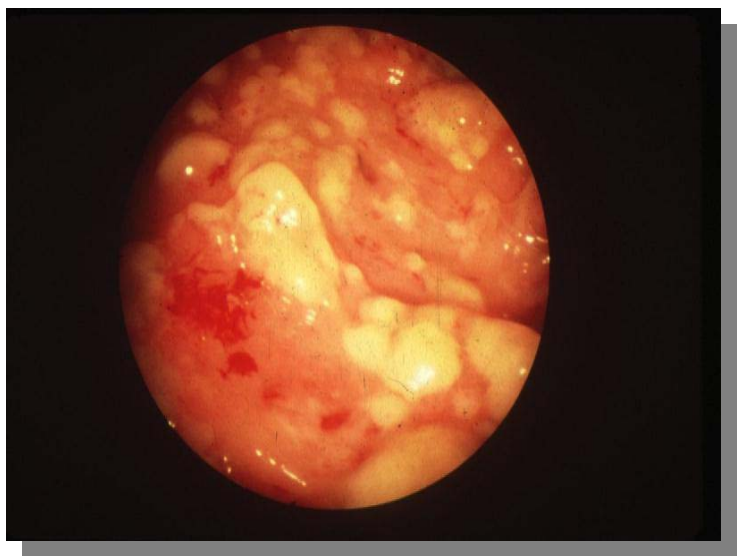
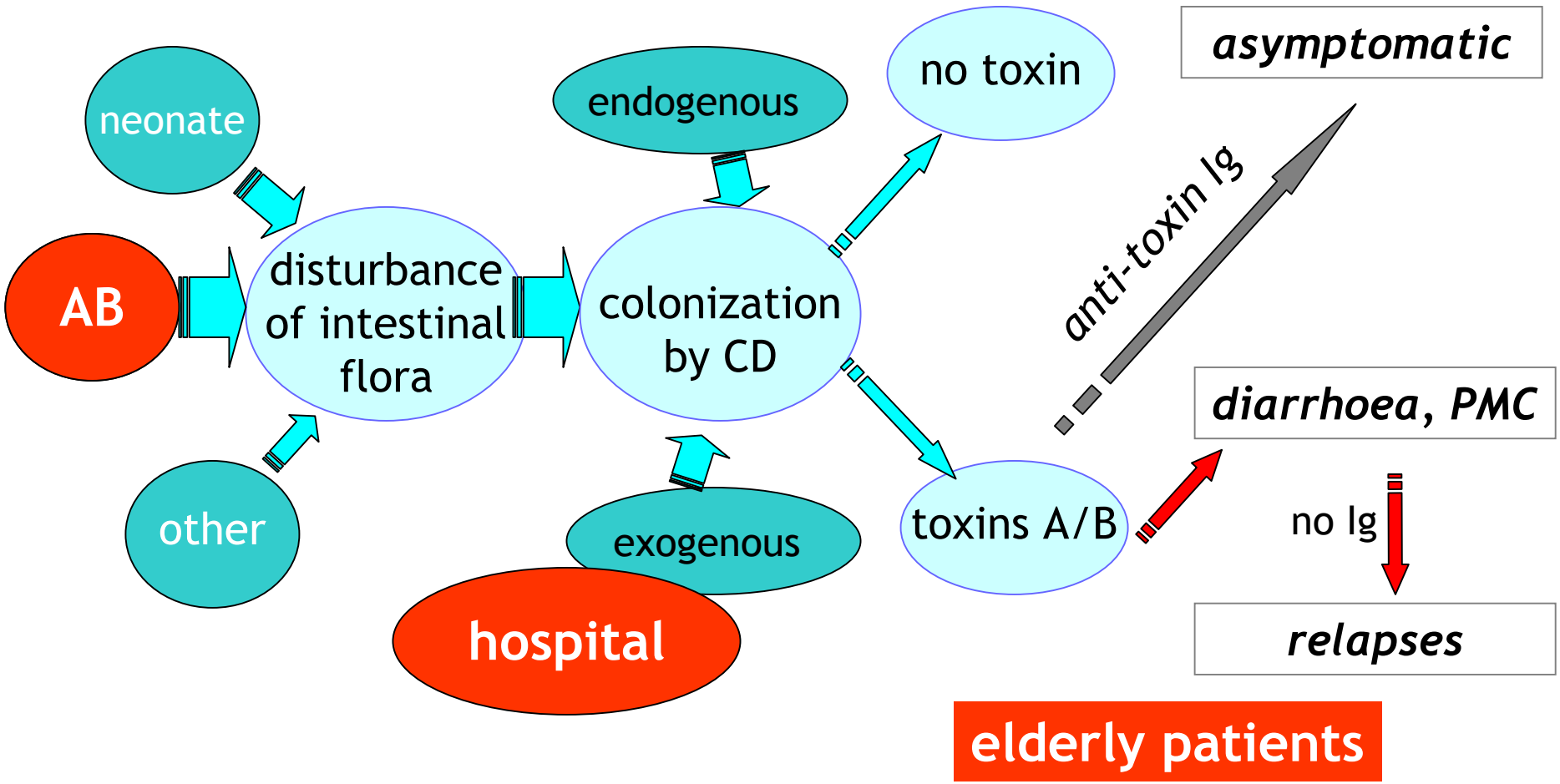
Les souches hyper-virulentes de *Clostridium difficile*

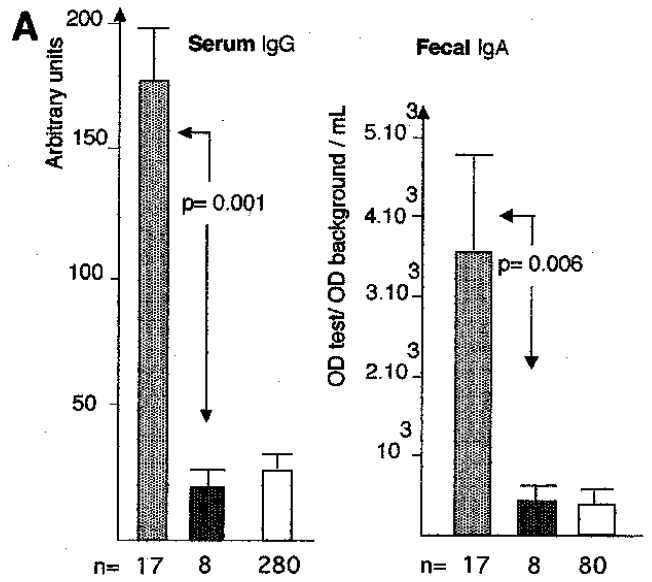
Michel Delmée



CDAD : Physiopathology



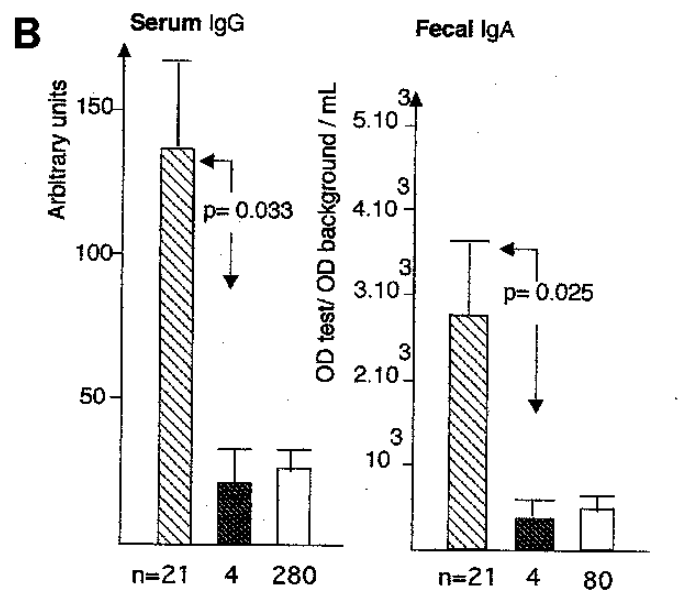
Immune response in CDAD



diarrhea of less than 2 w.

diarrhea of more than 2 w

controls



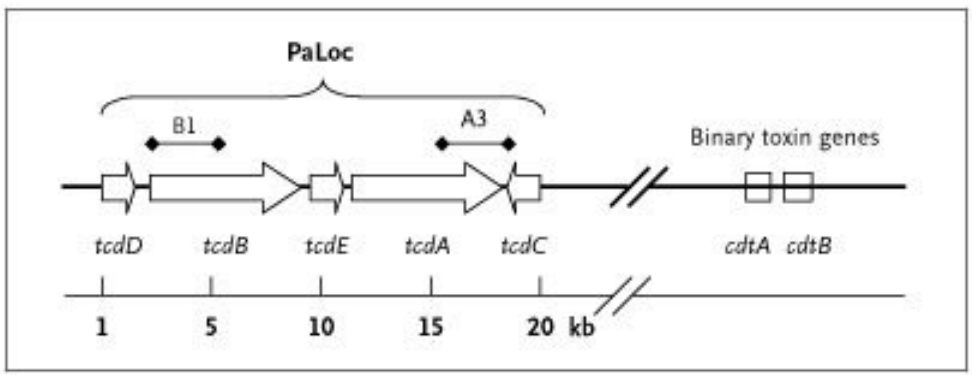
one episode of diarrhea

relapsing diarrhea

controls

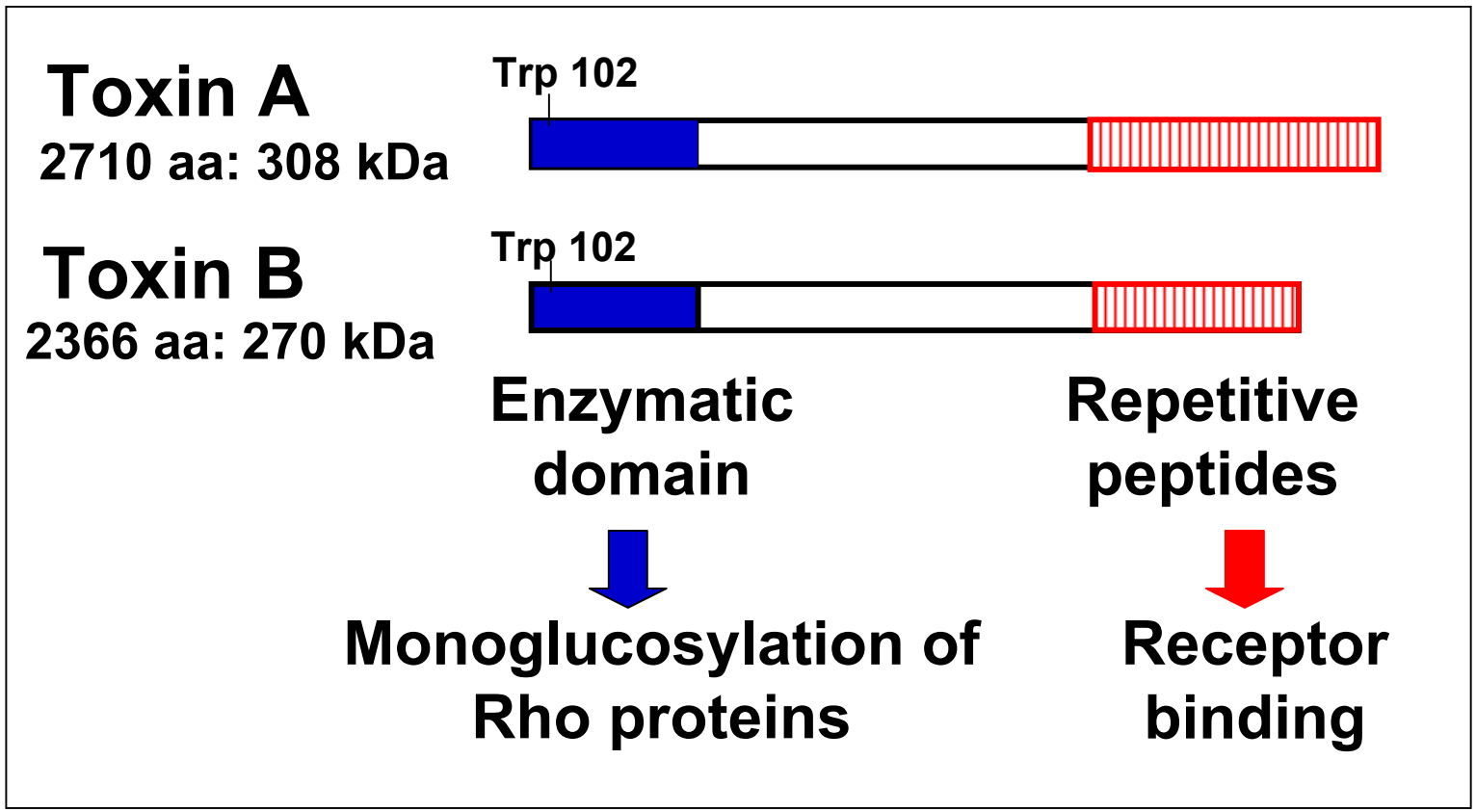
Warny et al. Infect. Immun. 1994;62:384

Pathogenicity locus (PaLoc)

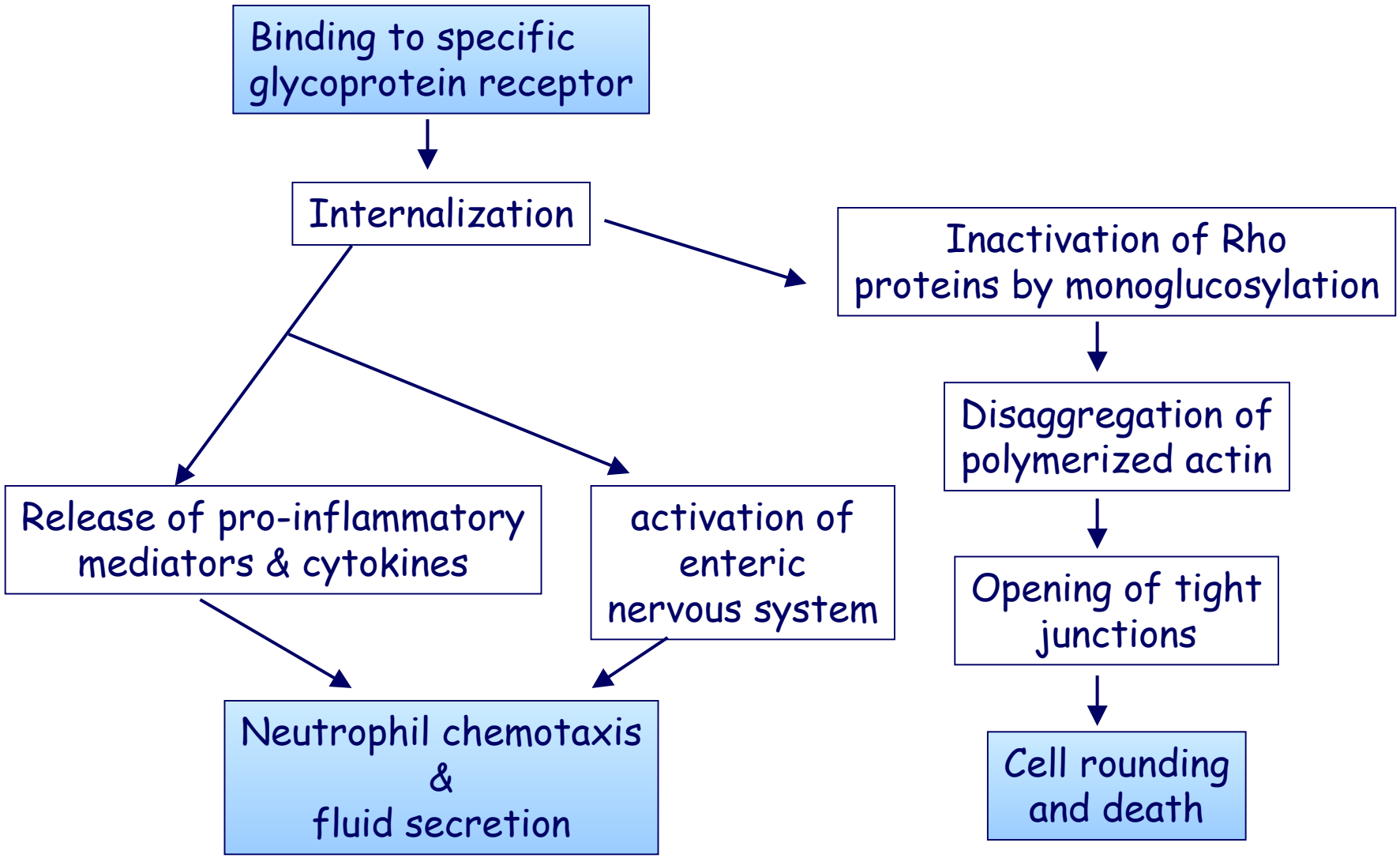


McDonald et al. NEJM, 2005; 353:2433-41

C. difficile Toxin A and Toxin B Are Glycosyltransferases



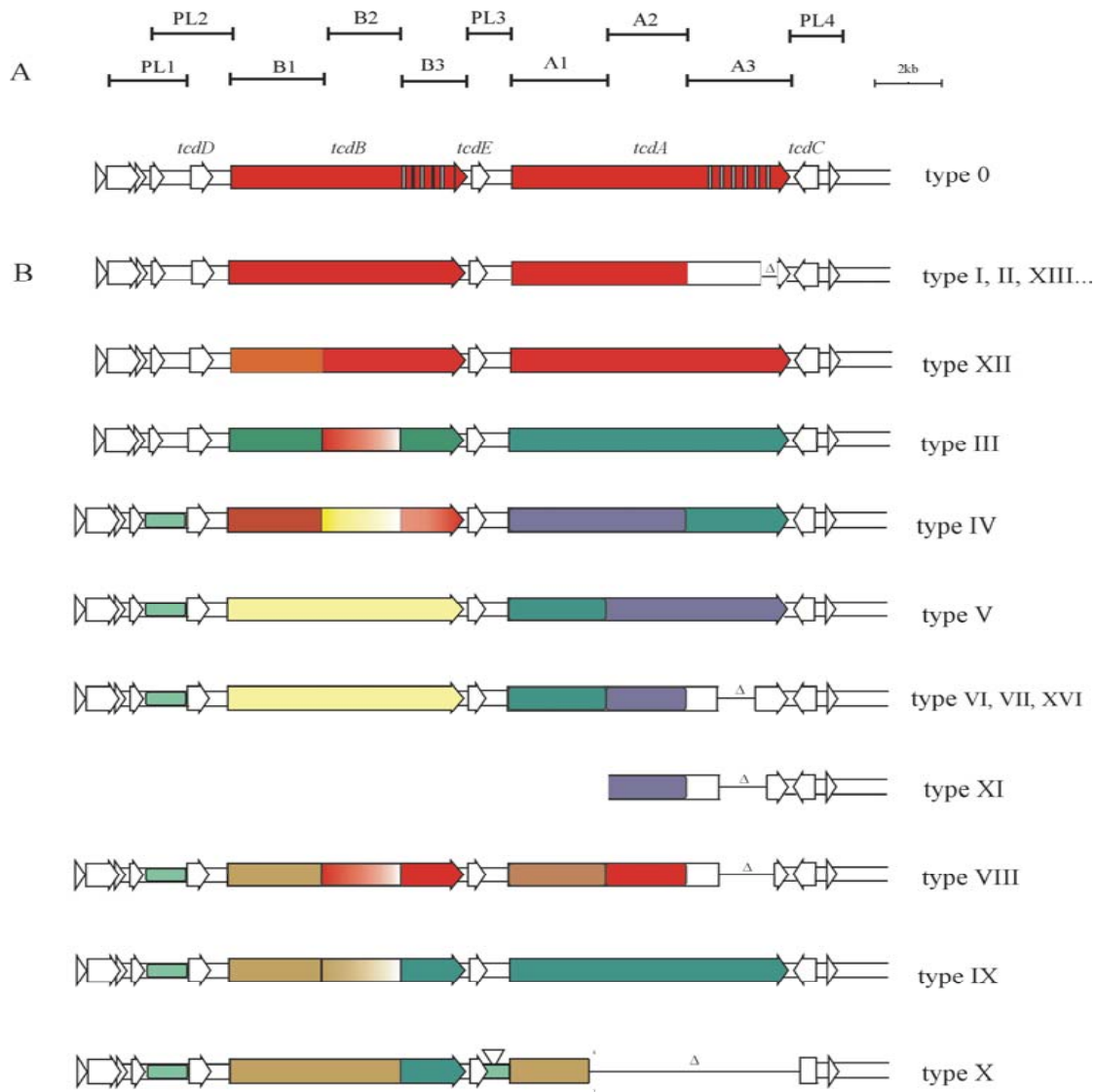
Toxins mode of action



Biological effects of *C. difficile* toxins

	Toxin A	**Toxin B**
• Enteritis in rodents	yes	no
• Mucosal damage in human colon	10^{-8} M	10^{-9} M
• Cytotoxicity in cultured cells	10^{-10} M	10^{-13} M

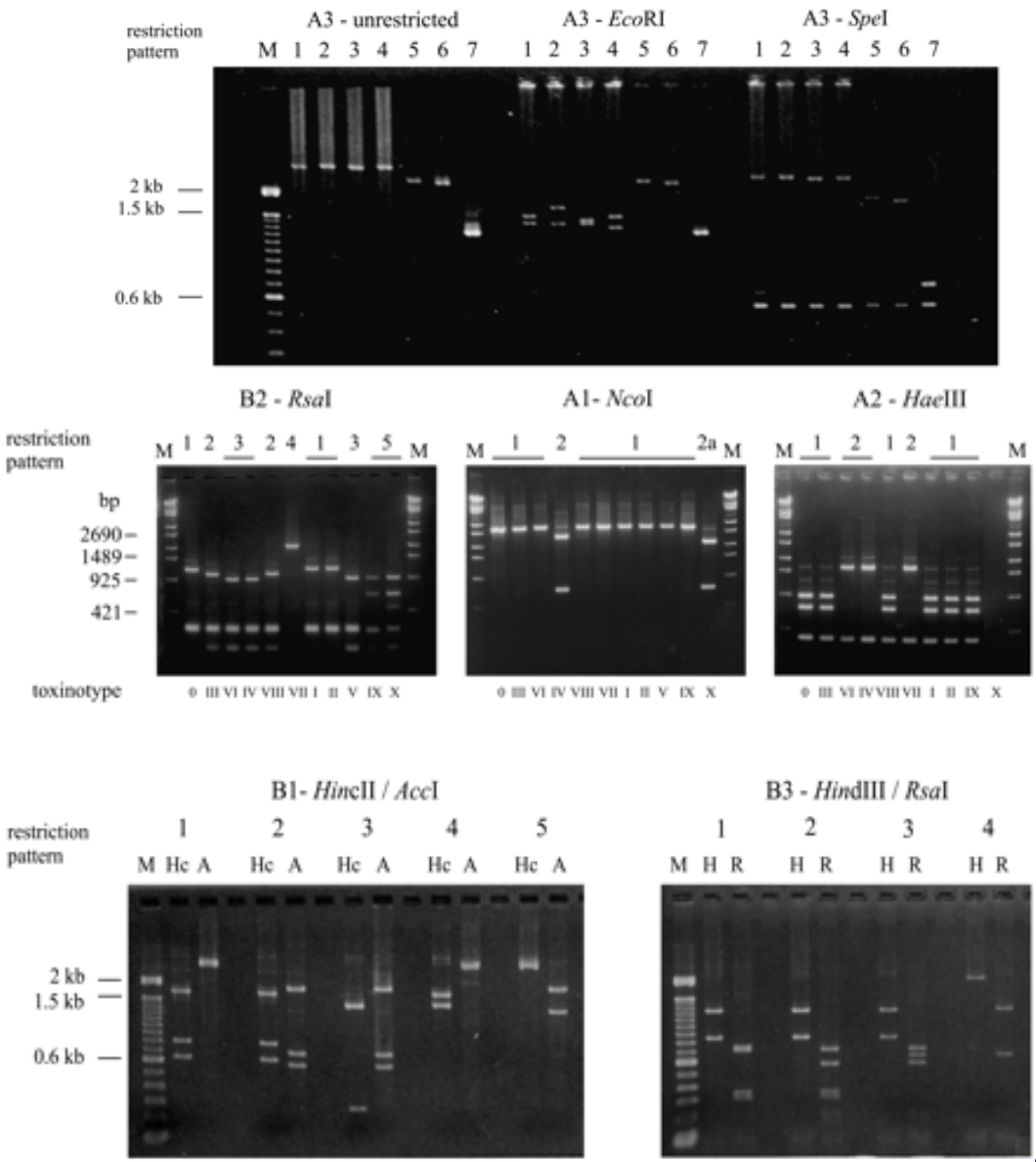
toxinotypage



Seminfect 27/04/2006

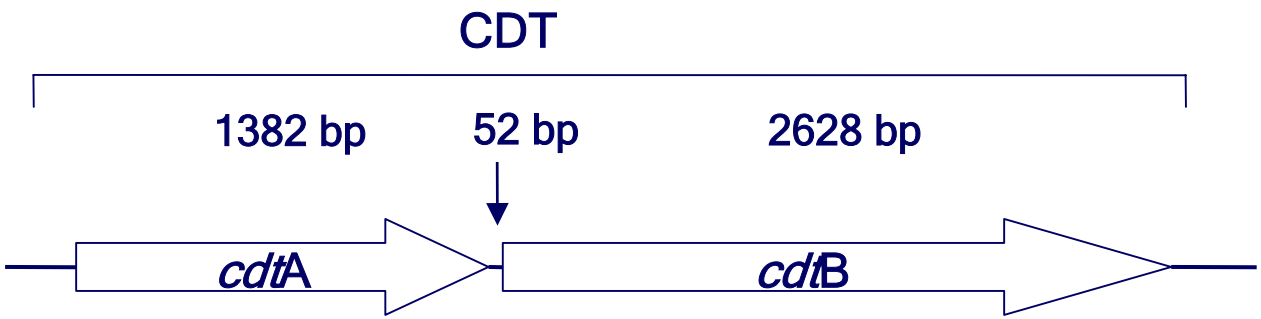
Rupnik et al. J. Clin. Microbiol. 1998; 36 : 2240-2247

toxinotypage



Rupnik et al. J. Clin. Microbiol. 1998; 36 : 2240-2247

CDT locus for binary toxin CDT



Binary toxin

- A-B exo-toxin
- Actin specific adenosine diphosphate-ribosyltransferase
- Encoded by *cdtA* (enzymatic component) and *cdtB* (binding component)
- Related to *C. perfringens* type E and *C. spiroforme* toxins
- 0.5 to 5% of strains
- May increase the severity of the disease
  Barbut et al. JMM 2005;54:181-5
- Not pathogenic by itself in hamster model
  Geric et al. JID 2006;193:1143-50

C. difficile toxin production types

	large clostridial toxins		binary toxin
	TcdB	TcdA	CDT
type 1	+	+	-
type 2	+	+	+
type 3	+	-	-
type 4	+	-	+
type 5	-	-	+
type 6	-	-	-

Clostridium difficile-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity

Jacques Pépin, Louis Valiquette, Marie-Eve Alary, Philippe Villemure, Anick Pelletier, Karine Forget, Karine Pépin, Daniel Chouinard

CMAJ, 2004

Emergence of Fluoroquinolones as the Predominant Risk Factor for *Clostridium difficile*–Associated Diarrhea: A Cohort Study during an Epidemic in Quebec

Jacques Pépin,[1] Nathalie Saheb,[2] Marie-Andrée Coulombe,[1] Marie-Eve Alary,[1] Marie-Pier Corriveau,[1] Simon Authier,[1] Michel Leblanc,[3] Geneviève Rivard,[1] Mathieu Bettez,[1] Valérie Primeau,[1] Martin Nguyen,[1] Claude-Émilie Jacob,[1] and Luc Lanthier[2]

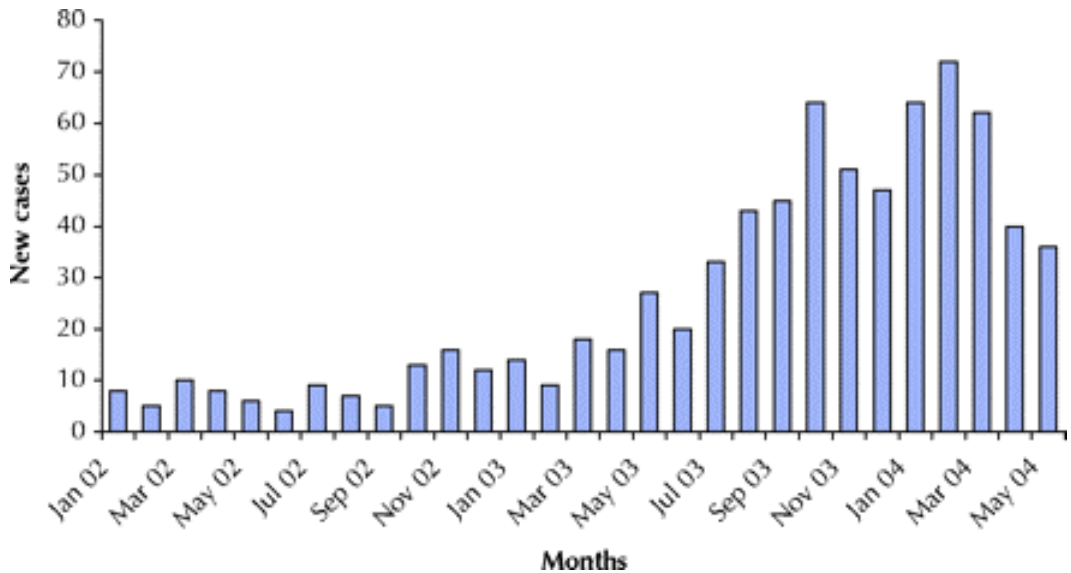
Clin. Infect. Dis., 2005

A Large Outbreak of *Clostridium difficile*–Associated Disease With an Unexpected Proportion of Deaths and Colectomies at a Teaching Hospital Following Increased Fluoroquinolone Use

Carlene A. Muto, MD, MS; Marian Pokrywka, MPH, BS, CIC; Kathleen Shutt, MS; Aaron B. Mendelsohn, PhD; Kathy Nouri, MPH, RN, BSN, CIC; Kathy Posey, MPH, BS, CIC; Terri Roberts, BS, CIC; Karen Croyle, Sharon Krystofiak, MPH, MS, CIC; Sujata Patel-Brown, BS; A. William Pasculle, ScD; David L. Paterso Melissa Saul, MS; Lee H. Harrison, MD

Infect. Control Hosp. Epidemiol., 2005

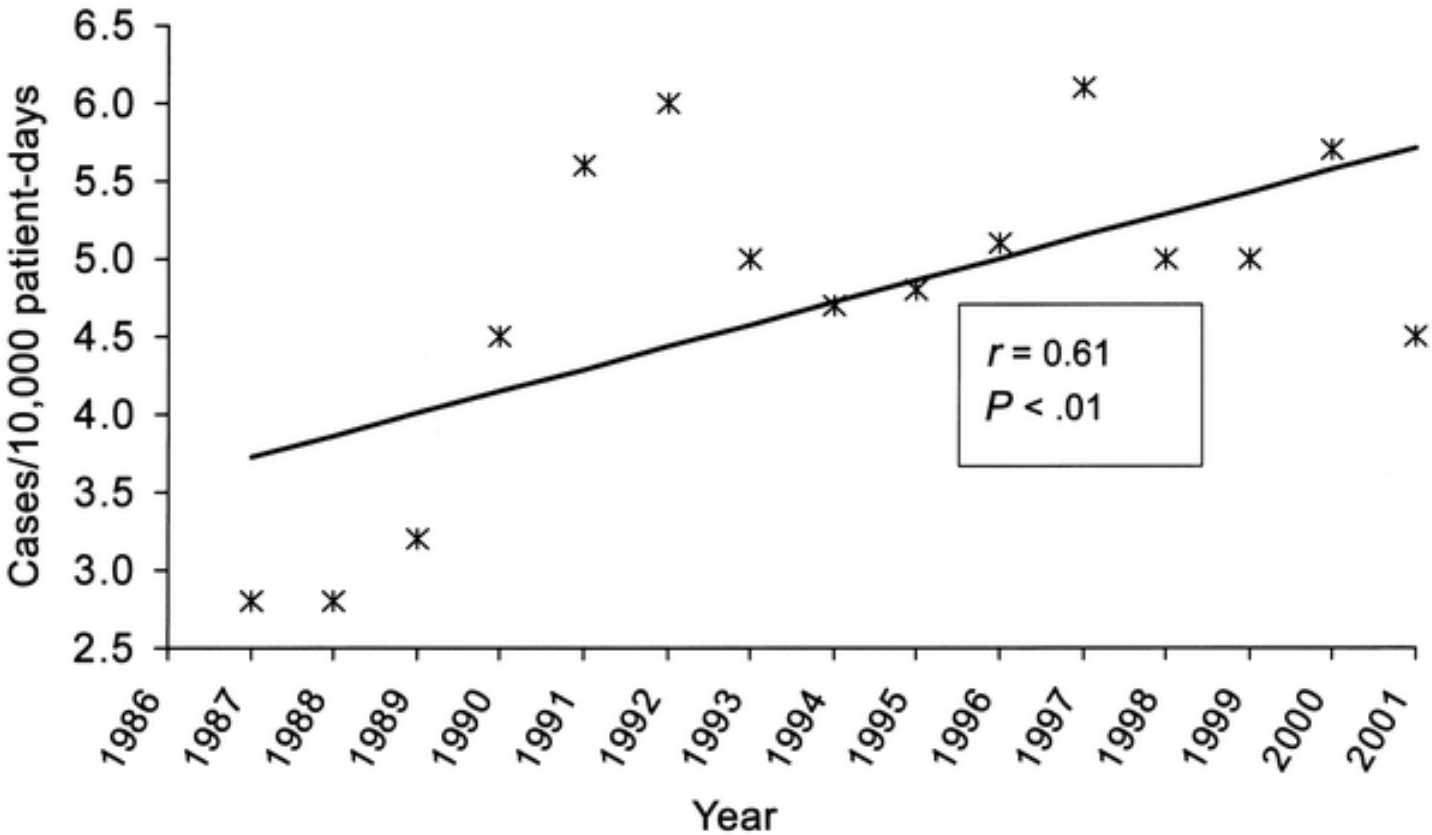
North America outbreak



New cases of nosocomial and community-acquired *Clostridium difficile*-associated diarrhea (CDAD; diagnosed by positive cytotoxin assay result) reported by the microbiology laboratory at the Centre hospitalier universitaire de Sherbrooke.

Valiquette et al. CMAJ 2004 171: 27-29

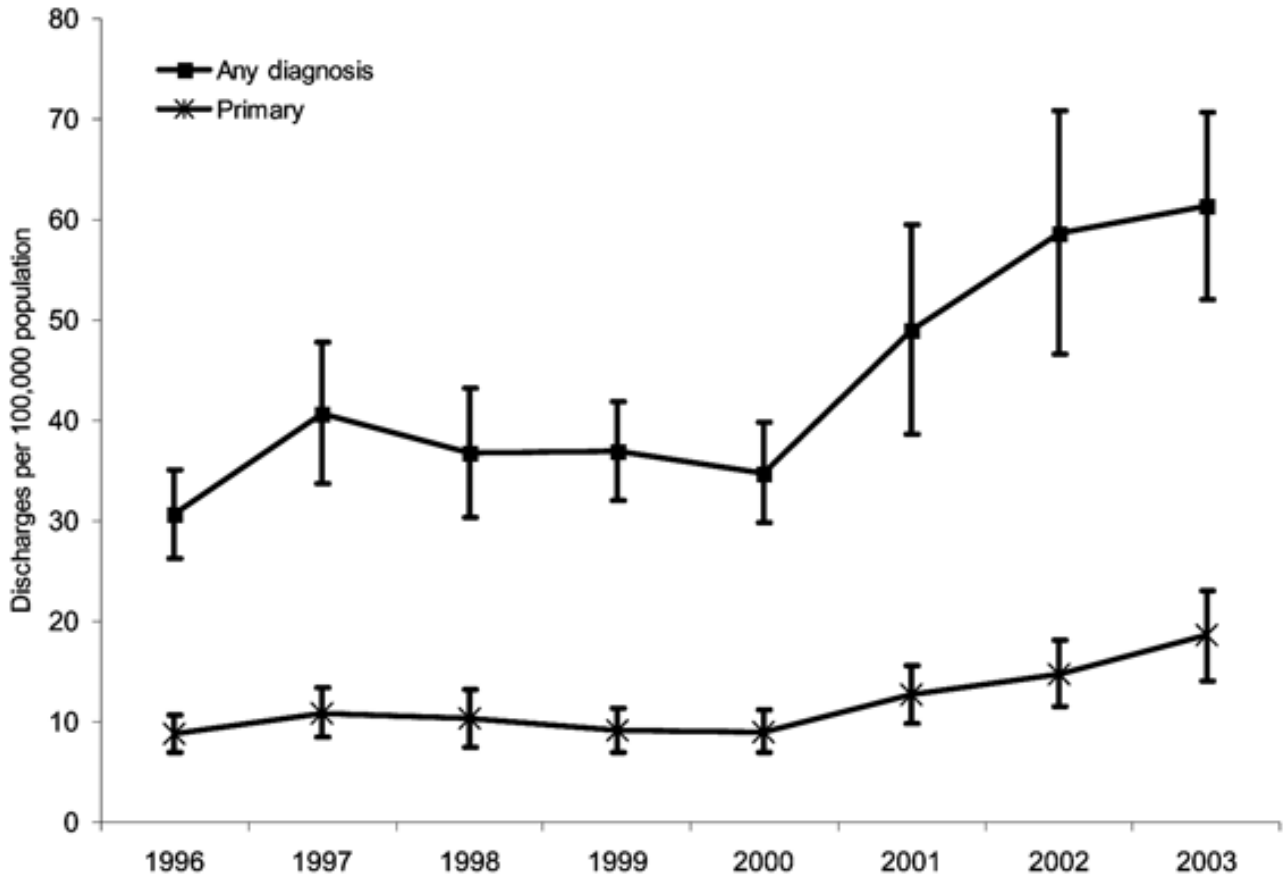
USA 1987-2001



Annual CDAD rates for hospitals with >500 beds, by intensive care unit surveillance component (NNISS, 1987 2001).

Archibald et al. JID 2004 189:1585-9

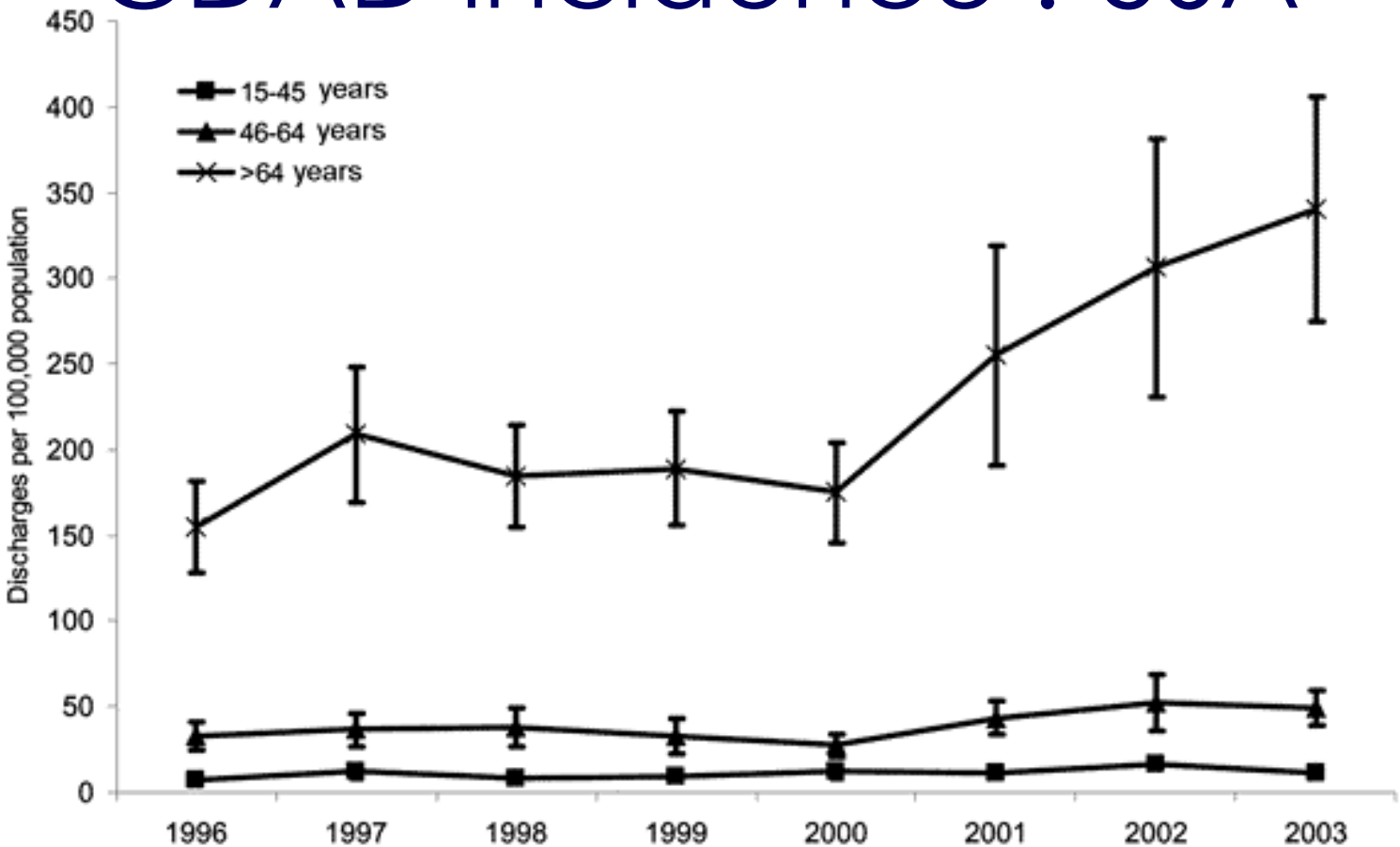
CDAD incidence : USA



National estimates of US short-stay hospital discharges with *Clostridium difficile* listed as primary or as any diagnosis.

McDonald et al. EID, 2006 12:409-16

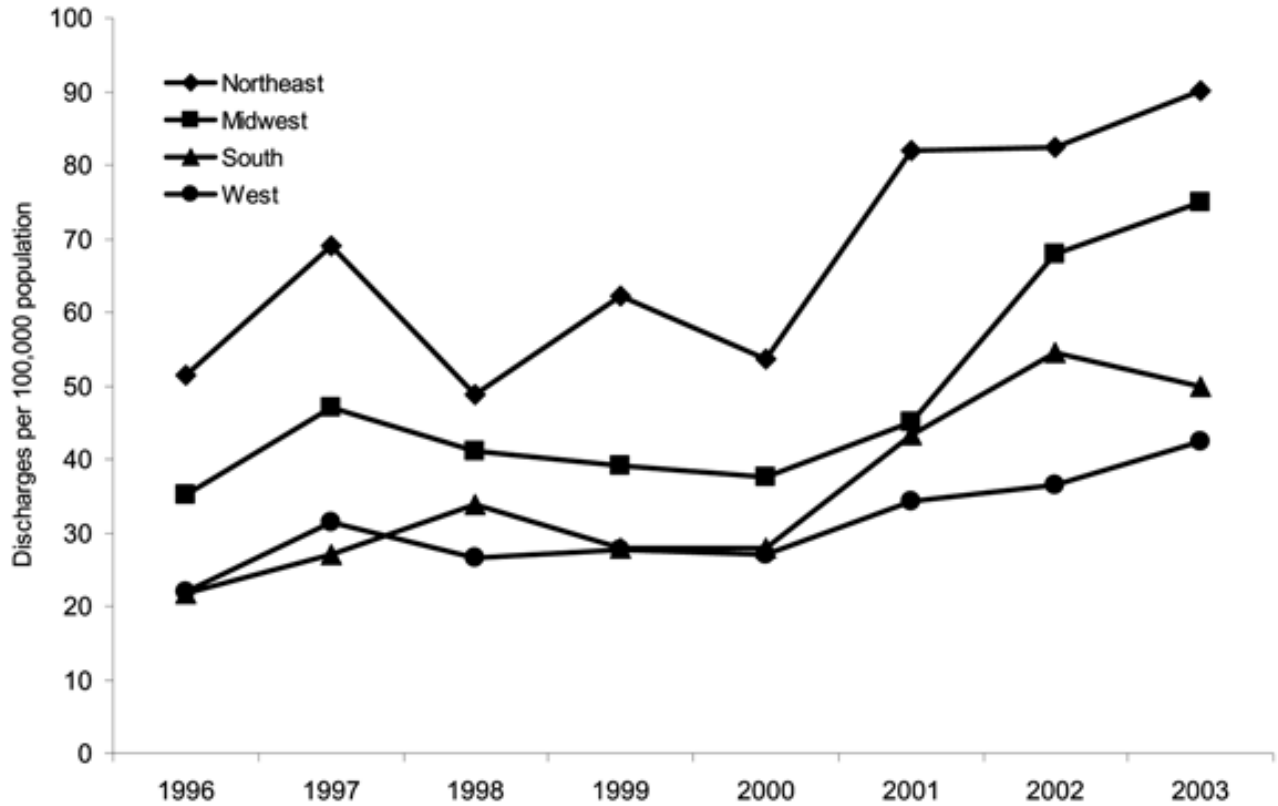
CDAD incidence : USA



Rates of US short-stay hospital discharges with *Clostridium difficile* listed as any diagnosis, by age..

McDonald et al. EID, 2006 12:409-16

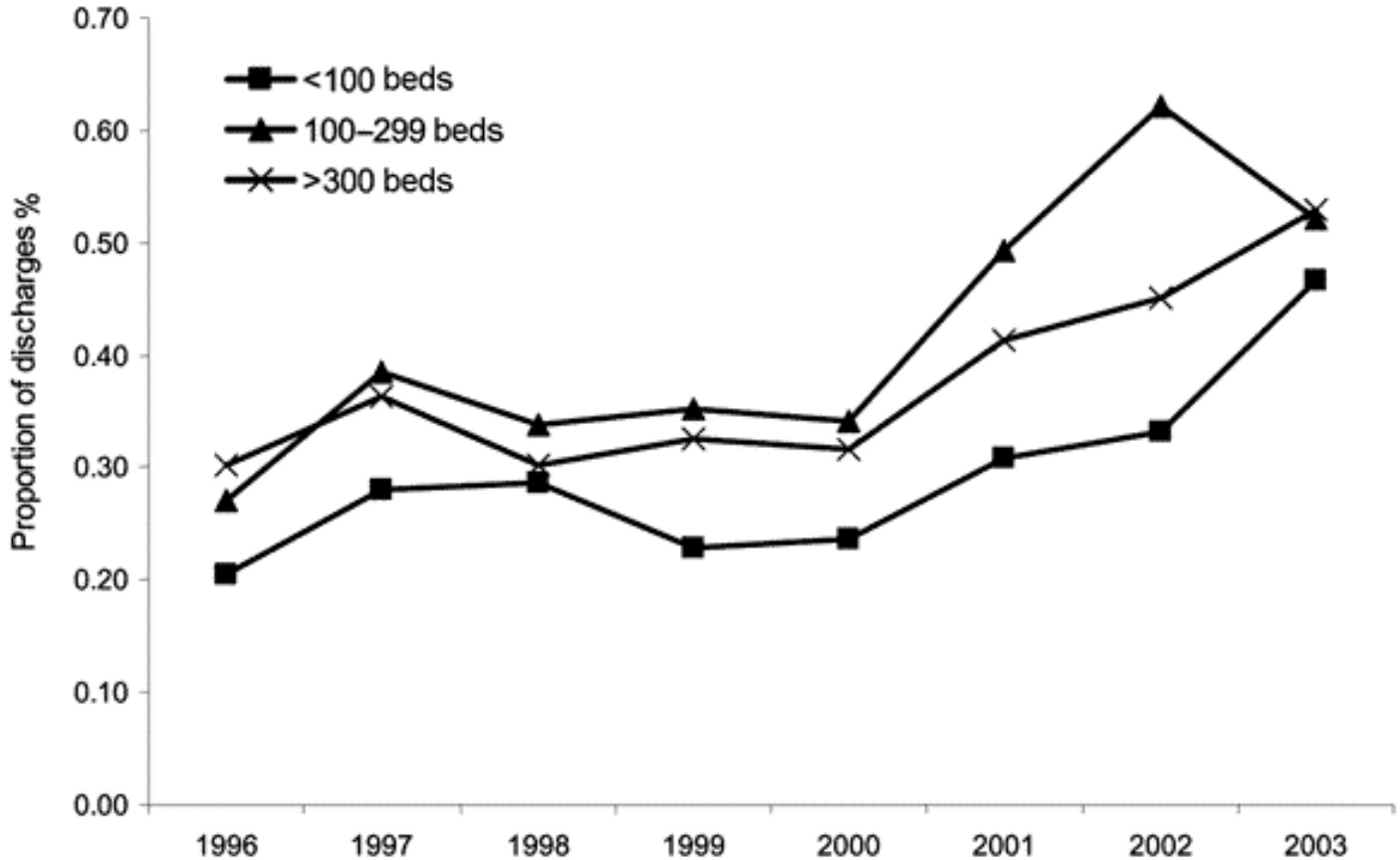
CDAD incidence : USA



Rates of US short-stay hospital discharges with *Clostridium difficile* listed as any diagnosis, by region

McDonald et al. EID, 2006 12:409-16

CDAD incidence : USA



Rates of US short-stay hospital discharges with *Clostridium difficile* listed as any diagnosis, by hospital size

McDonald et al. EID, 2006 12:409-16

CDAD incidence and severity

Table 1: Reports of increases in incidence and severity of *Clostridium difficile*-associated diarrhea (CDAD)

Source	Period	Outcome measure	Results
US National Nosocomial Infection Surveillance System[9]	1987–2001	CDAD among patients admitted to intensive care unit in hospitals with > 500 beds	Doubling of incidence
UK Health Protection Agency Communicable Disease Surveillance Centre[10]	1986–2001	Voluntary laboratory reporting system of stools positive for *C. difficile* toxin	From < 2000 positive test results per year in 1986/87 to > 12 000 per year in 2000/01
Oregon[11]	1994–2000	All-cause 90-day mortality among patients with CDAD	3.5% in a previous 10-year cohort v. 15.3% in 1994–2000 cohort
		Colectomy	7.6% in 1994–2000 cohort
Pittsburgh[12]	1989–2000	Fulminant colitis	From 0% to 3.2% (mean 1.6% over 10 years)

Valiquette et al. CMAJ 2004 171: 27-29

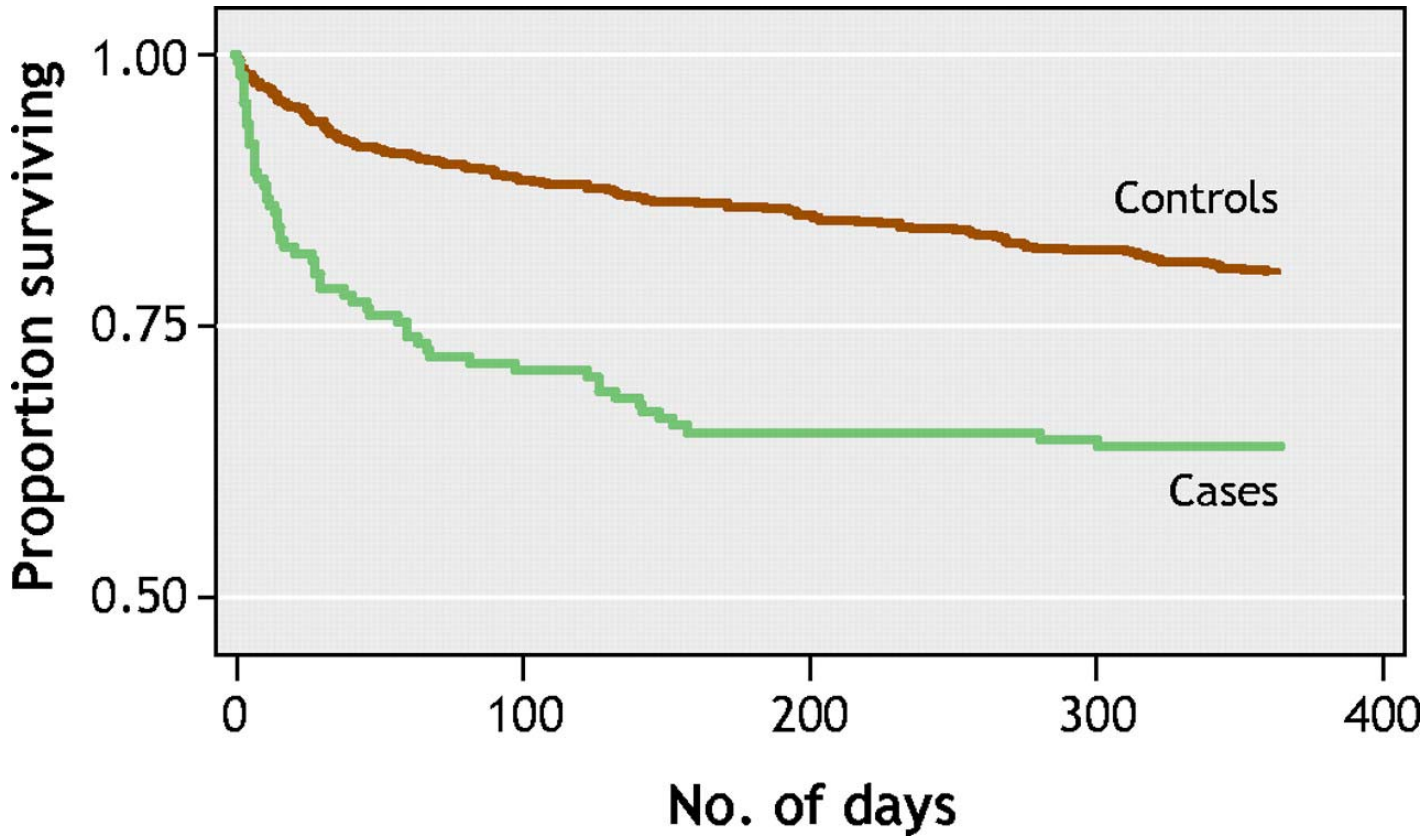
CDAD incidence : Canada

Table 2. Age-Specific Incidence and Mortality Attributed to *Clostridium difficile*–Associated Diarrhea.

Age	No. of Cases	No. of Cases/ 1000 Admissions*	Attributable 30-Day Mortality Rate
yr			%†
<40	76	3.5	2.6
41–50	85	11.2	1.2
51–60	191	20.0	3.2
61–70	272	24.4	5.1
71–80	523	38.3	6.2
81–90	458	54.5	10.2
>90	114	74.4	14.0

* Values are based on 1719 episodes of nosocomial *C. difficile*–associated diarrhea.
† Values are based on data from 1703 patients with nosocomial *C. difficile*–associated diarrhea.

Loo et al. NEJM, 2005; 353:2442-49



Pépin et al. CMAJ, 2005; 173: 1037-1042

NAP1/027 isolate

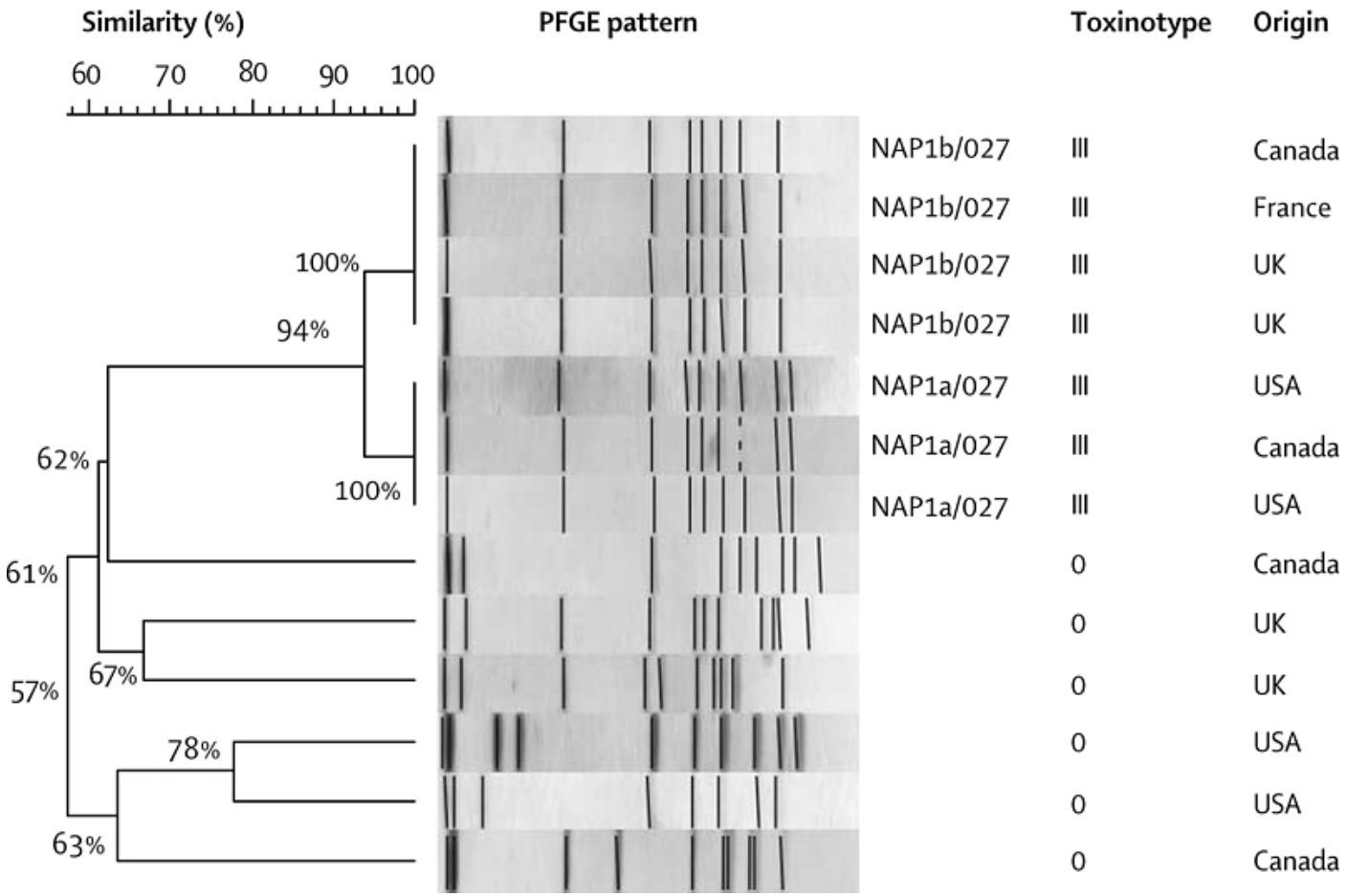
Table 1. Isolates of *Clostridium difficile* According to Health Care Facility and the Proportion of Isolates Belonging to the BI/NAP1 Strain.

Health Care Facility	Date of Onset of Outbreak	No. of Isolates Tested	BI/NAP1 Strain
			no. (%)
Georgia	Oct. 2001	46	29 (63)
Illinois	July 2003	14	6 (43)
Maine, Facility A	March 2002	13	9 (69)
Maine, Facility B	July 2003	48	30 (62)
New Jersey	June 2003	12	9 (75)
Oregon*	April 2002	30	3 (10)
Pennsylvania, Facility A	2000–2001	18	7 (39)
Pennsylvania, Facility B	Oct. 2003	6	3 (50)
Total		187	96 (51)

* Isolates were not collected until after the peak of the outbreak.

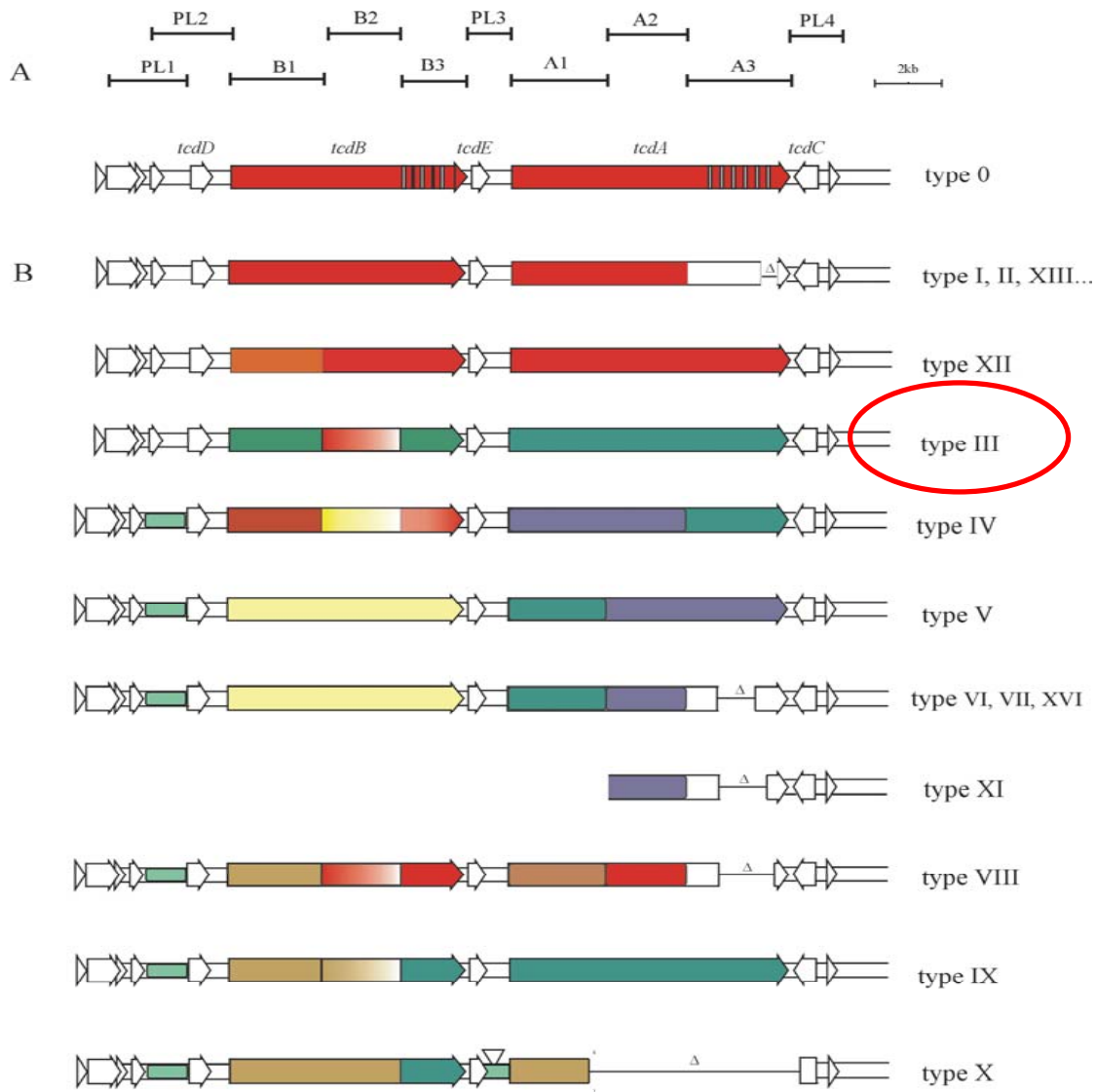
McDonald et al. NEJM, 2005; 353:2433-41

NAP1/027 isolate



Warny et al. Lancet, 2005; **366**:1079-1084

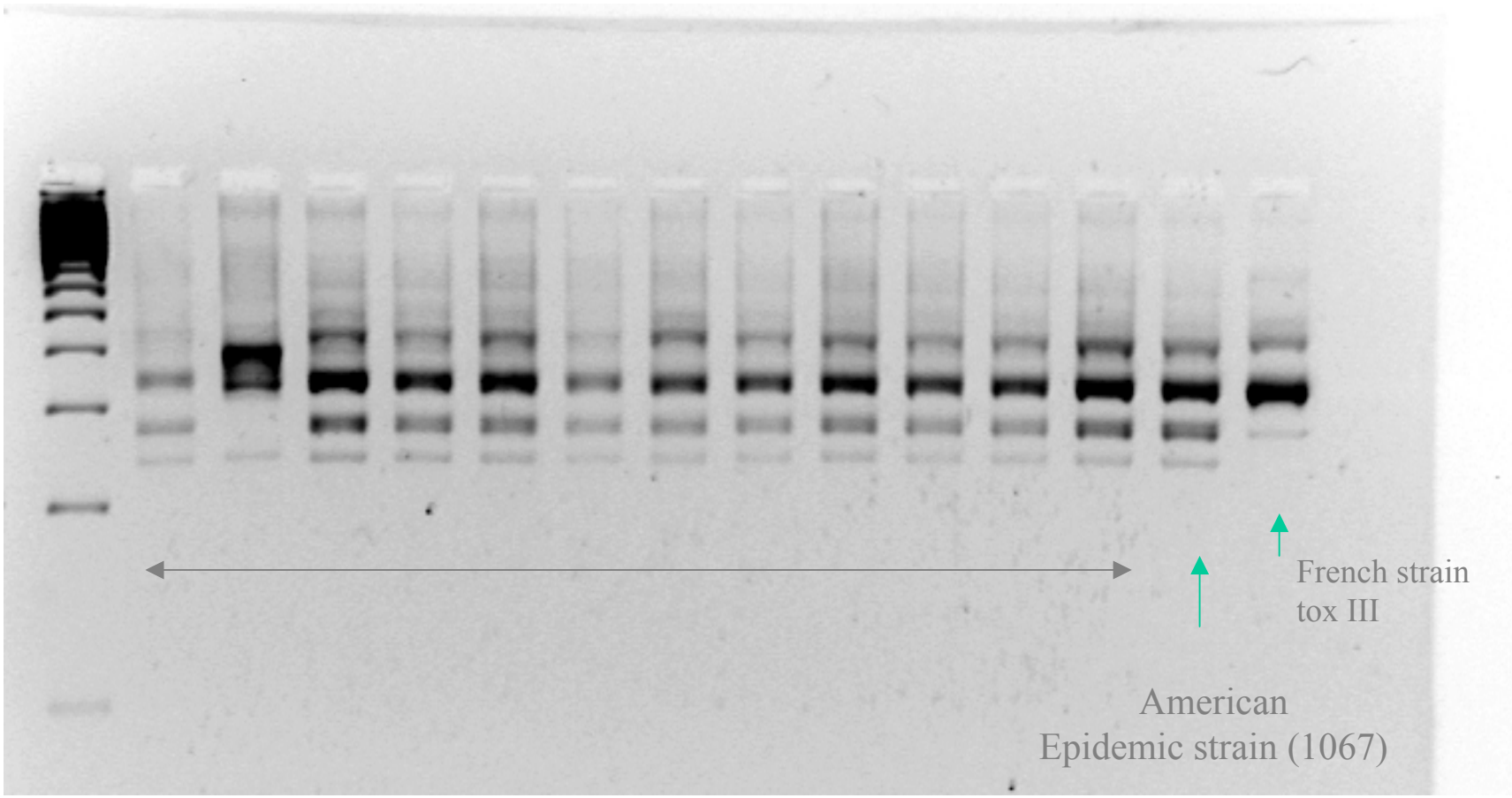
toxinotypage



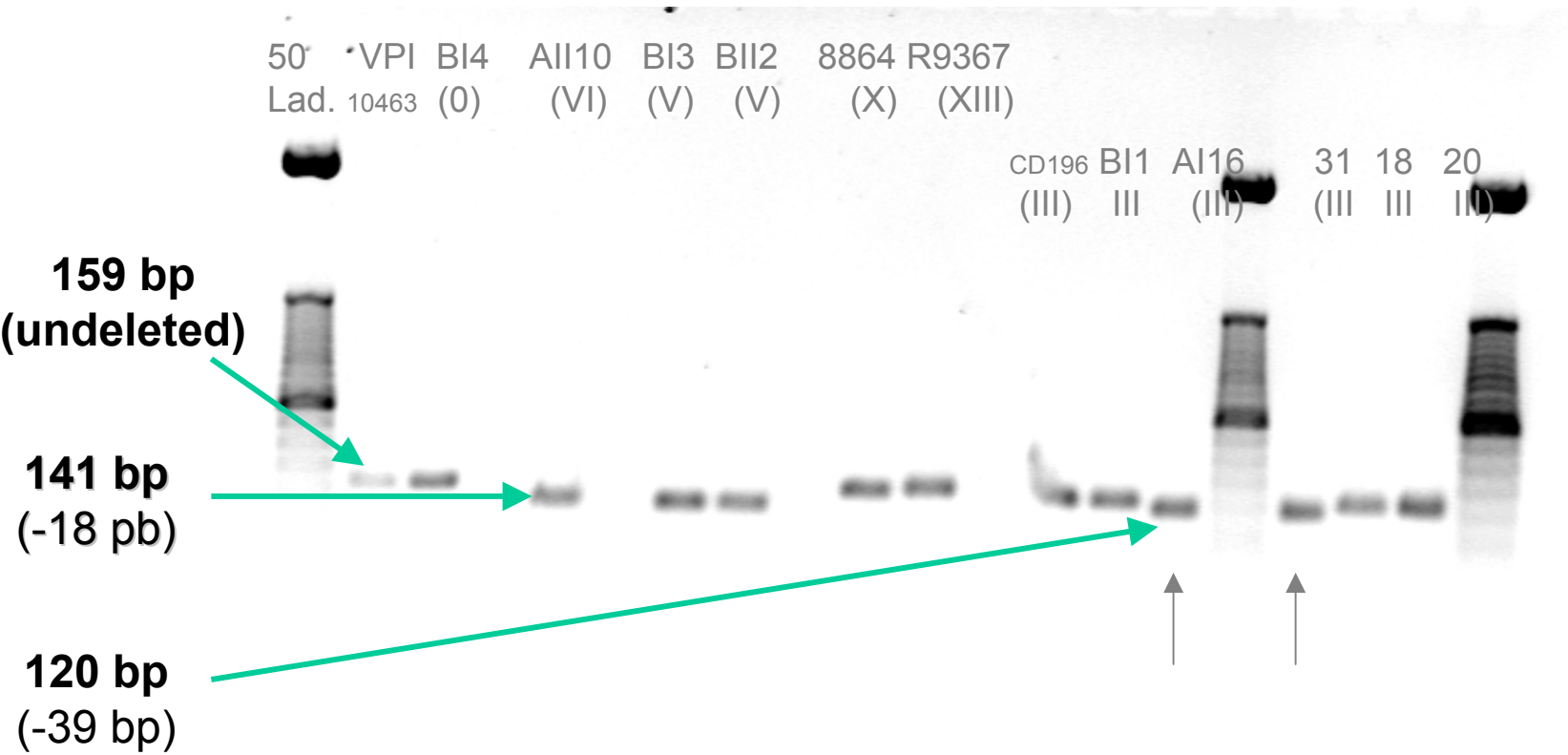
Rupnik et al. J. Clin. Microbiol. 1998; 36 : 2240-2247

Seminfect 27/04/2006

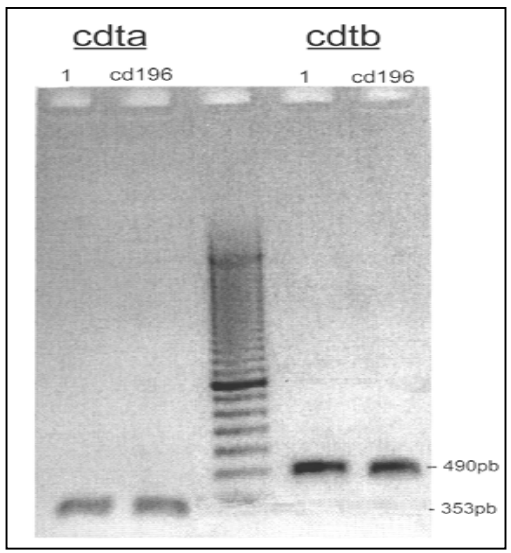
ribotypage



tcdC polymorphism

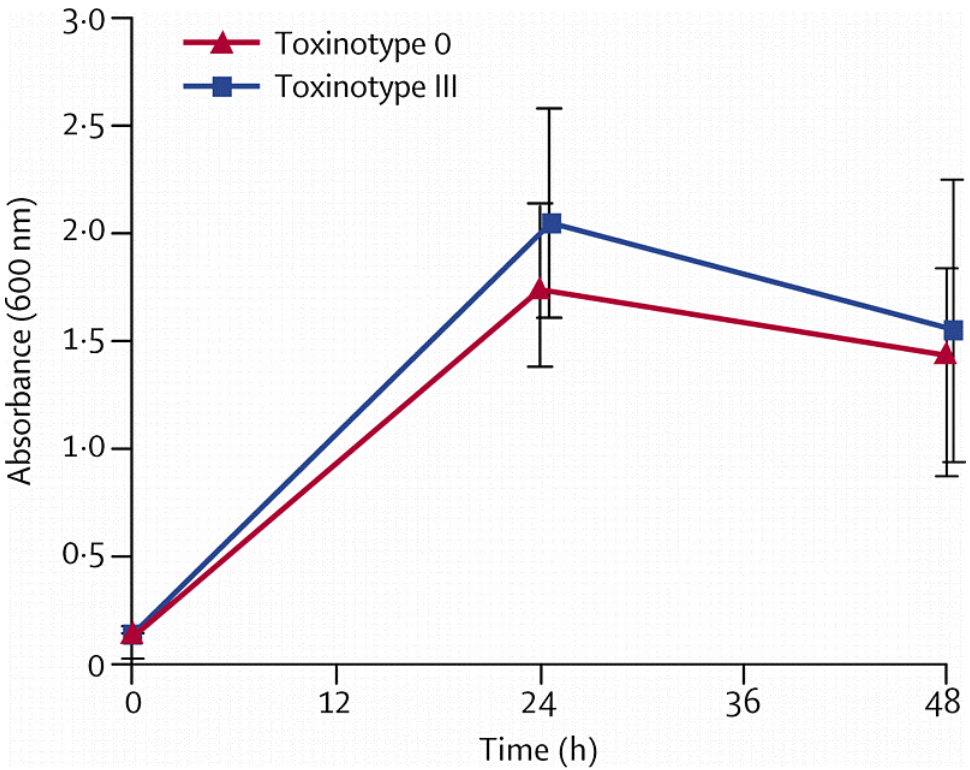


Binary toxin



NAP1/027 isolate

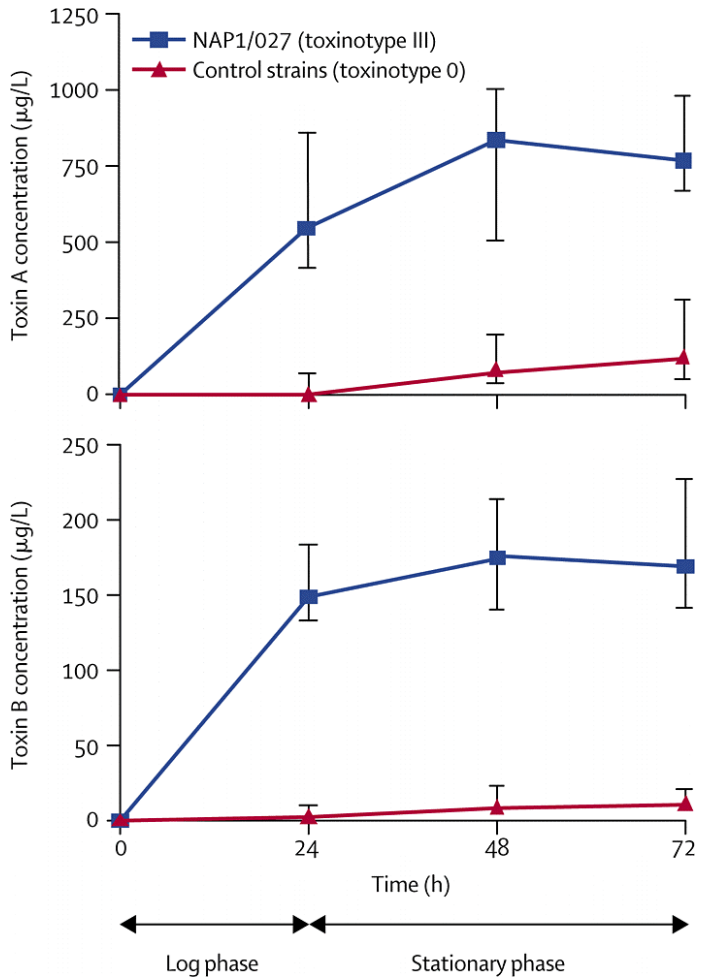
Growth curves of toxinotype 0 and toxinotype III (NAP1/027)



Warny et al. Lancet, 2005; **366**:1079-1084

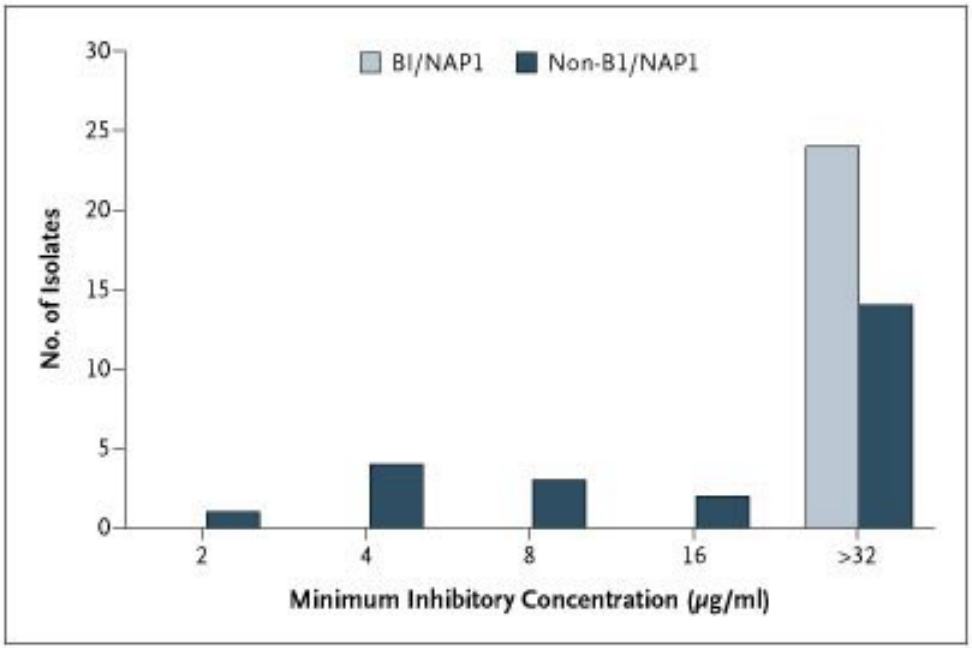
NAP1/027 isolate

In vitro production of toxins A and B by *C difficile* isolates



Warny et al. Lancet, 2005; **366**:1079-1084

NAP1/027 isolate



Distribution of MIC of Levofloxacin for Current (Obtained after 2000) BI/NAP1 and Non-BI/NAP1 *Clostridium difficile* Isolates.

McDonald et al. NEJM, 2005; 353:2433-41

Table 3. Characteristics of Case Patients and Control Patients.*

Characteristic	Case Patients (N=237)	Controls (N=237)	P Value
Age — yr			0.48
Median	75	75	
Interquartile range	66–82	66–82	
Male sex — no. (%)	115 (48.5)	126 (53.2)	0.3
Charlson index†	2.6±1.9	2.6±2.0	0.66
Ward			0.82
Medicine	133 (56.1)	142 (59.9)	
Surgery	78 (32.9)	70 (29.5)	
Geriatrics	17 (7.2)	15 (6.3)	
Oncology	9 (3.8)	10 (4.2)	
Community hospital — no. (%)	68 (28.7)	67 (28.3)	0.9
Days at risk for *C. difficile*–associated diarrhea			0.02
Median	13	16	
Interquartile range	6–25	8–29	
No. of antibiotics received	1.9±1.1	1.3±1.3	<0.001
Any exposure to antibiotics — no. (%)	188 (79.3)	141 (59.5)	<0.001
Cephalosporins	115 (48.5)	65 (27.4)	<0.001
Clindamycin	19 (8.0)	6 (2.5)	0.007
Fluoroquinolones	128 (54.0)	75 (31.6)	<0.001
Chemotherapy — no. (%)	17 (7.2)	13 (5.5)	0.45
Proton-pump inhibitors — no. (%)	112 (47.3)	111 (46.8)	0.92
Histamine H_2–blockers — no. (%)	47 (19.8)	47 (19.8)	1.0
Enteral feeding — no. (%)	44 (18.6)	28 (11.8)	0.04

* Plus–minus values are means ±SD.
† Scores for the Charlson index can range from 0 to 37, with higher scores indicating more coexisting conditions.

Loo et al. NEJM, 2005; 353:2442-49

NAP1/027 isolate

- Toxinotype III
- 18bp deletion *tcd*C
- Increased production tox A and B
- Ribotype 027/ PFGE NAP1
- Binary toxin
- FQ resistance

EUROPEAN PROSPECTIVE STUDY OF *CLOSTRIDIUM DIFFICILE* STRAINS: PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF THE ISOLATES FROM DIFFERENT CLINICAL STATUS : INTERIM RESULTS

F. Barbut, P. Mastrantonio, M. Delmée, G. Ackermann,
E. Bouza, C. Balmelli, D. Drudy, E. Kuijper, H. Ladas,
E. Nagy, H. Pituch, M. Somolinos, E. Urban, M. Wullt, M. Yücesoy, M. Rupnik and I. Poxton

for the European Study Group on *Clostridium difficile* *(ESGCD)*

16th ECCMID, Nice, France, 2-4 april 2006

OBJECTIVES

Main objectives :

- To establish a well defined European collection of *C. difficile* strains
- To study and to compare the phenotypic and genotypic markers of isolates *(prevalence of toxinotype III ?)*
- To get an estimation of the incidence of *C. difficile* infections

Other objectives :

- To correlate clinical presentations with phenotypic and genotypic features *C. difficile*.

- To get the baseline characteristics of *C. difficile* strains isolated in 2005 making further investigations possible to follow trends in antimicrobial susceptibility, serogroups, genotypic patterns...

MATERIALS AND METHODS

- Design
  - Prospective European-wide study on *C. difficile* strains isolated during a 2-month period with phenotypic and genotypic characterization of isolates

- Requirements for participating hospitals:
  - to have an on-site laboratory of microbiology
  - to systematically perform culture each time a test for *C. difficile* is requested.
  - to be able to detect toxins A or B from stools or from strains
  - to fill out a questionnaire (=data form) for each isolated CD strain (whatever the toxin result is)

MATERIALS AND METHODS

- Inclusion criteria:

  All the *C. difficile* strains (including the non toxigenic strains) isolated from inpatients (with community or nosocomial diarrhea)

- Exclusion criteria:

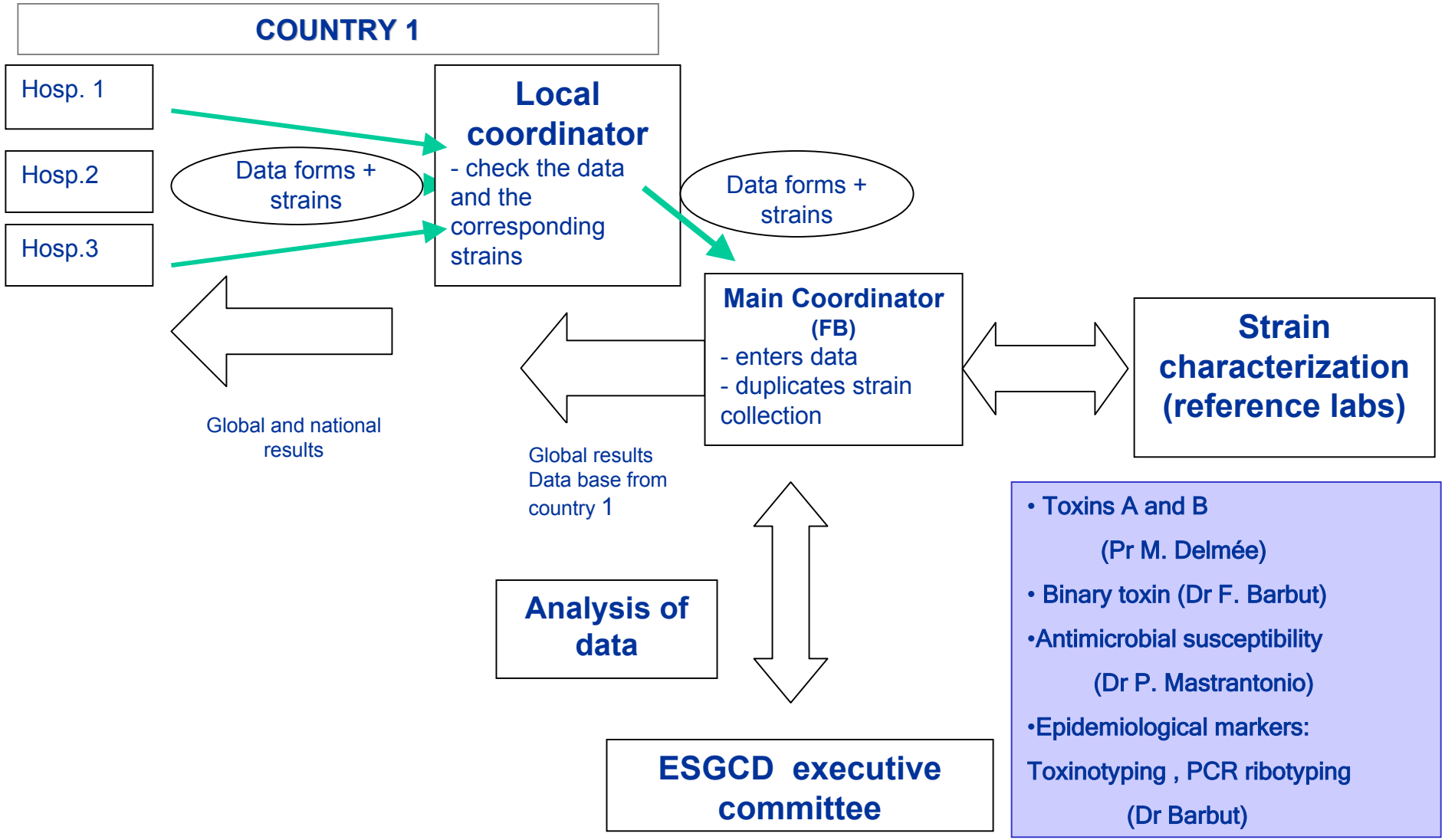
  Strains from outpatients or day-care patients.

  Strains from children under 2 years old

- Length of the study :

  a 2 month-period (or 1 month for every hospital which reaches 30 *C. difficile* strains at the end of the first month) between April and June.

GENERAL ORGANIZATION OF THE STUDY



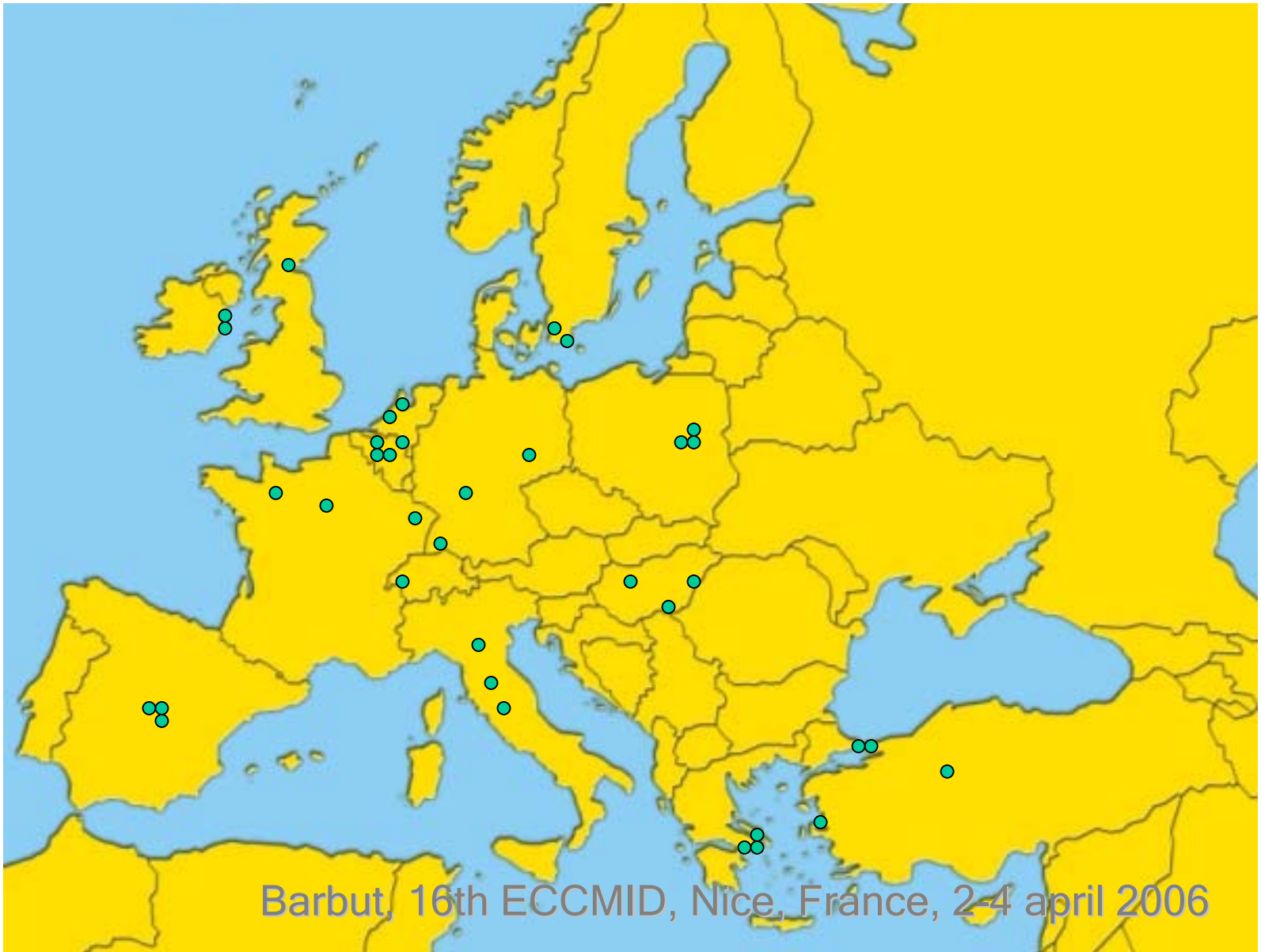
PARTICIPATING COUNTRIES

14 countries, 38 hospitals, 486 strains

COUNTRY	Letter	No. Hospitals	Local coordinator	No. Strains
Belgium	A	3	M. Delmée	73
France	B	3	F. Barbut	53
Germany	C	3	G. Ackermann	86
Hungary	E	4	E. Nagy	48
GB	R	1	I. Poxton	9
Italy	F	3	P. Mastrantonio	27

COUNTRY	Letter	No. Hospitals	Local coordinator	No. Strains
Netherlands	G	3	E. Kuijper	29
Poland	H	3	H. Pituch	20
Spain	K	3	E. Bouza M. Somolinos	39
Switzerland	L	1	C. Balmelli	15
Sweden		3	M. Wult B. Andersson	22
Turkey	N	4	M. Yucesoy	9
Greece	P	3	H. Ladas	24
Ireland	D	1	D. Drudy	32

Distribution of participating hospitals



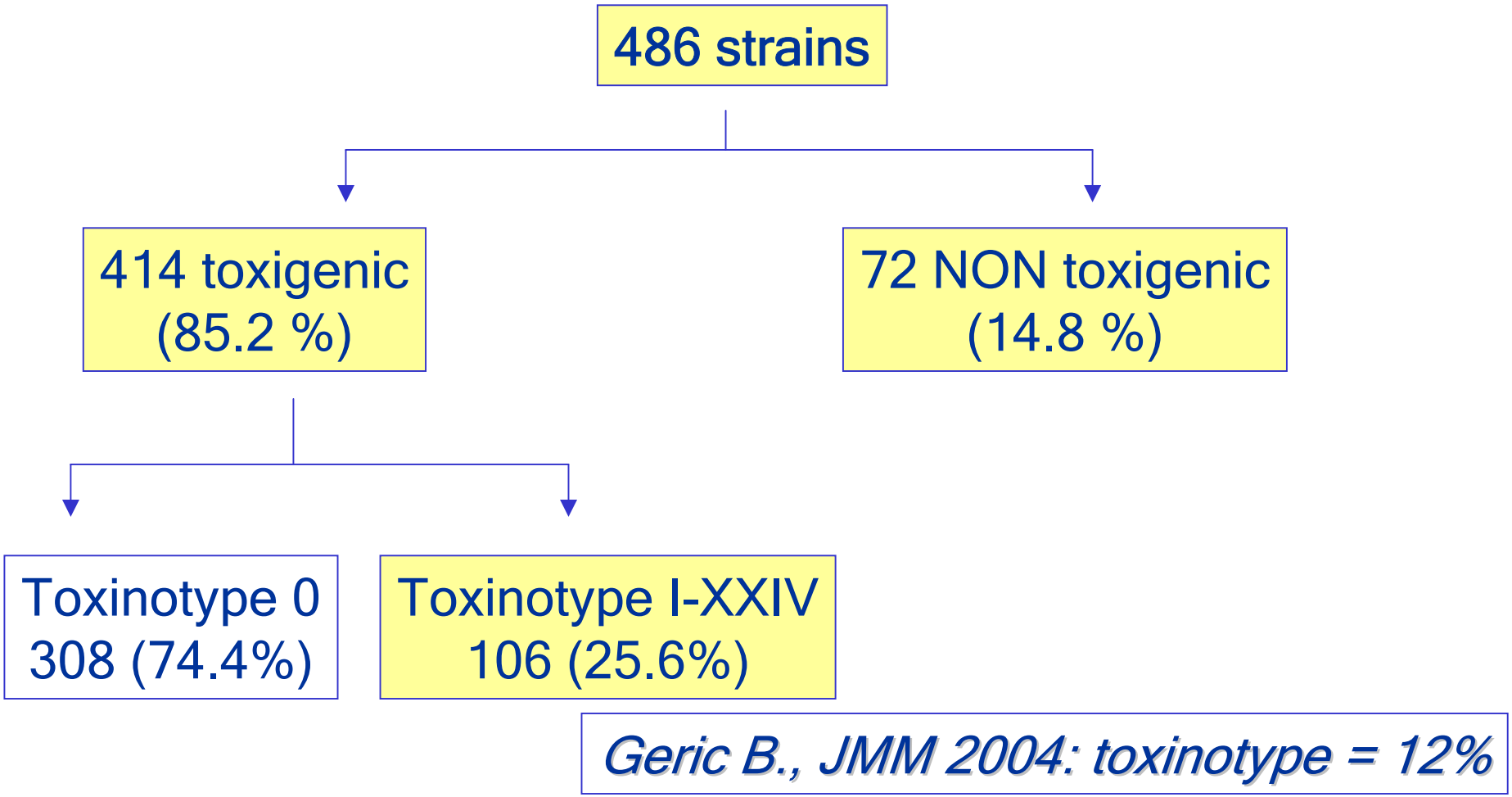
clinical data

Incidence varies widely (23 hosp.):
2.45 ± 1.8 CDAD/10,000 patient-days
(range : 0.14- 7.1)

Hospitals N : 0.13-0.14 CDAD/10,000 patient-days
Hospitals F: 0.5-1.2 «
Hospitals G, B: 2-7 «

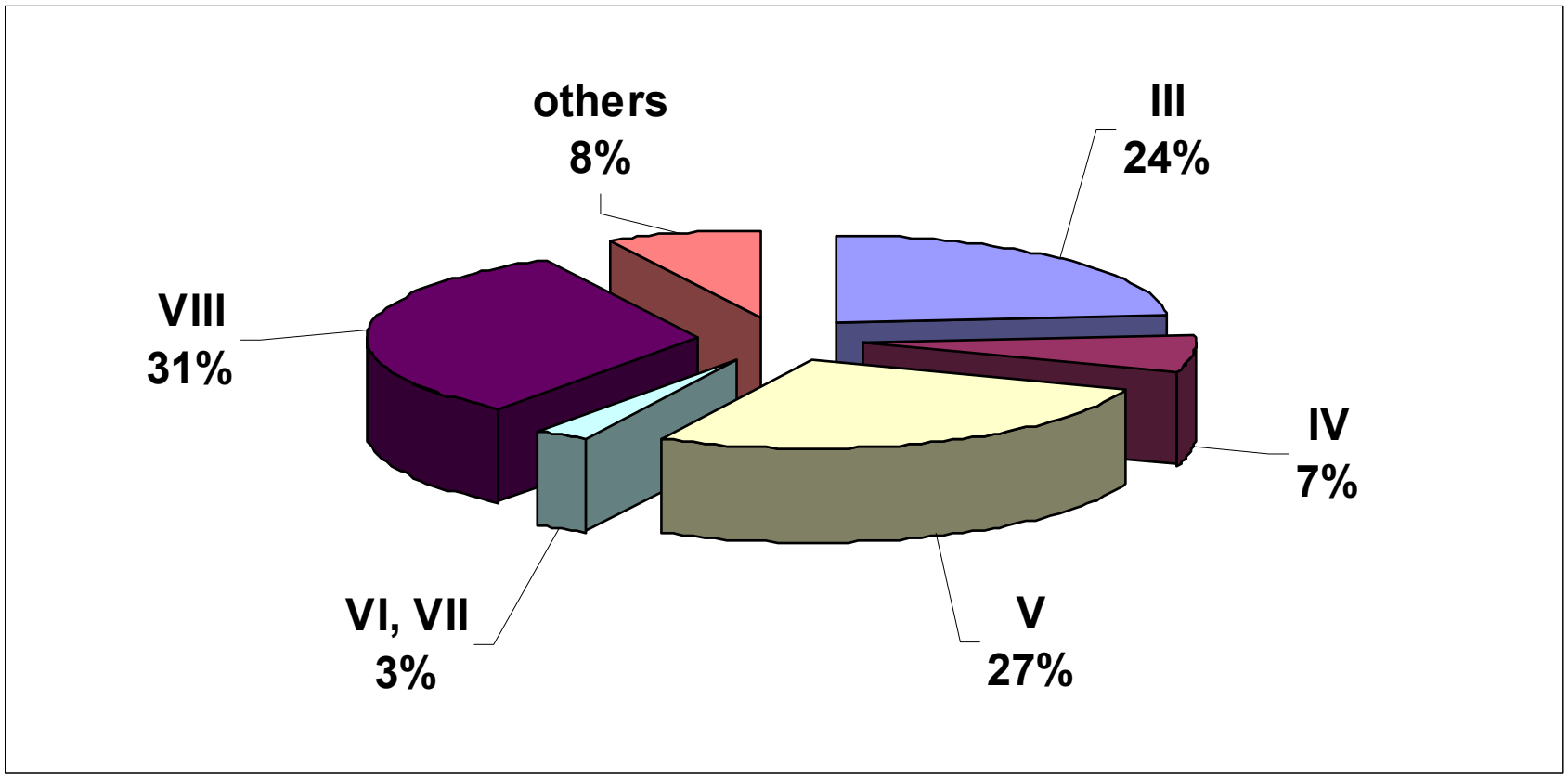
USA : 12.1 CDAD /10,000 patients days (range 3-25.1)
(Sohn, ICHE 2005)
USA : 5 /10,000 patient-days
(Archibald, CDC, JID 2004)
Québec : 12.8 /10,000 patients-days

Toxigenicity



Geric B., JMM 2004: toxinotype = 12%

Distribution of toxin variant strains (N=106)



USA (Geric, JMM 2004) : III (17%), V (6%), VIII (12%)

Barbut, 16th ECCMID, Nice, France, 2-4 april 2006

ANTIMICROBIAL SUSCEPTIBILITY

ATB	MIC50 (mg/l)	MIC90 (mg/l)	Range (min. max)	% R
VA	0.5	0.75	0.25-2	0
MZ	0.06	0.125	0.012-0.75	0
EM	1	>256	0.047->256	46.7
CM	4	>256	0.016->256	50.0
TC	0.032	0.38	0.023->32	8.8
MX	0.5	>32	0.032->32	33.9

Barbut, 16th ECCMID, Nice, France, 2-4 april 2006

ANTIMICROBIAL SUSCEPTIBILITY

Country	No. strains	% R EM+CM+TC+MX	% S EM+CM+TC+MX
Belgium	72	1.4	33.3
France	50	2	42
Germany	85	8.2	37.6
Ireland	31	9.7	32.3
Hungary	47	17	55.3
Italy	27	0	14.8
Netherlands	31	0	45.2
Poland	19	5.3	26.3
Spain	39	0	12.8
Switzerland	15	0	80
Sweden	22	0	81.8
Greece	23	8.7	21.7
Turkey	9	11.1	77.8
UK	8	0	12.5

Barbut, 16th ECCMID, Nice, France, 2-4 april 2006

Toxinotype III

- 25 strains isolated in 6 different countries
- 2 types of toxinotype III isolates by PCR-ribotyping
  - « 027 » (n=20) : AI13, AII6, AII9, AII12, AII25, AII27, AIII2, AIII3, AIII5, AIII8, AIII14, GII1, GII3, GII5, GII6, GII7, GII8, GII9, GII 10, DI12
  - Non « 027 » (n=5) : AI16, KIII3, BI1, MI27, MI23
- Epidemic « 027 » strains were clustered in 2 countries (Netherlands and Belgium) and isolated once in Ireland.
- Non « 027 » toxinotype III isolates were found once in France, Belgium, Spain and Sweden (2 strains)

Toxinotype III

- Epidemic «027» strains (n=20):
  - MIC EM > 256 mg/l
  - MIC MX ≥ 12 mg/l (except 1 strain with MIC= 6 mg/l)(AIII8)
- Non «027» toxinotype III strains (n=5) :
  - MIC EM = 0.064->256 mg/l (1 strain R)
  - MIC MX < 2 mg/l
- Resistance to MX is not specific of the epidemic clone : 31% of strains different from toxinotype III are resistant to MX

Toxinotype VIII

- 34 strains isolated in 6 countries
- All the strains were A-B+
- High prevalence in countries:
  - Poland (HII) : 80% (4/5)
  - Poland (HI) : 46.7 % (5/11)
  - Ireland (DI) : 30.7% (4/13)
  - Sweden (MI) : 25% (3/12)
  - Germany (CIII) : 14.2% (7/49)
  - Ireland (DII) : 15.9% (3/19)
- PCR-ribotyping is under investigation

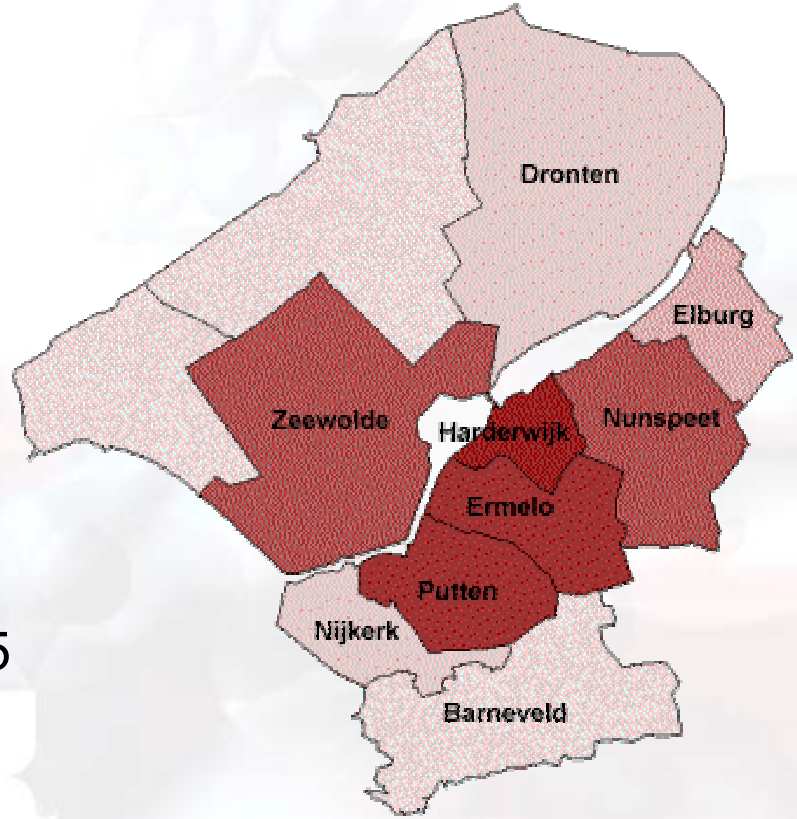
Binary toxin

- Binary toxin was found in 72 strains (17.4% of toxigenic strains)
- Binary toxin was not detected in non toxigenic strains

Stubbs (Wales):	*6.4%*
Goncalves (France) :	*6%*
Rupnik (Asia) :	*1.6%*
Geric (USA) :	*6.1%*

Conclusion

- So far, the epidemic strain toxinotype III, PCR -ribotype « 027 » has been isolated in 3 countries
- In 4 hospitals, this clone represents 30% to 70% of all the toxigenic isolates
- Resistance to MX is not a good screening method for detecting the epidemic clone
- There is a polymorphism among toxinotype III strains



The hospital

St. Jansdal hospital in Harderwijk

General hospital, 341 beds

Number of patients admitted in 2004: 25,625

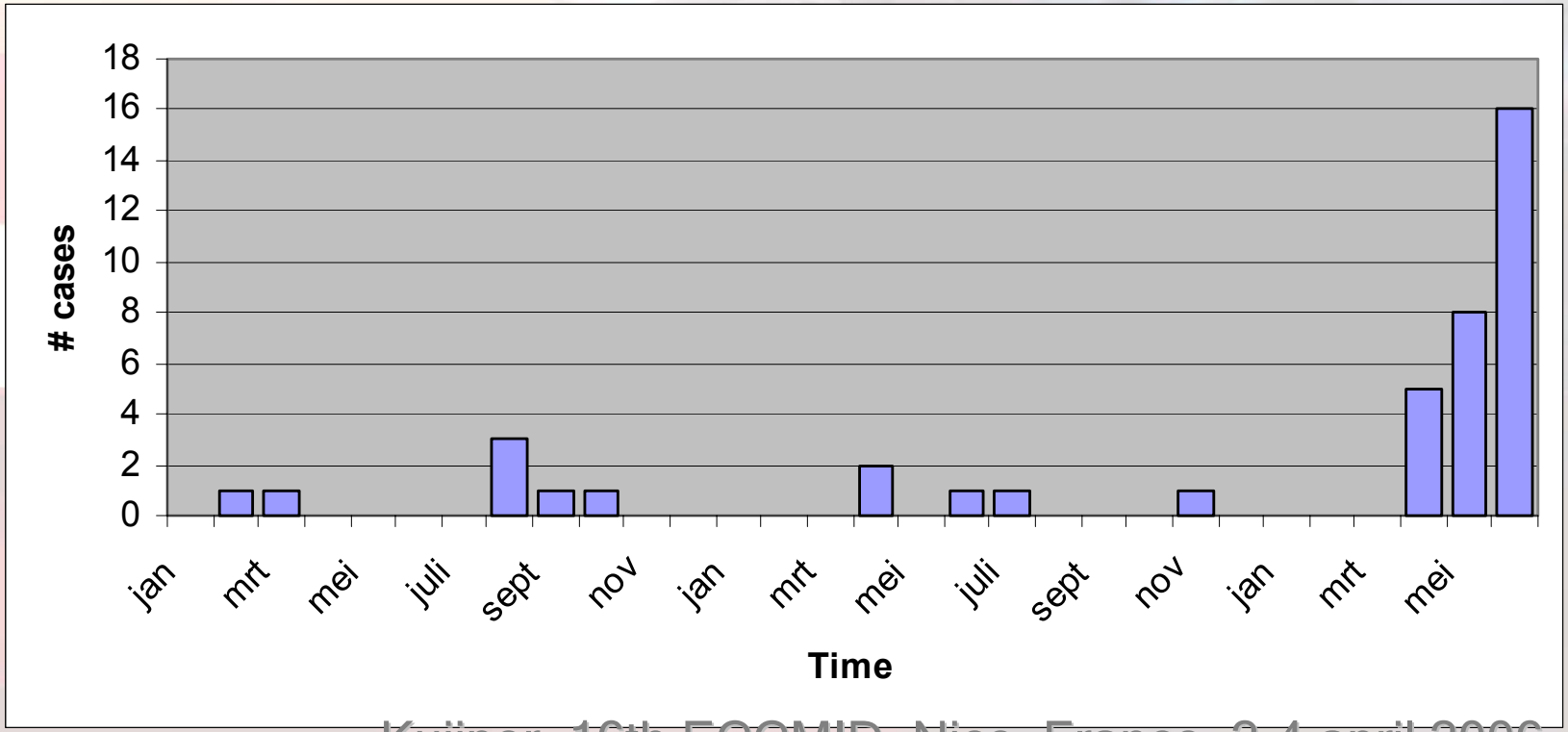
The laboratory

Before march 2005 testing for CDAD referred to Meander Medical Centre, Amersfoort (cytotoxicity test)

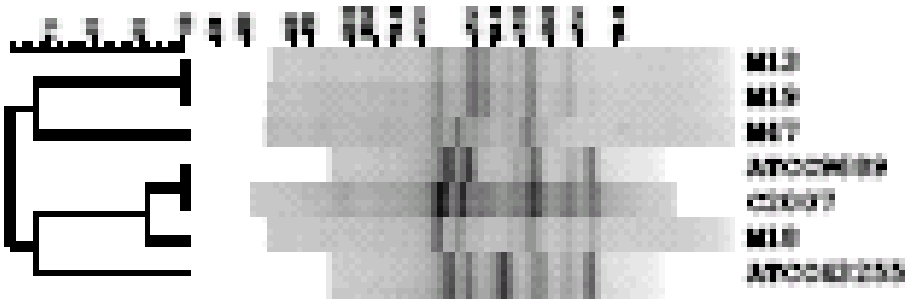
March: local introduction of a new diagnostic test in St Jansdal:

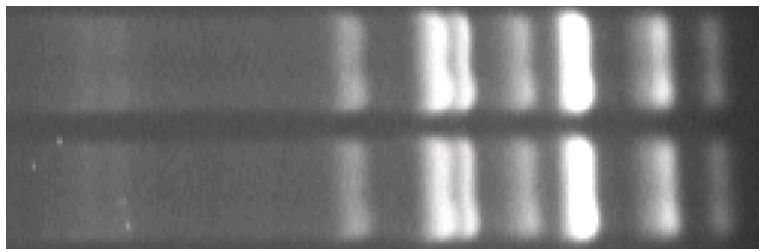
Rapid enzyme-immuno-assay (ICTAB, Meridian)

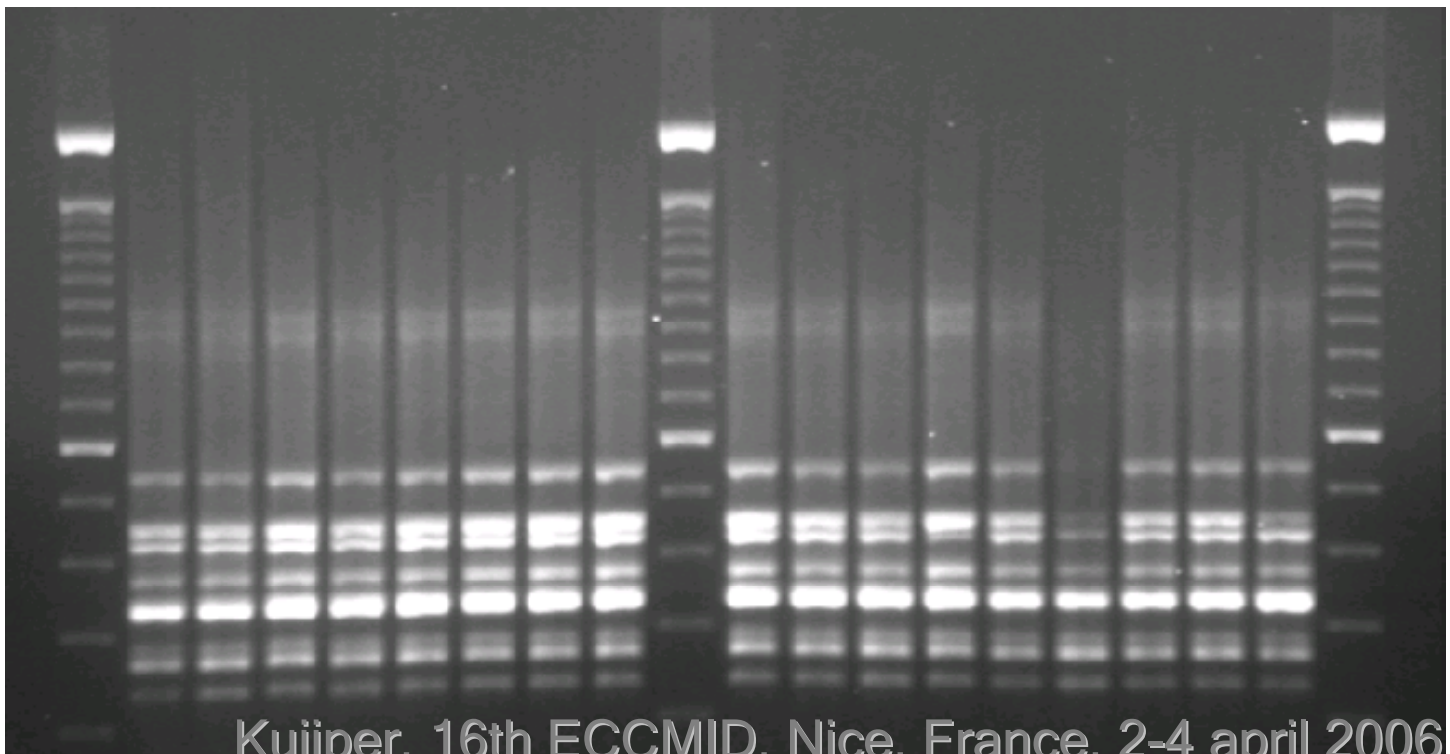
Year	# cases	incidence
2003	7	5.4 / 10.000 admissions
2004	5	3.8 / 10.000 admissions
April 2005	5	43 / 10.000 admissions
May 2005	8	79 / 10.000 admissions
June 2005	16	142 / 10.000 admissions



Ribotypering: 027



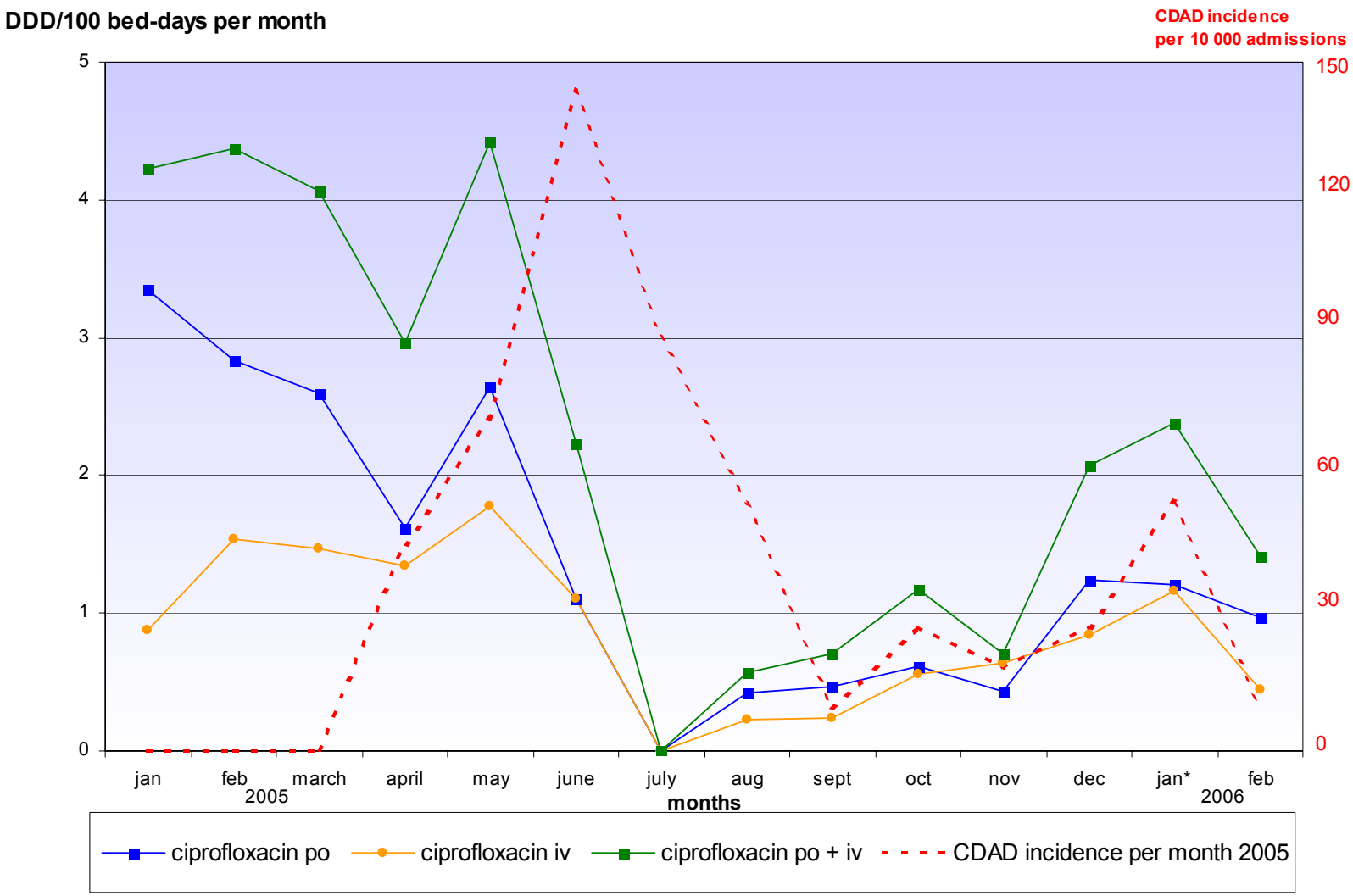




Kuijper, 16th ECCMID, Nice, France, 2-4 april 2006

Ziekenhuis St JANSDAL

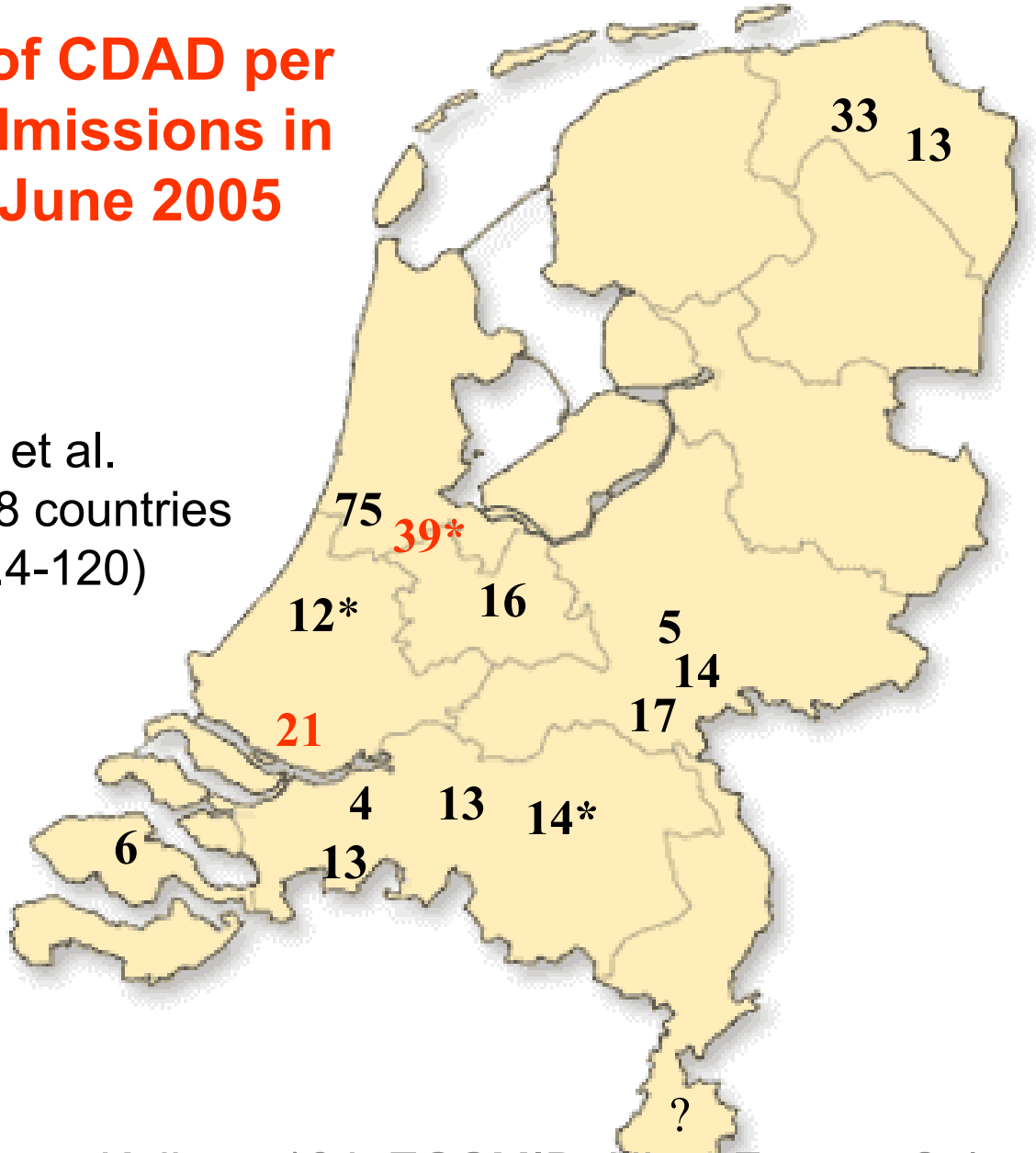
Use of ciprofloxacin in St. Jansdal hospital and patients with CDAD



Kuijper, 16th ECCMID, Nice, France, 2-4 april 2006

Number of CDAD per 10,000 admissions in May and June 2005

2003, Barbut et al.
N=112 labs, 8 countries
11/10,000 (0.4-120)



Kuijper, 16th ECCMID, Nice, France, 2-4 april 2006

Situation at March 29th, 2006

1 isolate of 027

multiple isolates

Epidemic ended at St. Jansdal:

Strict hand hygiene with water and soap

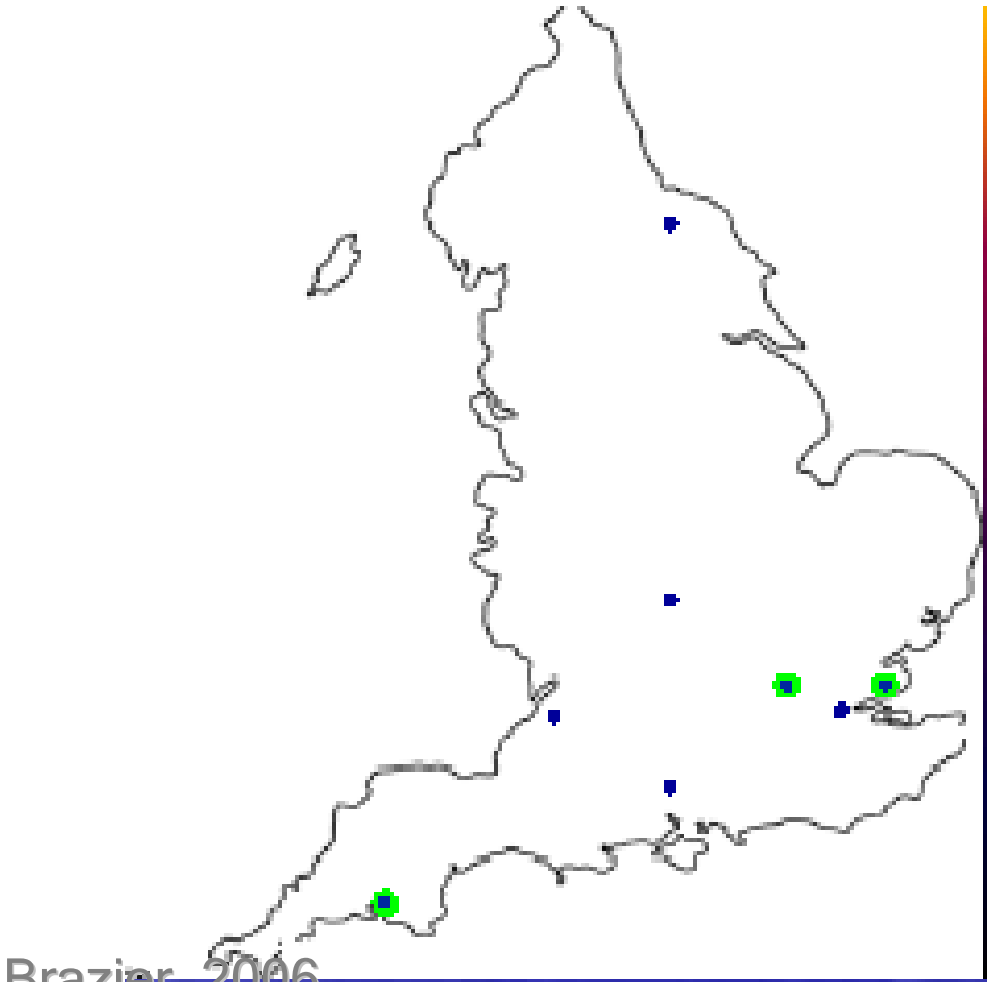
Gloves and apron

Cohorting of patients with CDAD

Complete banning of fluoroquinolones and restriction of cephalosporins

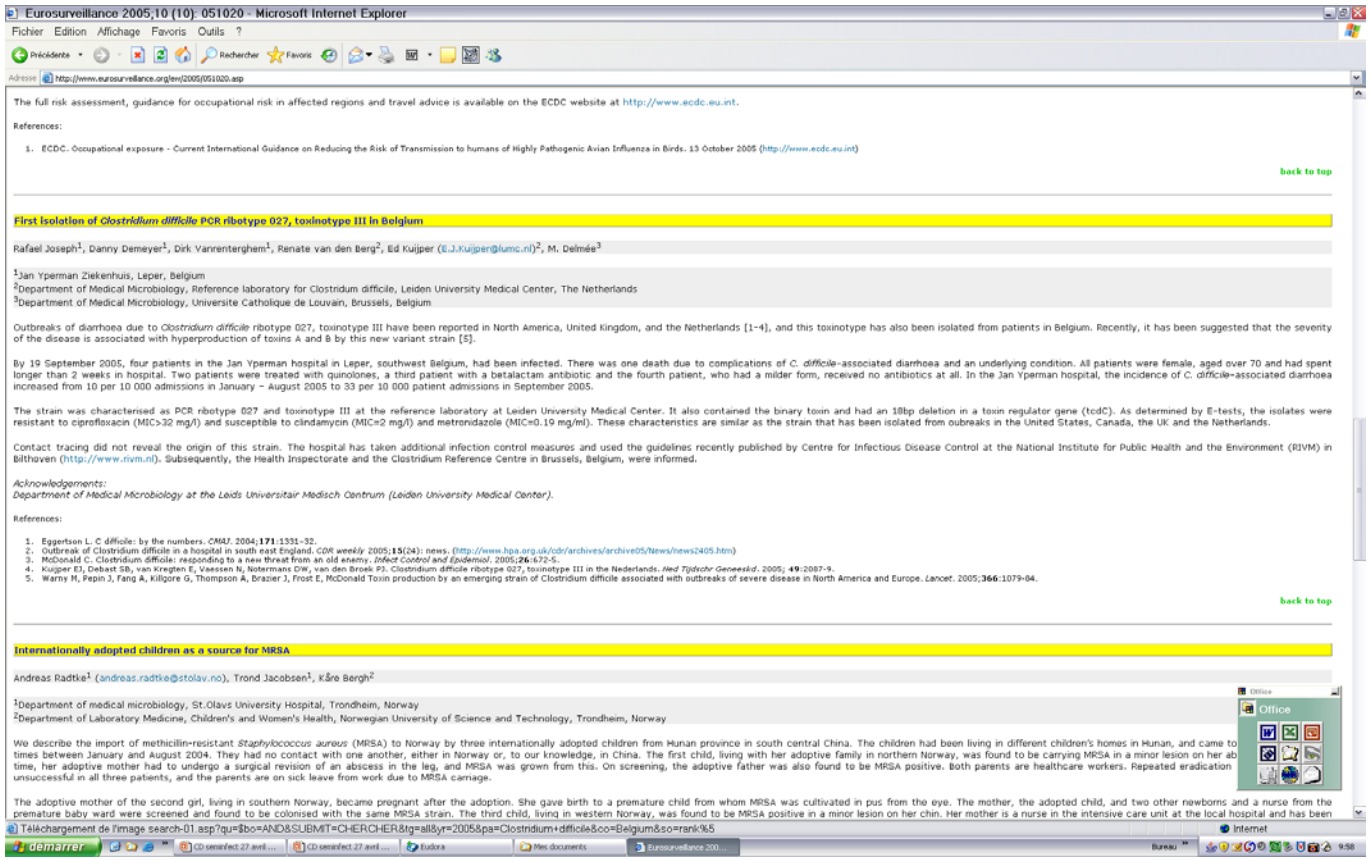
Effective environmental cleaning with chlorine containing desinfectants

GB : known locations with PCR ribotype 027
(as of January 2006)

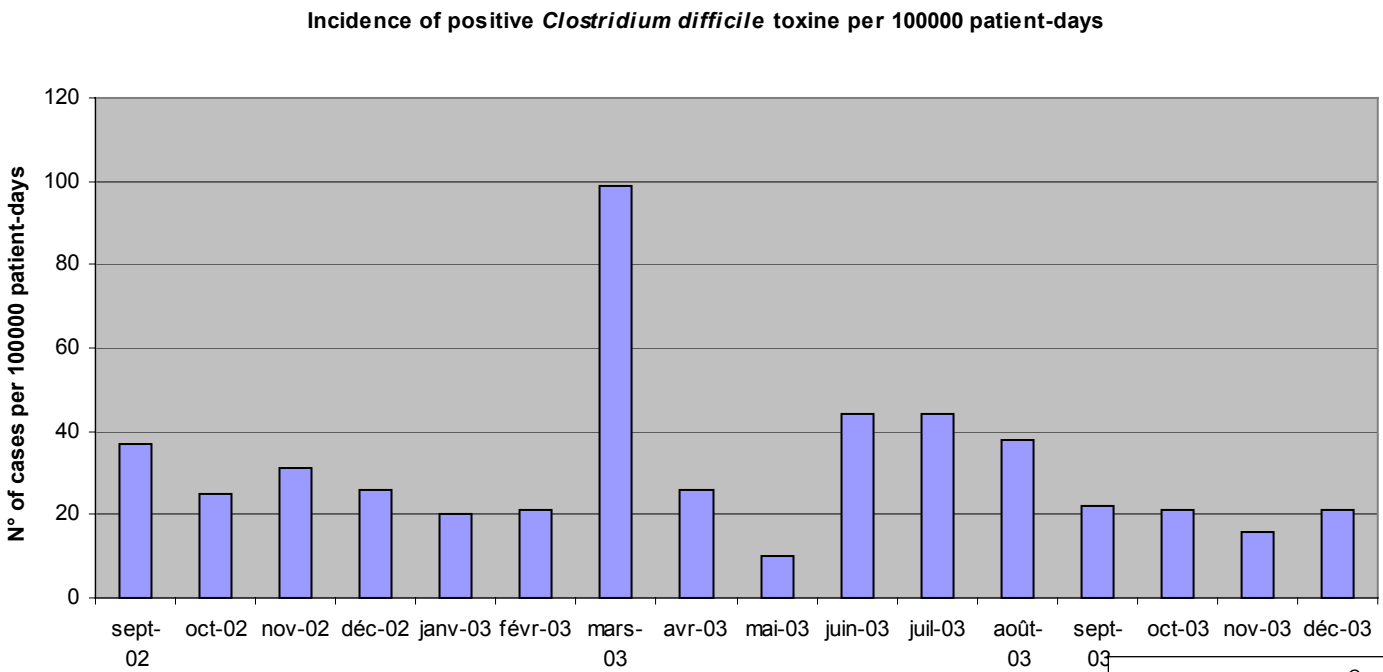


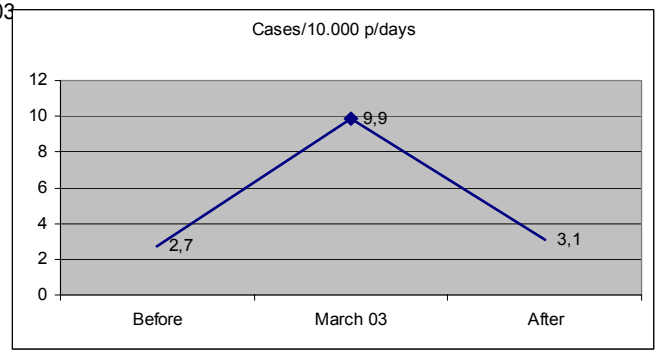
Brazier, 2006

027 in Belgium



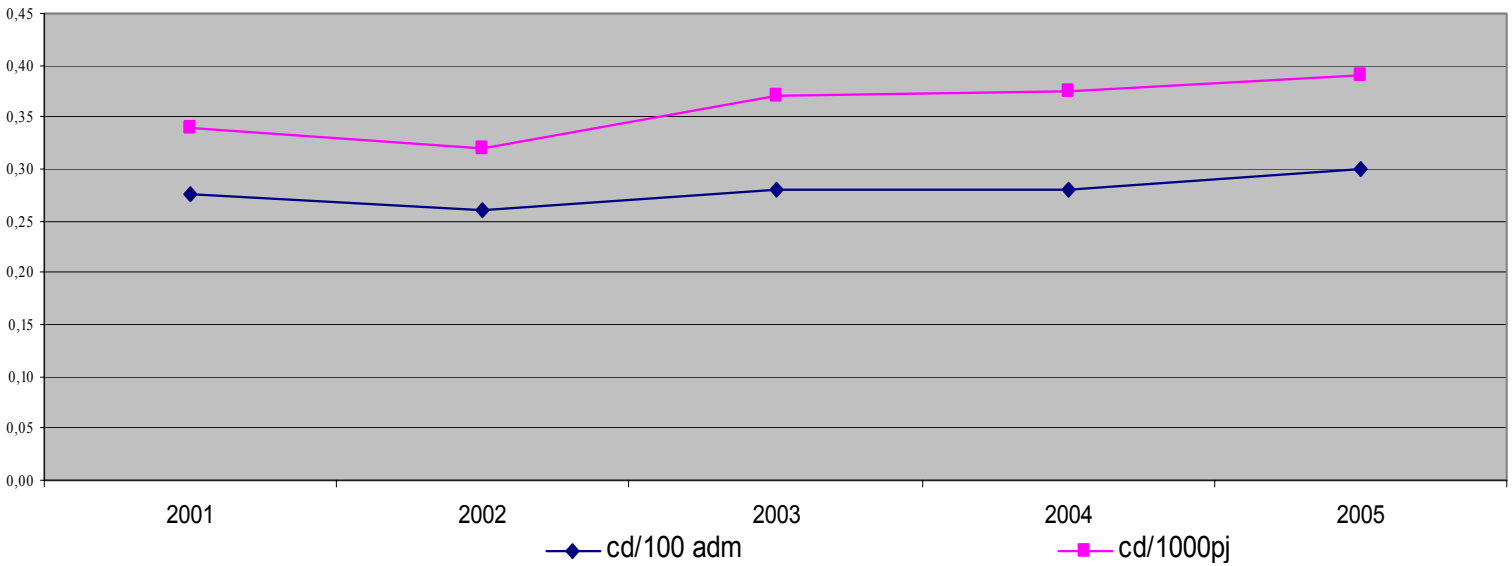
027 in Belgium



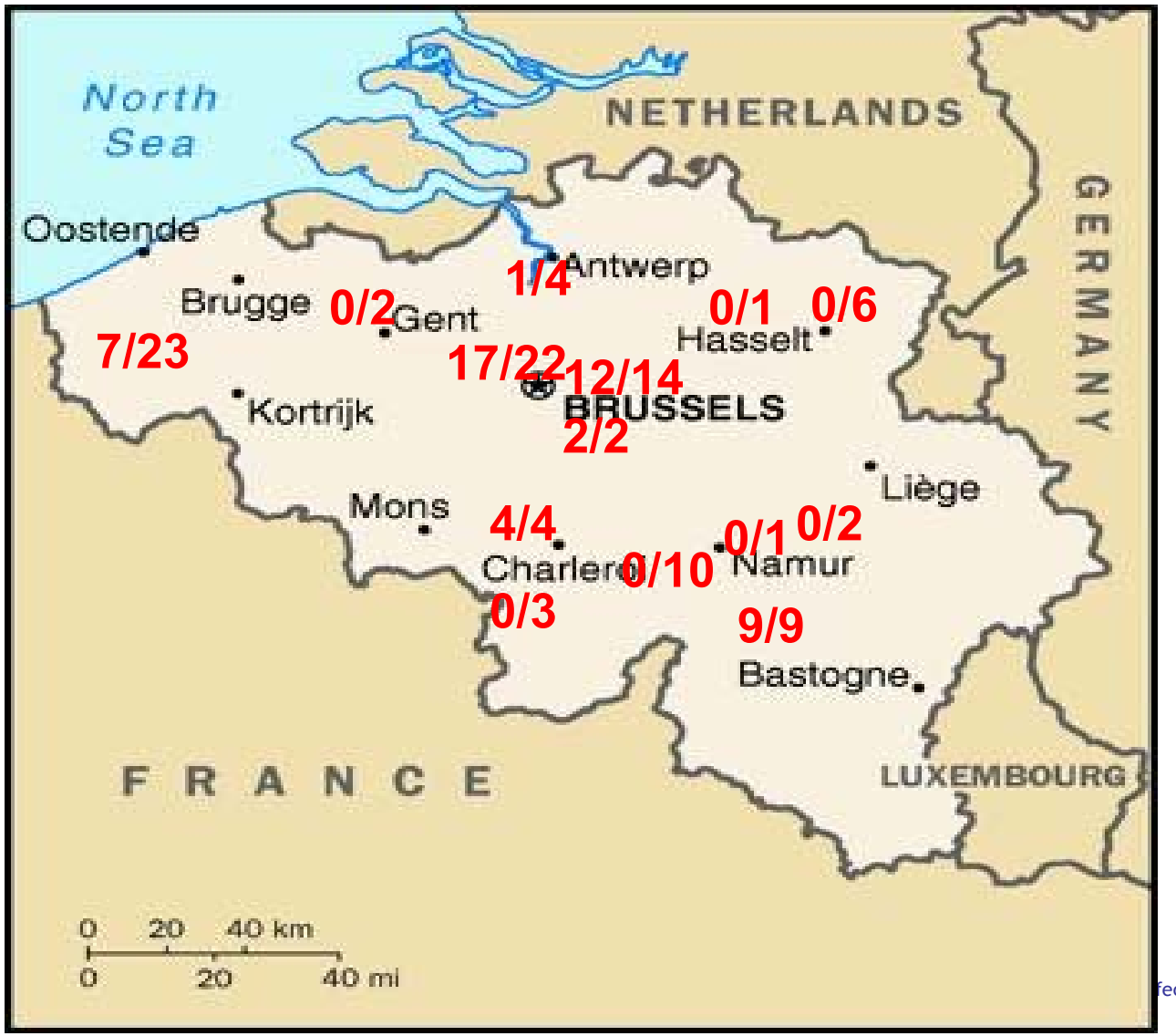


Cherifi et al. ICHE, 2006; accepted for publication

Densité d'incidence de *Clostridium difficile*



Belgium ref center 2006



n 027/
total

52/103
(50.5%)

fect 27/04/2006

027 : what to do ?

- Diagnosis
- Surveillance
- Treatment
- Prevention