Typing of *Clostridium difficile* infections in Europe

Typing methods for *C. difficile*

- Restriction Enzyme Analysis (REA)
- Arbitrarily primed PCR (AP-PCR)
- Pulsed-field gel electrophoresis (PFGE
- PCR-ribotyping
- Amplified Fragment Length Polymorphism
- Toxinotyping (PCR-RFLP)
- *SlpA* PCR-RFLP (S-layer precursor protein)
- Multilocus sequence typing (MLST, 7-12 genes)
- Multilocus variable number of tandem repeat analysis (MLVA)
- Tandem repeat sequence analysis

PCR ribotyping

- Robust, stable and reproducible
- Interlaboratory comparison can be improved by capillary gel electrophoresis
- Standardization of the library is necessary
- ECDC-Brazier collection, Leeds-LUMC-Ages collection

ARU collection of >15,000 *C. difficile* isolates

- 345 distinct ribotypes recognised
  - >1000 isolates of types 001, 027 & 106.
  - 100-1000 isolates of 13 ribotypes.
  - 11-100 isolates of 53 ribotypes.
  - <5 isolates of 226 ribotypes.
- Most common types are in the ECDC/Leeds collection.

Pulsed field gel electrophoresis
(standard at CDC, Canada)

- macrorestriction of whole genomic DNA with Sma1, increase of restriction pattern
- Labor intensive, time consuming, good interpretation,
- **Of 39 of the most frequently found PCR RTs, only 16 NAP field types were obtained**

MLST

- **Knetsch CW, et al. Infect Genet Evol. 2012 Oct;12(7):1577-85**

- Discriminatory power of MLST and PCR ribotyping is comparable
- At lest five well separated lineages and possibly a sixth monophyletic lineage
- MLST is an appropriate tool for studying the phylogeny of C. difficile

Supporting capacity building for surveillance of *Clostridium difficile* infections at European level *(2010-2013)*

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ECDIS-Net

Supporting capacity building for surveillance of C. difficile

ESGCD ESCMID STUDY GROUP FOR CLOSTRIDIUM DIFFICILE

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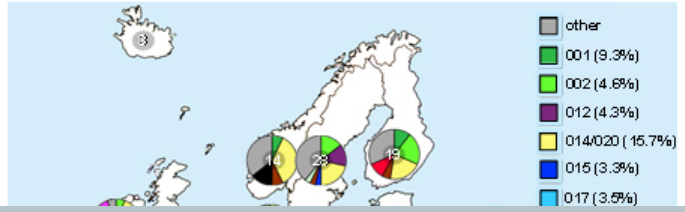
Supporting capacity building for surveillance of Clostridium difficile infections at European level (2010-2014)

Clostridium difficile infections (CDI) are an important healthcare problem across Europe. To improve recognition and awareness, and to enable surveillance at a European level, the European Centre of Disease Prevention and Control (ECDC) funded an upcoming project to enhance laboratory capacity for CDI detection and surveillance in Europe (2010-2014). This project will not be a duplication of the previous European *Clostridium difficile* infection study (ECDIS), but instead will be used to strengthen the network and capacity building for CDI surveillance on national and European level. We have therefore called the new project "European Clostridium difficile infection surveillance network (ECDIS-net)".

Background

After the recognition of a new hypervirulent *Clostridium difficile* strain, PCR ribotype 027, in 2005 in Europe, the ESCMID Study Group on *Clostridium difficile* (ESGCD) contacted ECDC leading to several actions. A background document on CDI was written, guidance documents were published, and a first pan-European surveillance study, the "European Clostridium Infection Survey (ECDIS)" was performed in 2008-2009. Results of this study have been published in Lancet (Bauer et al. *Clostridium difficile* infection in Europe: a hospital-based survey. Lancet. 2011;377:63-73). Based on the results of the ECDIS study, it was decided to provide support for further capacity building for surveillance of CDI across Europe.



ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

National Institute for Public Health and the Environment Ministry of Health, Welfare and Sport

The Leeds Teaching Hospitals NHS NHS Trust

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LUMC

Supporting capacity building for surveillance of *Clostridium difficile* infections at European level

- To enhance the laboratory capacity for detection and surveillance of *Clostridium difficile* in European Member States (MS), Norway, Iceland and Liechtenstein.
- To build up and maintain a European ribotyping nomenclature reference database for *Clostridium difficile*.
- To develop a enhanced CDI surveillance protocol.

Diagnostics and typing of *Clostridium difficile* infections in Europe

Cross-sectional survey

National coordinators active in National Health Services of **32 European countries** were requested to select 10% of microbiological laboratories to participate (minimum of 3).

In total, **206 laboratories** were invited to report on the local incidence and diagnostic testing practice of CDI in 2009/11.

Results

126 laboratories (61%) from 31 countries completed a questionnaire; 95% (n=120) performed diagnostic testing for *C. difficile*.

Of the 31 participating countries 39% (n=12) had a complete response rate and 68% (n=21) had a response rate >60%. Two had a response rate <30%.

Results

Laboratories tested a median of 1032 (IQR 462-1,934) patients for *C. difficile* per year. The median proportion of patients tested for *C. difficile* from which CDI was diagnosed was **10%** (IQR 5-16%).

The estimated CDI incidence was **17,9 per 10,000** admissions (IQR 8,2-37,3) and 3,7 per 10,000 patient-days (IQR 1,2-6,3)

CDI is diagnosed approximately **once every 500 admissions across Europe**.

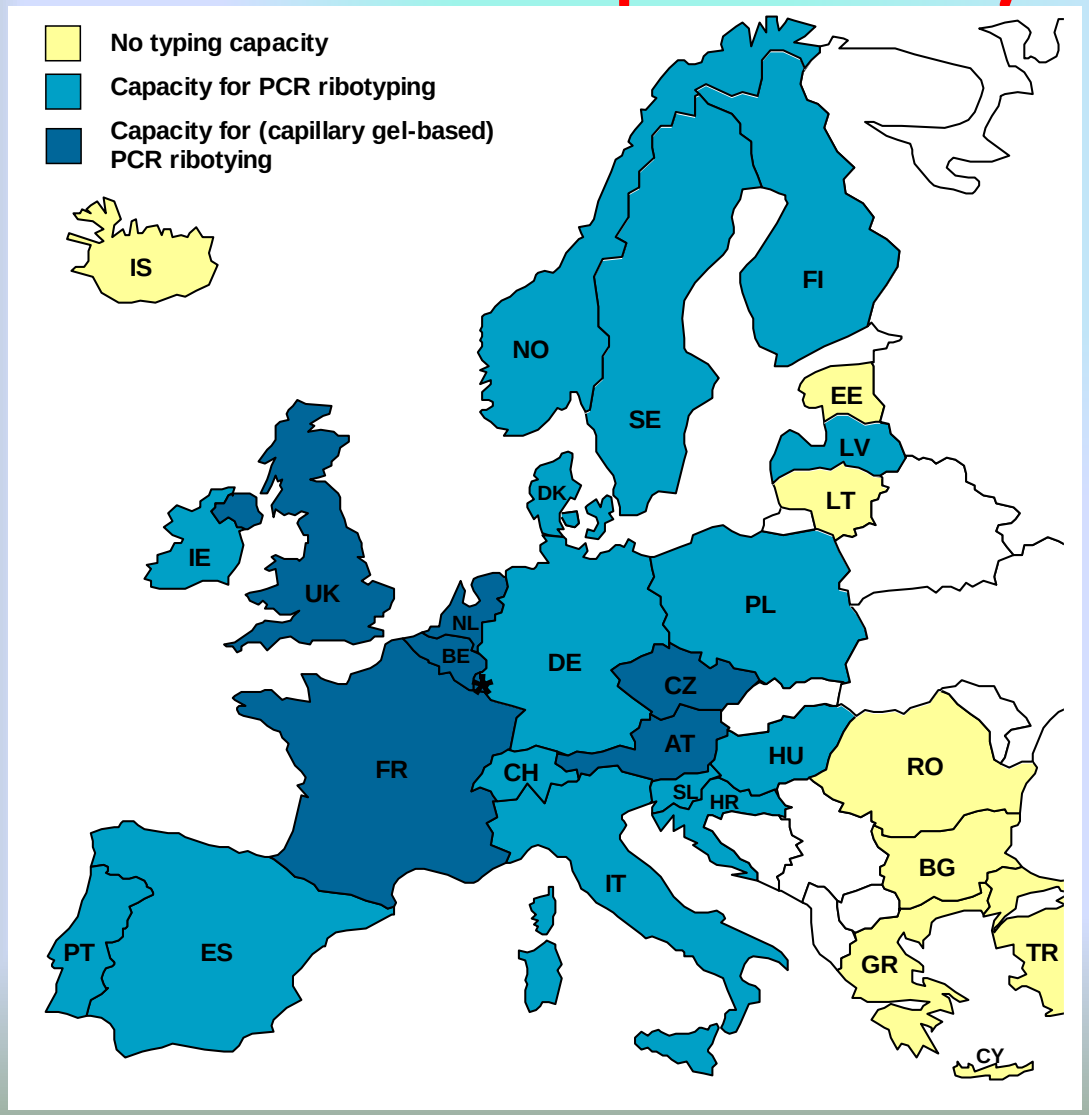
Typing capacity

Country level: 23 countries **(74%)** at least one laboratory was capable to perform typing

Polymerase chain reaction (**PCR) ribotyping** was most freqently (n=49, 89%) performed method to type isolates at the laboratories; 36 laboratories reported performance of only conventional agarose gel-based ribotyping on their samples compared to 10 for capillary gel based ribotyping (7 countries)

Other methods: PFGE, MLVA, MLST, TcdC typing, diversilab, rep-PCR.

Typing capacity and capacity for (capillary gel-based) PCR ribotyping, demonstrated per country



Conclusion

Across Europe CDI is diagnosed approximately once every 500 admissions: increased with 5 patients per 10,000 admissions compared to 2008

Virtually all European laboratories had diagnostic tests available for CDI, but reasons for testing varied widely

The majority of the laboratories were capable to culture, but half had capacity for typing

Future of molecular typing methods to study *Clostridium difficile* infections

C.W. Knetsch[1], T.D. Lawley[2], M.P.M. Hensgens[1], J. Corver[1], M. W. Wilcox[3] and E. J. Kuijper[1*].

Global epidemiology: CE-PCR ribotyping

Transmission: in general sequence based methods to determine whole genome single nucleotide polymorphism

Acknowledgements

Marjolein Hensgens (LUMC)
Sofie van Dorp (LUMC)
Martijn Bauer (LUMC)
Jeroen Corver (LUMC
Hans van Leeuwen (LUMC)
Wiep Klaas Smits (LUMC)
Dennis Bakker LUMC
Wilco Knetsch (LUMC)

Celine Harmanus (LUMC)
Ingrid Sanders (LUMC)

Daan Notermans, RIVM
Birgit van Benthem, RIVM

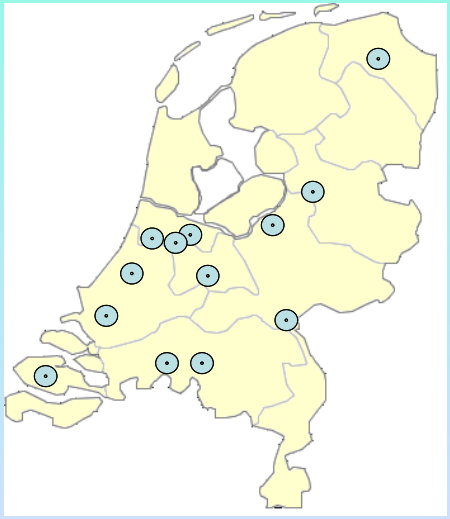
Liny Keessen (Utrecht)
Len Lipman (Utrecht)

Clostridium difficile ribotype does not predict severe infection

Seth T. Walk[1,2], Dejan Micic[1], Ruchika Jain[1,2], Eugene S. Lo[1], Itishree Trivedi[1], Eugene W. Liu[1], Luay M. Almassalha[1], Sarah A. Ewing[1], Cathrin Ring[1,2], Andrzej T. Galecki[1,3,4], Mary A. M. Rogers[1], Laraine Washer[1,5], Duane W. Newton[6,7], Preeti N. Malani[1,2,9], Vincent B. Young[1,2,8], and David M. Aronoff,[1,2,8,*]

Study

- 2006-2009 all hospitalized CDI pt in 13 hospitals
- CDI: diarrhoea & positive *C. difficile* toxin test
- *C. difficile* typed by PCR ribotyping



Data

- At time of diagnosis: age, sex, medication and co-morbidity
- After 1 year check survival in Dutch Civil Registration System

Analysis

- CDI-related mortality → **subset:** compare CDI patients to controls
  - ↓ non-diarrhoeal control; matched on: hospital, ward of diagnosis, duration of hospitalization
  - → **all:** death certificates (national registry)

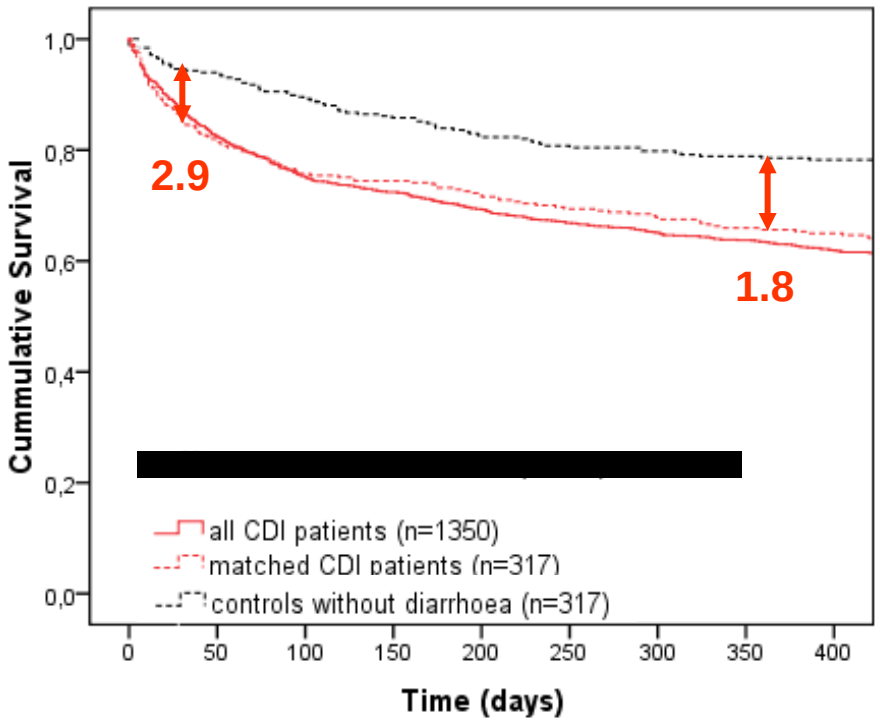
- Nummerous different PCR ribotypes

CDI pt vs. Controls

- Older
- More comorbidity
- More AB usage

	CDI patients (n=1366)	Subgroup of CDI patients (n=317)	Matched controls without diarrhoea (n=317)
Mean age, yr (±SD)	62,6 (±21.6)	61,9 (±21.1)	59,6 (±21.2)
Age > 65 years	57,0%	55,2%	47,6%
Male sex	50,7%	53,0%	52,1%
Hospital service			
Internal medicine	62,6%	61,8%	60,6%
Surgery	20,5%	22,1%	24,0%
Health-care association	86,0%	86,1%	-
Underlying diseases			
Neoplasms	27,6%	30,3%	28,8%
Respiratory system diseases	30,0%	25,1%	20,3%
Digestive system diseases	30,4%	30,1%	20,9%
Circulatory system diseases	43,9%	54,4%	50,6%
Genitourinary system diseases	32,8%	35,1%	24,8%
Endocrine diseases	22,2%	25,0%	26,3%
Antibiotic therapy	82,4%	83,5%	58,6%
Cytostatic agents	15,5%	16,2%	11,1%
Immunosuppressive agents	35,5%	43,6%	33,1%
Most common PCR ribotypes	**(n=689)**	**(n=172)**	
014	16,3%	18,6%	-
078	11,0%	12,2%	-
001	8,3%	4,7%	-
027	8,0%	7,6%	-

Prognosis of CDI patients vs. Cont



	Rate Ratio, HR (95% CI)
	Unadjusted
< 30 days	2.9 (1.7-5.1)
<1 year	1.8 (1.3-2.4)

	< 30 days	**< 1 year**
Death, no. (%)		
matched CDI patients	14,8%	34,4%
controls without diarrhoea	5,4%	21,5%

CDI-related mortality

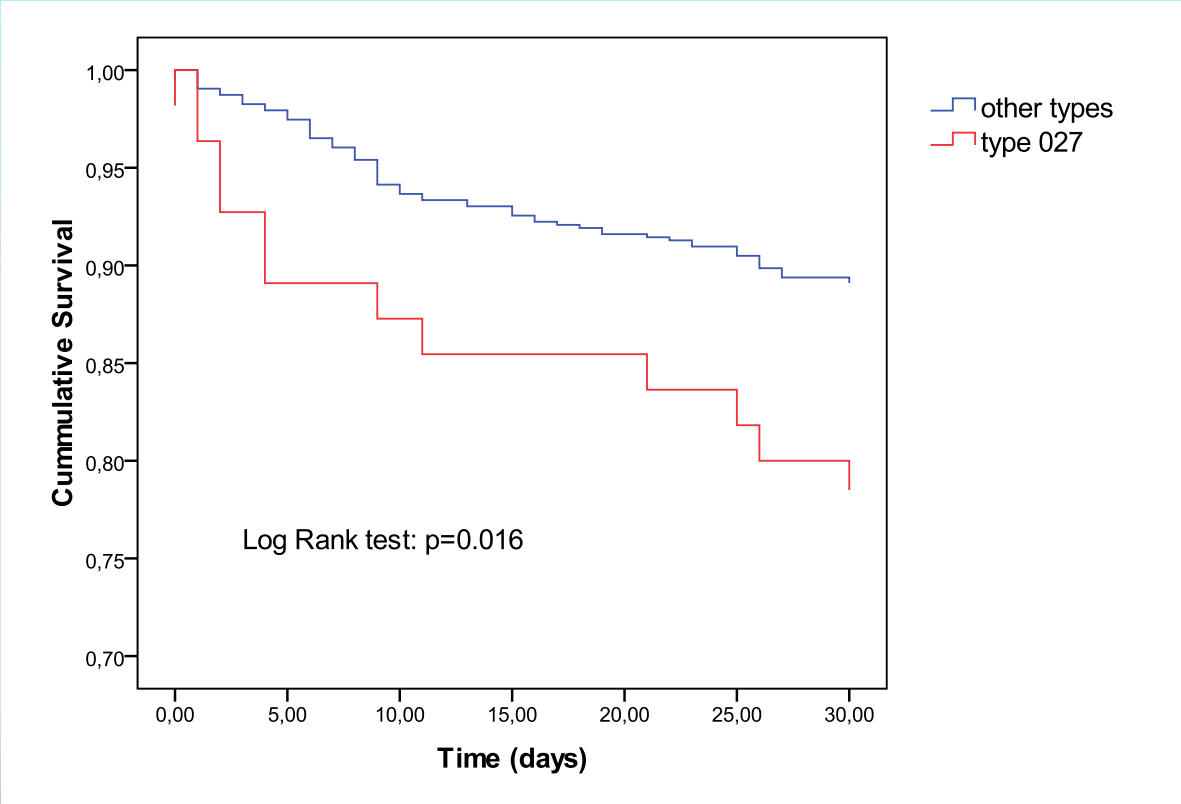
About 8% after 30 days

(5.4 * 2.5 – 5.4)

Prognosis per PCR ribotype

Types

- 027 n=55 (8%) → >20% died after 30 days
- Other types n=634 (92%) → <10% died after 30 days



Limitations and Conclusions

Conclusion

- CDI-related mortality is high, even in an endemic setting
  → 2.5 times higher
- The majority of the deaths occur within 30 days
- Death certificates underestimate this rate

Oake. Ann Intern Med. 2011

Limitation

- Non-diarrhoeal patients as control group
  → Hospitalized patients: 10% has diarrhoea
- Residual confounding due to underlying diseases can be present
  → Chronic underlying diseases (Charlson)
  → Acute disease (duration of hospitalization)