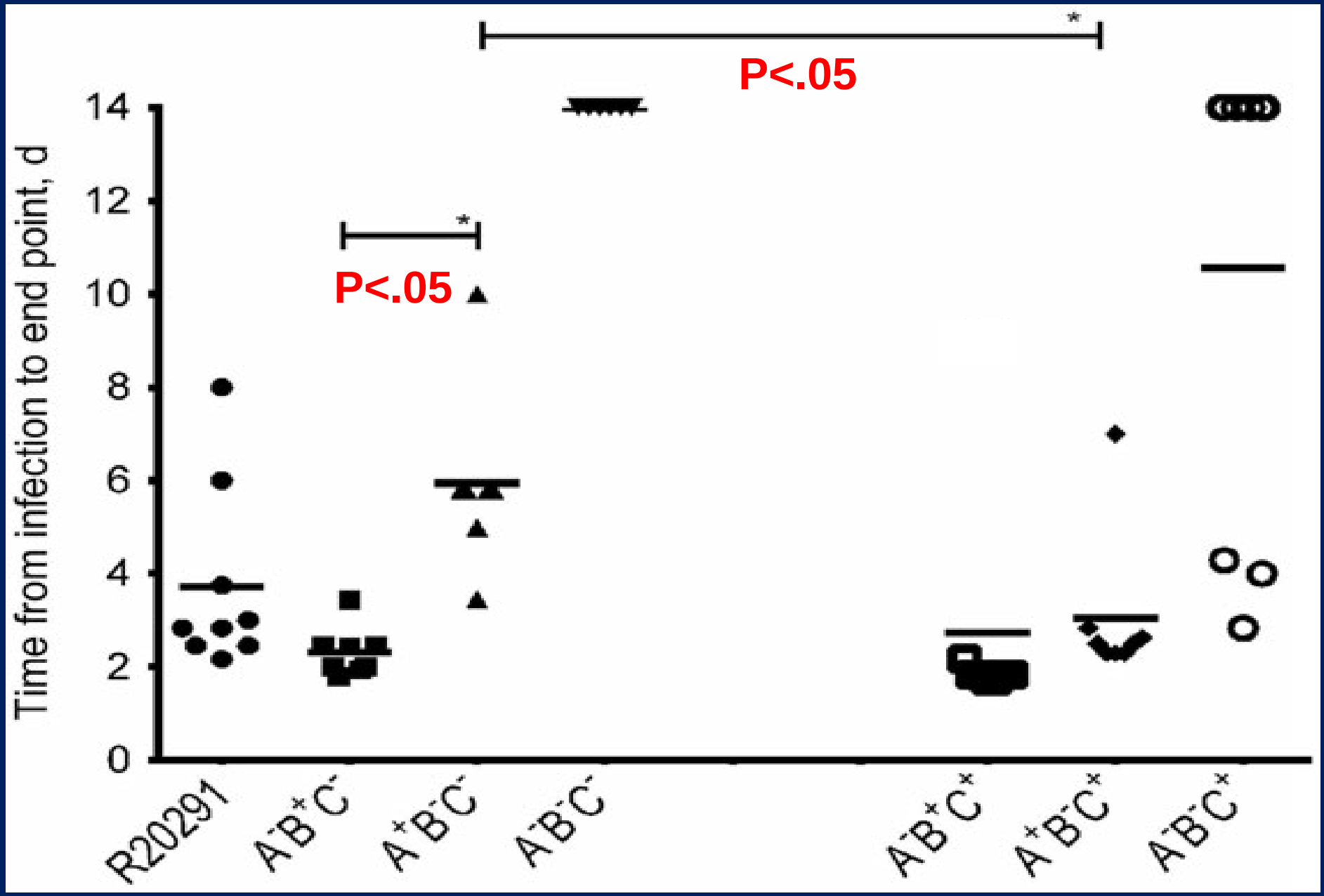


- 1990: Toxin A & B genes cloned and sequenced
  Dove CH, et al. *Infect Immun* **1990**;58:480
  von Eichel-Streiber, et al. *Med Microbiol Immunol*. **1990**;179:271-9.
- 1997: Transcription and toxin production described
  Hundsberger T, …von Eichel-Streiber C, et al. *Eur J Biochem* **1997**;244:735
  Hammond GA, et al. *Microb Pathogen* **1997**;22:143
- 2001: *tcdR* is a positive toxin regulator: Alternative RNA polymerase sigma factor
  Mani N and Dupuy B. *Proc Natl Acad Sci U S A*. **2001**;98:5844-9.
- 1988: Binary Toxin discovered long before clinical importance
  Popoff MR, et al Actinspecific ADP-ribosyltransferase produced by a *Clostridium difficile* strain. *Infect Immun* **1988**;56:2299-306

Basic Milestone Studies of *C. difficile* Toxins

- Toxin B is Most Important: Toxin B is essential for virulence of *Clostridium difficile*.
  Lyras D…Rood J et al Nature. **2009**;458:1176-9.
- No, Both Toxins A and B are important: The role of toxin A and toxin B in *Clostridium difficile* infection.
  Kuehne SA .. Minton NP et al Nature **2010**;467:711-3.
- No, A, B, and Binary Toxin are important: Importance of toxin A, toxin B, and CDT in virulence of an epidemic *Clostridium difficile* strain.
  Kuehne SA…Minton NP et al J Infect Dis. **2014**;209:83-6.

Kuehne et al J Infect Dis. **2014**;209:83-6



Animal Model Milestones

- "The protective effect of vancomycin on clindamycin-induced colitis in hamsters" (clindamycin alone induced fatal hamster colitis which was prevented by vancomycin, but relapsed after vancomycin) Browne R et al Johns Hopkins Med J **1977**:141:183-92
- Identical organisms and toxin effects from hamsters and humans with clindamycin induced colitis
  Chang TW et al Infect Immun. **1978**;20:526-9
- There is no *C. difficile* in normal hamster stool, isolation of hamsters + clindamycin = No CDI, *C. difficile* + no clindamycin = No CDI, two distinct events are required for CDI to occur, evidence of aerial spread of *C. difficile* spores
  Larson HE, Price AB & Borriello SP J Infect Dis **1980**;142:408-13
- "Antagonism of toxigenic *Clostridium difficile* by nontoxigenic *C. difficile*" Wilson KH & Sheagren JN, J Infect Dis **1983**;147:733-736
- "Protection of hamsters against *Clostridium difficile* illeocaecitis by prior colonisation with non-pathogenic strains"
  Borriello SP & Barclay FE, J Med Microbiol **1985**;19:339-351

Animal Model Milestones

- Mouse model for CDI opened the field to far more investigators and expanded immunologic studies.
  Chen X, … Kelly CP. A novel mouse model of *Clostridium difficile* associated disease. Gastroenterology 2008:135:1984-92 .
- Distinctive profiles of infection and pathology in hamsters infected with *Clostridium difficile* strains 630 and B1.
  Goulding D, …. Douce GR. Infect Immun. 2009;77:5478-85.

Epidemiologic Milestones

- *C. difficile* contamination of hospital environment
  Mulligan M et al Curr Microbiol **1979**;3:173-5
- *C. difficile* contamination of personnel hands, home environment, and persistence for 20 weeks in environment after seeding
  Kim K-H et al J Infect Dis **1981**;143:42-50

- Prospective case-control study of 149 CDI cases: 87% nosocomial, significant clindamycin & multiple antibiotic use risk, not H-2 blockers
  Gerding DN et al Arch Int Med **1986**;146:95-100
- Hospital acquisition of *C. difficile* by 21% of patients, 63% asymptomatic, clustering by immunoblot typing suggested patient to patient spread
  McFarland et al NEJM **1989**;320:204-10.
- Hospital acquisition of *C. difficile* by 21% of patients, 82% asymptomatic, 18 unique REA types, all disease caused by 2 REA types B1 and B2
  Johnson S et al Lancet **1990**;336:97-100
- Asymptomatic patient admissions carrying REA types of *C. difficile* preceded acquisition of that type by other patients in 85% of cases
  Clabots C et al J Infect Dis **1992**;166:561-7
- Patients who are asymptomatically colonized with *C. difficile* are at significantly lower risk of CDI than uncolonized patients on same wards
  Shim JK et al Lancet **1998**;351:633-6

Typing, Molecular Epidemiology and NAP1/BI/027

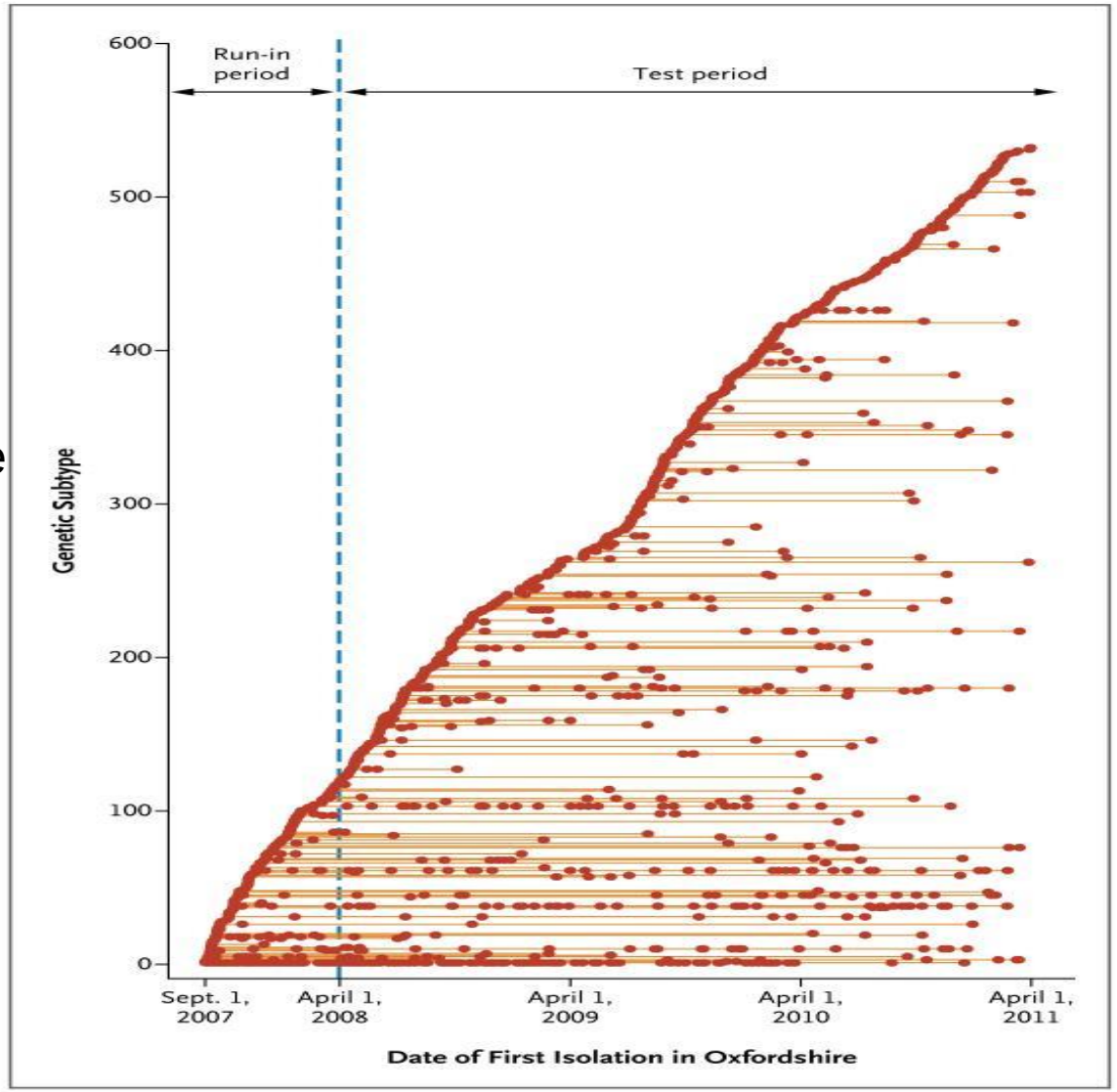
- **Development of REA typing: Application of whole-cell DNA restriction endonuclease profiles to the epidemiology of Clostridium difficile-induced diarrhea.** Kuijper EJ et al J Clin Microbiol. **1987**;25:751-3. **Development of a rapid and efficient restriction endonuclease analysis typing system for Clostridium difficile and correlation with other typing systems.** Clabots et al J Clin Microbiol. **1993**;31:1870-5.
- **PCR ribotyping System: PCR targeted to the 16S-23S rRNA gene intergenic spacer region of *Clostridium difficile* and construction of a library consisting of 116 different PCR ribotypes.** Stubbs, Brazier, O'Neill, Duerden. J Clin Microbiol **1999**;37:461-3.
- Recurrences of CDI are caused by new strains ~50% of the time Johnson S J Inf Dis **1989**;159:340-3
- Wide diversity of *C. difficile* types (55 by REA) causing CDI Samore MH et al J Inf Dis **1994**;170;615-21
- REA "J" group isolates resistant to clindamycin have increased CDI incidence with clindamycin use in US Johnson et al NEJM **1999**;341:1645-51

Typing, Molecular Epidemiology and NAP1/BI/027

- Epidemic of fluoroquinolone resistant NAP1/BI toxinotype III *C. difficile* in US Hospitals
  McDonald LC…Gerding DN et al NEJM **2005**;353:2433-41
- Increased fluoroquinolone risk and mortality with NAP1 outbreak in Quebec Loo V et al NEJM **2005**;353:2442-9
- Infants are repeatedly colonized in the first year of life with a variety of *C. difficile* strains. Is it an immunizing event?
  Clostridium difficile carriage in healthy infants in the community: a potential reservoir for pathogenic strains. Rousseau C…Collignon A et al. Clin Infect Dis 2012;55:1209-15.
- Emergence and global spread of epidemic healthcare-associated *Clostridium difficile*.
  He M…..Lawley TD et al. Nature Genet. **2013**;45:109-13.
- Diverse sources of *C. difficile* infection identified on whole-genome sequencing Eyre DW et al N Engl J Med. **2013**;369:1195-205.

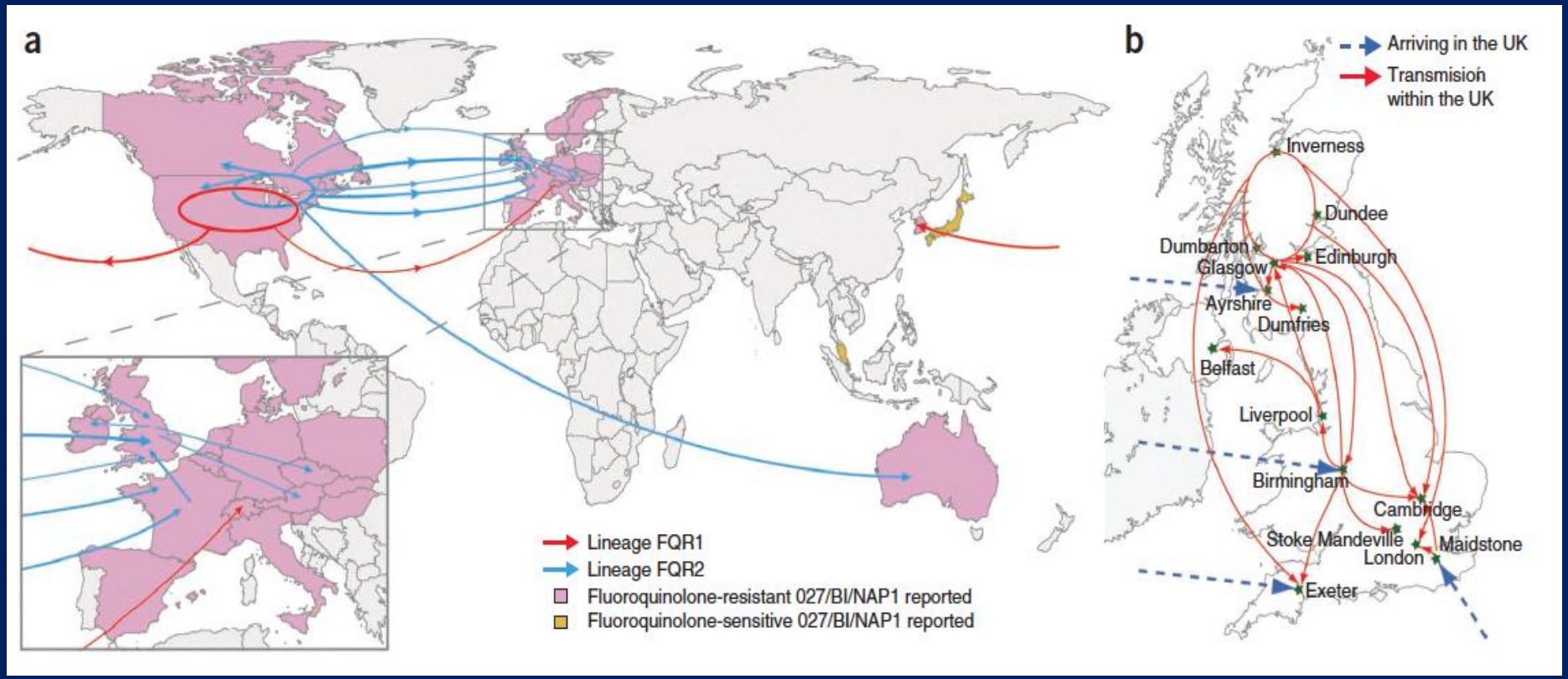
Distinct Genetic Subtypes (> 10 SNVs) by size of Clusters over time

Distinct subtypes identified throughout the study suggesting cases arose from a considerable reservoir.



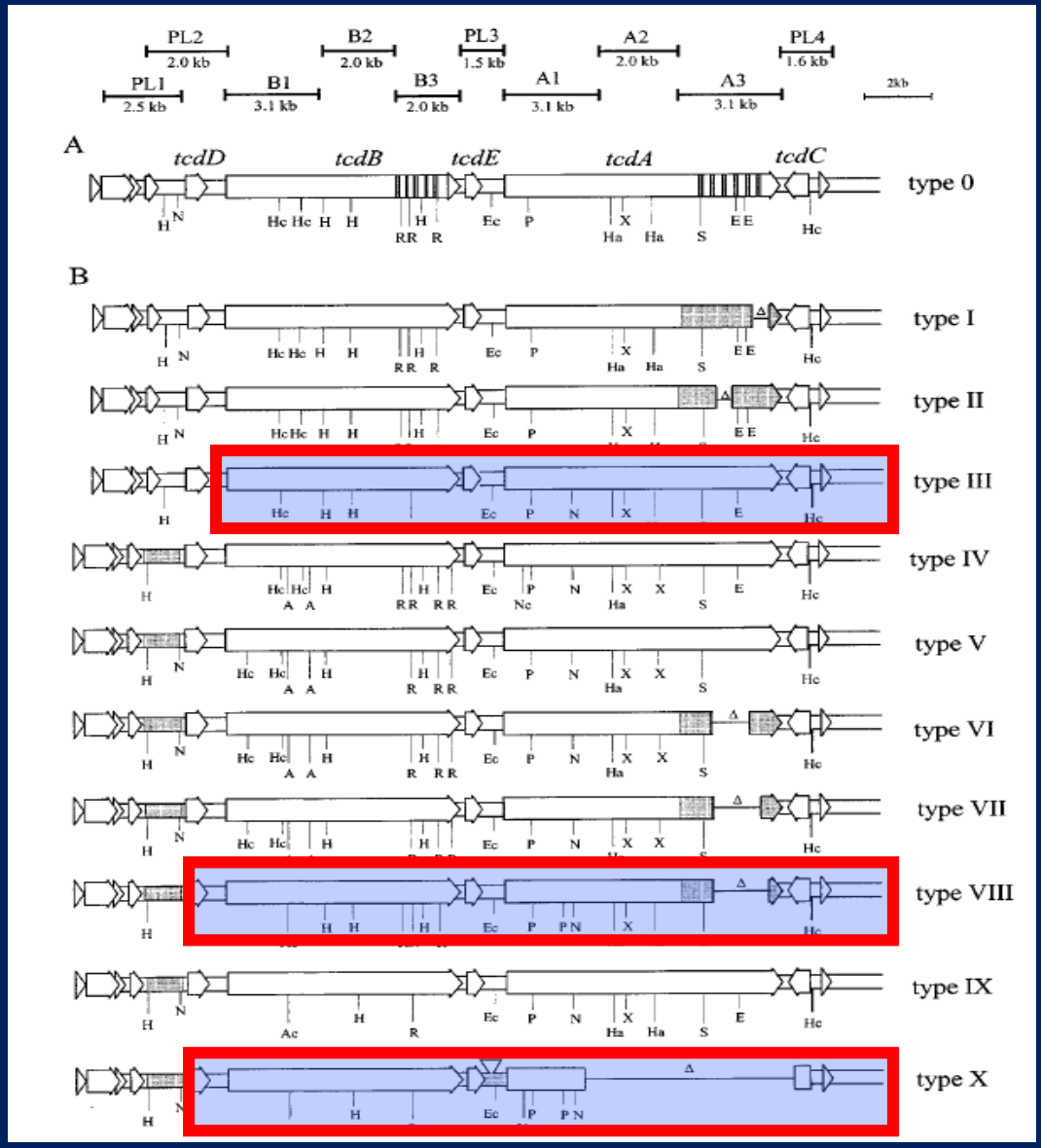
Eyre DW et al. N Engl J Med 2013;369:1195-205

Inferred Global Spread of the 027/BI/NAP1 Strain of *C. difficile*



He M…..Lawley TD et al. Nat Genet. **2013**;45:109-13

Toxinotyping Milestone



BI/027/NAP1, toxino III

< Tox A-Neg, B-Pos

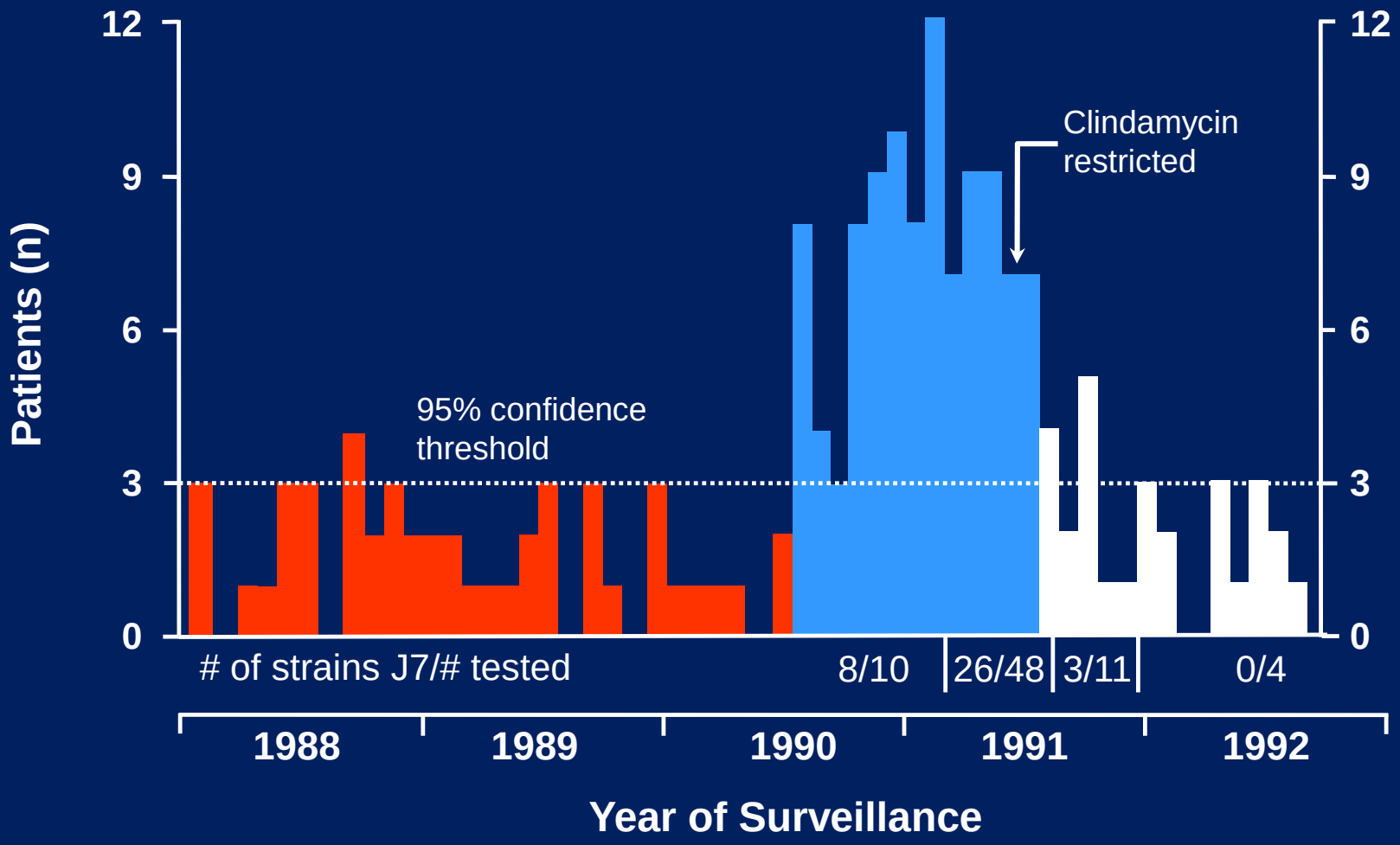
< Tox A-Neg, B-Pos

Rupnik M, et al. *J Clin Microbiol* 1998;26:2240

Milestone Infection Control Studies

- Use of gloves by healthcare workers reduces CDI rates Johnson S et al Am J Med. **1990**;88:137-40
- Decreased CDI by replacement of electronic thermometers with disposables
  Brooks SE et al ICHE **1992**;13:98-103
- Restriction of clindamycin ended *C. difficile* outbreak
  Pear SM et al Ann Intern Med. **1994**;120:272-7.
- Cephalosporin control reduced CDI rates in a LTC Facility Wilcox MH et al J Antimicrob Chemother. **2004**;54:168-72.
- Use of bleach for environmental cleaning reduced CDI rates Mayfield JL et al Clin Infect Dis. **2000**;31:995-1000

CDI Outbreak in a VA Hospital Southern Arizona, 1990 - 1991



Pear SM, et al. *Ann Intern Med* 1994;120:272

CDI Antibiotic Treatment Milestones

- Vancomycin effectively treats CDI but recurrences are frequent Silva J Jr et al Am J Med. **1981**;71:815-22
- Prospective randomized trial showed no difference in vancomycin vs metronidazole Teasley et al Lancet **1983**;2:1043-6
- 125 mg qid = 500 mg qid of vancomycin for CDI treatment Fekety R et al Am J Med **1989**;86:15-9
- Decolonization of *C. difficile* colonized patients is best done with placebo vs vancomycin or metronidazole Johnson S et al Ann Int Med **1992**;117:297-302
- US CDC recommends oral vancomycin not be used in hospitals for CDI treatment in order to prevent VRE. 1995
- Vancomycin is superior to metronidazole for severe CDI Zar FA et al Clin Infect Dis. **2007**;45:302-7. Louie T et al ICAAC **2007**.
- Fidaxomicin: First new antibiotic for CDI treatment in 25+ years; reduced recurrence. Louie et al NEJM **2011** Feb 3;364:422-31
- Vancomycin is superior to metronidazole for all CDI Johnson S et al Clin Infect Dis **2014** Aug;59(3):345-54.

CDI Diagnostic Milestones

- Two "Gold Standards" established by 1979:
  - **Cell cytotoxicity assay** Chang TW et al Inf Immun.**1978**;20:526-9
  - ***C. difficile* selective media containing cefoxitin and cycloserine** George WL et al J Clin Microbiol. **1979**;9:214-9

- EIA for toxin A developed Lyerly DM. J Clin Microbiol **1983**;17:72-8.

(and toxin A EIA floodgates opened replacing CCA and Culture)

- Latex agglutination test for Toxin A detects glutamate dehydrogenase (GDH) and test is later resurrected as an EIA Lyerly DM J Clin Microbiol. **1991**;29:2639-42

- Use of PCR for diagnosis Kato H et al J Infect Dis. **1993**;167:455-8

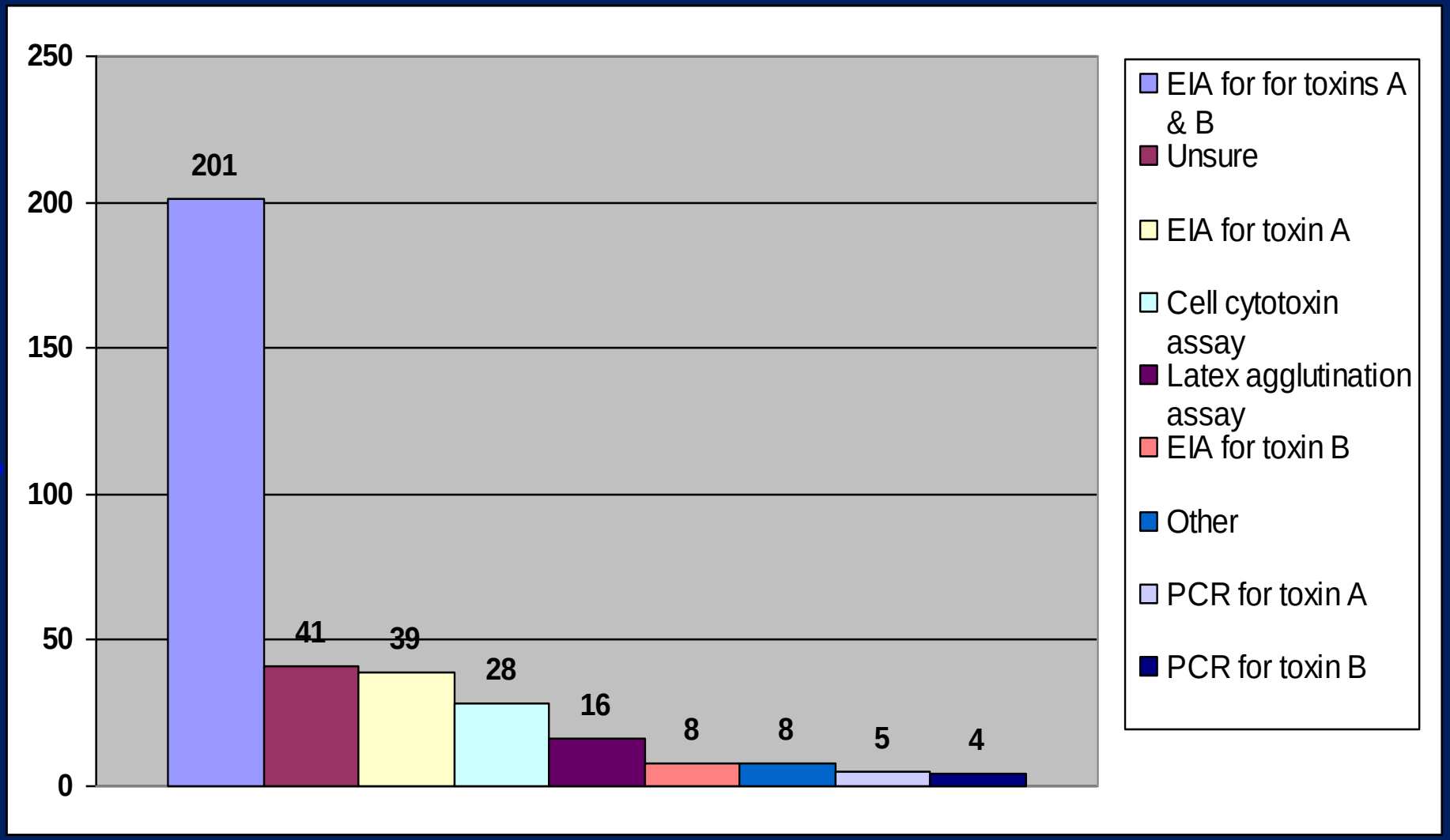
- Clinical outbreaks of *C. difficile* A-/B+ signal demise of toxin A EIA Alfa MJ et al J Clin Microbiol. **2000**;38:2706-14

CDI Antibiotic Treatment Milestones: In What Year and by Whom is this Quote?

"C. *difficile* can be recovered readily from feces of patients afflicted with antimicrobial agent related diarrhea by use of a recently developed selective medium. Such recovery of C. *difficile,* however, is not diagnostic because the organism may be present in the feces of normal subjects, in the feces of some subjects who are receiving antimicrobial agents but who do not have gastrointestinal symptoms, or in the feces of some individuals with antimicrobial agent-related diarrhea who do not have detectable fecal toxin."

GEORGE WL, ROLFE RD, MULLIGAN ME, FINEGOLD SM. **Infectious Diseases 1979-Antimicrobial Agent-Induced Colitis:** An Update Editorial: J Infect Dis **1979** Aug;140:266-8.

Method of Laboratory CDI Diagnosis



Gelone et al Late Breaker Abstract SHEA Ann Mtg Chicago 2006

CDI Diagnostic Milestones

- Superior sensitivity of toxigenic culture vs cell cytotoxin assay Delmee M J Med Microbiol. **2005**;54:187-91
- Tests for toxin in stool are more predictive of patients with CDI (based on outcome complications of CDI episode)
  Longtin et al Clinical Infect Dis **2013**;56:67–73
  Beaulieau C et al Clin Microbiol Infection online: **2014**;20:1067-73
- Toxin in stool predicts higher 30-day mortality compared to toxigenic culture.
  Differences in outcome according to *Clostridium difficile* testing method: a prospective multicentre diagnostic validation study of *C difficile* infection. Planche TD,….Wilcox MH et al. Lancet Infect Dis. **2013**;13:936-45

PCR vs GDH-Toxin EIA + Cytotoxicity

Complications	CDI cases detected by PCR only, not EIA/CCA (n=29)	CDI cases detected by both PCR and EIA/CCA (n=56)	P value*
30-d mortality (%)	1 (3)	10 (18)	.09
Colectomy (%)	0 (0)	1 (2)	1.00
ICU Admission (%)	0 (0)	1 (2)	1.00
CDI Readmission (%)	0 (0)	11 (20)	.01
≥1 Complication (%)	1 (3)	22 (39)#	<.001

Abbreviations: CDI, *Clostridium difficile* infection; EIA/CCA, detection of glutamate dehydrogenase antigen and toxins A and B by enzyme immunoassay and cell culture cytotoxicity assay; PCR, detection of toxin B gene tcdB by polymerase chain reaction. * By Fisher exact test.

One patient with colectomy was admitted to the ICU.

Expanded study: fewer complications (6/31 versus 29/66; p 0.02), but whether these two populations can be managed differently remains to be determined.

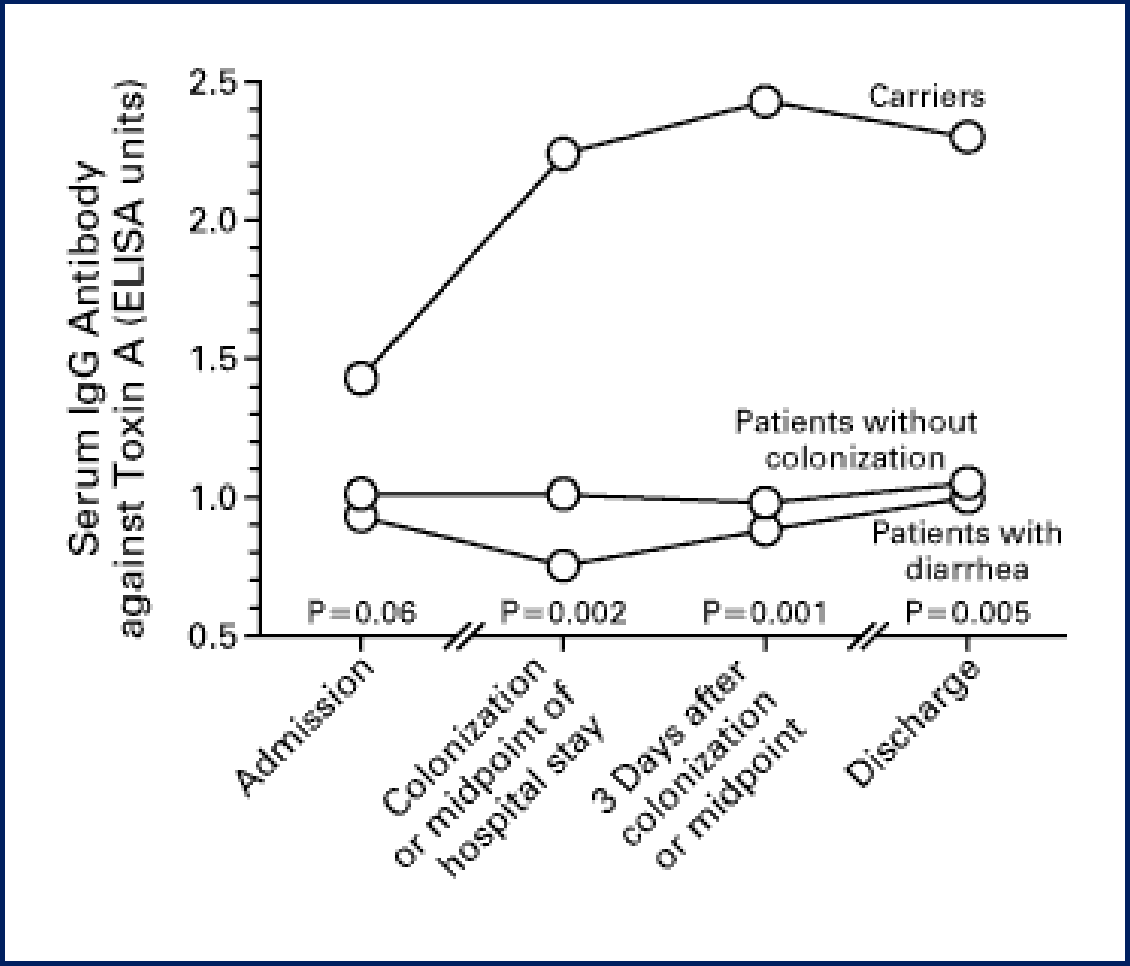
Longtin et al Clinical Infectious Diseases 2013;56:67–73

Beaulieau C et al Clin Microbiol Infection online: 2014;20:1067-73

Immunologic Milestones in CDI

- Antibody to toxin A in 64% and toxin B in 66% of adults; convalescent sera antibody to toxin B higher than controls (P<.001) Viscidi R et al J Inf Dis **1983**;148:93-100.
- Recovery from CDI and absence of relapse correlates with serum antitoxin B antibody and neutralization Aronsson B et al Infection. **1985**;13:97-101
- Rise in IgG antibody to toxin A with exposure to *C. difficile* results in colonization rather than CDI Kyne L …Kelly CP et al N Engl J Med. **2000**;342:390-7.
- Serum IgG antibody response to toxin A protects against recurrent CDI Kyne L ..Kelly CP et al Lancet. **2001**;357:189-93.
- Anti surface layer (SLP) antibody and reduced CDI recurrence Drudy D et al FEMS Imm Med Micro. **2004**;41:237-42.
- Treatment with monoclonal antibodies to *Clostridium difficile* toxins A and B prevent recurrent infection. Lowy I et al New Engl J Med 2010;362:1-9.

Antibody Response to toxin A Determines Outcome after Exposure



Kyne L, et al. N Engl J Med **2000**;342:390

CDI Vaccine Milestones

- Formalin inactivated *C. difficile* culture filtrates protected hamsters when given by nasal, peritoneal, and subcutaneous routes

  Torres JF et al Infect Immun **1995**;63:4619-27

- Inactivated *C. difficile* toxin (toxoid) protects hamsters when given by the IM and rectal routes

  Giannasca PJ et al Infect Immun **1999**;67:527-38

- IM toxoid vaccination of 3 recurrent CDI patients; 2/3 showed rises in serum IgG antitoxin A and B; all resolved diarrhea

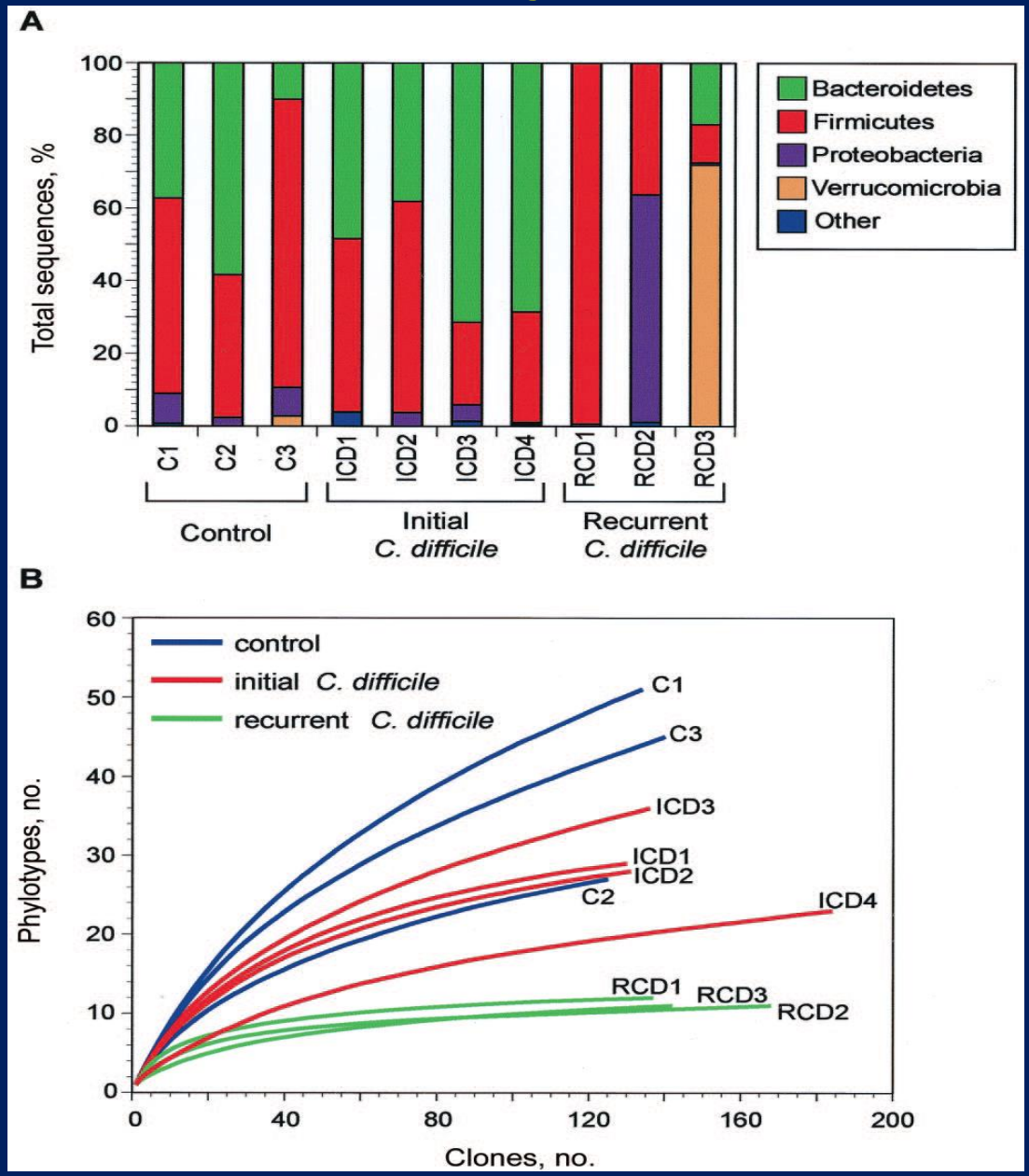
  Sougioultzis S ….Kelly CP et al Gastroenterol **2005**;128:764-70.

- Is toxin A or toxin B the more important antigen? Will elderly respond to vaccine? How quickly? Who should receive vaccine and when?

Biotherapeutic and Preventive Milestones and the Microbiota

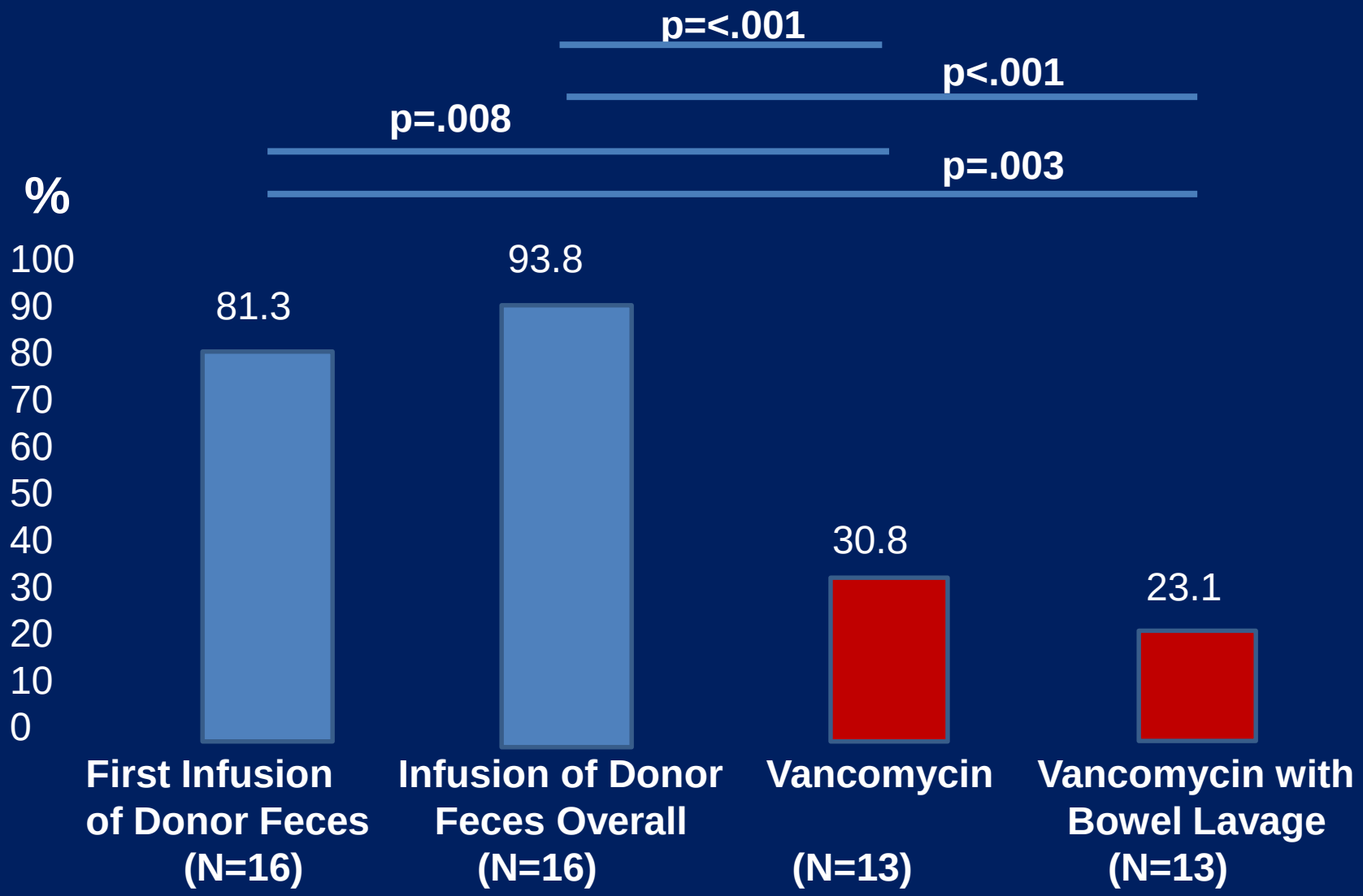
- Non-toxigenic *C. difficile* for recurrent CDI in 2 patients
  Seal D et al Eur J Clin Microbiol. **1987**;6:51-3.
- Rectal instillation of 10 bacterial species prevented CDI recurrence Tvede M & Rask-Madsen J, Lancet **1989**;1:1156-60.
- High-dose vancomycin combined with *Saccharomyces boulardii* reduced recurrent CDI (P=.05)
  Surawicz CM et al Clin Infect Dis. **2000**;31:1012-7.
- Decreased microbiome diversity in multiply recurrent CDI patients Chang JY…Young VB et al. J Infect Dis 2008;197:435–8
- Fecal Microbiome Transplant (FMT) prevents recurrent CDI in multiply recurrent CDI patients.
  Duodenal infusion of donor feces for recurrent *Clostridium difficile*. **van Nood** E et al N Engl J Med. **2013**;368:407-15

Decreased Microbiome Diversity in Recurrent CDI



Chang JY et al
J Infect Dis
2008;197:435–8

FMT Percentage Cured without Recurrence



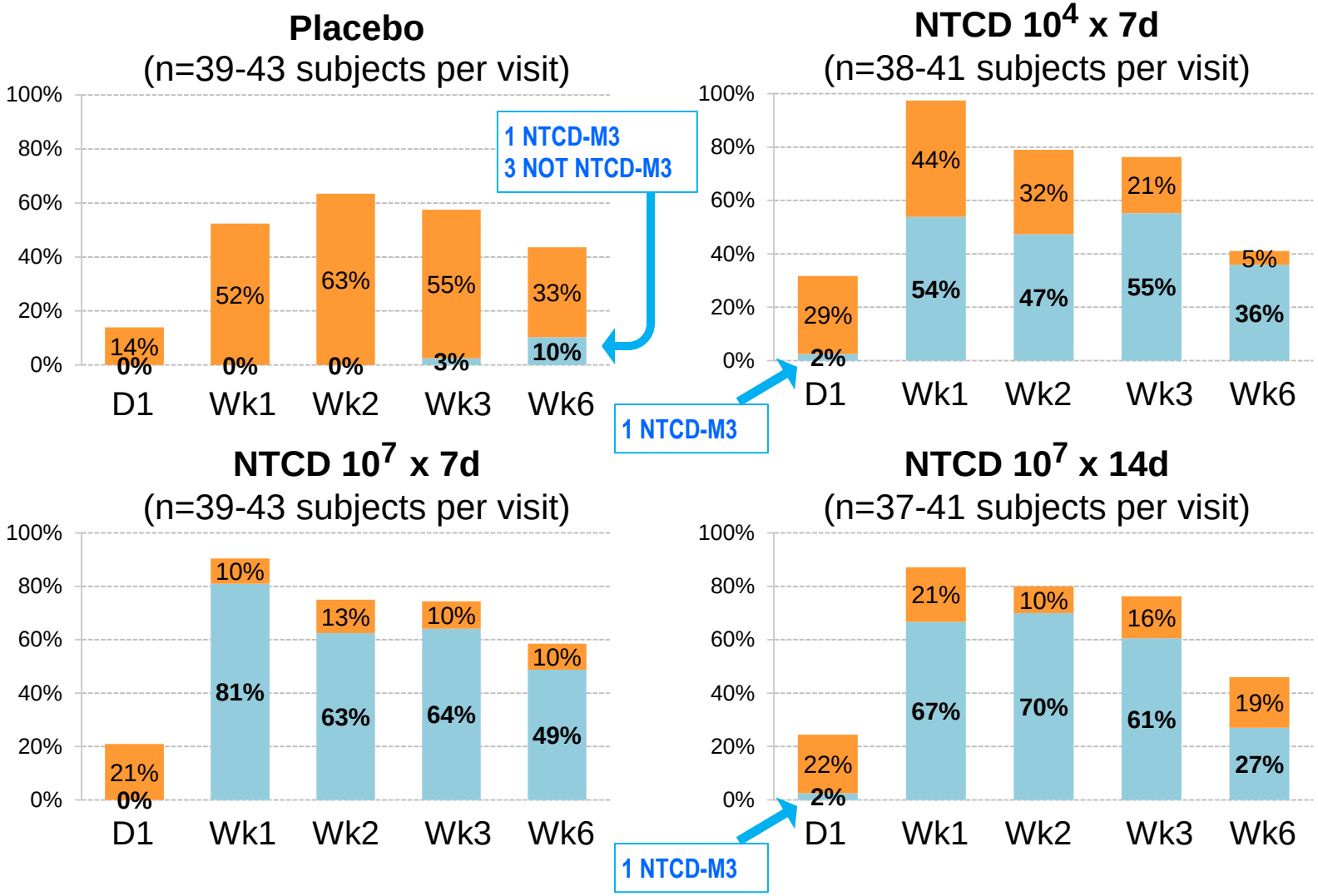
van Nood et al N Engl J Med. 2013;368:407-15.

Biotherapeutic and Preventive Milestones and the Microbiota

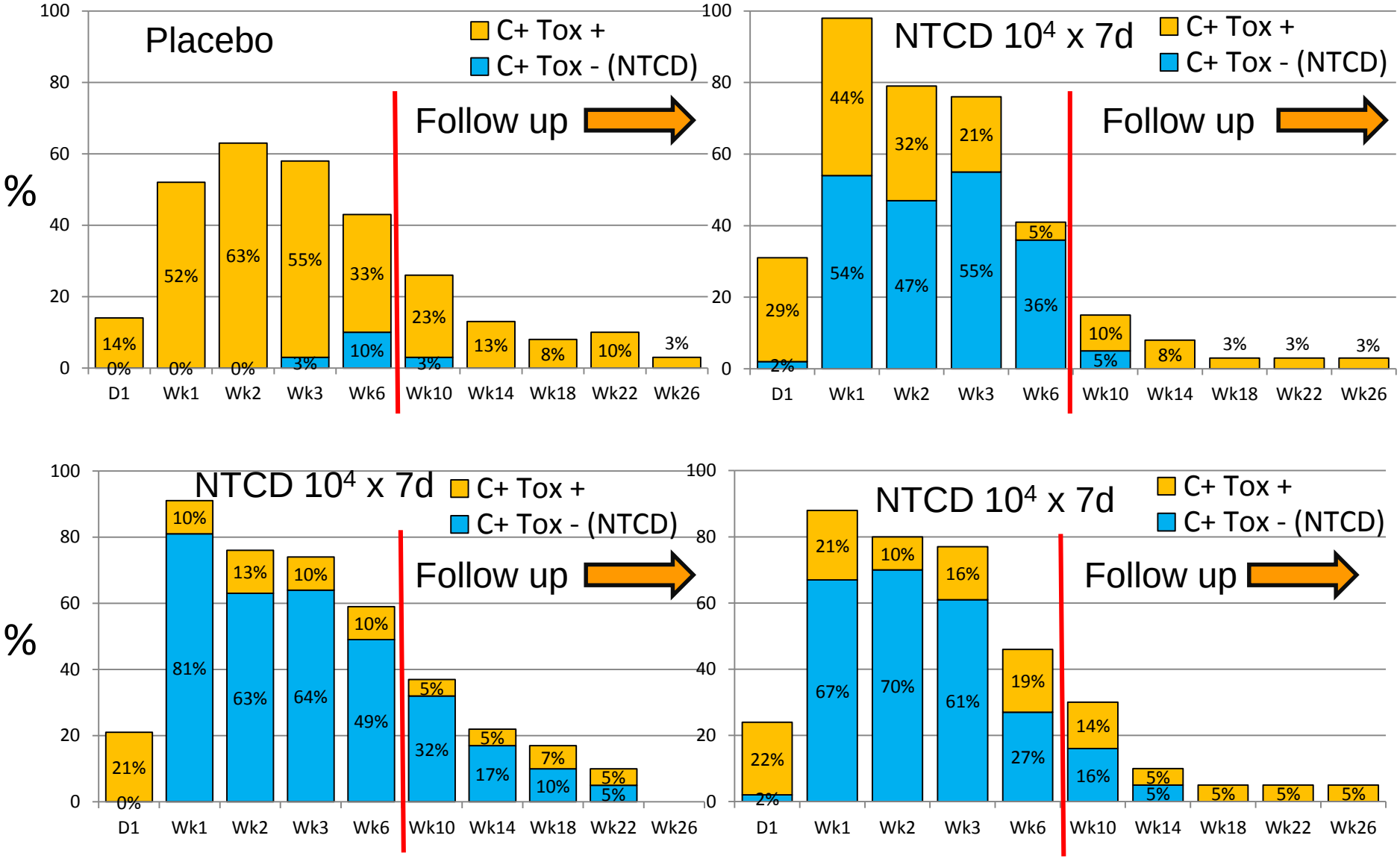
- Is *Clostridium scindens* and members of the cluster XIVa Clostridia the key to *C difficile* colonization resistance?
  Buffie CG…Pamer EG Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile.* Nature. **2015**;517:205-8.

- Non-toxigenic *C. difficile* M3 for prevention of Recurrent CDI
  Administration of Spores of Nontoxigenic *Clostridium difficile* Strain M3 for Prevention of Recurrent *C difficile* Infection: A Randomized Clinical Trial
  Gerding DN…Villano S et al JAMA. **2015**;313:1719-1727.

C. difficile Stool Culture (Central Lab)



Gerding DN et al JAMA 2015;313:1719, May 5, 2015



Monthly Fecal *C. difficile* Detection from Week 6 to 6 Months

Gerding DN et al JAMA 2015;313:1719, May 5, 2015, Supplementary Material

Clinical Endpoints Through Week 6

	Placebo	10^4 x 7d	10^7 x 7d	10^7 x 14d	All NTCD
n	43	41	43	41	125
CDI Recurrence	**13 (30%)**	**6 (15%)**	**2 (5%)**	**6 (15%)**	**14 (11%)**
p value		**0.11**	**0.01**	**0.10**	**0.006**
Antibacterial Use for CDI Treatment	**14 (33%)**	6 (15%)	**4 (9%)**	7 (17%)	**17 (14%)**
p value		0.07	**0.02**	0.14	**0.009**
Any Event of Diarrhea (of any severity) or CDI	33 (77%)	23 (56%)	25 (58%)	23 (56%)	71 (57%)
p value		**0.05**	0.09	**0.02**	**0.020**

p values adjusted for pre-specified parameters: use of metronidazole vs. vancomycin, and primary episode vs. first recurrence

Gerding DN et al JAMA 2015;313:1719, May 5, 2015

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