日本嫌気性菌感染症学会

All About *Clostridium difficile* in the World

Chairpersons : Kazuhiro Tateda (Toho University)
Hiroshige Mikamo (Aichi Medical University)

Speakers : Thomas V. Riley (The University of Western Australia, Australia)
Ellen Jo Baron (Stanford University School of Medicine, US)
Mark H. Wilcox (University of Leeds, UK)
Haru Kato (National Institute of Infectious Diseases, Japan)

Date : 27 February, 2015

Place : Shinagawa Prince Hotel, Tokyo, Japan

The world-wide epidemiology *Clostridium difficile* infection: is the worst still to come?

Thomas V Riley [1) 2)]

Microbiology & Immunology, The University of Western Australia, Ned lands 6009, Western Australia [1)]
Department of Microbiology, PathWest Laboratory Medicine (WA), Queen Elizabeth II Medical Centre, Ned lands 6009, Western Australia [2)]

Clostridium difficile infection (CDI) has come to prominence as major human epidemics caused by the PCR ribotype (RT) 027 strain of *C. difficile* have occurred in North America and Europe over the last 15 years. The main virulence factors of *C. difficile* are two toxins, TcdA (an enterotoxin) and TcdB (a cytotoxin), while the role of a third "binary" toxin remains controversial. Contamination of the environment with *C. difficile* spores plays a critical role in transmission as spores remain in the environment for months. *C. difficile* also causes infectious diarrhoea in animals and is found in pigs, horses, and cattle, suggesting a potential reservoir for human infection, and in food, suggesting the possibility of food-borne transmission. It is likely that excessive antimicrobial exposure is driving the amplification of *C. difficile* in animals rather than the organism just being normal flora of the animal gastrointestinal tract. Outside Australia, RT 078 is the most common RT of *C. difficile* found in pigs (83% in one study in the USA) and cattle (up to 100%) and this RT is now the third most common RT of *C. difficile* found in humans in Europe. Human and pig strains of *C. difficile* are genetically identical in Europe confirming that a zoonosis exists. Rates of community-acquired CDI are increasing world-wide and environmental contamination may play a role in this. *C. difficile* spores survive in treated piggery effluent, the by-products of which are used to irrigate crops and pasture, and manufacture compost. There is abundant evidence that food products intended for human consumption contain toxigenic strains of *C. difficile* but food-borne transmission remains unproven. Thus world-wide there are three problems that require resolution: a human health issue, an animal health issue and the factor common to both these problems, environmental contamination.

The situation in Asia regarding CDI is less clear. RT 027, which is still a major RT in North America, has been reported only sporadically in Hong Kong, Japan, Korea, Singapore and, more recently, China. Similarly, RT 078 has only been reported in Korea and China. The RTs most commonly reported in Asia are 017, 018, 014, 002 and 001. These RTs are among the top 10 most commonly found RTs in Europe. Of note, RT 017, which is a toxin A-negative, toxin B-positive (A−B+) strain, has been the predominant strain in China and Korea, and is prevalent in Japan, Taiwan and Hong Kong. This RT has also caused epidemics in The Netherlands and Ireland, and is an emerging RT in Australia. Only one study investigating *C. difficile* in production animals in Asia has been published, reporting a low prevalence of 0.8% (2/250) among finishing pigs in Japan. While more work is required, there is good evidence that *C. difficile* in production animals is spilling over into humans by an as yet undetermined mechanism. While the organism will be new to many proponents of "One Health", the concept will not and it will require a multi-disciplinary approach to prevent animal strains of *C. difficile* infiltrating human health systems as appears to have happened in North America. Better surveillance for CDI in Asia is essential, and urgently required.

The world-wide epidemiology of *Clostridium difficile* infection: is the worst still to come?

Tom Riley

Microbiology & Infectious Diseases, PathWest Laboratory Medicine, Nedlands, WA, Australia.
Microbiology & Immunology, The University of Western Australia, Nedlands, WA, Australia.

The NEW ENGLAND JOURNAL of MEDICINE

N Engl J Med 2005;353:2442-9.

ORIGINAL ARTICLE

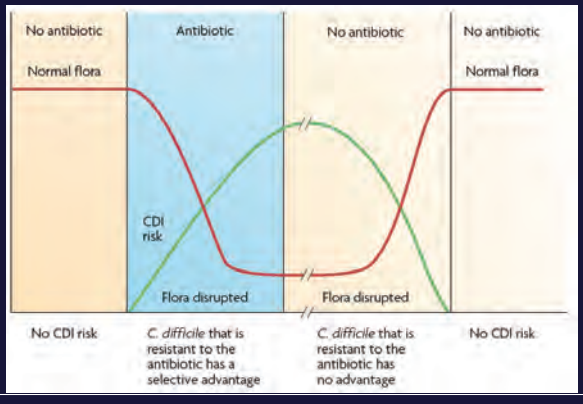
A Predominantly Clonal Multi-Institutional Outbreak of *Clostridium difficile*–Associated Diarrhea with High Morbidity and Mortality

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.

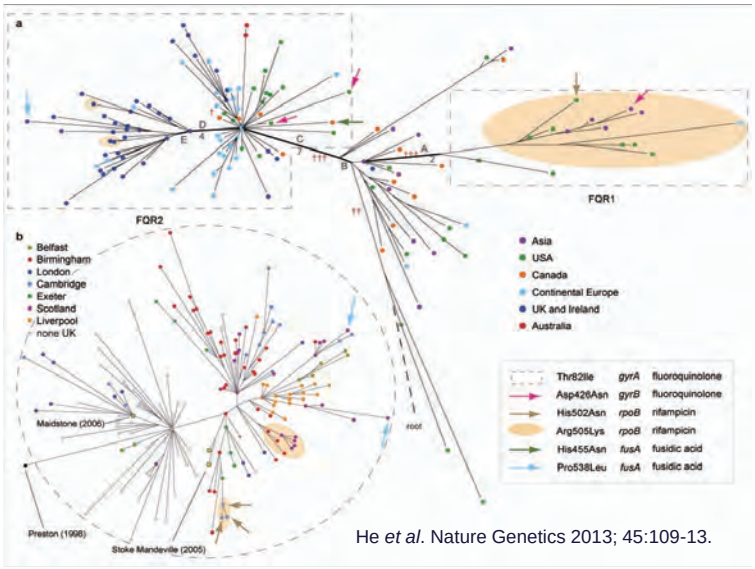
C. difficile PCR ribotype 027

- 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

Effect of antibiotics on normal flora

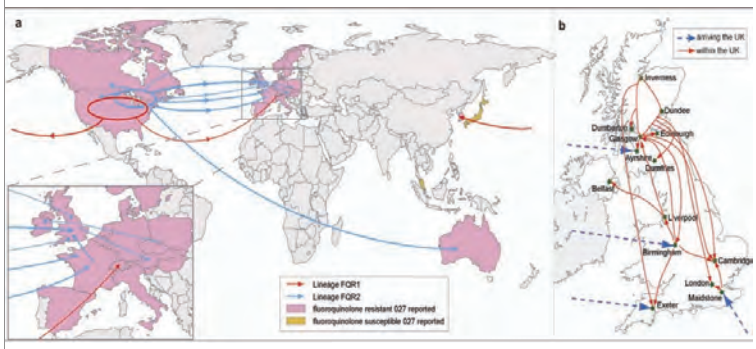


Rupnik *et al*. Nat Rev Microbiol 2009;7:526-36

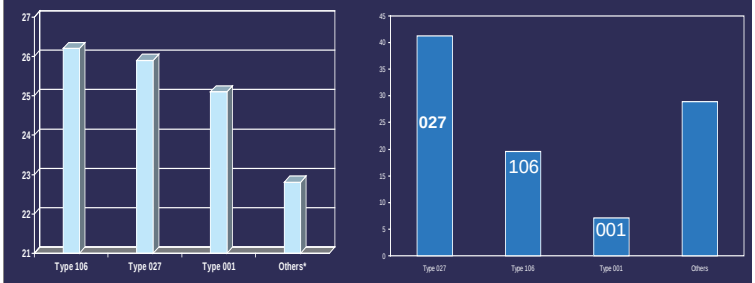


He *et al*. Nature Genetics 2013; 45:109-13.

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



England distribution of PCR ribotypes 2005/6 to 2007/8 as percentages



2005-6 (n=881)

2007-8* (n=677)

* Brazier et al. *Eurosurveillance* Vol.13;4. October 2008

Investigation

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

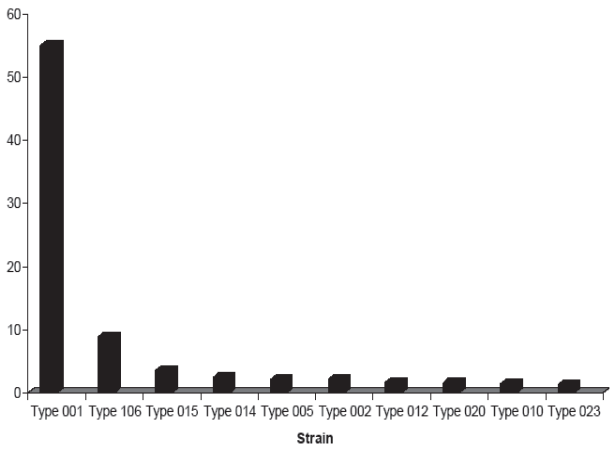
July 2006

Healthcare Commission

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

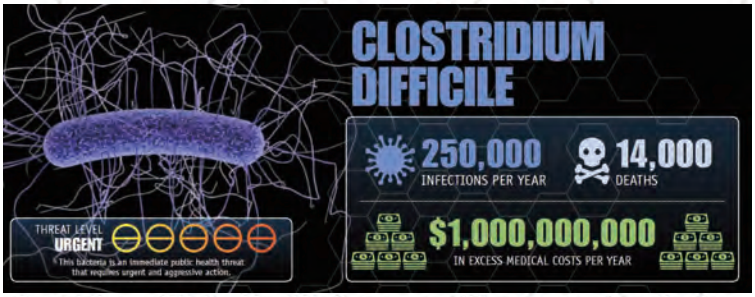
Rates in England 2008-11

Figure 5: PCR types of *C. difficile* in hospital patients in England and Wales: referrals to ARL 1995- 2003



Urgent Threats

- *Clostridium difficile*
- Carbapenem-resistant Enterobacteriaceae (CRE)
- Drug-resistant *Neisseria gonorrhoeae*



Clostridium difficile (C. difficile) causes life-threatening diarrhea. These infections mostly occur in people who have had both recent medical care and antibiotics. Often, *C. difficile* infections occur in hospitalized or recently hospitalized patients.

Clostridium difficile Ribotype Diversity at Six Health Care Institutions in the United States

Sheila Waslawski, Eugene S. Lo, Sarah A. Ewing, Vincent B. Young, David M. Aronoff, Susan E. Sharp, Susan M. Novak-Weekley, Arthur E. Crist Jr., W. Michael Dunne, Joan Hoppe-Bauer, Michelle Johnson, Stephen M. Brecher, Duane W. Newton and Seth T. Walk
J. Clin. Microbiol. 2013, 51(6):1938. DOI: 10.1128/JCM.00056-13.

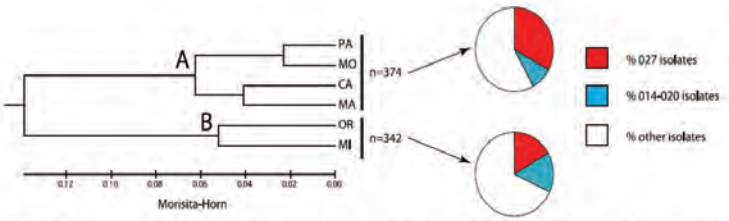


FIG 1 Ribotype-based *C. difficile* population diversity. An unweighted-pair group method using average linkages (UPGMA) dendrogram displays the similarity between six U.S. health care institutions based on the presence/absence and relative abundance of all ribotypes observed in this study (Morisita-Horn index). Pie charts display the percentages of two main ribotypes (027 and 014-020) and all remaining ("other") ribotypes for clusters A and B.

CDI in Asia

- Little is known about *C. difficile* in many areas of South-East Asia.
- Work from China (Shanghai), Taiwan, Singapore, Hong Kong and earlier work from Thailand.
- Still no useful data from Indonesia
- Nothing from Vietnam, Philippines, Cambodia, etc.
- Some data from Malaysia and India, none from Sri Lanka
- OK data from Japan/South Korea but relatively little

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, Jaap 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%)

Singapore

- 1985-89, 9.6% cases positive for *C. difficile* at NUS Hospital (Kumurasinghe *et al*. Trop Geogr Med 1992)
- Koh *et al*. (Pathology 2007) SGH 2002-03, 54 cases/100,000 patient-days
- RT 053 being most prevalent Tan et al. (*Int J Antimicrob Agents* 2014; 43: 47-51)

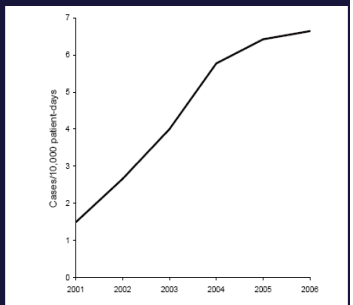
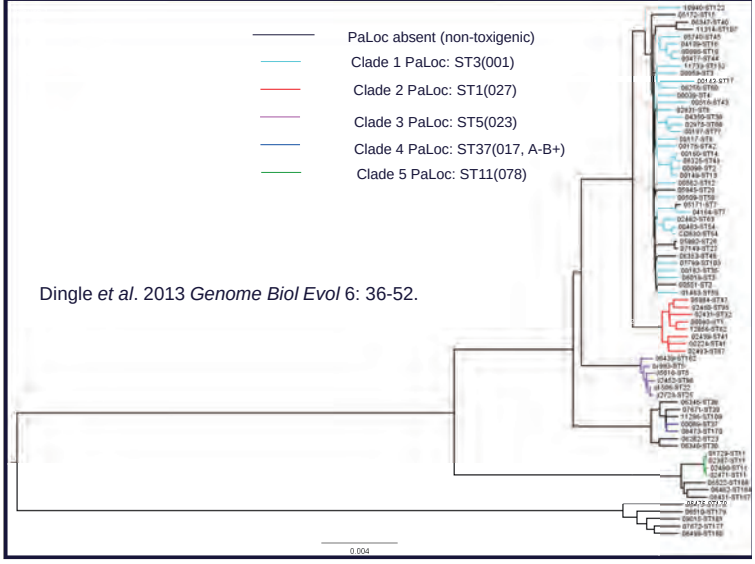


Figure. *Clostridium difficile*–associated disease incidence, Singapore, 2001–2006.

Lim *et al. Emerg Infect Dis* 2008; 14: 1487-9.



Dingle *et al*. 2013 *Genome Biol Evol* 6: 36-52.

Taiwan

- 1997 - 28/81 (34.6%) patients in ICU grew *C. difficile* (17 symptomatic). One isolate (type 1) from 47% (Cheng *et al*. Clin Infect Dis 1997)
- 2003 - 11/48 patients with AAD had *C. difficile* (Hsu *et al*. J Microbiol Immunol Infect 2006)
- 2007-08 – 42.6 cases/100,000 patient days (110.6 in ICU) (Chung *et al*. J Microbiol Immunol Infect 2010)

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journal homepage: www.elsevier.com/locate/anaerobe
ELSEVIER

Clinical microbiology

Clostridium difficile ribotype 126 in southern Taiwan: A cluster of three symptomatic cases

Yuan-Pin Hung [a, c, d, f], Hsiao-Ju Lin [a, c, d, f], Bo-Yang Tsai [e], Hsiao-Chieh Liu [a, b], Hsiu-Chuan Liu [b], Jen-Chieh Lee [c], Yi-Hui Wu [f], Mark H. Wilcox [g], Warren N. Fawley [h], Po-Ren Hsueh [i], Pei-Jane Tsai [e, j, k, **], Wen-Chien Ko [c, *]

Thailand

- 1990 - 26 month study, 269 diarrhoeal patients and 114 normal patients. *C. difficile* isolate from 13 (4.8%) of diarrhoeal patients & 3 (2.6%) normals. 52% diarrhoea & 22% normals had faecal cytotoxin! (Wongwanich *et al*. SEA J Trop Med Pub Health 1990)

Table 1 Recovery of *C. difficile* from asymptomatic individuals and diarrheal patients

Patient group	No. of persons	No. (%) of *C. difficile* positive	No. (%) of toxin A gene positive by PCR
Asymptomatic infants <12 months	235	28 (11.9)	2 (0.9)
Asymptomatic children 1–11 years	76	16 (21.1)	0
Adults	132	33 (25.0)	18 (13.6)
With diarrhea	84	13 (15.5)	8 (9.5)
With antibiotic-associated diarrhea	48	20 (41.7)	10 (20.8)
Total	443	77 (17.4)	20 (4.5)

- 2003 – Prevalence of *C. difficile* in AAD was 18.6% (Wongwanich *et al*. J Med Assoc Thai 2003)

South Korea

- Ribotype 027 described
- Ribotype 017 very common although now waning (Shin *et al*. J Med Microbiol 2008; Kim *et al*. J Clin Microbiol 2008)

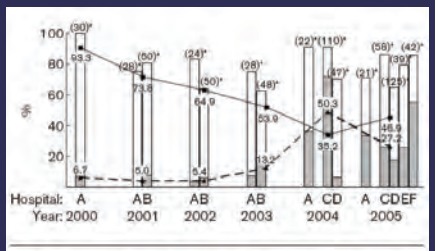

Fig. 2. The annual prevalence rate of *tcdA*$^+$*tcdB*$^+$, *tcdA*$^-$*tcdB*$^+$ and *tcdA*$^-$*tcdB*$^-$ *C. difficile* strains in each participating hospital

China

- 2007-08 – Clinical features of & risk factors for CDI in China similar to elsewhere in world (Huang *et al*. CID 2008)
- 2007-08 - Prevalence of *C. difficile* 12.6% in suspected CDI. High rates of resistance to fluoroquinolones. 25% due to A-B+ clone (Huang *et al*. IJAA 2009)
- Subsequently shown that A-B+ strain was ribotype 017 (Huang *et al*. Clin Microbiol Infect 2009)

Japan

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2001, p. 1391–1395 Vol. 39, No. 4
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.4.1391–1395.2001
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Analysis of *Clostridium difficile* Isolates from Nosocomial Outbreaks at Three Hospitals in Diverse Areas of Japan

HARU KATO,[1,2*] NAOKI KATO,[2] KUNITOMO WATANABE,[2] TOSHINOBU YAMAMOTO,[3] KANZO SUZUKI,[3] SHIOMI ISHIGO,[4] SEIKO KUNIHIRO,[5] ISAO NAKAMURA,[5] GEORGE E. KILLGORE,[6] AND SHINICHI NAKAMURA[1]

Department of Bacteriology, School of Medicine, Kanazawa University, Kanazawa 920-8640,[1] Institute of Anaerobic Bacteriology, Gifu University School of Medicine, Gifu 500-8705,[2] Nagoyashi-Kosein Geriatric Hospital, Nagoya 465-8610,[3] Ogaki Municipal Hospital, Ogaki 503-0864,[4] and Yamaguchi Prefectural Hospital, Hofu 747-8511,[5] Japan, and Nosocomial Pathogens Laboratory Branch, Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[6]

Eur J Clin Microbiol Infect Dis (2003) 22:525–529
DOI 10.1007/s10096-003-0992-5

ARTICLE

M. Komatsu · H. Kato · M. Aihara · K. Shimakawa · M. Iwasaki · Y. Nagasaka · S. Fukuda · S. Matsuo · Y. Arakawa · M. Watanabe · Y. Iwatani

High Frequency of Antibiotic-Associated Diarrhea due to Toxin A-Negative, Toxin B-Positive *Clostridium difficile* in a Hospital in Japan and Risk Factors for Infection

Author, year	No. isolates/ No. samples	No. hospitals	Region	Time (y)	A+B+	A-B+	A-B-	cdt+	*PCR ribotype
Dong, 2013	60/NG	1	Shang Hai	1.5	25	28	7	1	NG
Dong, 2014	94/NG	1	Shang Hai	2.3	63	31	0	1	NG
Fang, 2014	82/400	3 cancer	Zhe Jiang	0.8	82	0	0	0	001, 017, 017/1
Lam, 2012	10/32	1	Hong Kong	0.1	NG	NG	NG	NG	002
Huang, 2009	74/587	1	Shang Hai	1.0	43	13	13	0	SH II
Huang, 2014	65/206	1	Shang Hai	1.0	NG	NG	NG	NG	H, 012, 017
Cheng, 2011	345/3528	1 HCF	Hong Kong	1.0	345	0	0	0	002,og 39,012
Cheng, 1997	68/81	1 ICU	Tai Wan	0.4	68	0	0	0	NG
Huang, 2009	75/ NG	1 cancer	Shang Hai	0.9	50	25	0	1	017, 012, A
Zhu, 2014	57/ NG	1	Zheng Jiang	NG	38	14	5	0	NG
Huang, 2008	56/587	1	Shang Hai	1.0	NG	NG	NG	NG	SH II, 014
Pete, 2013	21/70	1	Chang Sha	1.0	9	10	2	UN	017, 012, 046
#Cheng, 2009	12/112	1	Beijing	0.6	5	3	4	0	ZR1
Chia, 2013	110/ NG	1	Taiwan	6.0	70	40	0	4	NG
Wei, 2013	57/149	6	Taiwan	0.7	29	NG	NG	NG	10, 106, 45
Wang, 2014	31/124	1 ICU	Cheng du	0.7	24	7	0	2	001, 046, 012

Eur J Clin Microbiol Infect Dis DOI 10.1007/s10096-014-2290-9 Published online: 04 December 2014

ARTICLE

Molecular epidemiology and antimicrobial susceptibility of *Clostridium difficile* isolated from a university teaching hospital in Japan

Y. Kuwata · S. Tanimoto · F. Sawabe · M. Shima · Y. Takahashi · H. Ushizawa · T. Fujie · R. Koike · N. Tojo · T. Kubota · R. Saito

Confirms the predominance of ribotype 18 (ST17) (21.8%) of 130 isolates – but ~25% non-toxigenic.

Tokyo Medical and Dental University

Eur J Clin Microbiol Infect Dis (2007) 26:699–703
DOI 10.1007/s10096-007-0355-8

ARTICLE

Molecular analysis of *Clostridium difficile* at a university teaching hospital in Japan: a shift in the predominant type over a five-year period

F. Sawabe · H. Kato · K. Osawa · T. Chida · N. Tojo · Y. Arakawa · N. Okamura

Documents the emergence of ribotype 18 in Japan.

Collins et al. Antimicrobial Resistance and Infection Control 2013, 2:21
http://www.aricjournal.com/content/2/1/21

ANTIMICROBIAL RESISTANCE & INFECTION CONTROL

REVIEW Open Access

Epidemiology of *Clostridium difficile* infection in Asia

Deirdre A Collins[1*], Peter M Hawkey[2,3] and Thomas V Riley[1,4]

Indonesia

Like Malaysia, reports on CDI in Indonesia are uncommon. An aetiology study of diarrhoea in Indonesian children identified *C. difficile* in 1.3% of stool samples tested. Toxin A EIA only was performed so the true prevalence of *C. difficile* may have been greater [59]. The only molecular study included eight isolates from Indonesia, five of which were toxinotype VIII and ribotype 017, and grouped with international 017 epidemic strains. Two were A^+B^+ toxinotype 0, and one A^-B^+ isolate was binary toxin-positive, toxinotype XVI [15].

59. Oyofo BA, Subekti D, Tjaniadi P, Machpud N, Komalarini S, Setiawan B, Simanjuntak C, Punjabi N, Corwin AL, Wasfy M, *et al.*: **Enteropathogens associated with acute diarrhea in community and hospital patients in Jakarta, Indonesia.** *FEMS Immunol Med Microbiol* 2002, **34:**139-146.

15. Rupnik M, Kato N, Grabnar M, Kato H: **New types of toxin A-negative, toxin B-positive strains among *Clostridium difficile* isolates from Asia.** *J Clin Microbiol* 2003, **41:**1118-1125.

CDI in Australia

- Not a notifiable infection
- But mandatory reporting by all hospitals since 2010 as part of hospital accreditation
- Reporting of "hospital identified" cases of CDI - a patient attending any area of a hospital i.e. admitted patients and those attending emergency and outpatient departments

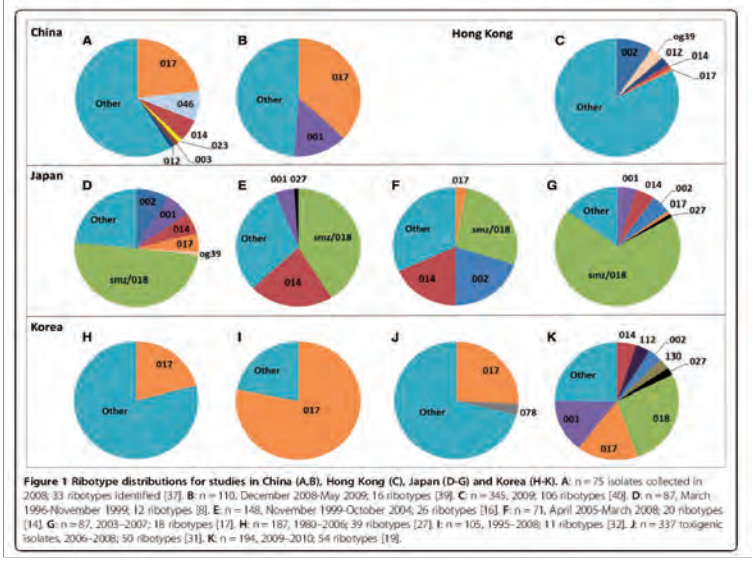
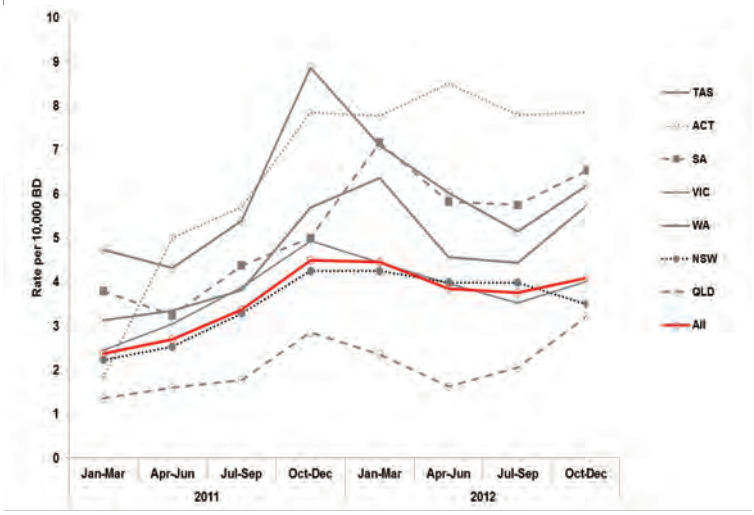


Figure 1 Ribotype distributions for studies in China (A,B), Hong Kong (C), Japan (D-G) and Korea (H-K). **A**: n = 75 isolates collected in 2008; 33 ribotypes identified [37]. **B**: n = 110, December 2008-May 2009; 16 ribotypes [39]. **C**: n = 345, 2009; 106 ribotypes [40]. **D**: n = 87, March 1996-November 1999; 12 ribotypes [8]. **E**: n = 148, November 1999-October 2004; 26 ribotypes [16]. **F**: n = 71, April 2005-March 2008; 20 ribotypes [14]. **G**: n = 87, 2003–2007; 18 ribotypes [17]. **H**: n = 187, 1980–2006; 39 ribotypes [27]. **I**: n = 105, 1995–2008; 11 ribotypes [32]. **J**: n = 337 toxigenic isolates, 2006–2008; 50 ribotypes [31]. **K**: n = 194, 2009–2010; 54 ribotypes [19].

Hospital identified CDI in Australia, 2011-2012

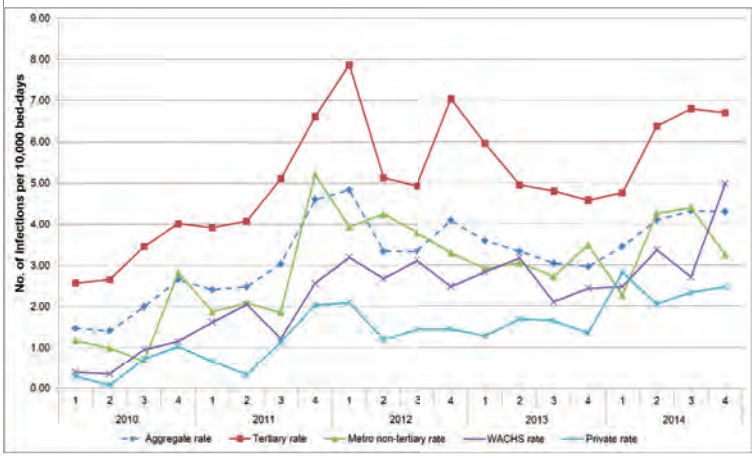
(Slimings et al. *Med J Aust* 2014; 200: 272-6)

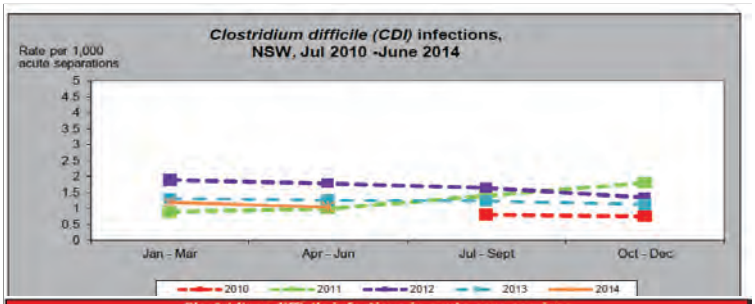


Asian overview

- *C. difficile* clearly present in region
- Found in research studies: 5-15% prevalence – maybe higher
- Not dissimilar to other regions of world
- Less data collected routinely
- Not a lot of good data on incidence
- Risk factors appear similar
- Very little data on animals

Quarterly HI-CDI rates by hospital group, Western Australia, 2010-14.





Clostridium difficile infections in acute care services per 1,000 acute separations

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
2014												
No. of infections	168	169	161	152	149	150						
Separations	133,750	136,354	147,645	141,031	150,702	140,367						
Rate/1,000 seps	1.3	1.2	1.1	1.1	1.0	1.1						
2013												
No. of infections	180	156	199	169	184	175	173	180	190	176	175	137
Separations	128,826	130,175	144,511	137,620	149,577	132,944	147,491	149,467	141,403	148,199	143,990	141,060
Rate/1,000 seps	1.4	1.2	1.4	1.2	1.2	1.3	1.2	1.2	1.3	1.2	1.2	1.0
2012												
No. of infections	268	210	217	220	225	230	238	240	215	196	184	187
Separations	111,606	123,470	132,847	119,165	133,186	126,009	139,542	145,705	136,128	143,699	142,911	135,699
Rate/1,000 seps	2.4	1.7	1.6	1.8	1.7	1.8	1.7	1.6	1.6	1.4	1.3	1.4
2011												
No. of infections	102	95	133	135	140	132	149	204	219	225	229	260
Separations	116,061	124,106	139,312	133,990	156,824	136,516	134,137	137,489	132,344	133,223	137,185	123,655
Rate/1,000 seps	0.9	0.8	1.0	1.1	0.9	1.0	1.1	1.5	1.7	1.7	1.7	2.1
2010												
No. of infections										102	100	102
Separations										11,033	115,742	108,894
Rate/1,000 seps										0.8	0.7	0.8

Is the worst still to come?

	Jan-May period		
Ribotype	2012	2013	2014
UK 014/020 [G]	30.8%	38.9%	40.6%
UK 002	12.3%	3.1%	7.1%
UK 056	2.3%	4.4%	6.0%
UK 054	1.2%	3.4%	1.5%
UK 046	1.2%	2.5%	2.6%
UK 010	3.8%	2.8%	1.9%
UK 018	2.3%	2.5%	1.1%
UK 017	4.1%	1.9%	1.1%
UK 070	2.6%	2.2%	1.5%
UK 103	0.6%	3.4%	2.6%
QX 158	-	0.3%	6.4%
UK 015 / UK 193	3.2%	0.3%	0.4%
UK 053	1.5%	2.5%	0.8%
UK 005	2.3%	1.9%	1.1%
QX 076	1.5%	3.1%	0.8%
UK 244	2.3%	0.6%	-

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 26, 2013 VOL. 369 NO. 13

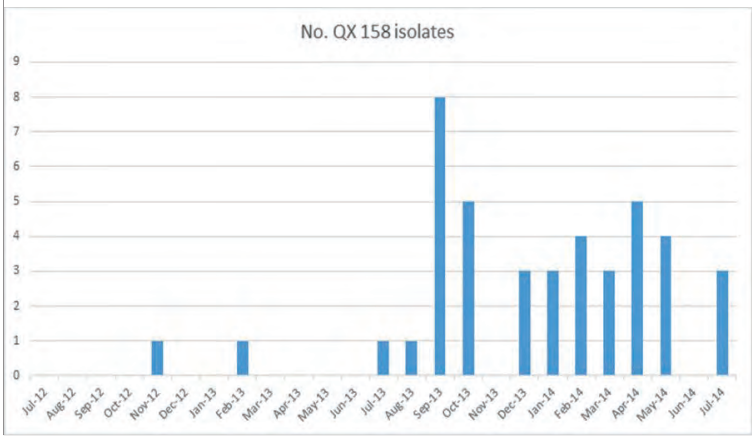
Diverse Sources of *C. difficile* Infection Identified on Whole-Genome Sequencing

David W. Eyre, B.M., B.Ch., Madeleine L. Cule, Ph.D., Daniel J. Wilson, D.Phil., David Griffiths, B.Sc., Alison Vaughan, B.Sc., Lily O'Connor, B.Sc., Camilla L.C. Ip, Ph.D., Tanya Golubchik, Ph.D., Elizabeth M. Batty, Ph.D., John M. Finney, B.Sc., David H. Wyllie, Ph.D., Xavier Didelot, D.Phil., Paolo Piazza, Ph.D., Rory Bowden, Ph.D., Kate E. Dingle, Ph.D., Rosalind M. Harding, Ph.D., Derrick W. Crook, M.B., B.Ch., Mark H. Wilcox, M.D., Tim E.A. Peto, D.Phil., and A. Sarah Walker, Ph.D.

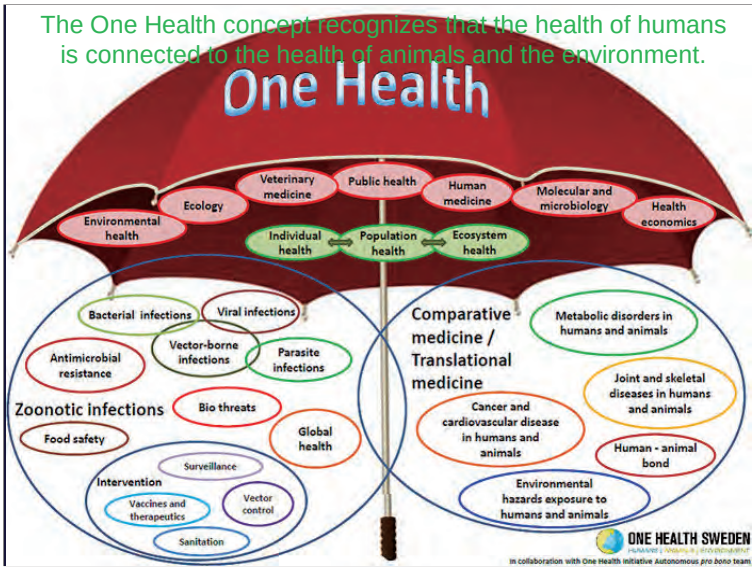
CONCLUSIONS

Over a 3-year period, 45% of *C. difficile* cases in Oxfordshire were genetically distinct from all previous cases. Genetically diverse sources, in addition to symptomatic patients, play a major part in *C. difficile* transmission. (Funded by the U.K. Clinical Research Collaboration Translational Infection Research Initiative and others.)

Emergence of QX158 in Western Australia

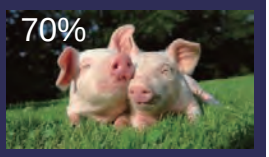


The One Health concept recognizes that the health of humans is connected to the health of animals and the environment.



Animal strains in Australia

- Ribotype 127 60%
- Ribotype 126 16%
- Ribotype 033 13%
- Ribotype 014 23%
- Ribotype 033 13%
- Ribotype QX009 12%
- Ribotype 237 10%



5-7days old

- Many new ribotypes from animals – CDT+

Knight *et al. Appl Environ Microbiol* 2013, 2014

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

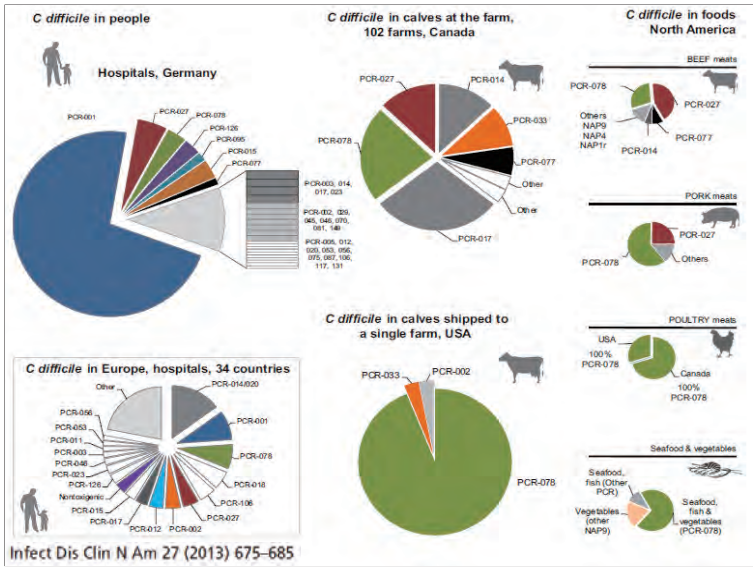
Meat product	No. samples cultured	Total no. (%) positive	Ribotype	Toxinotype	ΔtcdC, bp†	PFGE type	No. (%) pos
Ground beef (uncooked)	26	13 (50)	027	III	18	NAP1	1 (3.8)
						NAP1-related	2 (7.7)
			078	V	39	NAP7	8 (30.8)
						NAP8	2 (7.7)
Summer sausage (ready to eat)	7	1 (14.3)	027	III	18	NAP1	1 (14.3)
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.

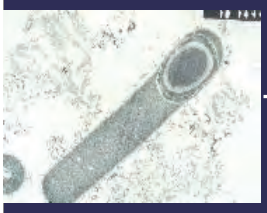
Songer *et al*. *Emerg Infect Dis* 2009; 15: 819-821

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)



Why is this happening?



+ antibiotics = disaster

Numbers of Danish pigs 1990-2006

Table 1. Production of food animals (including export of live animals) and the production of meat and milk, Denmark

DANMAP 2006

Year	Broilers		Turkeys a)		Cattle (slaughtered)		Dairy cows		Pigs		Farmed fish	
											Fresh water	Salt water
	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg milk	1,000 heads	mill. kg	mill. kg	mill. kg
1990	94,560	116	571	2.5	789	219	753	4,542	16,425	1,260	-	-
1992	107,188	137	761	5.4	862	236	712	4,405	18,442	1,442	35	7
1994	116,036	152	1,091	8.6	813	210	700	4,442	20,651	1,604	35	7
1996	107,895	149	961	9.3	789	198	701	4,494	20,424	1,592	32	8
1998	126,063	168	1,124	11.6	732	179	669	4,468	22,738	1,770	32	7
2000	133,987	181	1,042	10.3	691	171	636	4,520	22,414	1,748	32	7
2001	136,603	192	1,038	12.6	653	169	623	4,418	23,199	1,836	31	8
2002	136,350	190	965	11.5	668	169	610	4,455	24,203	1,892	32	8
2003	129,861	181	510	7.4	625	161	596	4,540	24,434	1,898	34	8
2004	130,674	181	55	1.0	632	165	563	4,434	25,141	1,965	34	9
2005	120,498	180	158	0.5	549	145	558	4,449	25,758	1,988	31	8
2006	105,888	163	32	0.1	509	140	563	4,492	25,763	1,957	-	-

Data from Statistics Denmark (www.dst.dk) and The Danish Directorate for Fisheries
a) From 2002, the export of live turkeys for slaughter increased. By 2004, 95% of all turkeys raised in Denmark were slaughtered abroad. For turkeys, data on export of live animals is not included in the table

Approx. 50% increase in numbers

Antimicrobial use in the Australian pig industry: results of a national survey

D Jordan,[a,b] JJ-C Chin[a], VA Fahy,[b] MD Barton,[c] MG Smith[b] and DJ Trott[a]

Aust Vet J 2009;87:222–229

Table 2. Percent of pig herds (n = 197) reported as having used particular antimicrobials in the 12 months prior to survey and the route of administration of those drugs

Antimicrobial	Importance rating for human health	Percent of herds within each category of use				
		Unanswered	Not used	Injection/oral	Oral only	Injection only
Apramycin/neomycin	Medium/low	1.0	47.3	2.5	42.1	7.1
Florfenicol (for respiratory disease)	Low	1.0	95.4	0.5	0.0	3.1
Florfenicol (for gut disease)	Low	2.5	93.4	0.0	0.5	3.6
Lincomycin and spectinomycin	Medium	2.0	64.5	6.1	19.3	8.1
Macrolide	Low	2.0	25.4	32.0	27.9	12.7
Penicillins[a]	Low	1.0	3.6	22.8	4.6	68.0
Ceftiofur	High	1.5	73.7	2.0	0.5	22.3
Tiamulin	Not yet rated	1.0	88.4	1.0	7.1	2.5
Olaquindox	Low	1.0	46.7	0.0	52.3	0.0
Virginiamycin	High	1.5	98.5	0.0	0.0	0.0
Dimetridazole	Medium	2.0	85.3	0.0	12.7	0.0
Sulfonamides	Low	2.0	28.9	5.6	10.7	52.8
Tetracyclines	Low	1.0	15.2	50.8	10.7	22.3
Other antimicrobials		3.1	86.2	0.0	7.6	3.1

[a]Includes amoxicillin and ampicillin.

Antimicrobial usage in Danish pigs 1990-2006

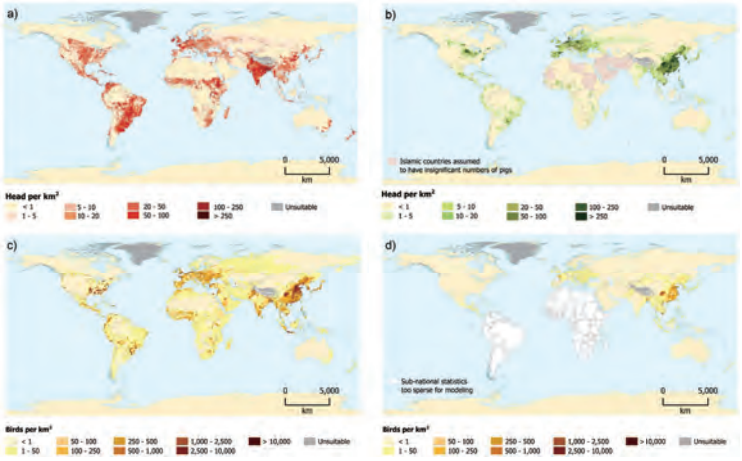
Table 4. Trends in the estimated total consumption (kg active compound) of prescribed antimicrobials for production animals, Denmark

DANMAP 2006

ATC_{vet} (group b)	Therapeutic group	1990	1992	1994	1996	1998	2000	2001	2002	2003	2004	2005	2006
QJ01AA	Tetracyclines	9,300 a)	22,900	36,500	12,900	12,100	24,000	28,500	24,500	27,300	29,500	30,050	32,650
QJ01CE	Penicillins, β-lactamase sensitive	5,000	8,700	9,400	7,200	14,300	15,100	16,400	17,400	19,000	20,900	22,250	22,600
QJ01C/QJ01DA	Other penicillins, cephalosporins	1,200	2,550	4,400	5,800	6,700	7,300	8,800	9,900	11,100	12,900	12,300	11,550
QJ01EW	Sulfonamides + trimethoprim	3,800	7,900	9,500	6,800	7,700	7,000	9,200	10,600	10,600	11,500	12,200	13,800
QJ01EQ	Sulfonamides	8,700	5,900	5,800	2,100	1,000	1,000	960	900	860	850	750	750
QJ01F/QJ01XX	Macrolides, lincosamides, pleuromulins	10,900	12,900	11,400	7,600	7,100	15,600	18,400	19,200	20,700	24,200	22,350	22,050
QJ01G/QA07AA	Aminoglycosides	7,700	8,500	8,600	7,100	7,800	10,400	11,600	11,700	11,700	11,600	10,800	10,500
	Others c)	6,700	5,800	4,400	600	650	300	900	1,800	1,500	1,000	1,950	1,250
Total		53,400	73,200	89,900	48,000	57,300	80,700	94,700	95,900	102,500	112,500	112,650	115,150

1990-2000: Data based on reports from the pharmaceutical industry of total annual sales. (Data 1990-1994: Use of antibiotics in the pig production. Federation of Danish pig producers and slaughterhouses. N. E. Rønn (Ed.). 1996-2000: Danish Medicines Agency). Data 2001-2006: VetStat. For comparability between VetStat data and previous data, see DANMAP 2000. Only veterinary drugs are included. Veterinary drugs almost exclusively used in pets (tablets, capsules, ointment, eye/ear drops) are excluded. Dermal spray with tetracycline, used in production animals, is the only topical drug included
a) Kg active compound rounded to nearest 50 or 100
b) Only the major contributing ATC_{vet} groups are mentioned
c) Consumption in aquaculture was not included before 2001

Approx. 400% increase in penicillins, β-lactamase sens
Other penicillins, cephalosporins 1000% increase



Robinson TP, Wint GRW, Conchedda G, Van Boeckel TP, Ercoli V, et al. (2014) Mapping the Global Distribution of Livestock. PLoS ONE 9(5): e96084. doi:10.1371/journal.pone.0096084

"This change in prescription habits suggests that the consumption of cephalosporins in pigs is changing from occasional prescription to more systematic prescription in herds producing 14-29% of the weaned pigs."
DANMAP 2007

DANMAP 2006

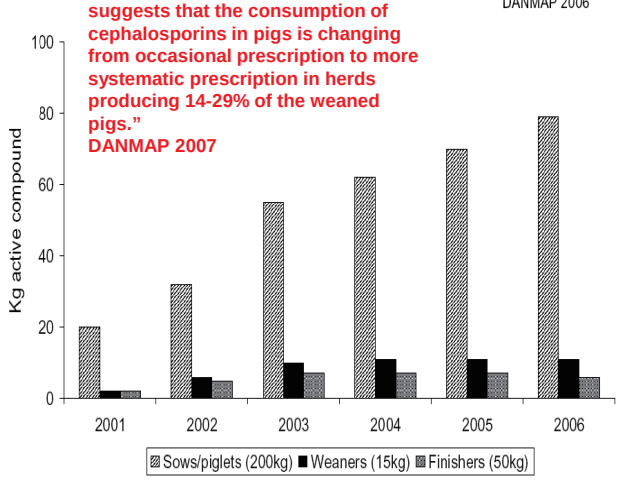


Figure 4 . Consumption of cephalosporins in pigs given as kg active compound from 2001 to 2006, Denmark

frontiers in MICROBIOLOGY
ORIGINAL RESEARCH ARTICLE

Genetic relatedness between Japanese and European isolates of *Clostridium difficile* originating from piglets and their risk associated with human health

*Masaru Usui[1], Yukie Nanbu[1], Kentaro Oka[2], Motomichi Takahashi[2,3], Takashi Inamatsu[4], Tetsuo Asai[5], Shigeru Kamiya[3] and Yutaka Tamura[1]**

Table 2 | Isolation and toxin gene profile of *Clostridium difficile* from Japanese piglets.

Farm	Location of prefecture	Samples	No. of positive samples	No. of isolates	Toxin A⁺B⁺		Toxin A⁻B⁻CDT⁻
					CDT⁺	CDT⁻	
A	Hokkaido	10	9	11	0	10	1
B	Hokkaido	10	7	8	0	8	0
C	Hokkaido	10	8	10	10	0	0
D	Hokkaido	10	7	13	0	0	13
E	Hokkaido	10	9	18	0	17	1
F	Miyagi	10	0	0	0	0	0
G	Miyagi	10	7	10	5	0	5
H	Yamagata	10	3	3	0	0	3
I	Fukushima	10	5	7	0	0	7
J	Tochigi	10	4	4	4	0	0
K	Chiba	10	7	9	6	0	3
L	Gifu	10	3	7	1	0	6
SubTotal					26	36	
Total		120	69 (57.5%)	100	61 (61.0%)		39 (39.0%)

To summarise the issues

- Community CDI a bigger problem than appreciated
- Need to prevent establishment of “new” virulent RTs in hospitals
- CDI a major animal health problem (pigs/horses)
- Gross contamination of the environment OUTSIDE hospitals - including contamination of food
- CDI epidemiology continually evolving
- Molecular typing probably at the WGS level essential for understanding
- CDI is a zoonosis - will require a One Health approach

Acknowledgments

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Deirdre Collins
Kerry Carson
Papanin Putsathit
Yuan Wu

Oxford University/PHL (Derrick Crook, David Eyre)
Leeds University (Mark Wilcox)
TechLab (Bob Carman, Matt Lyerly)

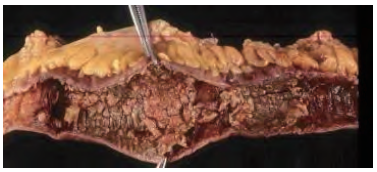
Diagnosis of *Clostridium difficile* infection

Ellen Jo Baron

Stanford University (Medicine; Pathology) Medical Affairs, Cepheid, USA

This presentation will outline the early discoveries that led to the recognition of *Clostridium difficile*, an anaerobic spore-forming rod, as the major microbial cause of antibiotic-associated diarrhea. Over time, *Staphylococcus aureus* was found to be less important and a *Clostridium* species was identified to cause disease in hamsters that were injected intracecally with patient samples or broth cultures containing the organism, soon after identified as *C. difficile* by pioneering work of Dr.'s John Bartlett, Sherwood Gorbach, and Andy Onderdonk. Now we recognize that the disease is a biofilm-based syndrome, which explains how fecal transplants have been the most successful treatment methods for otherwise intractable cases. The various pathogenic mechanisms of the organism will be described, including a discussion of all three important toxins: enterotoxin (toxin A), cytotoxin (toxin B), and binary toxin. Knowledge of toxin activity led to the development of various laboratory test methods for diagnosis of *C. difficile* infection (CDI). Each method, starting from the gold standard toxigenic culture, moving to cell culture cytotoxin neutralization, enzyme immunoassays for toxins A and B, enzyme immunoassays for glutamate dehydrogenase (GDH), loop-mediated nucleic acid amplification (LAMP), and finally other nucleic acid amplification methods for genetic markers of *C. difficile*, will be presented and briefly described. The presentation will end with an example of one healthcare system's efforts to control a *C. difficile* outbreak and the laboratory-based intervention that resulted in a turning point in decreasing cases and serious patient outcomes caused by this clever bacterium.

Diagnosis of *Clostridium difficile* infection

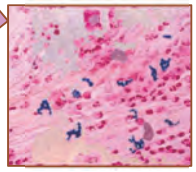


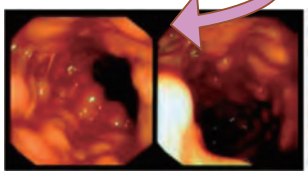
Ellen Jo Baron, Ph.D., D(ABMM)

- Professor Emerita, Pathology, Stanford University
- Executive Director of Technical Support, Cepheid

History of antibiotic-associated diarrhea

- **Altemeier et al. 1963.** Staphylococcal enterocolitis reported following antibiotic therapy. Ann Surg 157:847-58.
  - **Based on Gram stains and aerobic cultures**
  - **Vancomycin drug of choice**
- **Tedesco et al. 1974.** Clindamycin-associated colitis: a prospective study. Ann Intern Med 81:429-34.
  - **First use of endoscopy to identify plaques**
  - **Could not culture *Staph aureus* from most patients**



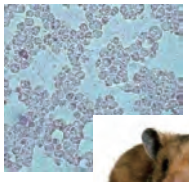


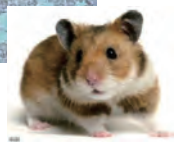
1978 Landmark publication

ANTIBIOTIC-ASSOCIATED PSEUDOMEMBRANOUS COLITIS DUE TO TOXIN-PRODUCING CLOSTRIDIA

James G. Bartlett, M.D., Ta Wen Chang, M.D., Moses Gurwith, M.D., Sherwood L. Gorbach, M.D., and Andrew B. Onderdonk, Ph.D.

- **Cytoxicity visualized in tissue culture treated with stool or with broth cultures of a *Clostridium* species found in patient stool**
- **Hamsters developed diarrhea after intracecal introduction of stool or broth**



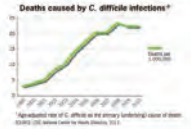


Clostridium difficile

- **Sporeforming anaerobic Gr+ rod**
- **Colonizes > 50% of newborns asymptomatically; even toxigenic strains**
- **Asymptomatic, colonized patients have some protection from CD disease**
- **Antibiotic treatment is a common risk factor**
- **Spores stable in environment; not destroyed by alcohol (alcohol hand gel)**

Lower incidence in Japan than in U.S.A.

- ❑ **In U.S.A. (per CDC)**
  - **CDI 82 per 10,000 patients**
  - **Mortality 7%**



- ❑ **In Japan (per Honda et al)**
  - **CDI 3 per 10,000 patients**
  - **Mortality 15%**

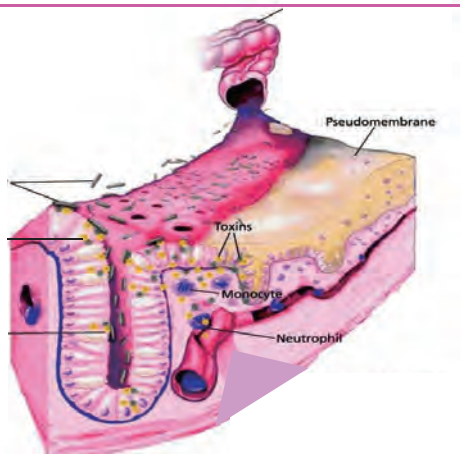
Incidence and mortality associated with Clostridium difficile infection at a Japanese tertiary care center. Honda H, Yamazaki A, Sato Y, Dubberke ER. Anaerobe. 2014 Feb;25:5-10.

Clostridium difficile: Pathogenesis

Binary toxin:
- Actin disruption
- Cytoskeleton disruption
- Increased bacterial adherence

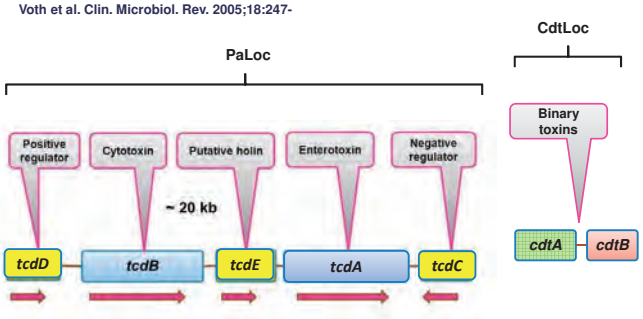
Toxin A:
- Enterotoxin
- Attracts WBCs

Toxin B:
- Cytotoxin
- Degrades colonic epithelium



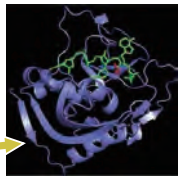
Genetic arrangement of the C. difficile pathogenicity locus (PaLoc)

Voth et al. Clin. Microbiol. Rev. 2005;18:247-



Pituch et al. J. Med. Microbiol. 2005; 55:143-. Bacci et al. 2011. EID; 17:976-

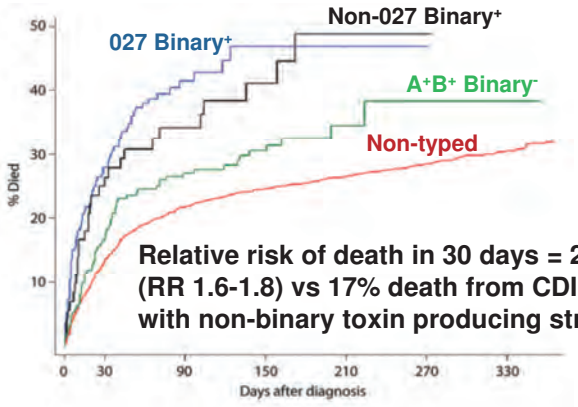
- Toxin A enterotoxin (*TcdA* gene)
- Toxin B cytotoxin (*TcdB* gene)
- Binary toxin (*CDT* gene) – found in ribotype 027, 078, and others



- Not all strains produce *Tcd A/B/CDT*
- Almost all strains that are *Tcd A+* are also *Tcd B+*
- ≤2% of strains that are *Tcd B+* are *Tcd A-*
- Toxin B antibody (not Toxin A antibody) lowers risk of recurrent disease

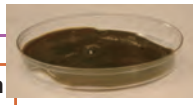
Patient Death vs Binary Toxin

Bacci et al. 2011. EID; 17:976-



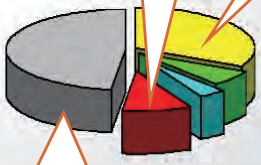
Relative risk of death in 30 days = 28% (RR 1.6-1.8) vs 17% death from CDI with non-binary toxin producing strain

Wide spectrum of CDI



Best treated with fecal transplant

Or carrier given laxative?

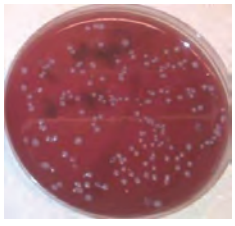


- Mild diarrhea
- Pseudomembranous colitis
- Fulminant / refractory colitis
- Recurrent diarrhea
- Asymptomatic carriage

Average 10-20% of all stools tested = Positive

Toxigenic culture is gold standard

- Grow the organism
- Test isolates for toxin



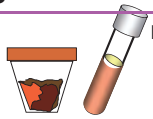


Plate direct or plate from broth enrichment

Anaerobic incubation

Cycloserine-cefoxitin-fructose agar with taurocholate

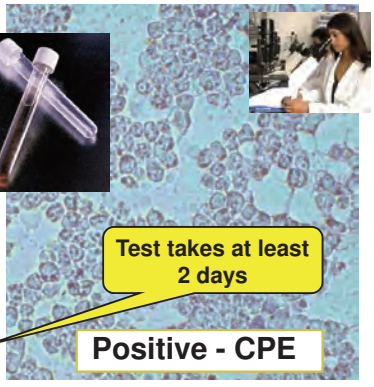
Test takes at least 4 days

Previous gold standard (2+ day TAT)

Cell culture cytotoxin neutralization (CCCN)

Detection of Cytotoxin B in toxin-sensitive cell culture monolayer

Stool supernatant or colony broth culture



Test takes at least 2 days

Normal, negative

or toxin + antitoxin = neutralized (no effect)

Positive - CPE

Rapid antigen detection CDI assays

Enzyme immunoassays and LFAs for toxins A&B or GDH

Vidas

Many labs <u>still</u> using this test type !!

C. difficile Test Result Sensitivities vs Comparator

	Cell culture Cytotoxin	Toxigenic Culture
Meridian Premier Toxins A & B EIA	92%	48%
Meridian Immunocard Toxins A & B	78%	48-67%
TechLab Toxins A & B	91%	74%
Remel Xpect	96%	48%
Wampole Tox A & B	95%	55%
TechLab GDH	90%	88%
BD GeneOhm PCR	92%	89%

LM Sloan et al, JCM, 2008 Jun;46(6):1996-2001
Eastwood et al. J. Clin. Microbiol. 2009. 47:3211-17.
L Alcalá et al, JCM, 2008 Nov;46(11):3833-3835

Clinical and Infection Control Implications of *C. difficile* Infection With Negative Enzyme Immunoassay for Toxin

Guerrero et al. 2011. CID 53:287-. (Cleveland VAMC)

- 132 PCR+ patients (unformed stools)
- 43 (32%) EIA negative for toxin A or B (would have been missed if only EIA used for testing or determining whom to treat)
- No difference in presentations: (9 pts had severe CDI and one patient died of fulminant CDI)
- All patients had equal shedding of spores onto body and environment (same ribotype)
- Of 150 strains typed, 50% were 027 <u>(significantly higher in EIA+ than EIA- patients)</u>

PCR tests improve Sensitivity without sacrificing Specificity; some offer rapid TAT

BD-GeneOhm
- PCR for tox B gene
- Usually batch 1-2/day
- 75-90 minutes TAT

Prodesse ProGastro™
- Need additional instruments
- Batch 1/day
- PCR for tox B gene
- ~ 3 hour TAT

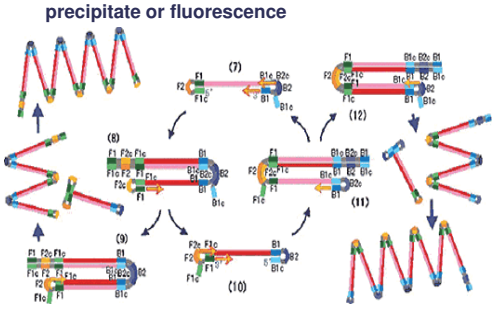
illumigene C. Difficile™
- Hands-on time <5 min
- Batch of 10
- LAMP for TcdA gene
- < 1 hour TAT

	Sens	Spec
GeneOhm	84%	98 %
Prodesse	87 %	99 %
illumigene	83 %	98 %
Xpert	94%	94%

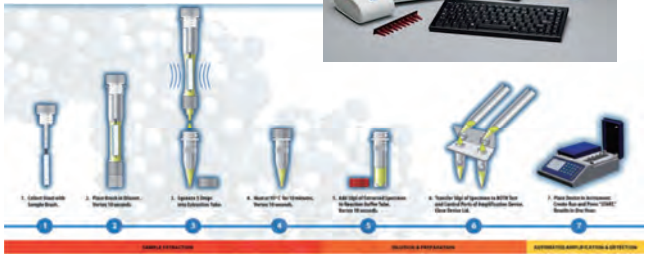
GeneXpert™ *C. difficile*
- Hands-on time 2 min
- Random access – can test 1 each
- PCR for tox B gene, cdtC gene, and Binary tox gene
- 47 min TAT

Loop mediated isothermal amplification (LAMP)

1. 4 sets of primers, inner and outer (first 95℃ heat, then 65℃ rest of reaction)
2. Primer tail loops back on itself
3. Double –loop structure amplifies on both sides, opens up on one side
4. Generates massive numbers of amplicons; detect by precipitate or fluorescence



Assay Procedure: illumigene



Cepheid Gene Xpert® *C difficile* Assay

Place swab with stool into buffer vial, vortex, pipette into cartridge.

Close top and place into instrument.

Detection of Toxigenic *Clostridium difficile*: Comparison of the Cell Culture Neutralization, Xpert® *C. difficile* & *C. difficile* /Epi and the Illumigene™ *C. difficile* Assays

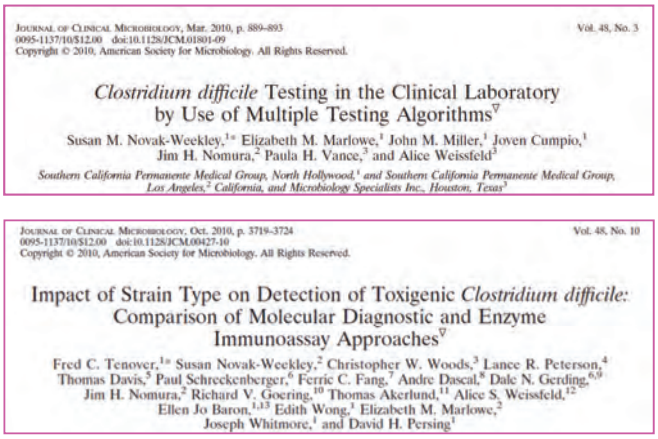
Pancholi et al. 2012. JCM 50:1331-.

Table 1: Comparison of Xpert CD, Xpert CD/Epi, Illumigene CD, CCNA and Toxigenic Culture in Prospective Stool Specimens

# Specimens	Xpert CD & Xpert CD/Epi [1]	Illumigene CD	CCNA	Toxigenic Culture
157	Negative	Negative	Negative	Not performed
20	Positive	Positive	Positive	Not performed
15	Positive	Positive	Negative	Positive
4	Positive	Negative	Negative	Positive
3	Positive	Negative	Positive	Positive
1	Positive	Invalid	Negative	Positive

In the prospective arm of the study, 10.5% specimens were positive overall by the CCNA compared to 17.5% (Illumigene) and 21.5% (Xpert)

Relevant publications



JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2010, p. 889-893 Vol. 48, No. 3
0095-1137/10/$12.00 doi:10.1128/JCM.01801-09
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Clostridium difficile Testing in the Clinical Laboratory by Use of Multiple Testing Algorithms▽

Susan M. Novak-Weekley,[1]* Elizabeth M. Marlowe,[1] John M. Miller,[1] Joven Cumpio,[1] Jim H. Nomura,[2] Paula H. Vance,[3] and Alice Weissfeld[3]

Southern California Permanente Medical Group, North Hollywood,[1] and Southern California Permanente Medical Group, Los Angeles,[2] California, and Microbiology Specialists Inc., Houston, Texas[3]

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2010, p. 3719-3724 Vol. 48, No. 10
0095-1137/10/$12.00 doi:10.1128/JCM.00427-10
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Impact of Strain Type on Detection of Toxigenic *Clostridium difficile*: Comparison of Molecular Diagnostic and Enzyme Immunoassay Approaches▽

Fred C. Tenover,[1]* Susan Novak-Weekley,[2] Christopher W. Woods,[3] Lance R. Peterson,[4] Thomas Davis,[5] Paul Schreckenberger,[6] Ferric C. Fang,[7] Andre Dascal,[8] Dale N. Gerding,[6,9] Jim H. Nomura,[2] Richard V. Goering,[10] Thomas Akerlund,[11] Alice S. Weissfeld,[12] Ellen Jo Baron,[1,13] Edith Wong,[1] Elizabeth M. Marlowe,[2] Joseph Whitmore,[1] and David H. Persing[1]

Rapid PCR and other assays compared to gold standard enriched toxigenic culture

Novak- Weekley et al. 2010. J Clin Microbiol 48:889-.

	EIA only	GDH + EIA + CCNA	GDH + PCR	PCR only
Sensitivity (n~72)	58.3%	83.1%	86.1%	94.4%
Specificity (n~360)	94.7%	96.7%	97.8%	96.3%
PPV	68.9% (61)	83.1% (71)	95.8% (432)	84.0% (81)
NPV (n~350)	91.9%	96.7%	97.2%	98.8%

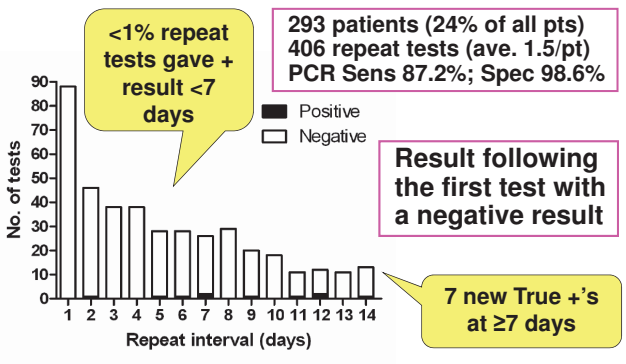
C. diff PCR vs GDH in Clinical Trials for 027 vs Non-027 Isolates

	Sensitivity		*P* value
Ribotype	PCR	GDH algorithm	
027 (11)	90.9%	90.9%	1.0
Non-027 (36)	91.7%	72.2%	0.001

Tenover, et al. 2010. JCM Vol. 48.

Repeat test NOT needed for the diagnosis of CDI if PCR is the method

Robert F. Luo, Niaz Banaei (Stanford UMC) J. Clin. Microbiol. 2010. 48:3738-



The Joint Commission Journal on Quality and Patient Safety

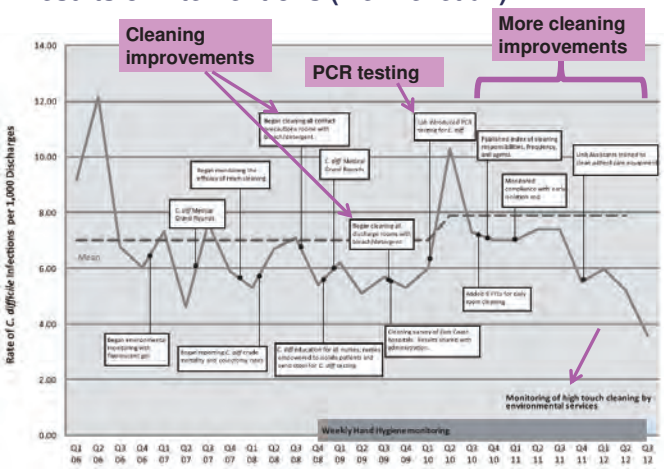
Infection Prevention and Control

Reducing *Clostridium difficile* Incidence, Colectomies, and Mortality in the Hospital Setting: A Successful Multidisciplinary Approach

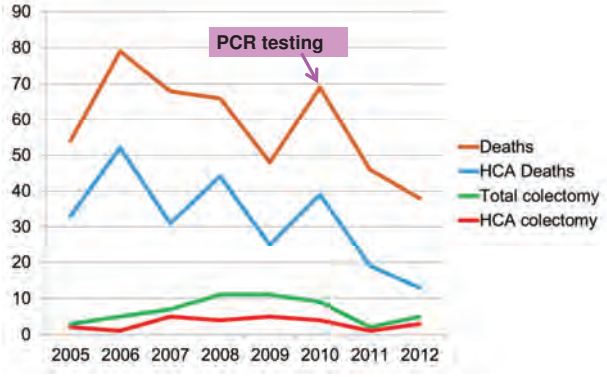
Leonard A. Mermel, DO, ScM; Julie Jefferson, RN, MPH; Kerry Blanchard BS; Stephen Parenteau, MS; Benjamin Mathis, MD; Kimberle Chapin, MD; Jason T. Machan, PhD

- Annual education for caregivers
- Enhance environmental services – practices & supplies
- Single-use devices in isolation rooms
- Portable equipment cleaning policies updated
- Improved access to personal protective equipment at rooms
- Robust antibiotic stewardship
- Identify patients at high risk sooner
- Allow nurses to initiate contact precautions
- Change C. difficile testing to molecular method (tox B PCR)
- Increase frequency of C. difficile testing in laboratory
- Develop guidelines for C. difficile patient management
- Create med/surgical rapid response team for severely ill patients
- Continue isolation of C.difficile patients for entire hospital stay

Results of interventions (Mermel et al.)



C. difficile patient outcomes over time (Mermel et al.)



Summary

- *C. difficile* strains impact test results for Toxin EIA and GDH EIA
  - Lower sensitivity for non-027 strain types (most common)
  - GDH screening utility depends on strain
  - EIA monoclonals were not developed with all strains
- Toxigenic culture and PCR are accurate for all strains
  - Best tests for infection control
  - Toxin B PCR is the best target
  - Rapid results improve outcomes, decrease costs
- Extreme Infection Control measures are necessary when outbreaks occur
- Multiple interventions, including PCR testing, can lead to lower incidence and severity of *C. difficile* disease in a healthcare institution

Treatment of *C. difficile* infection (CDI)

Mark H. Wilcox

Leeds Teaching Hospitals & University of Leeds, Leeds, UK
Public Health England

CDI should be managed according to disease severity and risk of recurrence. Severe CDI is characterised by at least one of: white blood cell count >15 x 10^9/L, acute rising serum creatinine (i.e. >50% increase above baseline), temperature >38.5°C, or abdominal or radiological evidence of severe colitis. There are currently two main treatment options for severe CDI; either oral vancomycin 125 mg qds for 10–14 days, or fidaxomicin. Fidaxomicin should be considered for patients with severe CDI who are considered at high risk for recurrence (e.g. elderly patients with multiple comorbidities who are receiving concomitant antibiotics). Metronidazole monotherapy should be avoided in patients with severe CDI because of increasing evidence that it is inferior to vancomycin (or fidaxomicin). In severe CDI cases who not responding to oral vancomycin 125 mg qds, oral fidaxomicin 200 mg bd is an alternative; or high-dosage oral vancomycin (up to 500 mg qds, if necessary administered via a nasogastric tube), +/- iv metronidazole 500 mg tds. Evidence is lacking regarding the efficacy of oral rifampicin or iv immunoglobulin as adjunctive options in severe CDI. Tigecycline has been used to treat severe CDI not responding to conventional options, but is an unlicensed indication.

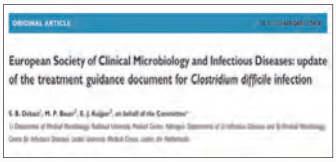
In life-threatening CDI (i.e. hypotension, partial or complete ileus or toxic megacolon) oral vancomycin up to 500 mg qds for 10–14 days via naso-gastric and/or rectal installation plus iv metronidazole 500 mg tds are used, but there is a poor evidence base in such cases. These patients require close monitoring, with specialist surgical input. Colectomy should be considered, especially if caecal dilatation is >10 cm, or for perforation or septic shock. Colectomy is best performed before blood lactate rises > 5 mmol/L, when survival is extremely poor. Total colectomy with end ileostomy has been the preferred surgical procedure. An alternative approach, diverting loop ileostomy and colonic lavage, has been reported to be associated with reduced morbidity and mortality.

There is a healthy pipeline of novel treatment (and prevention) options for CDI.

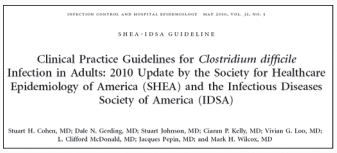
C. difficile infection (CDI) treatment

Professor Mark Wilcox

Leeds Teaching Hospitals,
University of Leeds,
Public Health England


ORIGINAL ARTICLE

European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection


SHEA-IDSA GUIDELINE

Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA)

Public Health England website:
https://www.gov.uk/government/publications/clostridium-difficile-infection-guidance-on-management-and-treatment

Debast SD, et al (ESCMID). Clin Microbiol Infect 2014

Cohen S et al. ICHE 2010

Unmet CDI treatment needs

- *Reduced recurrence*
- *Improved sustained cure rate*

- Time to resolution of symptoms
- Severe CDI
- Prediction tools to optimise treatment options
- Reduced mortality

How is CDI currently managed?

- Until now treatments have included metronidazole and vancomycin but these are sub-optimal
  - Failure in ~10-20% of cases[1]
  - According to severity of infection
  - Recurrence occurs in ~20% of cases
    & ~45% subsequently recur again[1]
  - Death
    17% 30-day mortality (~7% attributable)[2]
    24-48% mortality rate from severe CDI[3]

1. Kelly and LaMont. N Engl J Med 2008;359:1932–40
2. Planche TD et al. Lancet Infect Dis 2013.
3. Health Protection Agency. Mandatory Surveillance of Healthcare Associated Infections

'We recommend using any of the following to indicate severe CDI and so to use oral vancomycin (or fidaxomicin) in preference to metronidazole.'

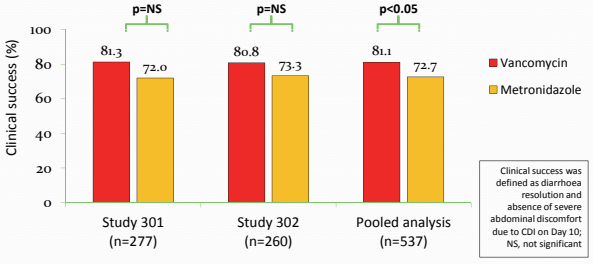
- **WCC >15 109/L**
- **Acutely rising blood creatinine**
  (>50% increase above baseline)
- **Temperature >38.5°C;**
- **Evidence of severe colitis**
  (abdominal signs, radiology)

Note variable definitions of CDI severity

Wilcox MH. *Clin Infect Dis* 2014. PMID: 24799325

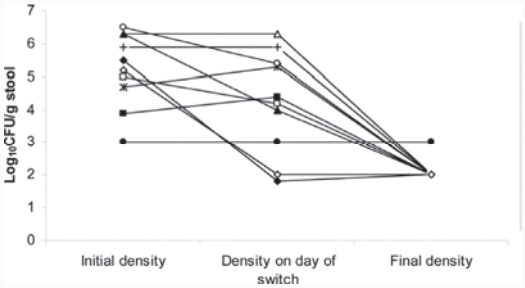
Rates of clinical success for metronidazole and vancomycin

Two identical multicentre, randomised, double-blind, parallel-group clinical trials



Johnson et al. Clin Infect Dis 2014.

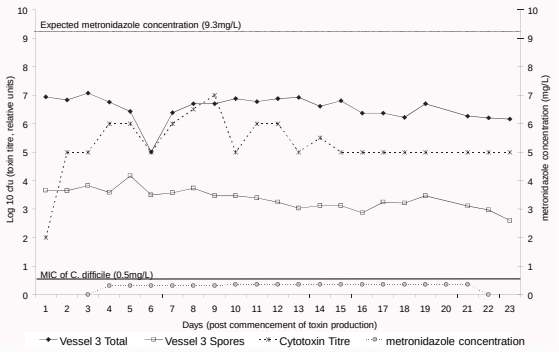
Concentration of *Clostridium difficile* in stool of 10 patients whose therapy was changed from metronidazole to vancomycin



Al-Nassir W N et al. Clin Infect Dis. 2008;47:56-62

Activity of metronidazole against *C. difficile* ribotype 027 in gut infection model

Baines, Freeman, Wilcox. *J Antimicrob Chemother* 2007.



Determinants of recurrence risk

- Flora inhibition (antibiotics)
- Spore persistence
- Antibody deficit
- Previous recurrence
- Strain type
- Host biomarkers e.g. albumin
- Age
- Co-morbidities

Determinants of recurrence risk

- Flora inhibition (antibiotics)
- Spore persistence
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- Age
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Effect of CA on outcome after treatment of CDI with fidaxomicin or vancomycin

	No CA	CA	p value
Clinical cure (%)			
CA during treatment*	93%	84%	<0·001
Recurrence (%)			
CA during treatment*	18%	24%	0·11
CA during follow-up†	18%	25%	0·06
CA at any time‡	18%	23%	0·08
Sustained response (%)			
CA at any time‡	75%	66%	0·005
Median time to resolution of diarrhoea (h)			
CA during treatment*	54	97	<0·001

CA=concomitant antibiotics. *Days 1–10. †Days 11–40. ‡Days 1–40.

Mullane KM, *et al. Clin Infect Dis* 2011;53:440-7.
Wilcox MH. Lancet Infect Dis 2012.

Fidaxomicin

- Reduced recurrence by ~50%
- Less effective against CD 027 (but same true for vancomycin)
- Some resistance emergence in VRE (not in CD)
- No fidaxomicin resistance in CD, but one isolate (cure patient) MIC = 16 mg/L
- Concomitant antibiotics
- Cost

Fidaxomicin vs Vancomycin Phase 3 CDI Studies

Per protocol, microbiologically evaluable	Fidaxomicin 200 mg bd	Vancomycin 125 mg qds	P value	95% C.I.
Clinical Cure	92.1% (244/265 pts) 91.7%	89.8% (254/283 pts) 90.6%	NA	(-2.6,)*
Recurrence	13.3% (28/211) 12.8%	24.0% (53/221) 25.3%	0.004 0.002	(-17.9, -3.3)
Sustained Cure	77.7% (206/265) 79.6%	67.1% (190/283) 65.5%	0.006 0.001	(3.1, 17.9)

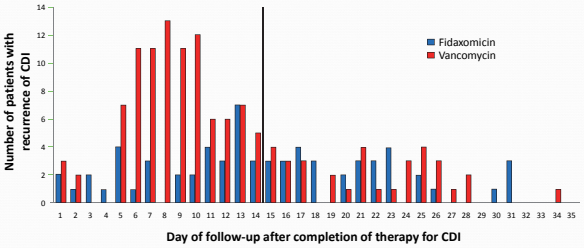
* one-sided 97.5% CI
NA= Not Applicable (trial met non-inferiority endpoint)

http://www.optimerpharma.com/news.asp?news_story=69&page_num=11.10.2008 SAN DIEGO, CA
http://www.optimerpharma.com/pipeline.asp?pipeline=1

Fidaxomicin pivotal phase 3 trials: time to recurrence

Early recurrence (relapse):
Fidaxomicin: 7.4%
Vancomycin: 19.3%
p<0.001

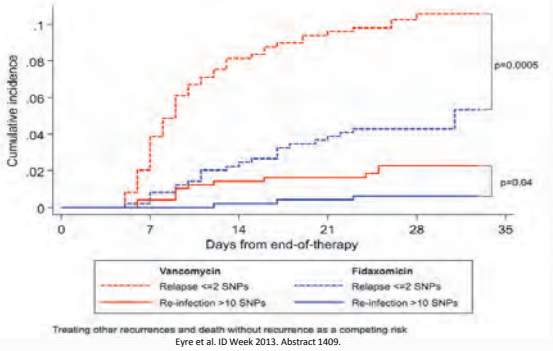
Late recurrence (relapse/reinfection):
Fidaxomicin: 7.3%
Vancomycin: 8.4%
p=0.560



European Public Assessment Report (EPAR) EMA/857570/2011, September 2011.

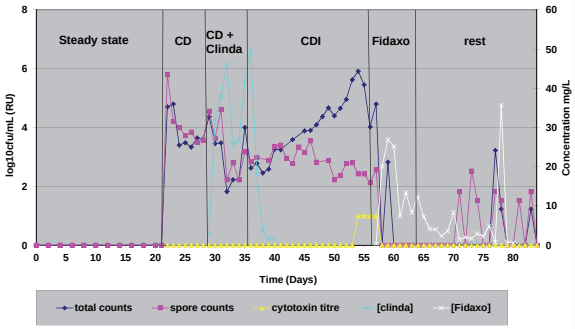
Fidaxomicin prevents CDI relapse & re-infection

whole genome sequencing data



Treating other recurrences and death without recurrence as a competing risk
Eyre et al. ID Week 2013. Abstract 1409.

Persistence of fidaxomicin



Chilton C *et al*. ECCMID 2013. LB-2817.

'Fidaxomicin should be considered for patients with severe CDI who are considered at high risk for recurrence; these include elderly patients with multiple co-morbidities who are receiving concomitant antibiotics.'

'Fidaxomicin should be preferred for patients with recurrent CDI, whether mild, moderate or severe, because of their increased risk of further recurrences.'

'The efficacy of fidaxomicin in patients with multiple CDI recurrences is unclear. Depending on local cost-effectiveness based decision making, oral vancomycin is an alternative.'

Weak evidence base for probiotics

Cochrane Review 2013

Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children

Joshua Z Goldenberg[1], Stephanie SY Ma[2], Jane D Saxton[1], Mark R Martzen[3], Per O Vandvik[4], Kristian Thorlund[5], Gordon H Guyatt[5], Bradley C Johnston[6,7]

[1]Bastyr University, Seattle, USA. [2]Division of Plastic & Reconstructive Surgery, McMaster University, Hamilton, Canada. [3]Bastyr University Research Institute, Bastyr University, Kenmore, USA. [4]Norwegian Knowledge Centre for the Health Services, Oslo, Norway. [5]Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Canada. [6]The Hospital for Sick Children Research Institute, Toronto, Canada. [7]Institute of Health Policy, Management and Evaluation, University of Toronto, Toronto, Canada

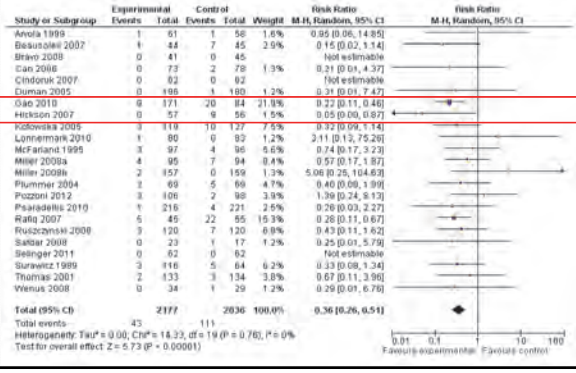
Contact address: Bradley C Johnston, bradley.johnston@sickkids.ca, bjohnst@mcmaster.ca.

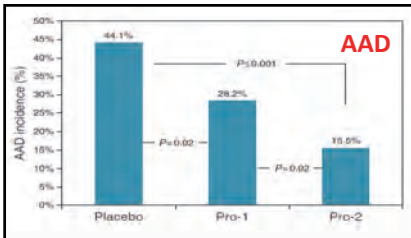
Editorial group: Cochrane Inflammatory Bowel Disease and Functional Bowel Disorders Group.
Publication status and date: New, published in Issue 5, 2013.
Review content assessed as up-to-date: 21 February 2013.

Cochrane Review 2013

- ***C. difficile* associated diarrhoea (CDAD)**
  Diarrhoea and
  positive stool cytotoxin/culture for *C. difficile*
- ***C. difficile* infection (CDI)**
  Positive stool cytotoxin/culture for *C. difficile*

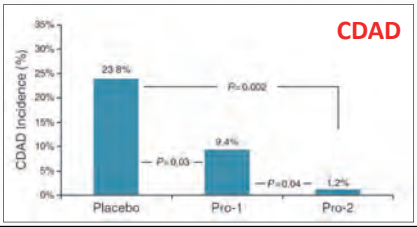
Cochrane Review 2013 probiotics & 'CDAD'





Gao *et al.*
Am J Gastroenterol
2010; 105: 1636-41.

Of the 1120 patients who were eligible to participate in the study, 865 were excluded from participation .
The remaining 255 patients were enrolled in the trial between January 2009 and March 2009.



Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial

Stephen J Allen, Katrina Wareham, Dinesh Wang, Caroline Bradley, Hayley Hutchings, Wyn Harris, Anjan Dhar, Helga Brown, Alwyn Foden, Michael B Gravenor, Dietrich Mack

Summary

Background Antibiotic-associated diarrhoea (AAD) occurs most frequently in older (≥65 years) inpatients exposed to broad-spectrum antibiotics. When caused by *Clostridium difficile*, AAD can result in life-threatening illness. Although underlying disease mechanisms are not well understood, microbial preparations have been assessed in the prevention of AAD. However, studies have been mostly small single-centre trials with varying quality, providing insufficient data to reliably assess effectiveness. We aimed to do a pragmatic efficacy trial in older inpatients who would be representative of those admitted to National Health Service (NHS) and similar secondary care institutions and to recruit a sufficient number of patients to generate a definitive result.

Interpretation We identified no evidence that a multistrain preparation of lactobacilli and bifidobacteria was effective in prevention of AAD or CDD. An improved understanding of the pathophysiology of AAD is needed to guide future studies.

Published online August 8, 2013 http://dx.doi.org/10.1016/S0140-6736(13)61218-0

‘We cannot at present recommend the use of probiotics for the prevention of AAD or CDI.’

Faecal transplantation

- the ultimate probiotic?

- Eiseman et al 1958, pts with severe AAD
- 160 cases (largest n=18), 15 failures i.e. 90% success
- Aas *et al*. *Clin Infect Dis* 2003;36:580-5.
- Randomised, sham-procedure-controlled clinical trial in the Netherlands

FDA letter requirement for IND/NDA

Fecal transplants may stall as FDA cracks down on docs

http://vitals.nbcnews.com/_news/2013/06/04/18659576-fecal-transplants-may-stall-as-fda-cracks-down-on-docs

FDA intends to exercise enforcement discretion

- **Consent**
- **Known donor**
- **Screening**

‘FDA does not intend to exercise enforcement discretion for the use of an FMT product when the FMT product is manufactured from the stool of a donor who is not known by either the patient or the licensed health care provider treating the patient, or when the donor and donor stool are not qualified under the direction of the treating licensed health care provider.’

http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm387023.htm

Clin Infect Dis. 2011 Nov;53(10):994-1002.

Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection.

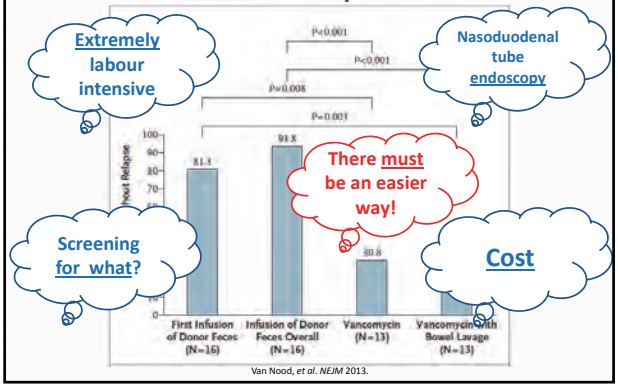
Gough E, Shaikh H, Manges AR.

Department of Epidemiology Biostatistics and Occupational Health, McGill University, 1020 Pine Avenue West, Montreal, Quebec, Canada.

Abstract

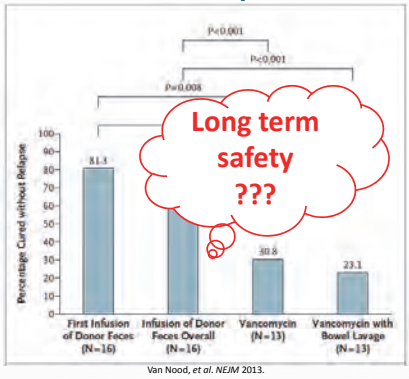
Clostridium difficile infection (CDI) is a gastrointestinal disease believed to be causally related to perturbations to the intestinal microbiota. When standard treatment has failed, intestinal microbiota transplantation (IMT) is an alternative therapy for patients with CDI. IMT involves infusing intestinal microorganisms (in a suspension of healthy donor stool) into the intestine of a sick patient to restore the microbiota. However, protocols and reported efficacy for IMT vary. We conducted a systematic literature review of IMT treatment for recurrent CDI and pseudomembranous colitis. In 317 patients treated across 27 case series and reports, IMT was highly effective, showing disease resolution in 92% of cases. Effectiveness varied by route of instillation, relationship to stool donor, volume of IMT given, and treatment before infusion. Death and adverse events were uncommon. These findings can guide physicians interested in implementing the procedure until better designed studies are conducted to confirm best practices.

Rates of cure without relapse for recurrent CDI



Van Nood, *et al*. *NEJM* 2013.

Rates of cure without relapse for recurrent CDI



Van Nood, *et al*. *NEJM* 2013.

Role of microbiome in human health

- Inflammatory bowel diseases
- Metabolic disorders, diabetes
- Hypertension
- Obesity
- Cancer
- Infection

particularly *Clostridium difficile* infection (CDI)

Regulatory positions on FMT

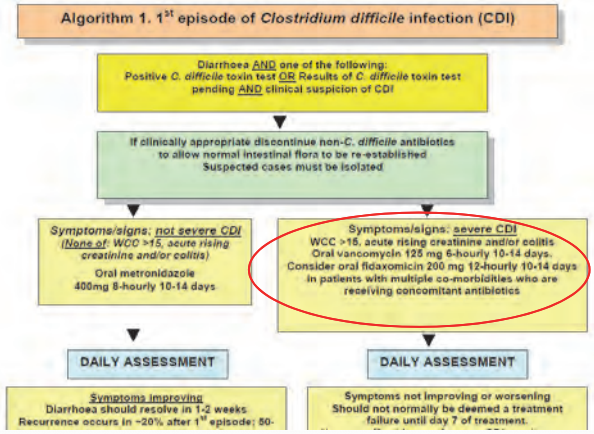
Health Canada, 2014

- "Since no company or individual has sought market authorizations for materials used in fecal therapy," Health Canada said, "the therapy is considered investigational, meaning that fecal therapy can only be conducted in the context of an authorized clinical trial."

NICE, 2104

- 'This procedure should only be considered for patients with recurrent *C. difficile* infections that have failed to respond to antibiotics and other treatments.'

'A cost-effectiveness evaluation of donor faeces transplantation has not been performed, which is notable considering the complexity of the procedure (donor testing, consenting, sample processing and endoscopy). antibiotics.'

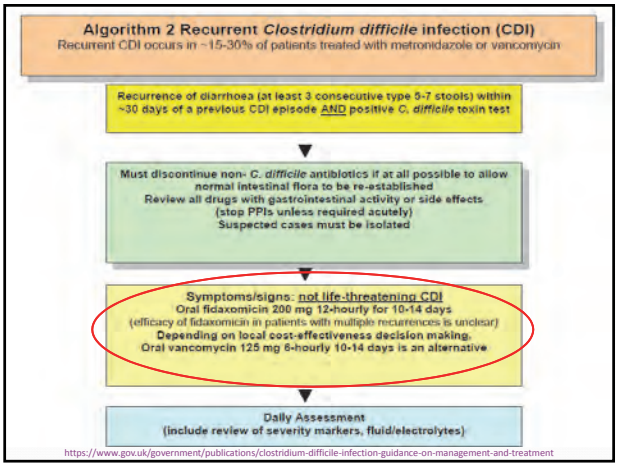


https://www.gov.uk/government/publications/clostridium-difficile-infection-guidance-on-management-and-treatment

Life-threatening CDI

(hypotension, partial or complete ileus or toxic megacolon)

- Poor evidence base
- Vancomycin 500 mg qds via naso-gastric and/or rectal installation + metronidazole 500 mg tds iv
- Very close monitoring; specialist surgical input
- Colectomy should be considered if
  - caecal dilatation is >10 cm
  - for perforation or septic shock
  - before blood lactate rises > 5 mmol/L
- Total colectomy with end ileostomy has been the preferred surgical procedure
- Diverting loop ileostomy and colonic lavage



https://www.gov.uk/government/publications/clostridium-difficile-infection-guidance-on-management-and-treatment

Clinical trial development phase	Drug/product	Indication. Notes.
Phase III	MK-3415 and MK-6072 (Merck)	Treatment of CDI. Anti-toxin A (MK-3415) and B (MK-6072) monoclonal antibodies given iv as adjunctive to standard treatment.
	Surotomycin (CB-183,315) (Cubist)	Treatment of CDI. Lipopeptide antibiotic related to daptomycin but given orally.
	Cadazolid (Actelion)	Treatment of CDI. Hybrid antibiotic molecule, comprising fluoroquinolone and oxazolidinone moieties, given orally.
	C. difficile vaccine (Sanofi Pasteur)	Primary prevention of CDI. Vaccine containing toxoids of toxin A and B.

Clinical trial development phase	Drug/product	Indication. Notes.
Phase II	Non-toxigenic C. difficile (Viropharma)	Prevention of recurrent CDI.
	Ramoplanin (Nanotherapeutics)	Treatment of CDI.
	LFF571 (Novartis)	Treatment of CDI. LFF571 is a novel semi-synthetic thiopeptide.
	SER-109 (Seres)	Treatment of recurrent CDI. Oral microbiome therapeutic (mixture of bacterial spores) tested in a single-arm, open-label clinical trial.
	SMT 19969 (Summit)	Treatment of CDI. SMT19969 is a novel, oral small molecule antibiotic that is active against some (including C. difficile) but not all clostridial species.
	C. difficile vaccine, PF-06425090 (Pfizer)	Primary prevention of CDI.

Clinical trial development phase	Drug/product	Indication. Notes.
Phase I	PolyCAb (MicroPharm)	Treatment of severe CDI. Polyclonal antibodies against C. difficile given iv.
	SYN-004 (Synthetic Biologics)	Prevention of CDI. SYN-004 is a class A B-lactamase.
	CRS3123, previously known as REP3123. (Crestone)	The National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, has launched an early-stage clinical trial of CRS3123, an investigational oral antibiotic intended to treat CDI.[143]
	IC84 vaccine (Valneva)	Prevention of CDI. Vaccine comprises a recombinant protein consisting of two truncated toxins A and B from C. difficile.

Summary CDI treatment issues

- **Need to identify patients**
  - **with severe infection**
  - **at risk of recurrence**
- **Detrimental effect of concomitant antibiotics**
- **Use accordingly metro', vancomycin or fidaxomicin**
- **Multiple new drugs / interventions under investigation**
- **Need to improve evidence base for when to use different CDI treatment options**
- **Future role of metronidazole in CDI**
- **Can new treatment options reduce mortality?**

What is going on about *Clostridium difficile* infection in Japan?

Haru Kato

Department of Bacteriology II, National Institute of Infectious Diseases, Japan

Clostridium difficile is well known as one of the most common organisms to cause healthcare-associated infection. A global spread of a hypervirulent strain, PCR ribotype 027 (BI/NAP1/027) that is resistant to new fluoroquinolones has been reported in recent decades although variation on the prevalence of 027 among countries was noted. In Japan, while outbreaks due to 027 *C. difficile* have not documented so far, there were a few reports of sporadic cases. Of these, a report documented that a young woman without any previous medical history suffered from fulminant colitis caused by 027 *C. difficile*, which was susceptible to gatifloxacin and moxifloxacin. It was suggested that PCR ribotype 027 has the potential virulence factors that are not associated with a fluoroquinolones resistance-conferring mutation.

We have identified a PCR ribotype, named as type smz, which has been prevalent in multiple Japanese hospitals since the 1990s. PCR ribotype smz strain, which is toxin A-positive, toxin B-positive, binary toxin-negative was found to be epidemic as well as endemic in Japan. Also, PCR ribotype trf strain, which is toxin A-negative, toxin B-positive, binary toxin-negative has caused outbreaks at some Japanese hospitals. Our recent study showed that among 120 isolates collected from 15 hospitals in non-outbreak settings, only 5 (4%) were binary toxin-positive and one of these was 027. Nucleic acid amplification test (NAAT) detecting presumptive 027 *C. difficile* may be introduced to Japan soon. The test results should be read in view of the difference in molecular epidemiology between in countries where 027 is epidemic and in Japan.

At Japanese hospitals, enzyme immunoassay (EIA) detecting toxin A and/or toxin B, EIA detecting glutamate dehydrogenase (GDH), and *C. difficile*-culture are available as laboratory tests for CDI. According to the results of questionnaire study (General Meeting of Japanese Society for Clinical Microbiology, 2013), GDH test is performed in 128 (77%) of 166 clinical laboratories responding. However, results of GDH test are not necessarily reported to physicians at some hospitals, because some physicians cannot read the test results. It may highlight the fact that laboratory testing is not always performed properly.

Since awareness of CDI considerably varies among hospitals in Japan, not negligible CDI cases may be overlooked. Recently new therapies, new laboratory tests, vaccines and so on are being introduced to Japan from one to the next. Before introducing those, there is an urgent need to increase awareness of CDI.

What is going on about *Clostridium difficile* infection in Japan?

Haru Kato
Department of Bacteriology II,
National Institute of Infectious Diseases, Japan

CDI incidence in Japan

Retrospective cohort study in patients with CDI was conducted at a 550-bed hospital in Sapporo from 2010 through 2012, and CDI incidence was 3.11 cases per 10,000 patient-days. Honda, H. et al. 2014. Anaerobe 25:5:10.

At another 340-bed hospital in Chiba, CDI incidences were 2.15, 2.41, 4.10, 3.59 and 3.36 cases per 10,000 patient-days in 2008, 2009, 2010, 2011 and 2012, respectively. Unpublished data by Satomura, H et al.

Numerous CDI patients may be undiagnosed? We need a national data, but are we ready to do a surveillance?

First of all, what kind of testing strategies are used in Japan ?

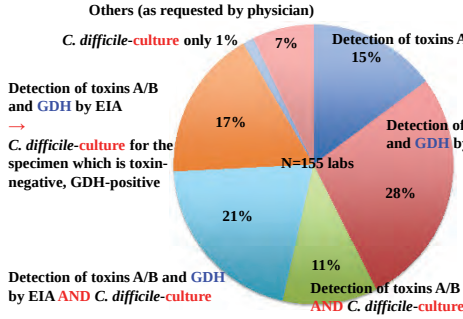
Rapid-tests evaluation on *Clostridium difficile* toxins and microbiological investigation. 2010. Kansensyogaku Zasshi 84:147-52.

?? Clinical CDAD was considered the detection gold standard ??

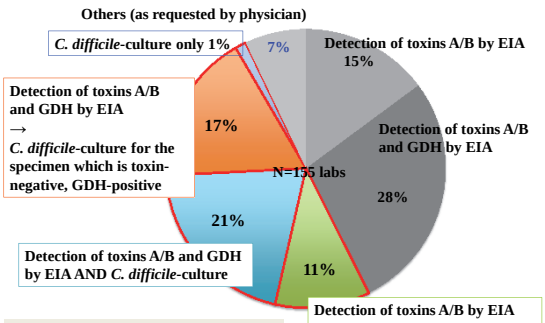
Abstract
We evaluated two rapid toxin tests for C. difficile combined with ... specimen cultures used from January 2006 to March 2009. Stool specimens numbered 877, 102 among which were from ... cases of diagnosed clinical C. difficile-associated diarrhea (CDAD). Rapid toxin A 'Uniquick' detection kits were used until October 2007 and toxin A&B 'TOX A/B' detection kits thereafter. Clinical CDAD was considered the detection gold standard. Uniquick sensitivity, specificity, and positive and negative predictive values were 54.3%, 99.1%, 90.5%, and 93.2% while those for TOX A/B were 46.2%, 97.6%, 85.2%, and 95.0% and for culture 42.2%, 95.5%, 55.1%, and 92.6%. Rapid toxin tests tended to have better sensitivity than culture results although not significantly so, and Uniquick showed significantly better positive predictive value than TOX A/B or culture results. Among clinical CDAD cases, concordance with culture was 24.3% for Uniquick and 53.1% for TOX A/B. For stored strains, 27 were typed toxin A+B+ (48.1%), toxin A-B+ (37.0%) and toxin A-B- (14.8%) with toxin gene detection by PCR. Eight of the 10 toxin A-B+ strains were classified into two cluster by ribotyping, and 7 of those were detected in two hospital wards, indicated the possibility of nosocomial toxin A-B+ strain spread. The rapid toxin test for both toxins A and B should be used if toxin A-B+ predominate. Simultaneous culture testing may be useful for detecting clinical CDAD more accurately, however

You do NOT need any laboratory tests, if you can give a diagnosis of CDI only by clinical suspicion, do you?

Laboratory tests used at clinical laboratories in Japan for the specimens from suspected CDI



Unpublished data from a questionnaire study (General Meeting of Japanese Society for Clinical Microbiology, 2013) by Moro, K. *et al.*



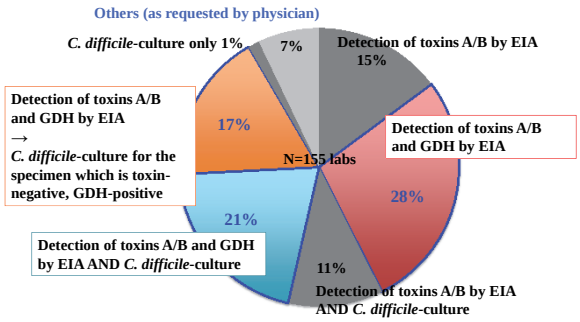
C. difficile-culture is performed at 50% of clinical laboratories responding

Unpublished data by Moro, K. et al.

Laboratory tests performed at clinical laboratories in Japan

According to the results of a questionnaire study…

1. **EIA detecting toxins A/B is routinely performed at all laboratories but one.**
2. ***Clostridium difficile*-culture is routinely performed at more than 50% of clinical laboratories.**
3. **Among 112 laboratories where *Clostridium difficile* is cultured, 58 (52%) routinely examine toxigenicity of recovered isolates, and 15 (13%) do it if it is required by physicians.**



Detection of GDH is routinely performed in 102/155 (66%) of clinical laboratories responding.

Unpublished data by Moro, K. et al.

We have NO data about the hospitals that did not respond to the questionnaire ---

An example

1. **An outbreak of CDI occurred at a 250-bed hospital.**
2. **Before they noticed the outbreak, almost NO stool specimens for *C. difficile* testing had been submitted.**

What they did during the outbreak ---

1. **While detection of toxins A/B and GDH by EIA was performed at the clinical laboratory of the hospital, the results of GDH were NOT reported to physicians.**

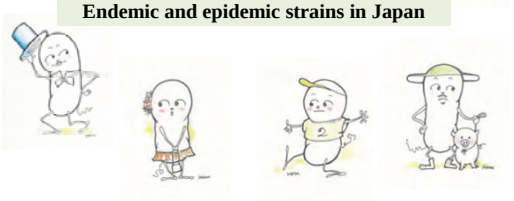
1. **Asymptomatic carriers were also tested and treated with metronidazole or vancomycin when the test results were positive.**
2. **In some CDI cases, vancomycin was given intravenously.**

THIS IS JUST THE REAL WORLD !!?

1. **A number of CDI should be undiagnosed because of an absence of clinical suspicion in Japanese hospitals.**

1. **Before introducing new laboratory tests, new therapies, and vaccines *etc*, there is an urgent need to increase awareness of CDI in Japan.**

Endemic and epidemic strains in Japan



Typing results of *C. difficile* isolates collected from 15 hospitals in non-outbreak settings

Toxigenicity	No. of isolates (%)	PCR ribotype	No. of isolates (%)
$A^+B^+CDT^-$	96 (80.0)	002	16 (13.3)
		014	13 (10.8)
		smz	47 (39.2)
		Other types	20 (16.7)
$A^-B^+CDT^-$	19 (15.8)	trf	19 (15.8)
$A^+B^+CDT^+$	5 (4.2)	027	1 (0.8)
		078	1 (0.8)
		Other types	3 (2.5)
Total No. of isolates			120

Of the 120 isolates tested, only 5 (4.2%) were binary toxin-positive, and of which only one was identified as PCR ribotype 027, with another as PCR ribotype 078.

1. **In Japan, while some reports have shown sporadic cases due to PCR ribotype 027 *C. difficile*, no outbreaks associated with type 027 have been reported so far.**

1. **The results of nucleic acid amplification test (NAAT) detecting *cdt*-positive or presumptive ribotype 027 *C. difficile* should be read in view of the difference in molecular epidemiology between in countries where 027 is epidemic and in Japan.**

A healthy young Japanese woman suffered from fulminant colitis due to fluoroquinolone-susceptible 027 strain. This case report suggested that *C. difficile* PCR ribotype 027 has the potential virulence factors that are not associated with a fluoroquinolone resistance-conferring mutation. Nishimura, S. et al. 2014. J Infect Chemother 20:729-731.

Identification of non-027 does not mean that you do not need to be careful of nosocomial transmission

1. **Isolation of PCR ribotype 078 from humans is infrequent in Japan.**

2. **Studies on animals in Japan**

- **Postoperative *C. difficile* infection with PCR ribotype 078 strain was found at necropsy in five Thoroughbred racehorses; the case sequences might have been health-care associated infection.** Niwa, H et al. 2013. Veterinary report 24:607-613.
- **Of 120 neonatal piglet fecal samples tested, 69 were positive for *C. difficile*; PCR ribotype 078 was the third dominant PCR ribotype.** Usui, M et al. 2014. Front Microbiol 5:513-521.

3. **Further molecular studies on community-acquired CDI and food surveillance are required in Japan.**

Typing results of *C. difficile* isolates collected from 15 hospitals in non-outbreak settings and from 2 outbreaks

Toxigenicity	PCR ribotype	No. of isolates (%) recovered from 15 hospitals in non-outbreak settings	No. of isolates (%) in an outbreak period at :	
			Hospital A (2010)	Hospital B (2009)
$A^+B^+CDT^-$	002	16 (13.3)	0	0
	014	13 (10.8)	5 (23.8)	0
	smz	47 (39.2)	9 (42.9)	9 (50.0)
	Other types*	20 (16.7)	1 (4.8)	0
$A^-B^+CDT^-$	trf	19 (15.8)	6 (28.6)	9 (50.0)
	017	0	0	0
$A^+B^+CDT^+$	027	1 (0.8)	0	0
	078	1 (0.8)	0	0
	Other types	3 (2.5)	0	0
Total No. of isolates		120	21	18

*15 different PCR ribotypes were identified.

Distribution of prevalence of 5 PCR ribotypes recovered from CDI patients in 4 studies and healthy volunteers in Japan

Period	1996-1999	2003-2007	2005-2008	2011-2013	1998-1999
PCR ribotype	3 hospitals	4 hospitals	1 hospital	15 hospitals	Healthy individuals
smz	51 (58.6)*	51 (58.6)	19 (26.8)	47 (39.2)	0
014 (hr)	6 (6.9)	4 (4.6)	13 (18.3)	13 (10.8)	17 (18.1)
002 (yok)	9 (10.3)	3 (3.4)	14 (19.7)	16 (13.3)	3 (3.2)
017 (fr)	5 (5.7)	1 (1.1)	2 (2.8)	0	4 (4.3)
trf	0	10 (11.5)	6 (8.5)	19 (15.8)	0
Other types	16 (18.4)	18 (20.7)	17 (23.9)	25 (20.8)	70 (74.5)
Total	87	87	71	120	94**
Reference	Kato et al. 2001	Kato et al. 2010	Iwashima et al. 2010	Unpublished data	Kato et al. 2001

*No of isolates (%);
**Of 1,234 volunteers tested, 94 (7.6%) were positive for *C. difficile*.

1. **PCR ribotype smz (toxin A-positive, toxin B-positive, CDT-negative) and PCR ribotype trf (toxin A-negative, toxin B-positive, CDT-negative) were noted as having particular association with both epidemic and endemic CDI in Japan.**

1. **PCR ribotype smz was already endemic since in the 1990s in Japanese hospitals.**
2. **Interestingly, in a healthy volunteer study in Japan, none of them carried type smz isolates in their intestinal tract**

3. **While PCR ribotype 017 was recovered from sporadic cases, ribotype trf has caused outbreaks.**

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