

Molecular detection of toxin genes and *C. difficile* toxinotyping

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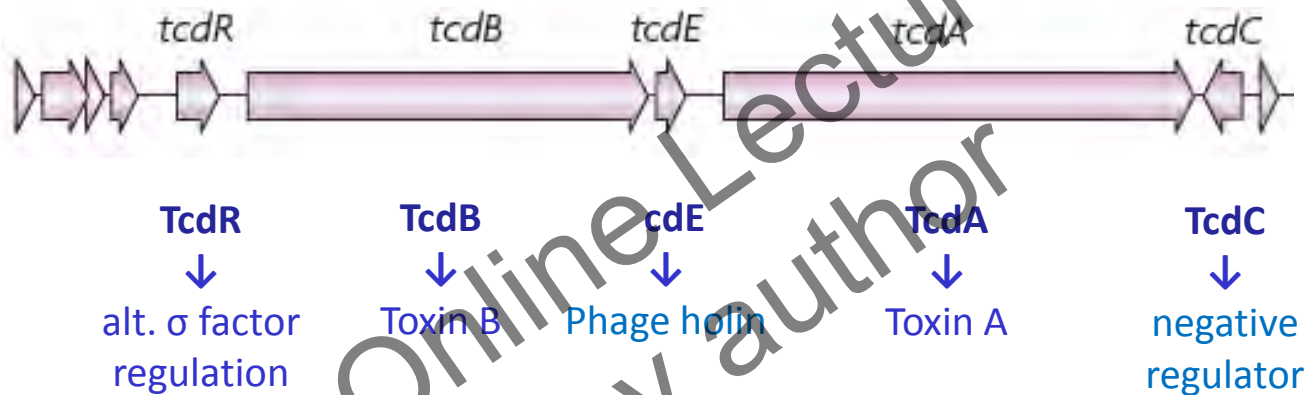


Objectives

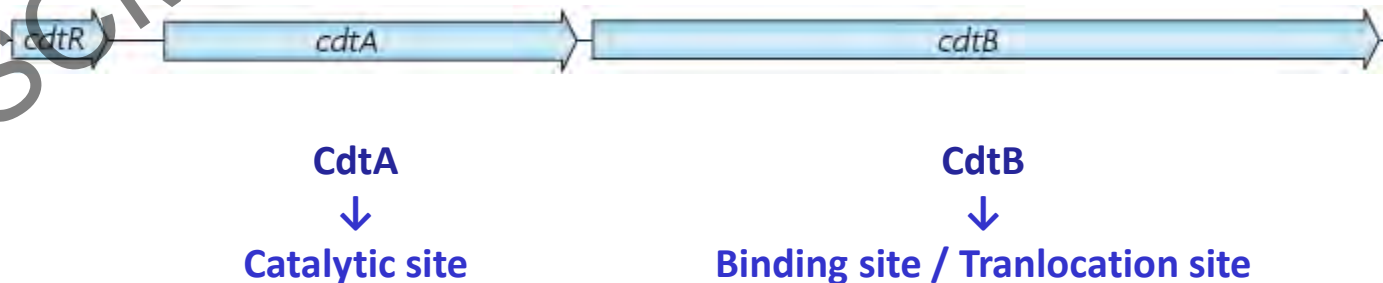
- *C. difficile* toxins and toxin coding regions
- detection of genes for binary toxin
- detection of genes for toxin A and B
- toxinotyping – variability of genes for toxins A and B

C. difficile toxins – genes and proteins

- **Pathogenicity Locus (PaLoc)**



- **Binary toxin LOCUS (CdtLoc)**

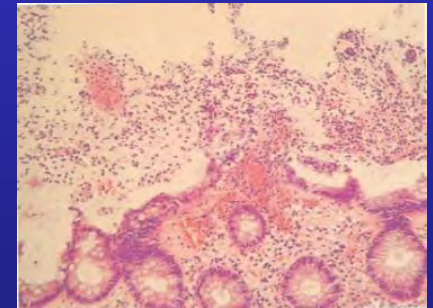
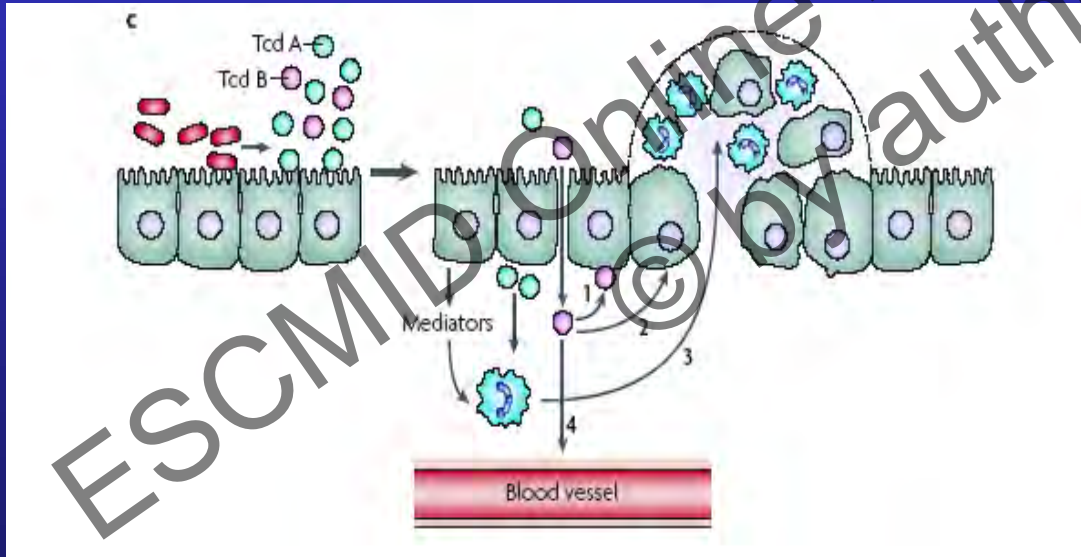


Role of the toxins in the pathogenesis – TcdA and TcdB

Main disease signs and symptoms

secretory diarrhoea

inflammation of colonic mucosa



Rupnik et al., Nature Rev Microbiol 2009

Lyras et al., Nature, 2009; Kuehne et al., Nature, 2010

Carter et al., mBio, 2015

Role of the toxins in the pathogenesis – binary toxin CDT

cytotoxicity

protrusions on cells - adhesion

Schwan et al., PLOS Pathog 2009

hamster model - mutants

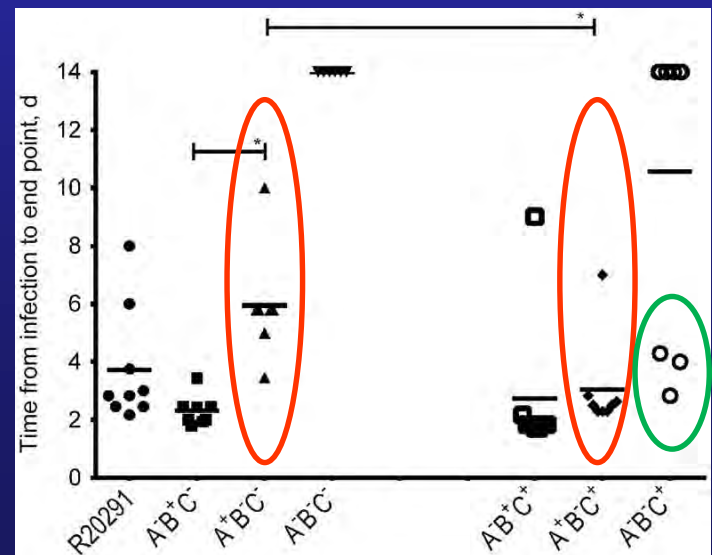
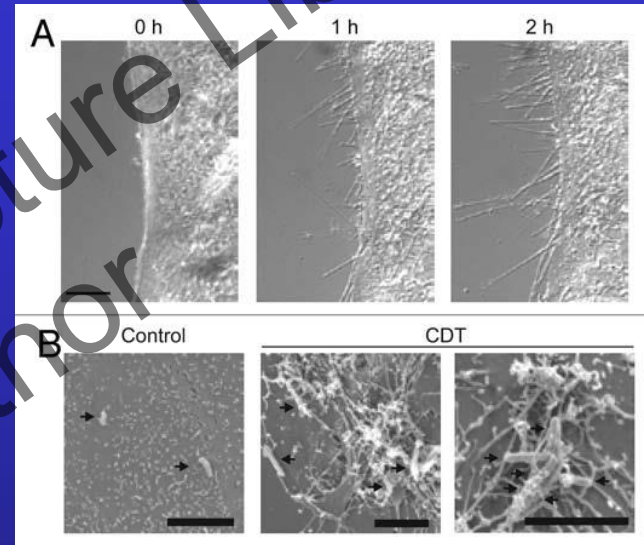
Kuehne, J Infect Dis, 2014

CDI in humans with A-B-CDT+ strains

Eckert et al., New Micr New Infect, 2015

Elliot et al., Anaerobe, 2009

Geric et al., J Clin Micr, 2003



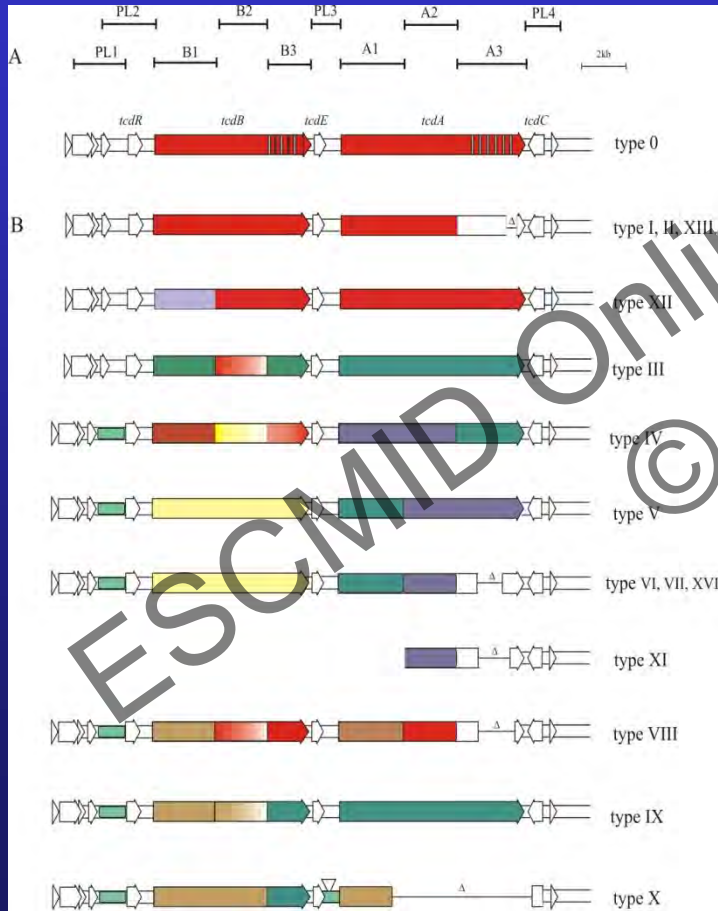
Detection of *C. difficile* toxin genes

	Toxins A and/or B	Binary toxin CDT
non-commercial methods	<ul style="list-style-type: none"> • many • often used Kato H et al., J Clin Micro, 1998 	most often used Stubbs et al., FEMS Microbiol Lett, 2000
commercial tests	<ul style="list-style-type: none"> • many (see talk by Barbut) • shown on PWS: <ul style="list-style-type: none"> -illumigene ® <i>C. difficile</i> (Meridian) -Xpert ® <i>C. difficile</i> (Cepheid) 	Xpert ® <i>C. difficile</i> (Cepheid) GenoType Cdiff (Hain lifescience) Verigene ® <i>C. difficile</i> test (Nanosphere) Genespeed ® <i>C. diff</i> OneStep (Greiner Bio-One)

Variability of *C. difficile* toxin loci

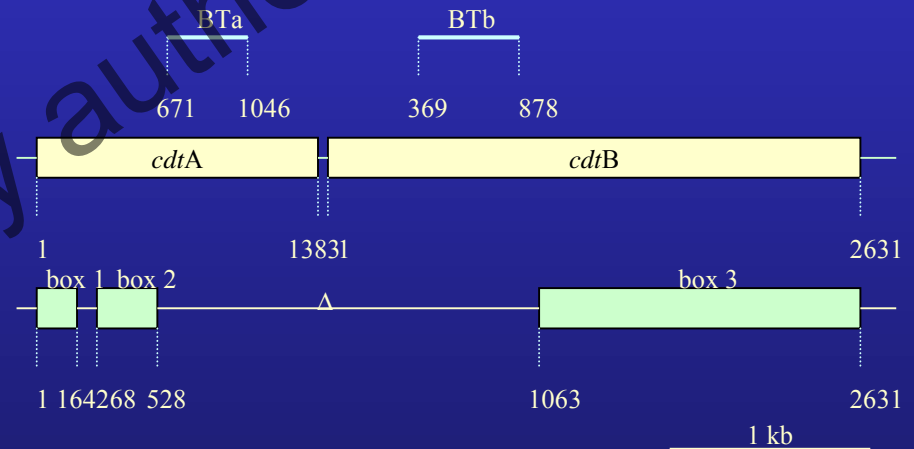
PaLoc (toxins A and B)

34 variants- toxinotypes

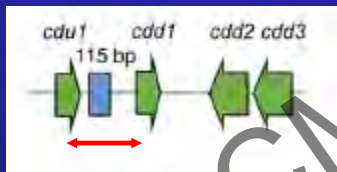
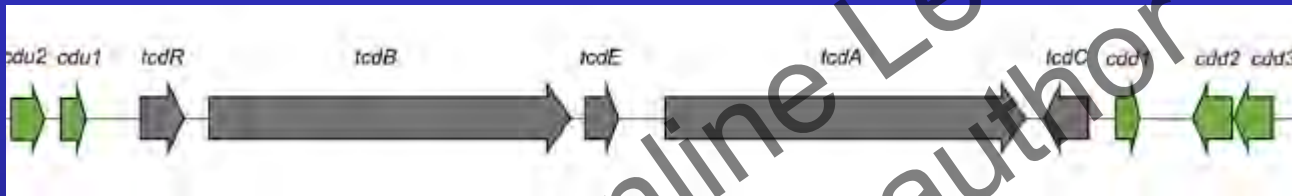


CdtLoc (binary toxin CDT)

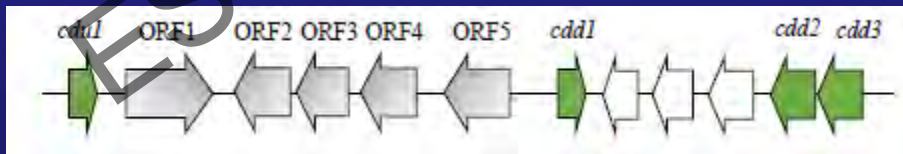
2 main variant forms



Molecular detection of *C. difficile* nontoxigenic (PaLoc neg) strains – Lok1/Lok3 PCR



Braun et al., 1996, Gene



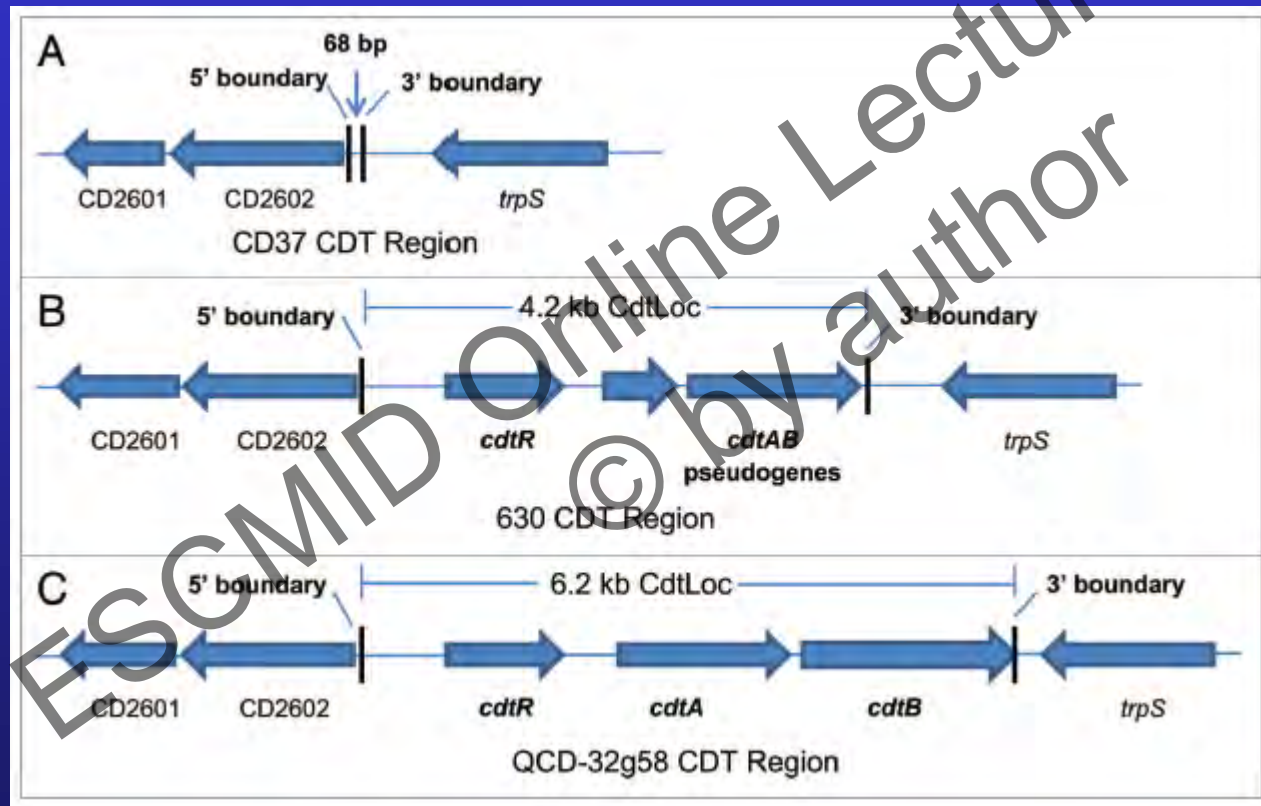
Lok1/3 PCR	strain
neg	toxigenic
poz	nontoxigenic
neg	<u>nontoxigenic</u>

Janezic et al., 2015, JCM

Dingle et al., 2014, Genome Biol Evol

Elliott et al., 2009, Anaerobe

Variant forms of region coding for binary toxin



CdtLoc negative

truncated
nonfunctional
CdtLoc

whole functional
CdtLoc

Combinations of two toxin loci in *C. difficile*

PaLoc (toxins A and B)

CdtLoc (binary toxin)

ordinary strains (toxintype 0)

truncated nonfunctional locus

variant toxinotypes

whole functional locus

nontoxigenic strains (PaLoc neg)

none

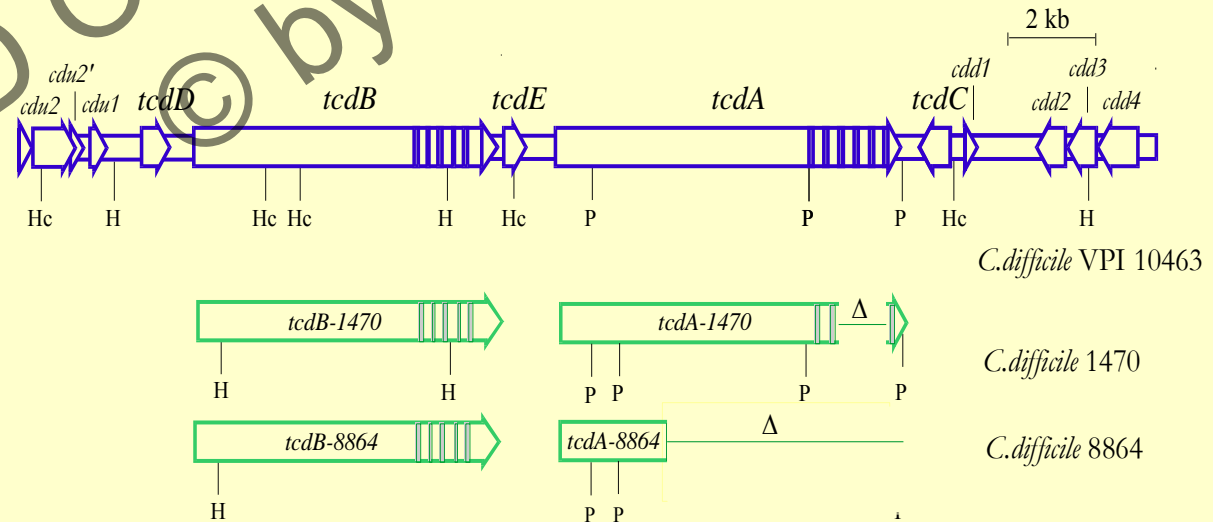
C. difficile toxin production types

	TcdB	TcdA	CDT	
Type 1	+	+	-	most prevalent
Type 2	+	-	-	0.2 - 12 %
Type 3	+	+	+	up to 35 % (non-outbreak)
Type 4	+	-	+	very rare
Type 5	-	-	+	1.6 %
Type 6	-	-	-	20 %
Type 7	-	+	-	two strains?

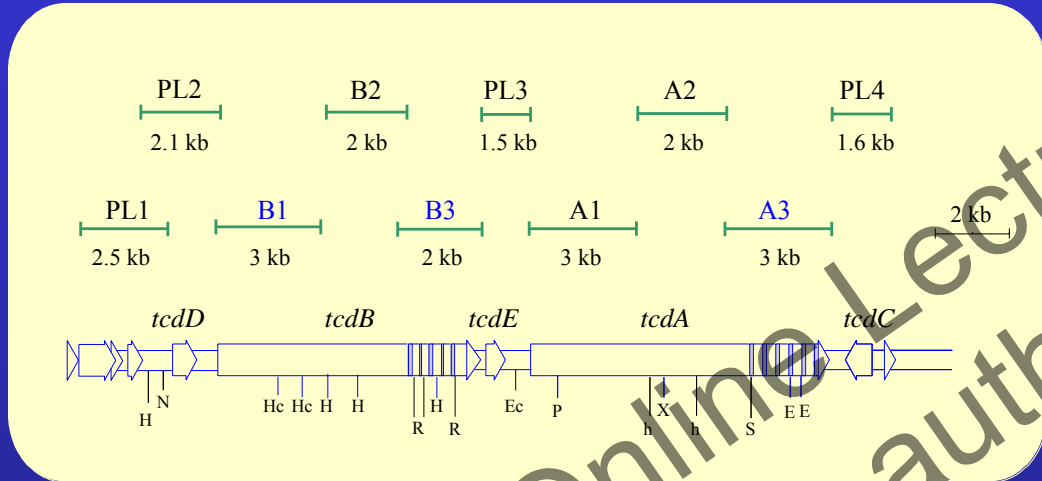
binary gene(s) positive – binary toxin positive?

First variant *C. difficile* strains

- TcdB-positive, TcdA-negative
- two groups
 - strain 8864
 - strains from serogroup F and X



Toxinotyping of *C. difficile* strains



PCR method for screening changes in PaLoc

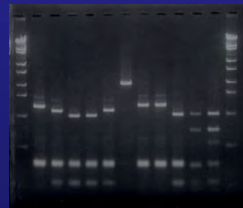
- RFLPs
- deletions
- insertions

B1 PCR

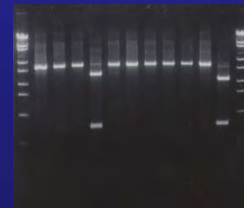


1 2 3 4 5 6 7

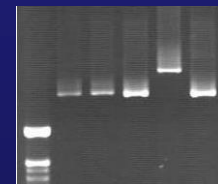
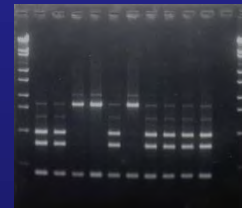
B2 PCR



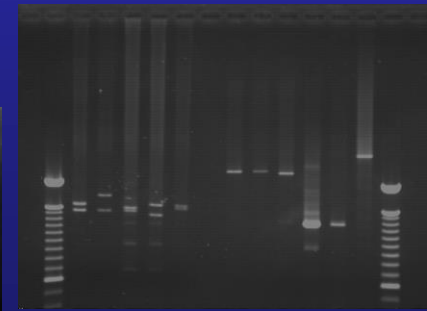
A1 PCR



A2 PCR



A3 PCR

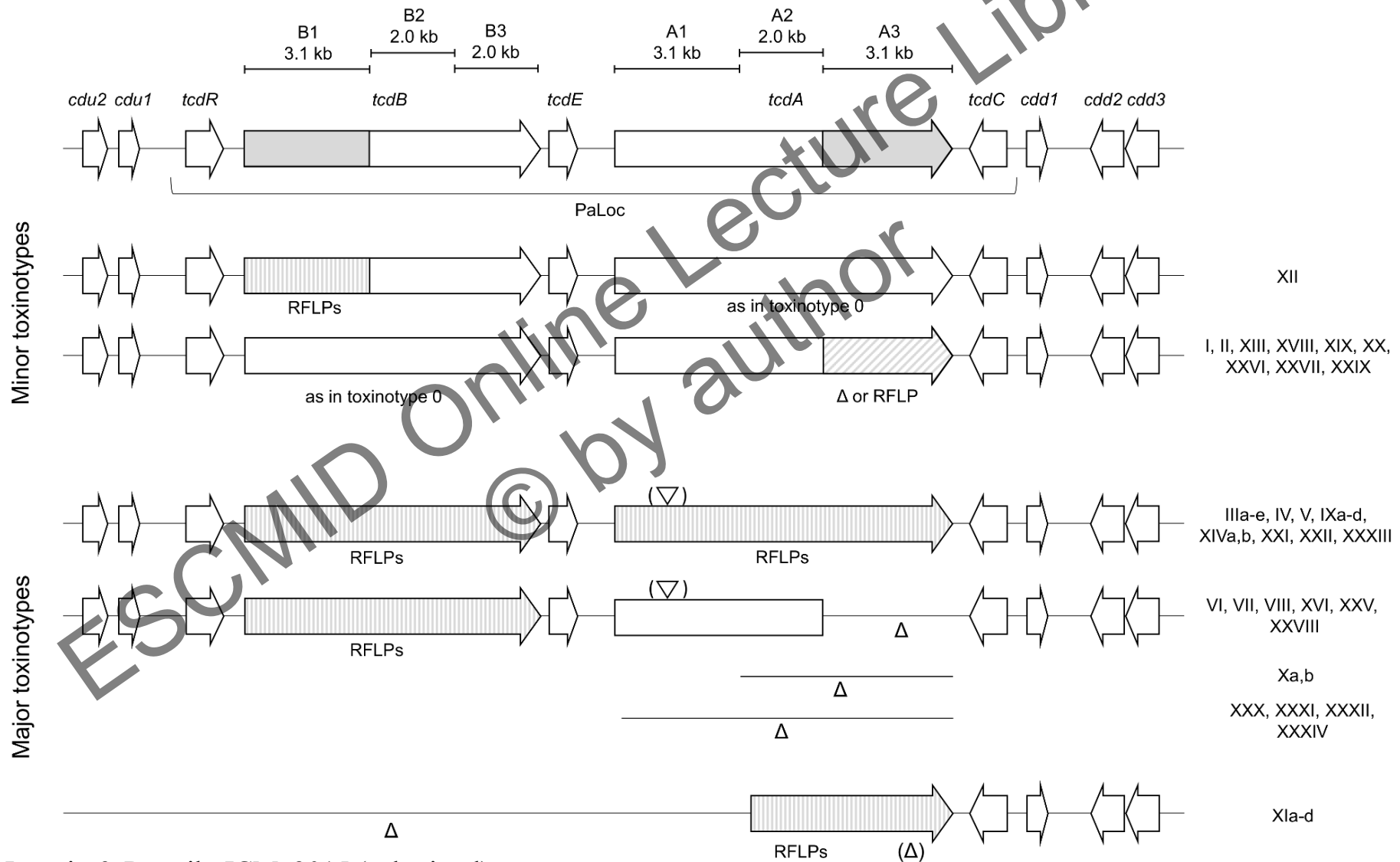


1 2 3 4 9 5 5 6 7 7 8

How to determine the toxinotype

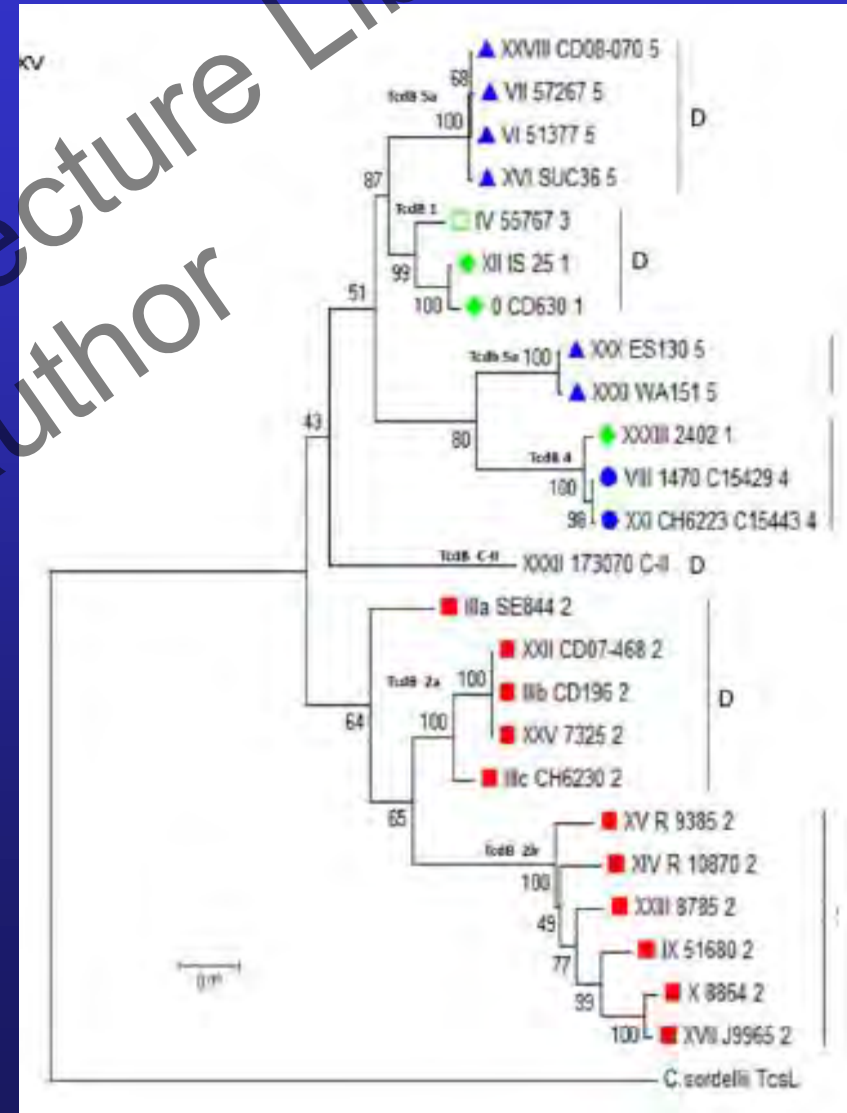
Toxinotype New designation	Toxinotype Previous designation	Type strain	Toxin production ^a	B1 ^b	A3 ^b	Type of tcdC gene ^c	Type of CPE ^d	PCR ribotype ^e	BTb PCR	Remarks
Xa	X	8864	A- B+ CDT+	5	neg	neg	S	nd	pos	<ul style="list-style-type: none"> in Xb longer A1 (compared to toxinotype 0) due to the presence of ISTRon
Xb	XVII	J9965	A- B+ CDT+	5	neg	neg	S	SLO 032	pos	<ul style="list-style-type: none"> no insertion in PL3 in Xb in Xa shorter A1 (compared to toxinotype 0) due to the presence of deletion
XXXI	XXXI	WA 151	A- B+ CDT+	5	neg	neg	S	SLO 098	pos	<ul style="list-style-type: none"> same as toxinotypes Xa and Xb in B1 and A3 RFLP in XXXI neg A1 and PL3 (both positive in Xa and Xb) difference to X also in B2 and B3 fragments
XXI	XXI	CH6223	A+ B+ CDT-	5	1	1	S	SLO 035	neg	<ul style="list-style-type: none"> new type of A2 in orig. publication B1 described as type 4
IXa	IX	51680	A+ B+ CDT+	5	2	1	S	019	pos	Differentiation of types IXa, IXb, IXc and IXd <ul style="list-style-type: none"> in IXc longer A1 due to the presence of ISTRon differences in B2 and B3 fragments
IXb	IX	TFA/V20-1	A+ B+ CDT+	5	2	2	S	244	pos	
IXc	XXIII	8785	A+ B+ CDT+	5	2	1	S	109	pos	
IXd	new	1732874	A+ B+ CDT+	5	2	3	nd	SLO 228	pos	
VIII	VIII	1470	A- B+ CDT-	5	7 d	1	S	017	neg	
XII	XII	IS 25	A+ B+ CDT-	6	1	1	D	258	neg	

Minor and major toxinotypes

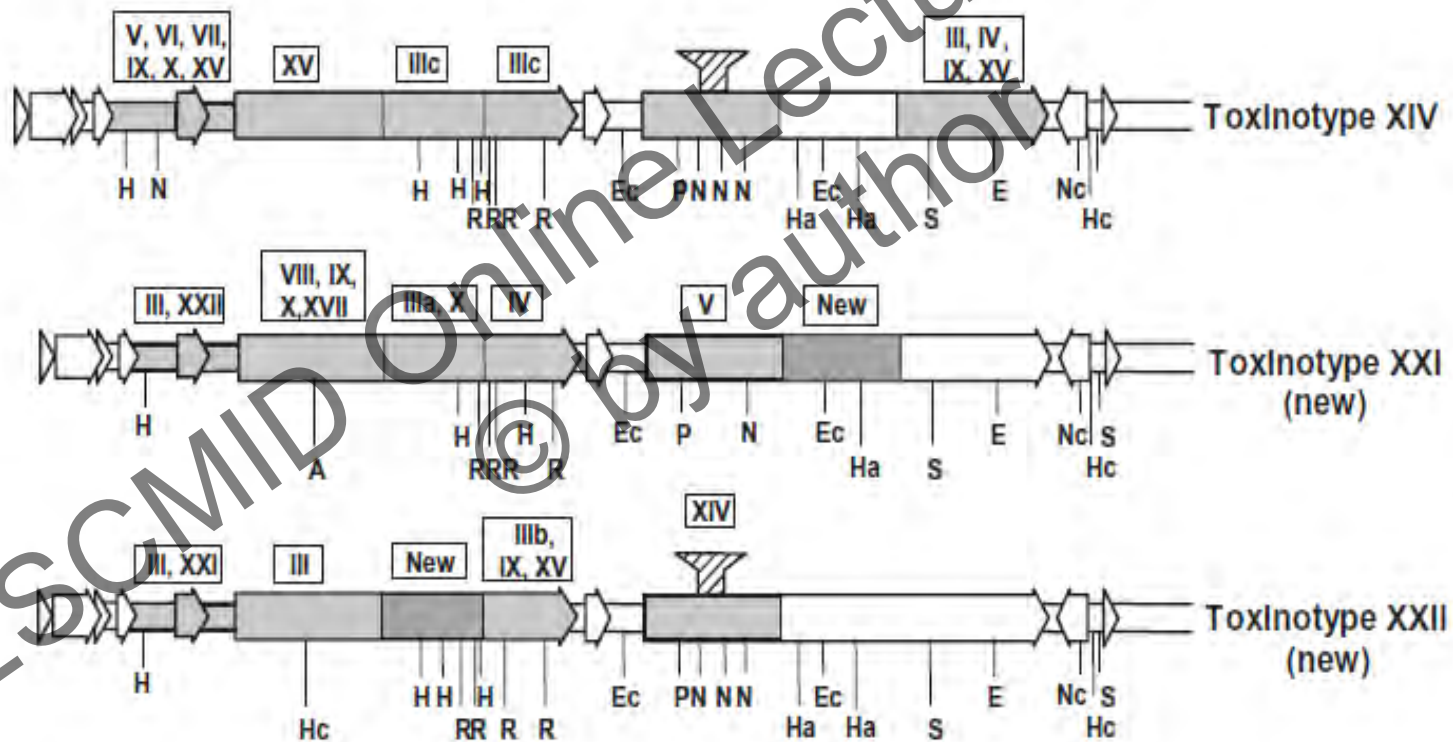


Toxinotypes

- groups of strains with changes in PaLoc
- toxinotype 0: reference strain VPI 10463
- toxinotypes I to XXXIV: variant strains
- clonal subpopulations or mutant strains



Modular structure of toxin genes

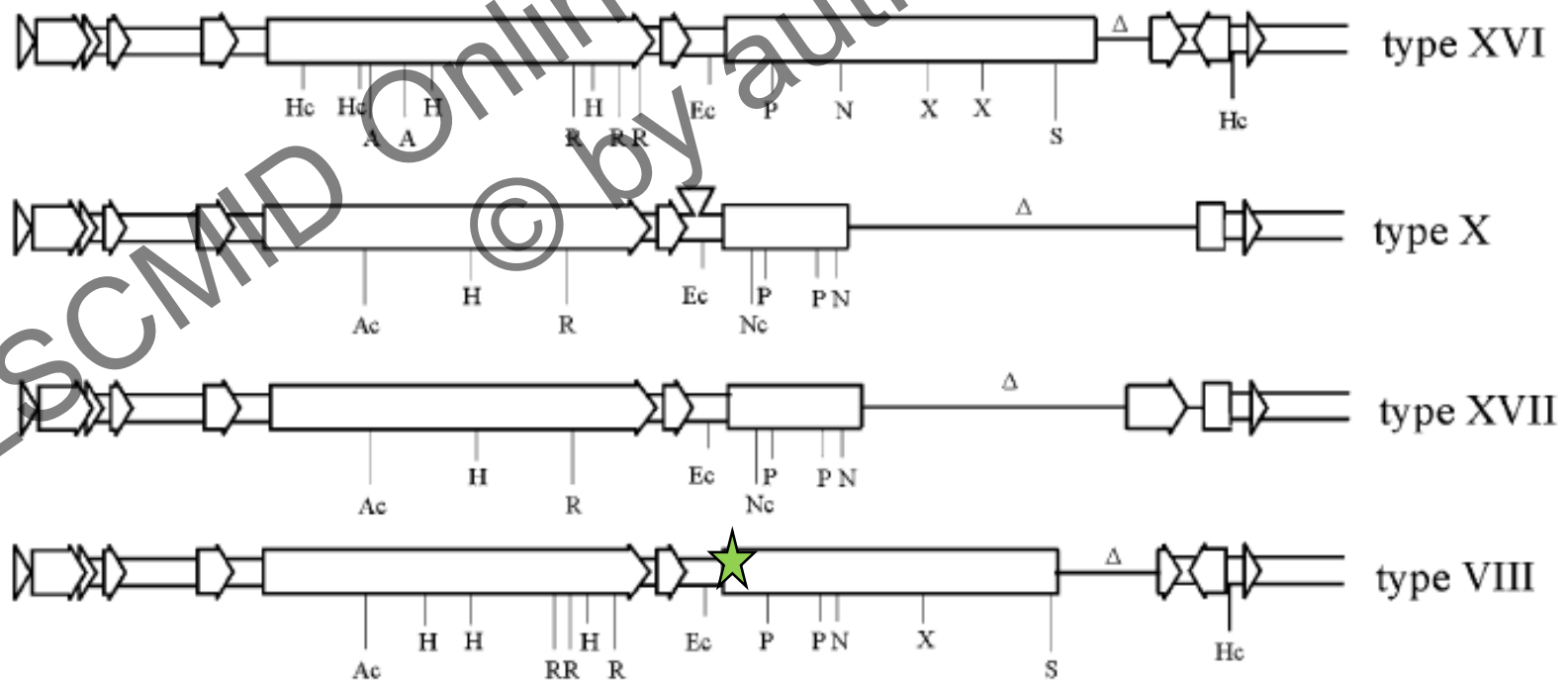


Toxinotypes - toxin production and ribotypes (some examples)

Toxin production type	Toxinotype	Ribotype
A + B+ CDT-	minor toxinotypes	
A + B+ CDT+	minor type 0/v most major toxinotypes IIIa-e IV V IXa-d XXII	131 027, 034, 075, 080 023, 063 078, 126, 045, 066 019, 244 027
A - B+ CDT-	VIII XXXII	017 (SLO148)
A - B+ CDT+	XVI XXX, XXXI	078 (SLO 101, SLO 098)
A - B- CDT-	XIa-d	033, 288, 251

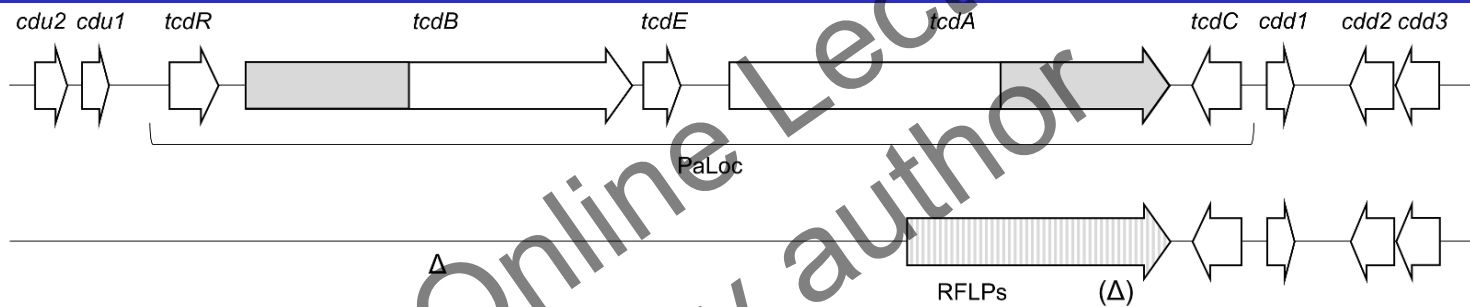
Toxinotypes and toxin A negative phenotype

- deletions of *tcdA*
- small deletions and effect on recognition by antibodies?
- mechanisms unknown
- point mutation at position 47 (toxinotype VIII)



Some unusual toxinotypes – XI

A negative, B negative, CDT positive
associated with clinical cases (France, USA, Danmark, Slovenia)

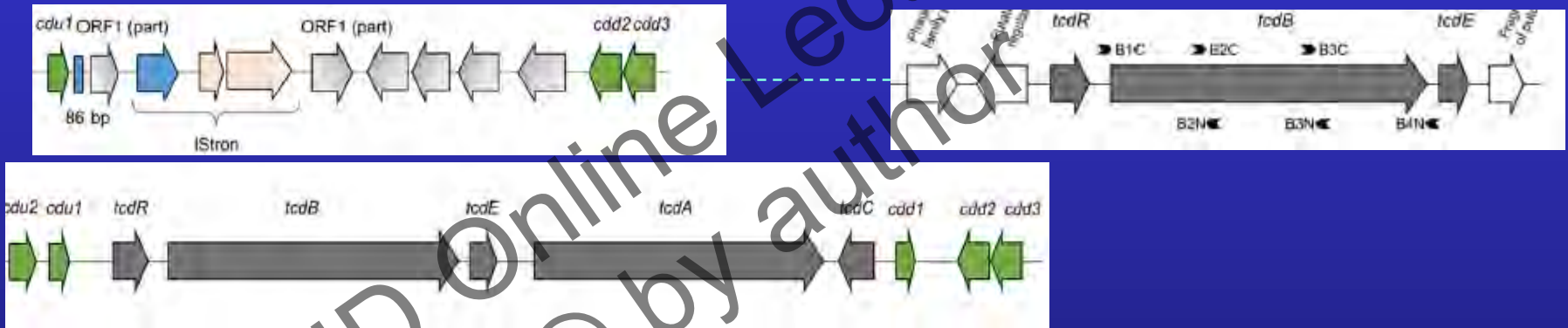


ORIGINAL ARTICLE

Prevalence and pathogenicity of binary toxin–positive *Clostridium difficile* strains that do not produce toxins A and B

C. Eckert^{1,2}, A. Emirian⁵, A. Le Monnier^{3,6}, L. Cathala⁷, H. De Montclos⁹, J. Goret¹⁰, P. Berger¹¹, A. Petit¹, A. De Chevigny¹, H. Jean-Pierre^{7,8}, B. Nebbad⁵, S. Camiade¹², R. Meckenstock¹³, V. Lalande^{1,4}, H. Marchandin^{7,8} and F. Barbut^{1,2,4}

Some unusual toxinotypes – XXXII



A negative, B positive, CDT negative
new clade
new chromosomal integration site

Distribution of variant strains in EU (2005 and 2009)

2 months period in 2005	1 month period in 2009
Barbut et al., CMI, 2007	Bauer et al., Lancet, 2012
14 countries, 38 hospitals	26 EU countries, 73 hospitals
486 isolates	395 isolates
85,2 % toxinogenic (LCT+)	98,5 % toxinogenic (LCT+)
17,4 % of toxinogenic strains were CDT+	23 % of toxigenic strains were CDT+
027, 078, 126, 023	078, 027, 126, 023

Summary

- toxinotypes are groups with changes in genes for toxins A and B
- toxin gene variant strains often have variant pattern of produced toxins: A+B+CDT+ or A-B+CDT+/-
- variant toxinotypes can be associated with increased virulence
- variation in toxin genes is important in molecular detection assays