



# Tipizzazione del *Clostridioides (Clostridium)* *difficile*

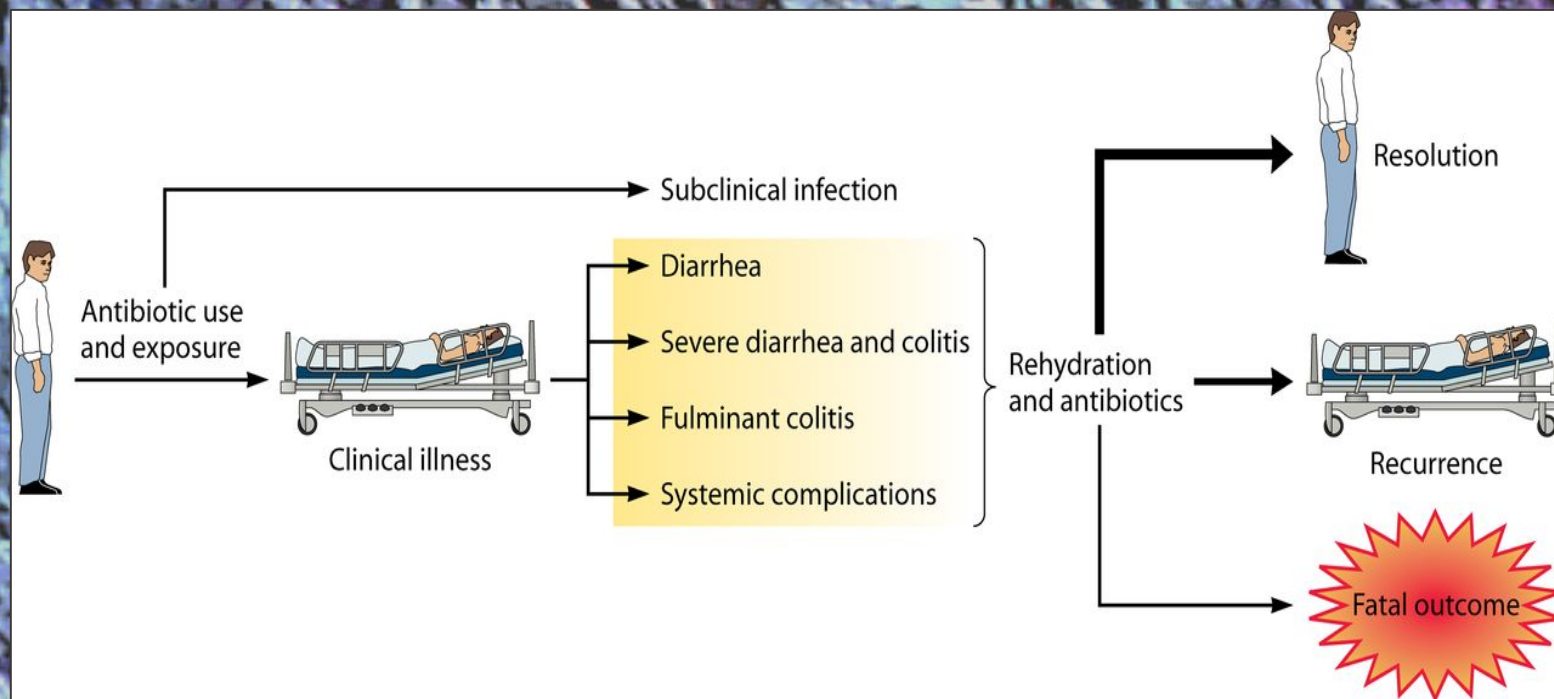
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# Infezione da *C. difficile* (CDI)



Microbiology and Molecular Biology Reviews. 2013. 77:567- 581



# HA-CDI

**Esordio dei sintomi in ospedale dopo 48 h dal ricovero  
oppure  
in comunità nelle 4 settimane successive a precedente dimissione ospedaliera**



## USA

- 453 000 CDI / anno
- 29 500 morti/ anno (diretta o indiretta)
- 1° agente responsabile di HAI

## EU

- 123 997 CDI / anno
- 9% mortalità (diretta o indiretta)
- 8° → 6° agente responsabile di HAI



[ecdc.europa.eu/en/publications/Publications/re-associated-infections-antimicrobial-use-](http://ecdc.europa.eu/en/publications/Publications/re-associated-infections-antimicrobial-use-)



# CA-CDI

**Esordio dei sintomi in comunità in assenza di ricovero nelle 12 settimane precedenti  
oppure  
in ospedale entro 48 dal ricovero in assenza di ricovero nelle 12 settimane precedenti**

**CA-CDI ↑**

**USA : 33% - 41% di tutti i casi di CDI**

**EU : 15%-23% di tutti i casi di CDI**

Journal of Hospital Infection 99 (2018) 436–442  
Available online at [www.sciencedirect.com](http://www.sciencedirect.com)  
Journal of Hospital Infection  
journal homepage: [www.elsevier.com/locate/jhin](http://www.elsevier.com/locate/jhin)

Healthcare Infection Society

Community-acquired *Clostridium difficile*:  
epidemiology, ribotype, risk factors, hospital and  
intensive care unit outcomes, and current and  
emerging therapies

Tripathi<sup>a</sup>, D. Ramai<sup>a,b,\*</sup>, M. Dhawan<sup>a</sup>, F. Mustafa<sup>a</sup>, J. Gasperino<sup>c</sup>, M. Reddy<sup>a</sup>

Journal of Hospital Infection. 2018. 99: 436e442

**In molti casi assenza dei tradizionali fattori di rischio**



# Cosa è cambiato?

Emergenza di nuovi tipi di *C. difficile*

Acquisizione di nuove caratteristiche

- Aumento numero casi e gravità delle infezioni
- Aumento delle ricorrenze / fallimenti terapeutici
- Elevata mortalità



# Le SOP dell'ECDC



TECHNICAL REPORT

Laboratory procedures for  
diagnosis and typing of  
human *Clostridium difficile*  
infection

[www.ecdc.europa.eu](http://www.ecdc.europa.eu)

European Centre for Disease Prevention and Control

Laboratory procedures for diagnosis and typing of  
human *Clostridium difficile* infection

Stockholm: ECDC; 2018



# La tipizzazione del CD

Alcohol shock o heat shock



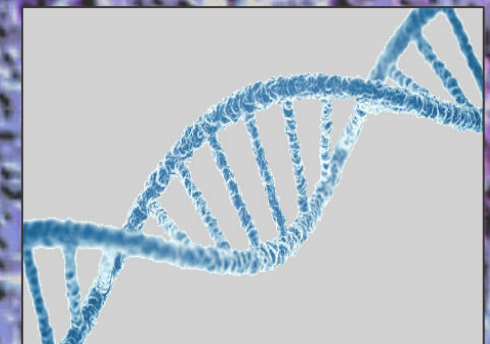
**Isolamento  
*C. difficile***



**Identificazione  
Test per le tossine**



**Estrazione DNA genomico**





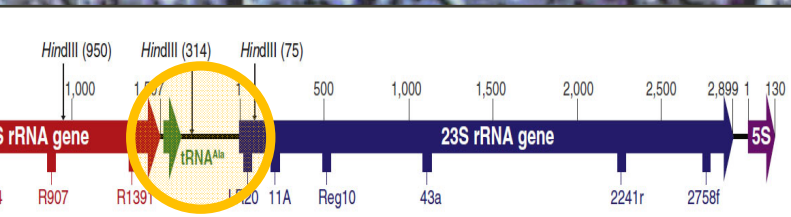
# La tipizzazione del CD

Method	Target	Discriminatory power	Typeability	Reproducibility	Ease of interpretation	Technical complexity	Transportability
Band-based							
REA	Whole genome	Good	Fair	Fair	Poor	Moderate	Poor
PFGE	Whole genome	Moderate	Fair	Moderate	Fair	Moderate	Moderate
PCR ribotyping	16S-23S ISR	Good	Moderate	Moderate	Moderate	Low	Moderate
Capillary PCR ribotyping	16S-23S ISR	Excellent	Moderate	Good	Good	Moderate	Good
MLVA	Whole genome, tandem repeats	Excellent	Poor	Moderate	Good	Moderate	Moderate
Sequence-based							
MLST 7HG	7 HG	Good	Moderate	Moderate	Excellent	Moderate	Excellent
SNP typing	Whole genome, SNPs	Excellent	Moderate	Moderate	Excellent	High	Good

veill. 2013;18(4):pii=20381

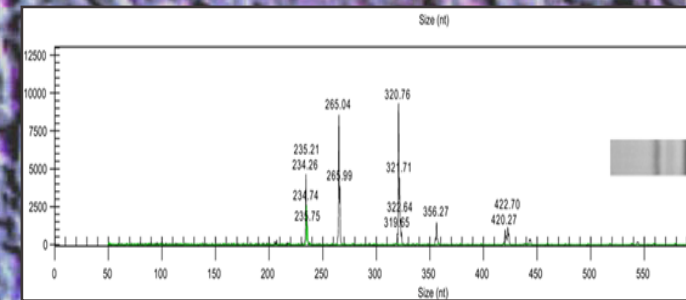
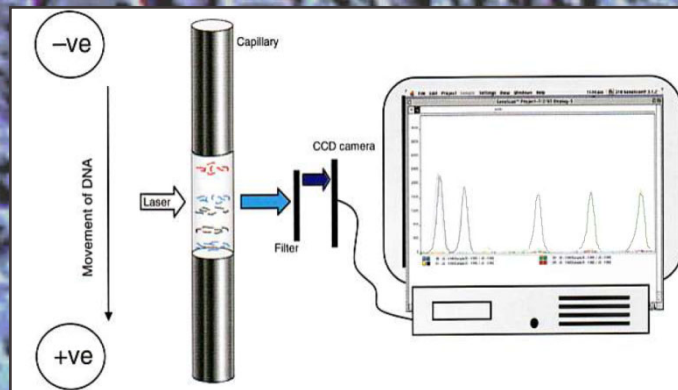


# Capillary PCR-ribotyping



Operoni rRNA

Amplificazione delle regioni inter-geniche



WEBribo database

The Austrian Agency for Health and Food Safety

Identificazione Ribo-Type (R)



# RT in Europa

PREVALENCE AND OUTBREAK REPORT

Diversity of *Clostridium difficile* PCR ribotypes in Europe: results from the European, multicentre, prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea (EUCLID), 2012 and 2013

Shawlin<sup>1</sup>, CM Longshaw<sup>2</sup>, DA Burns<sup>2</sup>, GL Davis<sup>1</sup>, MH Wilcox<sup>1</sup>, on behalf of the EUCLID study group<sup>3</sup>  
<sup>1</sup>Centre for Biomedical and Clinical Sciences, University of Leeds, Leeds, United Kingdom  
<sup>2</sup>EMA EMEA, Chertsey, United Kingdom  
<sup>3</sup>The EUCLID study group are listed at the end of the article  
 Correspondence: Mark Harvey Wilcox (mark.wilcox@nhs.net)

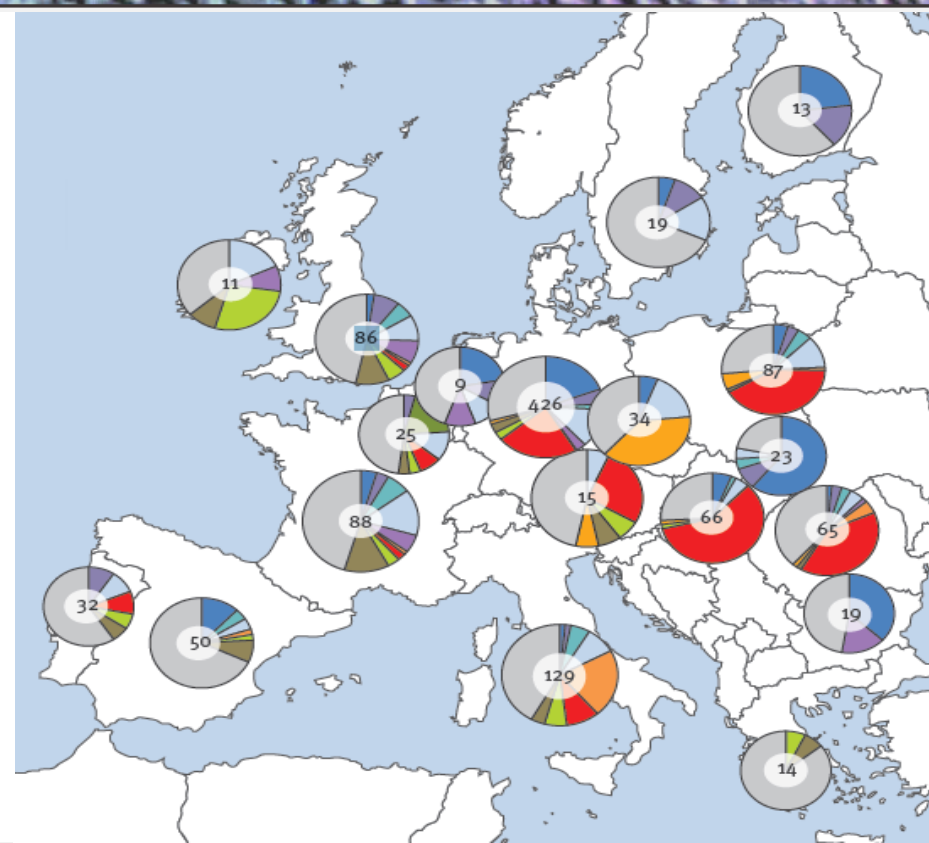
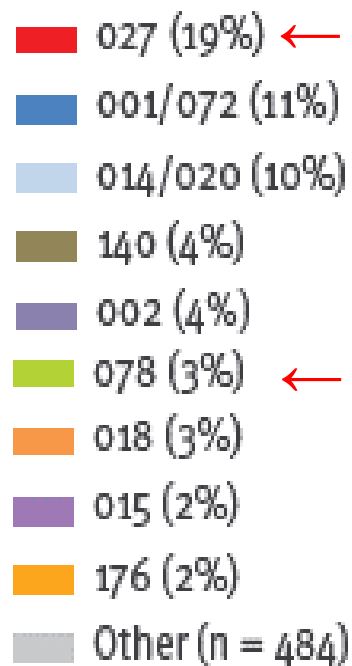
Journal: *Journal of Antimicrobial Chemotherapy*, 2016;21(29):pii=30294

19 paesi EU

1196 ceppi

125 RT identificati

## Overall ribotype prevalence



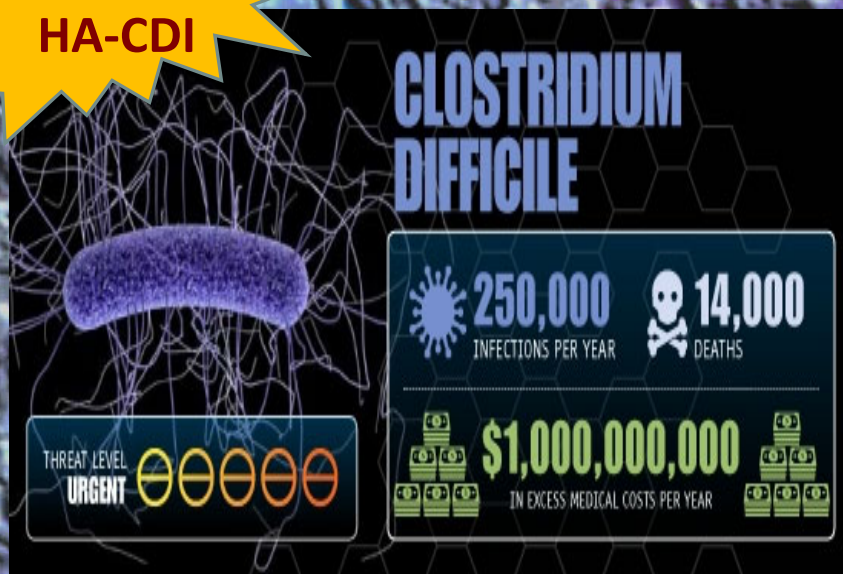


# “Highly virulent” RT

- Diffusione a livello mondiale
- Infezioni gravi / ricorrenze
- Elevata mortalità

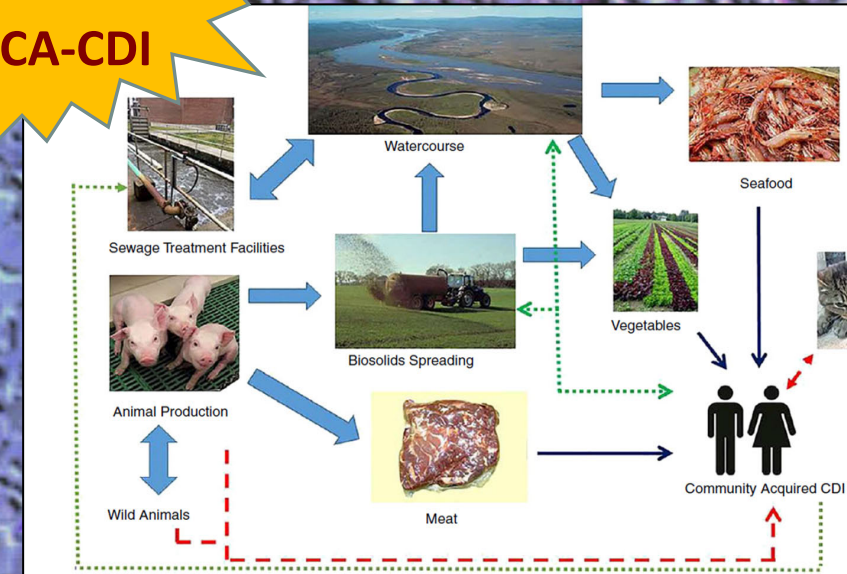
RT 027

HA-CDI



RT 078

CA-CDI



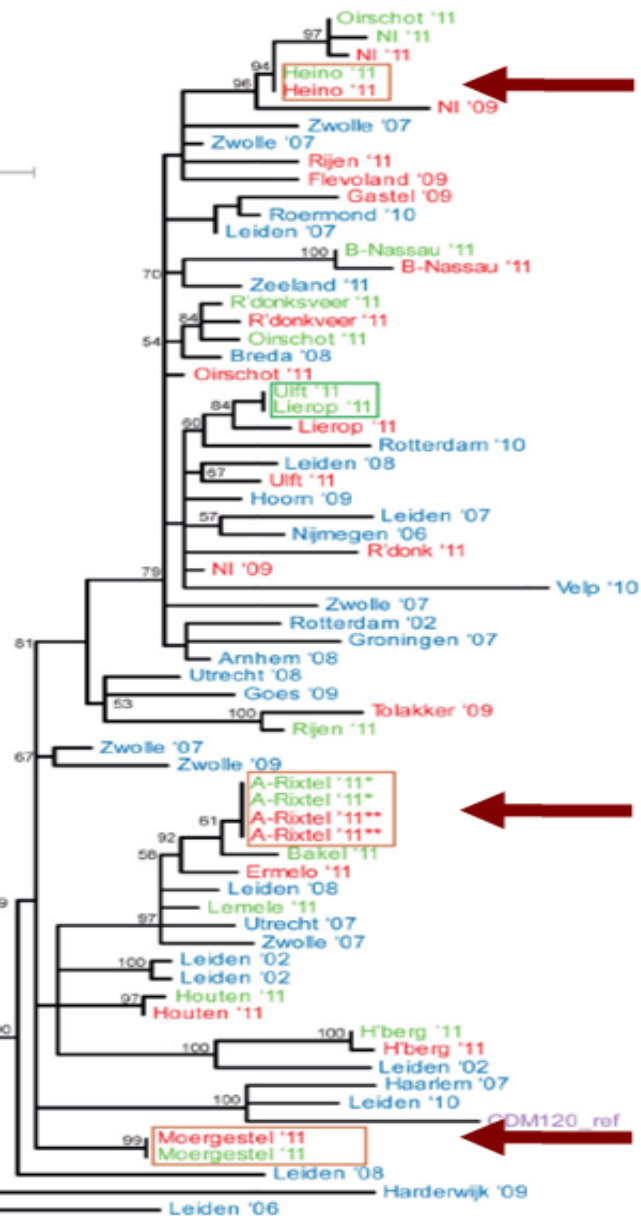
J Appl Microbiol. 2017. 122:542-553



B)

Pig
Farmer
Clinical

10 SNPs



WGS di 65 isolati RT078 isolati dal 2002 al 2011

Euro Surveill. 2014. 19, 20954.

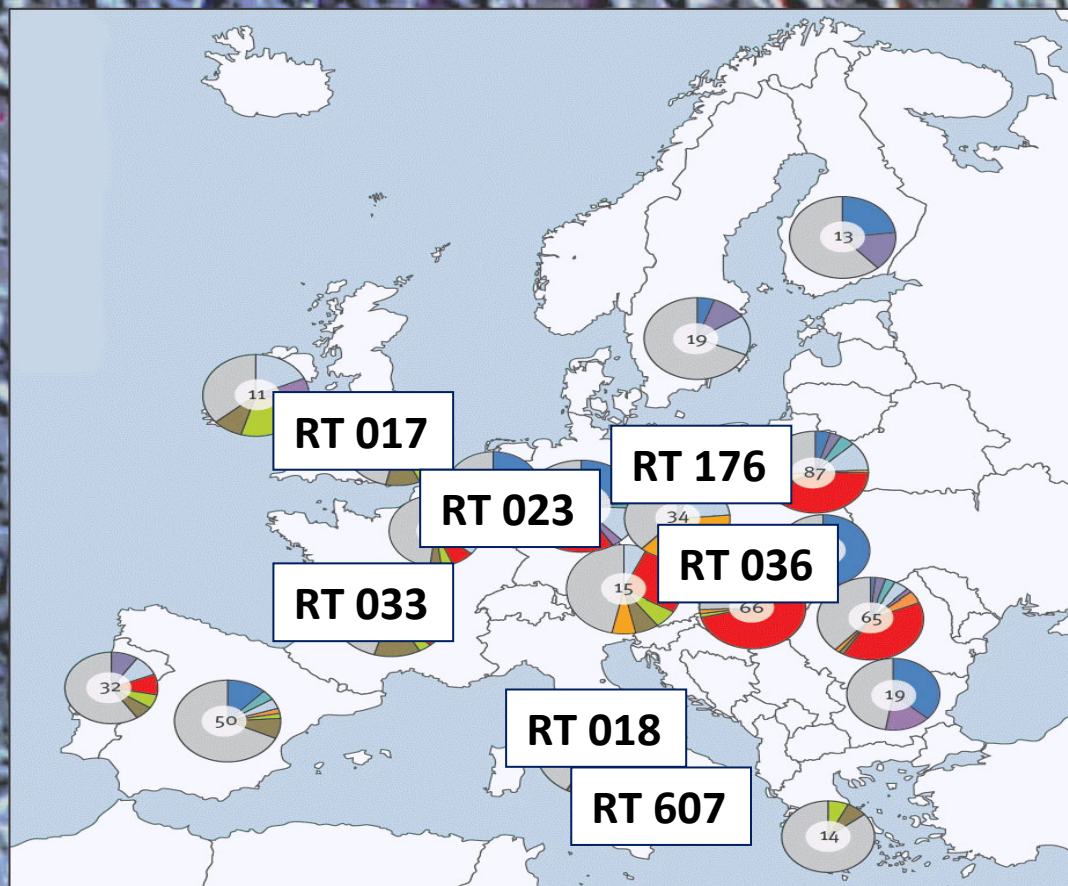
Whole genome sequencing reveals potential spread of *Clostridium difficile* between humans and farm animals in the Netherlands, 2002 to 2011

C W Knetsch<sup>1</sup>, T R Connor<sup>2</sup>, A Mutreja<sup>3</sup>, S M van Dorp<sup>1</sup>, I M Sanders<sup>1</sup>, Harris<sup>3</sup>, L Lipman<sup>4</sup>, E C Keessen<sup>4</sup>, J Corver<sup>1</sup>, E J Kuijper<sup>1</sup>, and T D Law

Stessi cloni RT 078 negli uomini e nei suini: possibile trasmissione interspecifica



# RT emergenti



**RT 036:** Ungheria

**RT 023:** Germania

**RT 176:** Polonia, Repubblica Ceca, Croazia

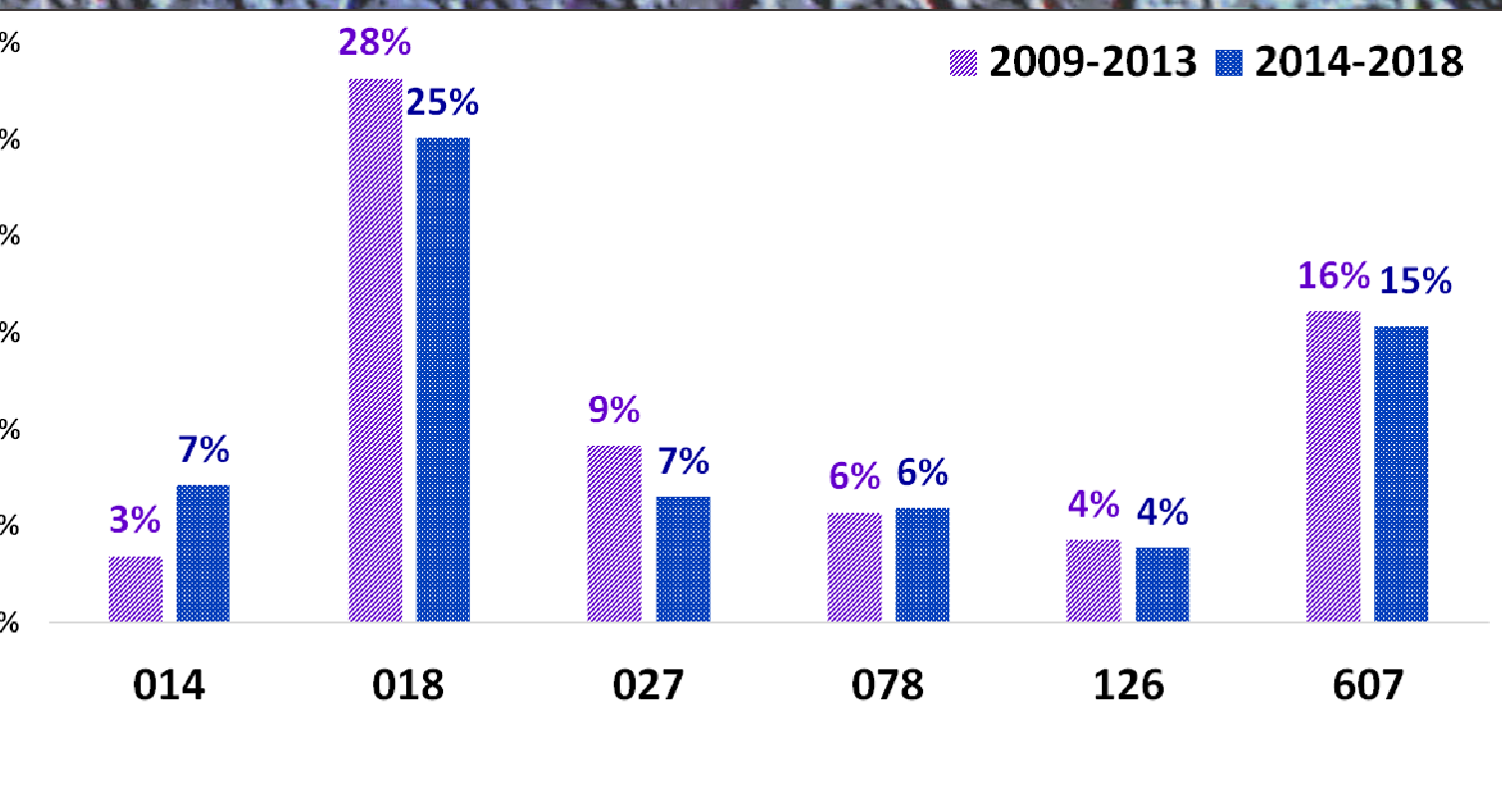
**RT 017:** UK, Olanda, Germania

**RT 033:** Francia

**RT 018 e RT 607:** Italia



# RT in Italia (dati ISS)



2009 – 2018

980 ceppi

100 RT identificati

RT 018 e RT 607  
predominanti e  
filogeneticamente correlati



# Le tossine

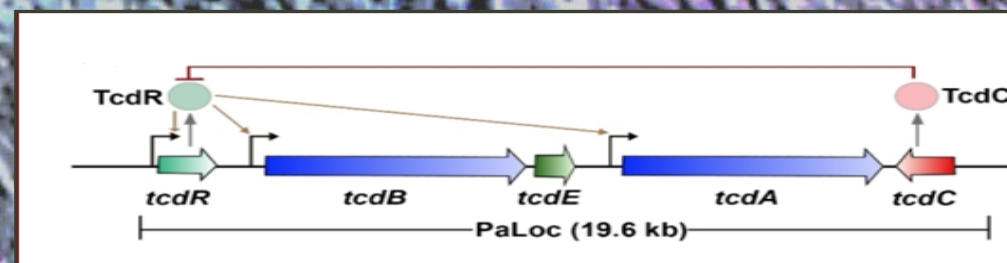
TECHNICAL REPORT

Laboratory procedures for diagnosis and typing of human *Clostridium difficile* infection

Standard operating procedure for  
multiplex PCR for characterisation of  
*Clostridium difficile*

Laboratory procedures for diagnosis and typing of human *Clostridium difficile* infection. Stockholm: ECDC; 2018.

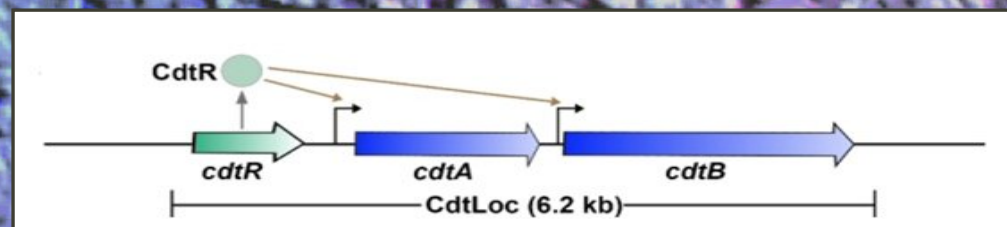
## PaLoc



Tossina B

Tossina A

## CdtLoc



Tossina Binaria (CDT)



# I profili tossinogenici

**+B+CDT-**  
più diffuso

**Clade 1**

(RT 001 - RT 014 - RT 018 - RT 607 e molti altri)

**-B+CDT-**  
solo tossina B → CDI

**Clade 4**

(RT 017)

**+B+CDT+**  
V e la molti RT emergenti

**Clade 2**

(incluso **RT 027-lineage**: RT 016 - RT 036 - RT 080 - RT 106 - RT 176 - RT 244 - RT 320)

**Clade 5**

(incluso **RT 078-lineage**: RT 066 - RT126 - RT 620)

**Clade 3**

(RT 023)

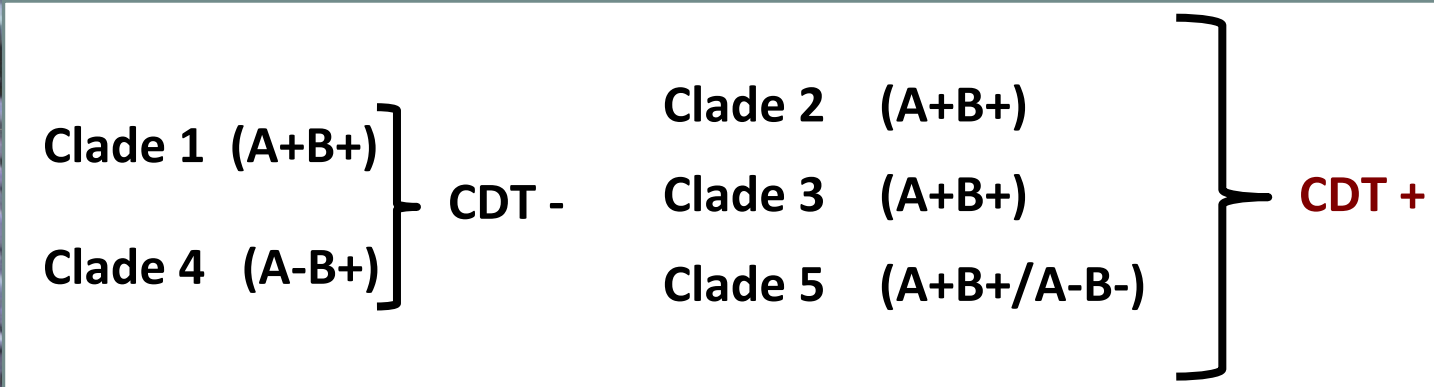
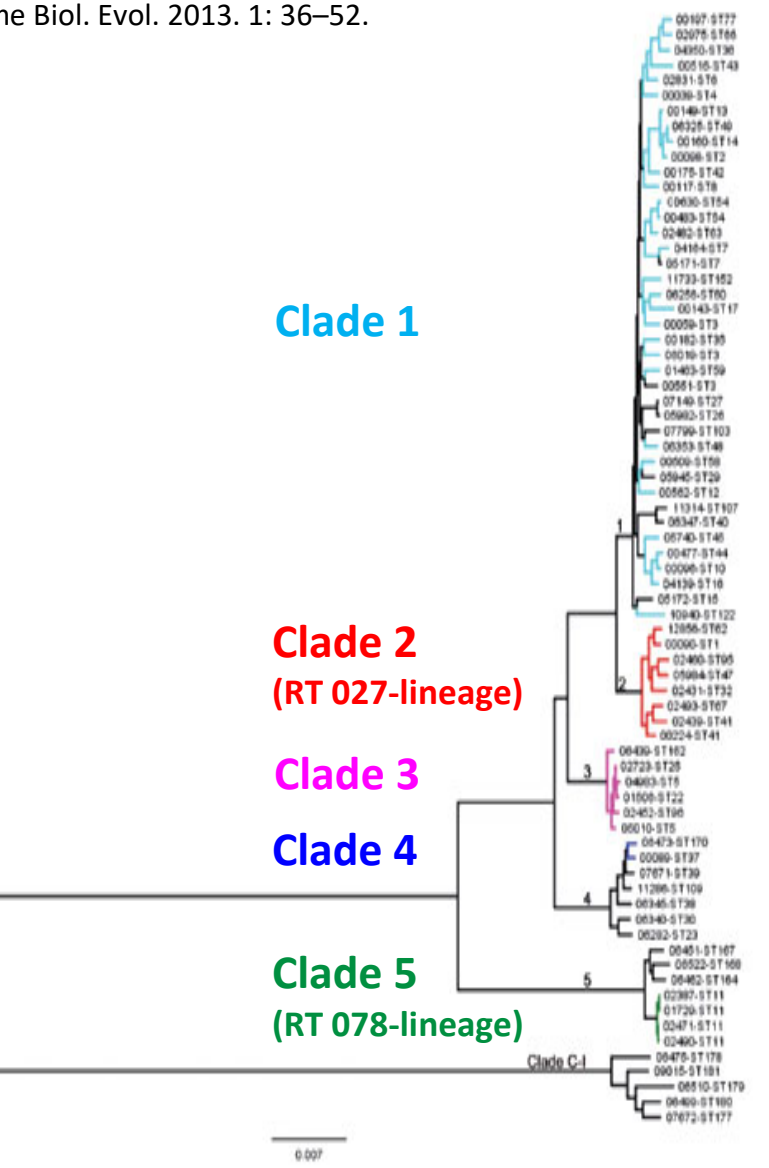
**-B-CDT+**  
solo CDT → CDI

**Clade 5**

(RT 033)

Identificazione solo tramite NAAT per CDT





**Ceppi CDT+**

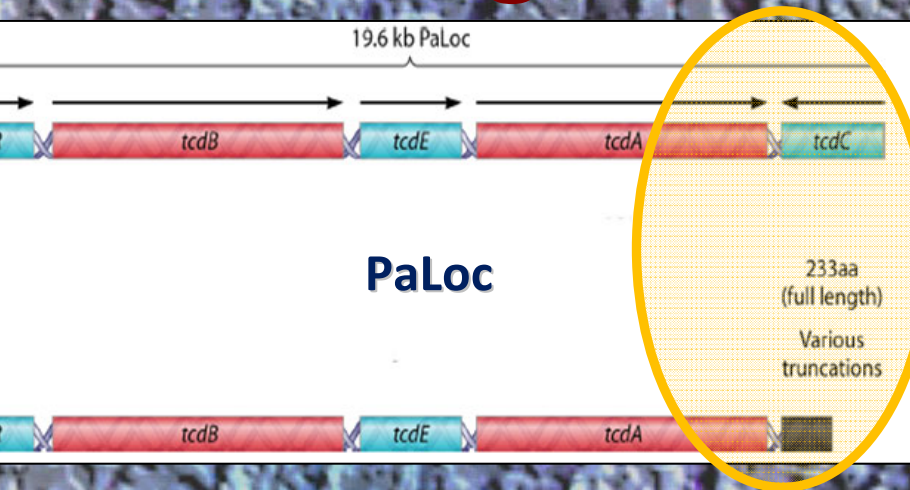
**USA 30% ↑ EU 23%**

**Associazione tra CDT e aumento della mortalità a 30 g indipendentemente da RT di appartenenza**

Gut Microbes. 2014. 5:1, 15–27



# Variazioni genetiche *tcdC*



azioni *tcdC*

No attività regolatore negativo

Iperproduzione tossine A e B

RT 078: C184T

RT 027:  $\Delta$ 117



$\Delta$ 117 : RT 016 - RT 036 - RT 080 - RT 328 - RT 176 - RT

I sistemi molecolari che usano  $\Delta$ 117 come marker molecolare non danno una tipizzazione dell'RT 027 ma solo una «identificazione presuntiva»

Solo la tipizzazione con Capillary PCR-ribotyping permette di identificare l'RT di appartenenza

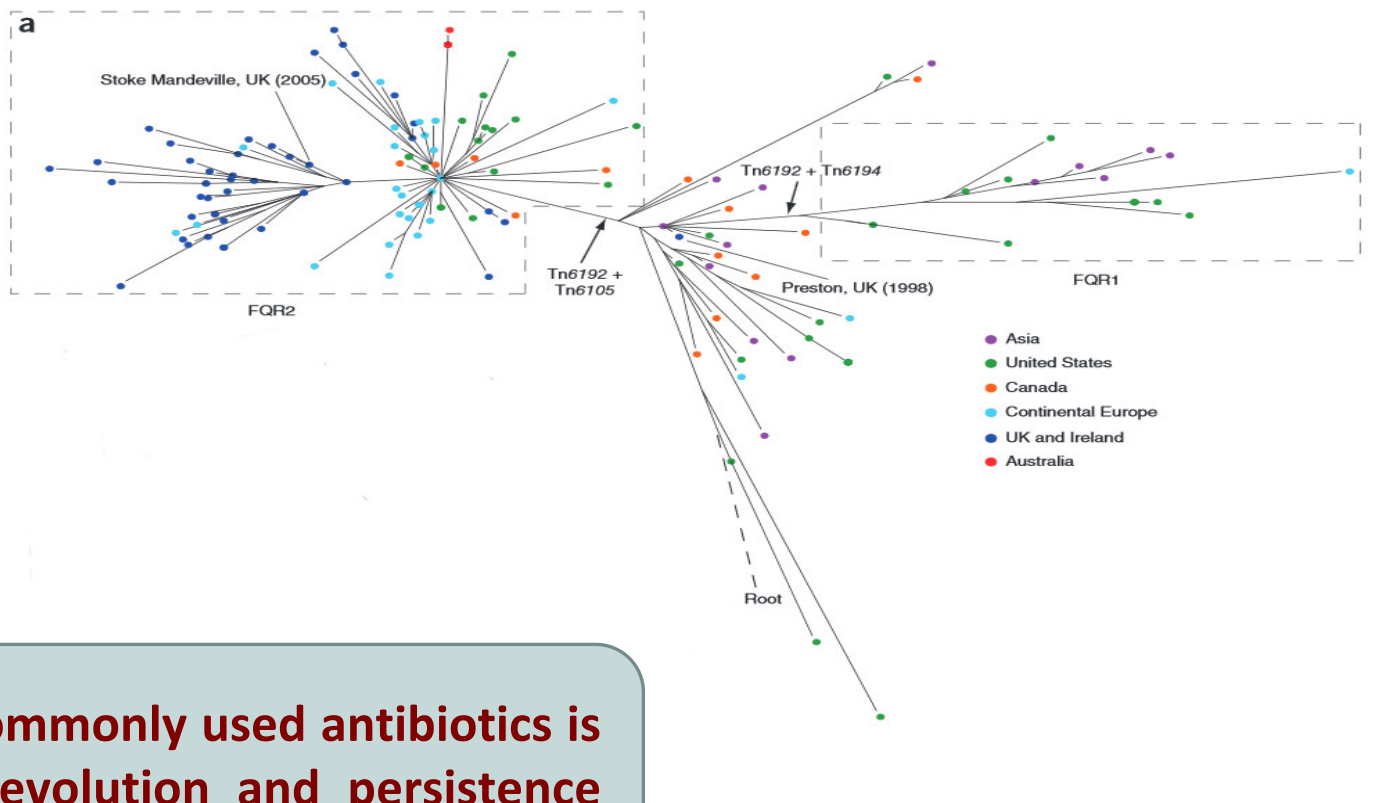


# WGS di 151 ceppi RT 027 isolati dal 1985 al 2010

Nat Genet. 2013. 45: 109-113

## Evolution and global spread of epidemic healthcare-associated *Clostridium difficile*

Yoshinori Miyajima<sup>2,3</sup>, Paul Roberts<sup>2,3</sup>, Louise Ellison<sup>1</sup>, Derek J Pickard<sup>1</sup>, Melissa J Martin<sup>4</sup>, Simon R Harris<sup>1</sup>, Derek Fairley<sup>5</sup>, Kathleen B Bamford<sup>6,7</sup>, Stephanie D'Arcy<sup>6,7</sup>, Jon Brazier<sup>8</sup>, John E Coia<sup>9</sup>, Gill Douce<sup>9</sup>, Dale Gerding<sup>10</sup>, Hee Jung Kim<sup>11</sup>, Tse Hsien Koh<sup>12</sup>, Haru Kato<sup>13</sup>, Junji Koh<sup>13</sup>, Tom Louie<sup>14</sup>, Stephen Michell<sup>15</sup>, Emma Butt<sup>15</sup>, Sharon J Peacock<sup>1,16-18</sup>, Nick M Brown<sup>17,18</sup>, Helen Songer<sup>20</sup>, Mark Wilcox<sup>21</sup>, Munir Pirmohamed<sup>2,3</sup>, Ed Kuijper<sup>22</sup>, Peter Hawkey<sup>23</sup>, Gordon Dougan<sup>1</sup>, Julian Parkhill<sup>1</sup> & Trevor D Lawley<sup>1</sup>

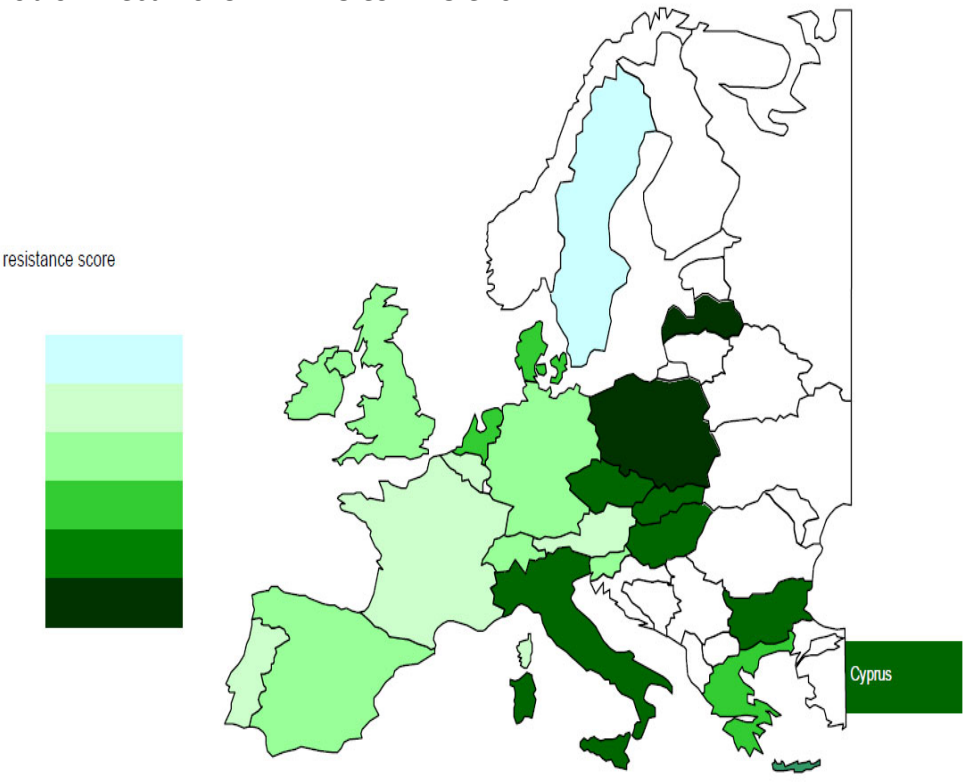


...the acquisition of resistance to commonly used antibiotics is a major feature of the continued evolution and persistence of *C. difficile* 027/BI/NAP1 in healthcare settings”



# La sensibilità agli antibiotici

Microbiol Infect. 2015. 21: 248.e9–248.e16



## Europa:

60% dei ceppi isolati da CDI sono MDR ↑

MDR pattern: FQs - CFs - MLS<sub>B</sub>

Adv Exp Med Biol. 2018;1050:137-159

## Italia (dati ISS):

62% dei ceppi è MDR:

RT 018 e RT 607 → 100% MDR

MDR pattern: ERY- CLI - MXF - RIF



# RT 018: un «insospettabile» RT di successo

- Non appartiene al Clade 2 o 5
- No CDT
- No variazioni TcdC

- Infezioni gravi ed elevata mortalità

RT018	11.7%
RT 027 (HV)	14.3%

BMC Infectious Diseases (2016) 16:656

European Journal of Internal Medicine 54 (2018) 21-26

- Indice di trasmissione durante outbreak  
>10 volte rispetto a RT 078 (HV)

J. Clin. Microbiol. 2015. 53: 2575–2580



AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements | CrossMark

Draft Genome Sequence of *Clostridium difficile* Strain IT1118, an Epidemic Isolate Belonging to the Emerging PCR Ribotype 018

François Wasels,\* Fabrizio Barbanti, Patrizia Spigaglia

Anaerobe 42 (2016) 123–129

Contents lists available at ScienceDirect

Anaerobe

journal homepage: [www.elsevier.com/locate/anaerobe](http://www.elsevier.com/locate/anaerobe)

*Clostridium difficile* (including epidemiology)

Characterization of *Clostridium difficile* PCR-ribotype 018: A problematic emerging type

Fabrizio Barbanti, Patrizia Spigaglia\*

## RT 018 vs RT 078

> adesione cellule CaCO<sub>2</sub> a 15 g  
e  
> produzione tossine a 48h

> sporulazione a 24h  
e  
> R agli antibiotici

RT 018 predominante a 24h  
nei test di competizione *in vitro*

### *In vivo*

Rapida colonizzazione  
dell'intestino

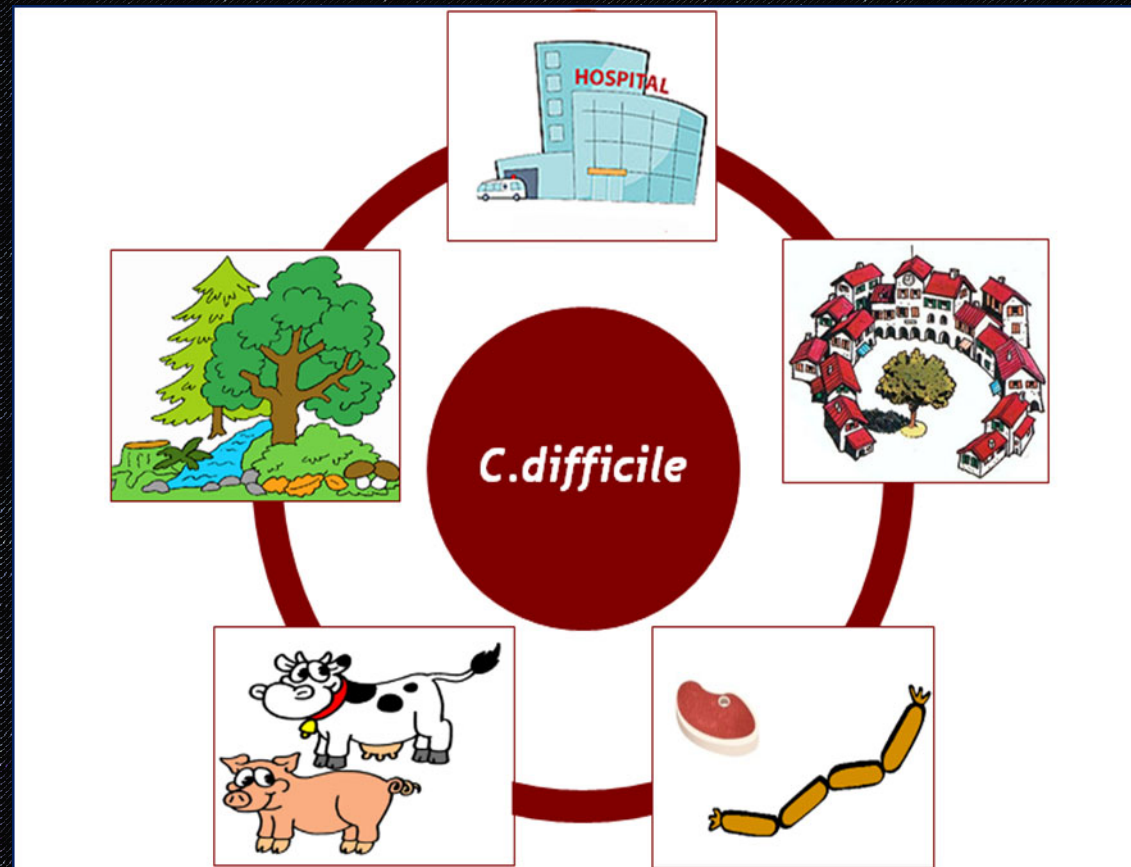
Elevata trasmissibilità  
e persistenza



“One Health sits firmly under the One Health umbrella, in which human health, animal health and the environment are inextricably linked”

L.E. Bloomfield – T.V. Riley

Caratteristiche che si modificano nel tempo



Lineage di successo  
RT di successo  
Cloni di successo



A microscopic view of numerous rod-shaped bacteria, likely E. coli, against a light blue background. The bacteria are rendered in a semi-transparent, glowing style, giving them a three-dimensional appearance. They are scattered across the frame, with some in sharp focus and others blurred in the background.

**Grazie per  
l'attenzione**