BIOMÉRIEUX

CLOSTRIDIUM DIFFICILE INFECTIONS

From Diagnosis to Outbreak Management

PIONEERING DIAGNOSTICS

INTRODUCTION

***Clostridium difficile* infection** (CDI) is now established as a worldwide threat, with major consequences for morbidity, mortality and healthcare costs. This educational booklet concentrates on the key information needed to understand, diagnose, control and treat CDI, taking into account the large expansion of knowledge that has occurred in response to the emergence of *C. difficile* in the first decade of this millennium as a global threat.

This transformation of ***C. difficile*** was likely driven by four main factors:

- firstly, the **spread of epidemic strains** and, in particular, a so-called 'hypervirulent' clone, variably referred to as *C. difficile* ribotype 027/NAP1/BI, which is associated with increased morbidity and mortality, especially in the elderly;
- secondly, **sub-optimal infection control precautions** in many different healthcare settings likely contributed to the transmission of *C. difficile* strains, in particular those with epidemic potential;
- thirdly, **sub-optimal antimicrobial stewardship**, which provided a selective pressure for some antibiotic-resistant *C. difficile* strains, particularly the **fluoroquinolone-resistant** *C. difficile* ribotype 027/NAP1/BI clone;
- and lastly, **confusion about when, where and how best to test** for evidence of *C. difficile* infection has contributed to under-detection/under-reporting of cases and so has fueled the spread of this opportunistic pathogen.

As a high proportion of hospitalized patients receive antibiotics, this means that there are large numbers of potentially susceptible hosts who may acquire, be colonized by, transmit and/or become infected by *C. difficile*.

In short, *C. difficile* is a nosocomial pathogen that has found and exploited 'weaknesses' in our healthcare systems. *C. difficile* infection can be considered as a **healthcare quality indicator**, potentially reflecting infection control and antimicrobial prescribing practice, as is already the case in some countries.

Improved control of *C. difficile* requires a greater understanding of the pathogen, the at-risk hosts and how transmission occurs, as well as improved use, improved use of detection and diagnosis methods, and optimized treatment options.

Professor Mark H. Wilcox
B Med Sci, BM, BS, MD, FRCPath.

Consultant / Head of Microbiology Research & Development,
and Infection Lead of the Leeds NIHR Diagnostic Technologies,
Medical Technology and *In Vitro* Diagnostic Co-operative,
Leeds Teaching Hospitals NHS Trust, UK

This booklet provides essential information on the diagnosis, treatment and prevention of *C. difficile* infections.

Although not exhaustive, it is intended as a succinct and practical reminder for laboratory professionals and clinicians.

For easy reading and reference, look for the colored boxes highlighting the key points in each chapter.

CONTENTS

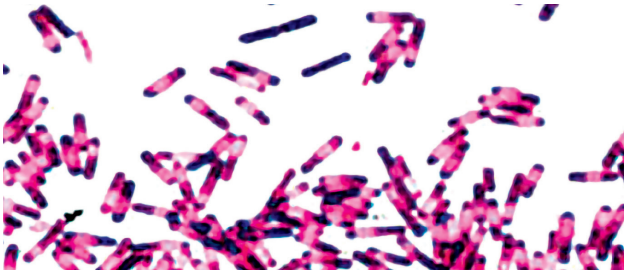
1 WHAT IS *CLOSTRIDIUM DIFFICILE* AND *C. DIFFICILE* INFECTION (CDI)?

What is *Clostridium difficile*?

Clostridium difficile is a naturally-occurring species of **Gram-positive bacteria** of the genus *Clostridium*. It has recently been proposed that the name *Clostridium difficile* is changed to *Clostridioides difficile*; either term is correct, and both abbreviate to *C. difficile* or "*C. diff*".

C. difficile is present in the large intestine of 1-3% of healthy adults and the majority of healthy infants (but these normally only remain colonized for 1-2 years at most).

Clostridia are motile, **anaerobic, spore-forming rods (bacilli)**. When stressed, the bacteria produce **spores** that are resistant to extreme conditions of heat, drying, and a wide range of antibiotics and chemicals, including some disinfectants.



Gram stain of *Clostridium difficile* (CDC)

How does *C. difficile* cause CDI?

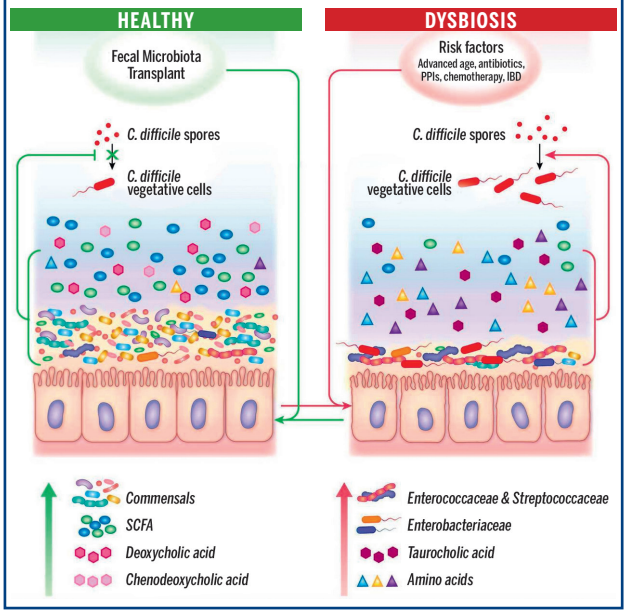
C. difficile proliferates in the human colon when there is a **modification of the normal/healthy microbiota** (bacterial intestinal flora).

A 'healthy colonic' microbiota contains commensal bacteria, short chain fatty acids, chenodeoxycholate and deoxycholate (**secondary bile acids**), which together inhibit germination of *C. difficile* spores, and growth of vegetative cells (**Figure 1 - Healthy**).

By contrast, **when the microbiota is damaged** (e.g. antibiotic exposure) *Enterobacteriaceae*, *Enterococcaceae* and *Streptococcaceae* increase, alongside an environment enriched with **amino acids** and **primary bile acids**, which promotes *C. difficile* germination, colonization and toxin production (**Figure 1 - Dysbiosis**).

Figure 1: Microbial and metabolite status during health and disease

Adapted from Ross CL, et al. *Anaerobe* 2016;41:37-43



Only **pathogenic (toxigenic) strains** of *C. difficile* cause CDI, due to the production of one or two distinct **toxins (A and B)**, which damage the colonic cells, causing cell breakdown and an inflammatory response. Recent evidence highlights a **key role for toxin B in humans** *[Wilcox et al, 2017]*. Another toxin, **binary toxin** is also expressed in some virulent strain groups, and may be associated with worse outcomes, including mortality *[Cartman et al, 2010; Berry et al, 2017]*.

Non-toxigenic strains do not cause clinical illness, but may provide protection against colonization with toxigenic strains and so against CDI *[Gerding et al, 2015; Crobach et al, 2018]*. Some but not all individuals develop a **host antitoxin antibody response**, which can provide some protection against CDI and/or recurrent CDI *[Kyne et al, 2000; Kyne et al, 2001]*.

What is the clinical presentation of CDI?

Clostridium difficile infection is most often an **antibiotic-induced illness**, often **contracted in hospitals or healthcare institutions**, due to presence of elderly, colonized patients with increased potential for transmission.

The usual symptoms of CDI may include any or all of the following (but especially the first two):

- **watery diarrhea**
- **lower abdominal cramps**
- abdominal bloating
- nausea
- fever

Mucus or pus (very occasionally blood) may be found in the stools. Leukocytosis, sometimes extremely high, may also accompany CDI.

Symptoms generally **start during antibiotic therapy**, or **up to 1 month after completion**.

Populations most at risk of CDI include:

- people who take antibiotics
- prolonged stay in healthcare facility
- the elderly (>65 yrs)
- those with a serious underlying illness
- the immunocompromised
- possibly patients taking proton pump inhibitors or other antacid medications, although doubt remains whether these are truly causal relationships *[Novack et al, 2014]*.

Which antibiotics are associated with an increased risk of CDI?

Almost all antibiotics can induce CDI, particularly because patients are often exposed to more than one antibiotic either simultaneously or sequentially *[Stevens et al, 2011]*. **Long duration antibiotic therapy** also increases the risk of CDI, probably as this means there is an extended risk period when *C. difficile* acquisition may coincide with antibiotic-induced damage to the colonic microbiota. For these reasons, **even 'low risk' antibiotics can still induce CDI**.

SEVERAL KEY FACTORS INTERACT TO DETERMINE THE ANTIBIOTIC RISK OF INDUCING CDI:

Extent of microbiome dysbiosis (Figure 1)

Antibiotics vary in both the **degree and duration of microbiome disturbance** they cause, thereby potentially providing a greater/longer opportunity for *C. difficile* strains to colonize, proliferate and cause disease. Antibiotics that cause profound dysbiosis of anaerobic components of the microbiome appear to be particularly associated with CDI.

Antibiotic penetration into the colon

Broad spectrum antibiotics that are **excreted predominantly via the gastrointestinal tract** are considered to be high CDI risk. This helps to explain the high CDI rates associated, for example, with ceftriaxone (which undergoes biliary excretion), as opposed to some renally-excreted antibiotics that have lower CDI risk. *[Khan et al, 2003; Dancer et al, 2013]*.

C. difficile strain antibiotic susceptibility

C. difficile strains that are **resistant** to an antibiotic have a competitive advantage and so may better survive antibiotic exposure and proliferate in the colon. Such strains may also be **more transmissible** if the antibiotic is commonly used in one particular setting. There is good evidence that fluoroquinolone prescribing is associated with CDI risk when the prevalent *C. difficile* strains are fluoroquinolone resistant *[Dingle et al, 2017]*. Clindamycin has been associated with CDI outbreaks in the context of a clindamycin-resistant *C. difficile* strain *[Pear et al, 1994; Climo et al, 1998]*.

In addition, frequently prescribed antibiotics may be more commonly associated with CDI (especially given multiple antibiotic exposure in an individual).

Taking all of these factors into consideration, **clindamycin, ampicillin, amoxicillin, cephalosporins and fluoroquinolones** have been most commonly associated with an increased risk of CDI.

Conversely, there is evidence that **tetracyclines** have a lower risk of CDI than most other antibiotics *[Tariq et al, 2018]*.

Why is CDI recurrence an important issue?

One of the major issues with CDI is the high recurrence rate.

Recurrence may occur due to:

- **relapse** (persisting infection with original strain)
- **re-infection** (infection with a new strain)

Recurrences usually occur within 4 weeks of the end of CDI treatment, but potentially up to 12 weeks. The risk of recurrences increases in the elderly and if (non-CDI treatment) antibiotics are administered during or after CDI treatment.

With each recurrence, the risk for further episodes increases even more: following treatment with metronidazole or vancomycin, CDI recurrence occurs in **~20% of first-time cases**, increasing to **40% to 60% after subsequent recurrences** *[Kelly and LaMont, 2008]*. The risk of death increases in patients with recurrent episodes *[Olsen et al, 2015]*.

Other risk factors for recurrence include **concomitant antibiotics, the frail elderly, immunodeficiency, strain type (e.g. ribotype 027), and a decreased host antibody response** against *C. difficile* toxins A and B *[Eyre et al, 2012; Debast et al,(ESCMID) 2014]*.

Patients with **inflammatory bowel disease**, especially ulcerative colitis, are at increased risk of both primary CDI and recurrences, and have increased associated morbidity and mortality *[Negron et al, 2016; Razik et al, 2016]*.

Several scoring systems have been proposed to determine the magnitude of risk of recurrent CDI, but these are not used in routine practice *[Hu et al, 2009; Eyre et al, 2012]*.

Currently, there are no commercial assays available to measure anti-*C. difficile* toxins A and B antibodies.

What is the clinical impact of CDI?

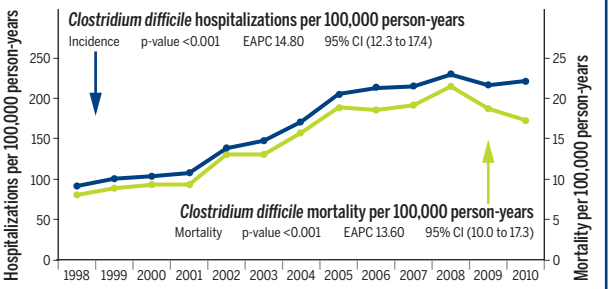
CDI is associated with excess morbidity, mortality and costs (**Figure 2**). **Between 1 in 6 and 1 in 16 patients die by day 30** after CDI diagnosis *[Lessa et al, 2012; Planche et al, 2013]*. Rapidly progressing, life-threatening CDI may require surgery (**colectomy**) because of **severe pseudomembranous colitis/toxic megacolon**.

In the US, *C. difficile* infections are linked to **~29,000 deaths per year** *[Lessa et al, 2015]*.

- Between 2000 and 2007, deaths related to *C. difficile* increased 400%, partly due to the increasing spread of the more virulent strain 027. Since this time, CDI cases in the US have peaked and started to decline.
- Over **90% of deaths** related to CDI occur in **patients aged 65 and older** *[CDC Vital Signs. March 2012]*.
- Reports show **all-cause mortality rates at 30 days** varying from **9-38%** *[Mitchell et al, 2012]*.

Figure 2: Burden of *Clostridium difficile*-Associated Hospitalizations in the United States

Adapted from McNabb-Baltar J, et al. *Gastroenterology* 2013;144(5)S1:S-239



EPAC: estimated annual percent change

KEY RISK FACTORS associated with mortality due to CDI include:

- increasing age
- concomitant antibiotics
- higher white cell count and creatinine levels at the time of CDI diagnosis
- lower albumin levels

What is the economic impact of CDI?

CDI has a considerable impact on healthcare resources, and the financial burden attributable to CDI is significant. Most excess cost is driven by **additional length of hospital stay** (**Figure 3**), which may account for >85% of patient management costs *[Wilcox et al, 2017, Tresman et al, 2018]*.

IN THE UNITED STATES, the annual economic burden of CDI on the U.S. healthcare system has been estimated to be as high as **$4.8 billion** in **excess costs in acute-care facilities** alone *[Dubberke et al., 2012]*.

Typical costs associated with a primary episode of CDI exceed **$10,000 per case** *[McGlone et al., 2012]*.

The attributable (US) inpatient cost of **recurrent CDI** by day 180 has been reported to be **$11,631 per case** *[Dubberke et al, 2014]*.

IN THE UK, a study in 6 acute hospitals on 64 adults hospitalized for recurrent CDI and 64 with a first episode only CDI demonstrated that the median total management cost for **recurrent CDI was £7,539 per patient and £6,294 for first time CDI** (cost difference, p=0.075); median length of stay (LOS) was **21 days** and **15.5 days**, respectively (p=0.269).

Subgroup analysis demonstrated the **highest median costs (£8,542/patient) in 43 severe recurrent CDI cases** *[Wilcox et al, 2017]*.

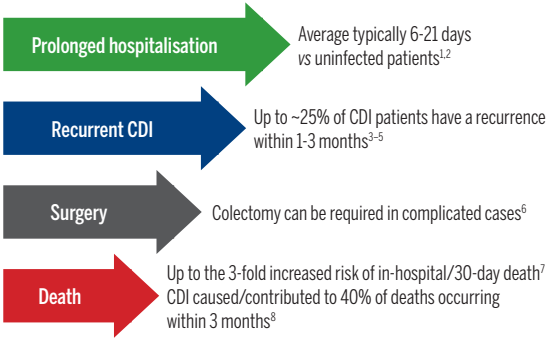
Compared with primary CDI, recurrences are associated with *[Olsen et al, 2015]*:

- **33% higher** mortality rate by day 180
- **2.5 times higher** hospital re-admission rates
- **4 times higher** hospital re-admission days

However, the total burden of disease is likely to be **significantly underestimated**, since the costs of recurrent CDI, adverse events caused by CDI, the cost of care in long-term care facilities, and societal costs have been poorly quantified. Furthermore, the burden of disease may rise significantly if CDI becomes increasingly common in the community.

Figure 3: CDI outcomes

Adapted from *personal presentation, M Wilcox*



1. Wilcox MH, et al. *J Hosp Infect* 1996;34:23–30
2. Forster AJ, et al. *CMAJ* 2012;184:37–42
3. Louie TJ, et al. *N Engl J Med* 2011;364:422–31
4. Cornely OA, et al. *Lancet Infect Dis* 2012;12:281–9
5. Vardakas KZ, et al. *Int J Antimicrob Agents* 2012;40:1–8
6. Bauer MP, et al. *Clin Microbiol Infect* 2009;15:1067–79
7. Hensgens M, et al. Abstract presented at ICAAC 2012; K-472
8. Bauer MP, et al. *Lancet* 2011;377:63–73

2 EPIDEMIOLOGY AND SURVEILLANCE

How frequent is CDI?

The epidemiology of CDI varies according to disease awareness (how frequently testing is carried out and the tests used – see **Laboratory Diagnostics** section, page 17), when epidemic strains appear, and how effectively they are controlled, including the implementation of effective antimicrobial stewardship programs.

> ***C. difficile*** **is the most common infective cause of diarrhea in healthcare settings, accounting for 15-25% of cases of healthcare-associated diarrhea, and is the primary cause of antibiotic-associated colitis.**
> ***[Bartlett JG, 2002; DuPont et al, 2011]***

CDI rates are often higher than the incidence of many other healthcare-associated infections such as catheter-associated intravascular (CAI) infections, vancomycin-resistant enterococcal (VRE) infections and ventilator-associated pneumonia (VAP) *[Miller et al, 2011]*.

There is a need for **constant vigilance (surveillance)** to recognize clusters/outbreaks and changes in epidemiology of CDI. Multiple countries/regions have introduced surveillance systems for CDI; for example:

- **UK (Public Health England)**
  https://www.gov.uk/government/collections/clostridium-difficile-guidance-data-and-analysis
- **Europe (ECDC)**
  https://ecdc.europa.eu/en/publications-data/european-surveillance-clostridium-difficile-infections-surveillance-protocol-1
- **US (CDC)**
  https://www.cdc.gov/hai/organisms/cdiff/tracking-cdiff.html

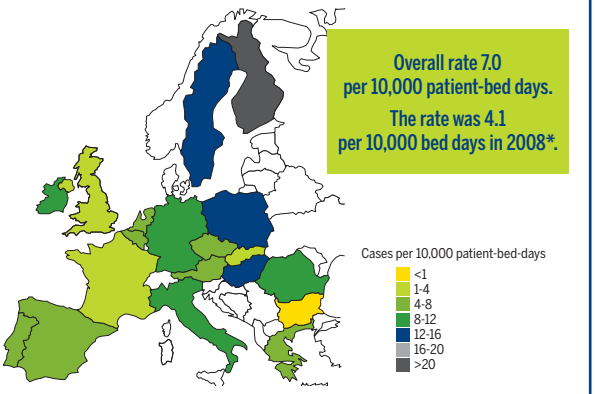
CDI incidence rates can be used as a valuable **indicator of healthcare quality** at both hospital and national levels *[Krutova et al, 2018]*. To increase comparability between clinical settings, it is recommended that **standardized case definitions for surveillance** are used:

- **healthcare facility-onset (HO) CDI**
  Specimen collected in >3 days after hospital admission
- **community-onset, healthcare facility–associated (CO-HCFA) CDI**
  Specimen collected in an outpatient setting or ≤3 days after hospital admission and documented overnight stay in a healthcare facility in the prior 12 weeks
- **community-associated (CA) CDI**
  No documented overnight stay in a healthcare facility in the prior 12 weeks

IN EUROPE, a large study across almost 500 hospitals in 19 countries recorded an **average CDI rate of 7.0 cases** (country range 0.7-28.7) **per 10,000 patient-bed days** in 2012-13 *[Davies et al, 2014]* (**Figure 4**). This rate was ~70% higher than that recorded in 2008 *[Bauer et al, 2011]*.

Figure 4: Observed CDI case rates in 19 European countries in 2013

Adapted from Davies KA, et al. *Lancet Infect Dis.* 2014;14:1208-19; Bauer MP, et al. *Lancet Infect Dis.* 2011;377:63-73



* Bauer MP, et al. Lancet 2011;377:63-73

IN THE UNITED STATES, in 2011, the **incidence of CDI was 147.2** (95% CI, 129.1-165.3) **cases/100,000 persons**; the incidence was highest among those aged ≥65 years (627.7) *[Lessa et al, 2015]*.

Of the total estimated annual number of CDI cases (453,000), two-thirds were considered to be healthcare-associated, of which 37% were hospital-onset, 36% had an onset in long-term care facilities, and 28% were community-onset healthcare-associated.

Of the estimated 159,700 community-associated CDI cases, 82% were associated with outpatient healthcare exposure, and so the great majority (94%) of all CDI cases had had a recent healthcare exposure.

The same study estimated a total of 83,000 first recurrences and 29,000 CDI associated deaths per annum in the US.

Recent data indicate that the total number of US hospital discharges with a diagnosis of CDI reached historic highs between 2011-13 *[Agency for Healthcare Research and Quality, 2016]*.

IN ASIA, ribotypes 027 and 078, which have caused significant outbreaks in other regions of the world, do not appear to have become established, whereas **ribotypes 017 and 018** have caused epidemics in several countries *[Collins et al., 2013]*. There are increasing reports highlighting increased recognition of CDI in Asian countries *[Tang et al, 2016; Ho et al, 2017; Choi et al, 2015]*.

IN OTHER REGIONS (Latin America, Africa), few data are generally available. However, there are increasing reports from some South American countries, including several documenting the existence and spread of *C. difficile* **ribotype 027** *[Aguayo et al, 2015; Salazar et al, 2017; Cejas et al, 2018]*.

How frequent is CDI in the Community and 'Low-Risk' Populations?

There has been increased recognition of CDI **in the community** and in populations thought to be at **low risk for CDI** (pregnant women, infants), without a history of hospitalization or antibiotic therapy *[Dubberke et al., 2012, Eckert et al., 2011, Kuntz et al., 2011]*.

The emergence of more virulent *C. difficile* strains, such as the 027 strain, may be a cause of more frequent and more severe disease in such populations. It is also possible that increased awareness has led to increased detection of **community-associated CDI (CA-CDI)** and similarly of cases occurring in long-term care and outpatient care settings *[Gupta et al, 2014; Lessa et al, 2015]*. However, an analysis of US CA-CDI cases between 2009–2011 found that most (82%) had some kind of healthcare exposure in the 12 weeks before CDI diagnosis *[Chitnis et al, 2013]*.

IN THE COMMUNITY, increases in CA-CDI in healthy individuals often with little or no history of hospitalization have been observed *[Wilcox et al, 2008]*.

Also, *C. difficile* is increasingly isolated from the **community** *[Hensgens MP et al., 2014]* and a significant number of hospital CDI could come from the community. Although controversial, between 30% and 50% of the CDI could have onset in the community *[Dubberke ER, et al. 2014, Lanzas C et al, 2014]*, as well as in animals or in the environment.

Pediatric CA-CDI has also been reported more frequently, with one US children's hospital reporting 25% of pediatric CDI cases to be community-acquired, of whom 65% had no recent exposure to antibiotics *[Sandora et al, 2011; Antonara S et al, 2016]*.

IN CHILDREN, a possible pathogenic role for *C. difficile* remains controversial. Although **asymptomatic carriage is high** in the pediatric population, some recent studies have claimed an **increased prevalence of CDI in both healthcare and community settings**, in particular in the 1-5 age-group *[Khalaf et al, 2012, Khanna et al, 2013]*.

In a large study in 38 US states, the incidence of CDI-related pediatric hospitalizations in the US was found to have almost doubled between 1997 and 2006, rising from 7.24 to 12.80 per 10,000 admissions *[Zilberberg et al, 2010]*. Another study also reported a 12-fold increase in pediatric CDI incidence compared with rates approximately a decade earlier *[Khanna et al, 2013]*. However, great care needs to be taken when interpreting such data given the possibility of ascertainment bias, due to **high colonization rates** and **different institutional sampling and testing policies**, which complicate interpretation of CDI trends in infants.

IN PERIPARTUM WOMEN, occasional acute CA-CDI cases have been reported, including some requiring emergency colectomy, and with fatal outcome *[Kelly and Lamont et al., 2008]*.

Evolving strain types causing CDI

Ribotyping has become the dominant *C. difficile* typing method and this has permitted a more detailed understanding of CDI epidemiology both within and between countries *[Wilcox et al, 2012; Fawley et al, 2016; Eyre et al, 2018]*. There are now approximately 1,000 distinct ribotypes, but most of these are rarely recovered from human CDI cases. The original agarose based ribotyping technique has largely been replaced by a capillary gel electrophoresis method that produces consistent results across laboratories *[Fawley et al, 2015]*.

It is likely that ribotyping will eventually be replaced by **whole genome sequencing (WGS)** based methods, given the enhanced discriminatory power of WGS, although cost differences still clearly favor the former method *[Eyre et al, 2013]*. At present, *C. difficile* ribotypes cannot be determined from (long read) WGS.

Early recognized epidemic ribotypes

The severity of CDIs increased dramatically from about 2002, initially in North America, and then in many countries in Europe. The emergence of 'hypervirulent' strains, particularly **ribotype 027, also known as PFGE type 1 (NAP1), and REA type BI**, was responsible for this transformation of CDI epidemiology.

The term hypervirulent is partially misleading as the same strain may cause no or mild disease in one individual but fatal disease in another. Hence, **host response** to challenge by *C. difficile* clearly affects **outcome severity** *[Walker et al, 2013]*.

Other hypervirulent strains include **ribotypes 078 and 244**. In addition, many other **epidemic strain types** have been described, e.g. 001, 014, 020, and 106, including some that are largely region specific, e.g. 017. **Ribotype 001** was epidemic in the UK in the 1990s, and was eventually supplanted by **ribotype 106**, neither of which were then reported in the US. Whilst ribotype 106 has declined substantially in the UK, it has recently displaced ribotype 027 as the most prevalent strain type in the US.

3 LABORATORY DIAGNOSTICS

A large European study observed that **clinical suspicion of CDI translates into a confirmed diagnosis in only about 10% of cases** (or less depending on the testing/sampling strategy being used) *[Davies et al, 2014]*. Thus, **relying on clinical diagnosis alone to make a diagnosis of CDI is not possible**.

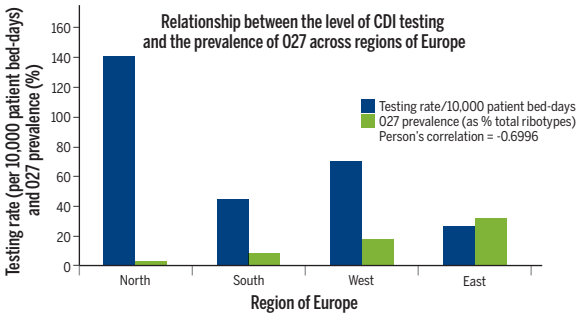
On average, ~80 CDI cases were not diagnosed per hospital per year, primarily because CDI testing was not performed on some/all diarrheal samples. Together, **absence of clinical suspicion and use of sub-optimal laboratory diagnostic methods** mean that an estimated 40,000 inpatients with *C. difficile* infection are **potentially undiagnosed each year** in 482 European hospitals *[Davies et al, 2014]*.

Notably, less than half of 500 European hospitals were using optimum testing methods for CDI, i.e. **two-stage testing including a fecal toxin test method, as defined by European guidelines** *[Crobach et al, 2016]* (**Figure 8**, page 24). However, the number of participating hospitals using optimum methods increased during the study period, from 152 (32%) of 468 in 2011-12 to 205 (48%) of 428 in 2012-13 *[Davies et al, 2014]*.

The same study found an association between countries/regions that had **higher testing rates and lower prevalence of ribotype 027** (**Figure 5**). The likely explanation here is that control of this epidemic, 'hypervirulent' strain is more likely if cases are not missed (and so appropriately managed).

Figure 5: Inverse correlation of testing and outbreaks of PCR ribotype 027

Adapted from Davies KA, et al. *Lancet Infect Dis* 2014;14:1208-19



What are the criteria for CDI testing?

The main clinical criterion for requesting a laboratory diagnosis for CDI is **symptomatic disease**.

- Testing for *C. difficile* or its toxins should be performed on all patients with **potentially infective diarrhea** (some guidelines define this as 3 or more **unformed or watery stools** in a 24 hour period or less; others recommend testing after a single unexplained diarrheal stool) *[PHE 2012, (ESCMID) Crobach et al, 2016, (IDSA/SHEA) McDonald et al, 2018]*.
- There is good evidence that relying on clinician requests (as opposed to routine testing of all diarrheal samples) is associated with **under-diagnosis** of CDI *[Davies et al, 2014]*.
- Testing of formed stools (i.e. those that do not adopt the shape of the container) is generally not recommended.
- **Diarrheal samples should be tested for *C. difficile* from:**
  - all hospitalized patients aged > 2 years with potentially infectious diarrhea
  - all patients aged > 65 years
  - all patients aged < 65 years if clinically indicated *[Dept. Health NHS/UK/DR/HAI, 2012]*
- **Repeat testing** during the same episode of diarrhea is of **limited value** and is generally not recommended unless symptoms/signs of possible CDI change.
- Stool samples should not be left at room temperature for more than 2 hours to prevent toxin degradation. Samples may be stored at 2-8°C for several weeks, but **freeze-thawing causes toxin degradation** *[Freeman & Wilcox, 2003]*.

What are the different laboratory techniques available?

Different commercial techniques are available for the laboratory diagnosis of *C. difficile* infection (Figure 6):

- detection of toxigenic and non-toxigenic *C. difficile* bacteria (GDH EIA and culture)
- detection of *C. difficile* toxins (Toxin EIA and CTA)
- detection of *C. difficile* toxin genes (molecular)

These different techniques are used in laboratory diagnostic strategies which are currently based on **2- or 3-step techniques** or **molecular testing as a stand-alone technique** (**Figures 7 and 8**).

Although identification, susceptibility testing and strain typing are not usually performed in routine, they are particularly important for **epidemiological studies** and in the event of **outbreaks** to determine the presence of specific strains.

Detection of *C. difficile* bacteria in stools

Glutamate dehydrogenase (GDH) immunoassay

- The enzyme GDH is produced in large quantities by *C.difficile*. Its presence indicates the **presence of *C. difficile* bacteria** in the sample with a **high negative predictive value** (a GDH-negative result can be used to rule out CDI) *[Crobach et al, 2016]*.
- For GDH positive stool specimens, confirmation by toxigenic culture/ toxin EIA or Nucleic Acid Amplification Technique (NAAT) is required, as GDH detects both toxigenic and non-toxigenic strains of *C. difficile*.

Culture

- **Highly sensitive method**
- Essential for typing if epidemiological studies are required or in case of outbreaks, and more rarely for antibiotic susceptibility testing.
  Culture of *C. difficile* is performed for at least 24 hrs on a selective medium (chromogenic medium or Cycloserine-Cefoxitin-Fructose Agar [CCFA]) in an anaerobic environment at 37°C.

Detection of *C. difficile* toxins in stools

Enzyme immunoassay (EIA) / Immunochromographic (IC) rapid tests

- ***C. difficile* toxins A and B** can be detected using monoclonal antibodies coated on a support (solid for conventional immunoassay and membrane for an immunochromatographic test). The sensitivities of available EIA assays vary considerably *[Eastwood et al., 2009; Planche et al, 2008]*.
- *C. difficile* **toxins A and B should both be tested**, due to the presence of toxin A-negative and toxin B-positive pathogenic strains.

Cell culture cytotoxicity assay (CTA)

- Traditionally, one of the **gold standard techniques** to which most methods have been compared. Usually only performed by reference laboratories.
- **CTA detects toxins directly in stool specimens**, using a cytopathic effect in cell cultures. Confirmation is carried out by neutralizing the cytopathic effect with *C difficile* toxin antibodies *[Planche et al., 2013]*.

Toxigenic culture (TC)

- Another **gold standard technique** for the diagnosis of CDI *[Planche et al, 2013]*. Usually only performed by reference laboratories.
- **Two-step technique: culture followed by detection of toxins** produced by the isolated strain either by CTA or EIA.
- This method can be useful in cases where patients have negative toxin stool results, but present with clinical symptoms suggestive of CDI.
- However, the TC method cannot differentiate 'colonization' from 'infection' by a toxigenic strain. It is also slow as it relies on culture first.

Magnetic bead based toxin immunoassay

- **Ultrasensitive fecal toxin detection** methods have recently been developed. These can detect much lower concentrations of *C. difficile* toxins in feces. This offers both potential advantages (sensitivity) and disadvantages, as toxin detection may occur in samples from individuals who do not have true CDI *[Pollock et al, 2015]*. At the time of writing, determining the clinical utility of such methods will require further studies.

Detection of *C. difficile* toxin genes in stools

Nucleic Acid Amplification techniques (NAAT)

- Molecular testing is based on **toxin B +/- toxin A gene detection and performed directly on a diarrheal stool sample**.
- High negative predictive value for CDI.
- Poor positive predictive value for CDI when used as a standalone test.
- It is specific for the presence of toxigenic *C. difficile* but cannot differentiate 'colonization' from 'infection' by a toxigenic strain.

Figure 6: Main features of *C. difficile* laboratory techniques

Adapted from Eckert et al, *Journal des anti-infectieux*, 2011;13:67-73; Gateau et al, *Clin Microbiol Infect*. 2018;24:463-468

METHOD	EIA GDH ENZYME DETECTION	CULTURE STRAIN ISOLATION	EIA TOXINS A&B / IC RAPID TESTS	CYTOTOXITY ASSAY (CTA) (TOXIN B)	NAAT TOXIN B DETECTION & TYPING	TOXIGENIC CULTURE
TARGET	Presence of *C. difficile* in stools		Detection of free toxins A & B in stool		Presence of a toxigenic *C. difficile* strain	
TIME TO RESULT	15 min - 2 hours	2 - 4 days	15 min – 2 hours	1 - 2 days	<2 hours	1 – 2 days
ADVANTAGES	High sensitivity/ High NPV	High sensitivity	High specificity	High sensitivity	High sensitivity / High NPV	High sensitivity
	Rapid	Antibiotic susceptibility testing	Rapid	High specificity		Gold standard
	Manual or automated	Typing	Standardized		Rapid	
			Manual or automated			
DISADVANTAGES	Low specificity	Low specificity	Lower sensitivity	Time consuming	Low specificity	Low specificity
		Manual		Lack of standardization	High cost	Time consuming
		Long time to result		Technical expertise required		Long time to result
INTERPRETATION	Toxigenic strain/or not ?		CDI diagnosis due to free toxin detection		CDI or carriage of a toxigenic strain?	
	Need to perform free toxins A & B test for definitive CDI diagnosis				Need to perform free toxins A & B test for definitive CDI diagnosis	

EIA: enzyme immunoassay - CDI: *C. difficile* infection - GDH: glutamate dehydrogenase - IC: Immunochromographic

NAAT: nucleic acid amplication test – NPV: negative predictive value

Why have guideline recommendations for the diagnosis of CDI changed?

Prior to the availability of **nucleic acid amplification tests (NAATs, e.g. PCR)** for toxin B +/- toxin A gene detection, the diagnosis of CDI relied mainly on the detection of toxin in feces, either using a biological assay (cytotoxicity test) or enzyme immunoassays (EIAs). Over the past decade, NAAT use has become commonplace in some settings, particularly North America, whereas in Europe fecal toxin detection methods have generally been favored. The uptake of toxin gene NAATs reflected concerns regarding the sub-optimal sensitivity of toxin detection tests (especially EIAs) *[Planche et al, 2008; Crobach et al, 2016]*.

The high sensitivity of NAATs has a key drawback, which is the **poor specificity/ positive predictive value for true CDI**. Increasing numbers of studies have found that NAATs, when used alone for CDI testing, can **inflate the true infection rate by 50-80%** *[Planche et al, 2013; Longtin et al, 2013; Polage et al, 2015; Marra et al, 2017]*. However, NAATs have an excellent negative predictive value i.e. utility to rule out CDI.

The over-diagnosis of CDI has important potential consequences for patients, including **unnecessary treatment, isolation, and label/stigma**, which could affect their future medical management. Furthermore, healthcare institutions may be penalized for having such (apparently) high CDI rates.

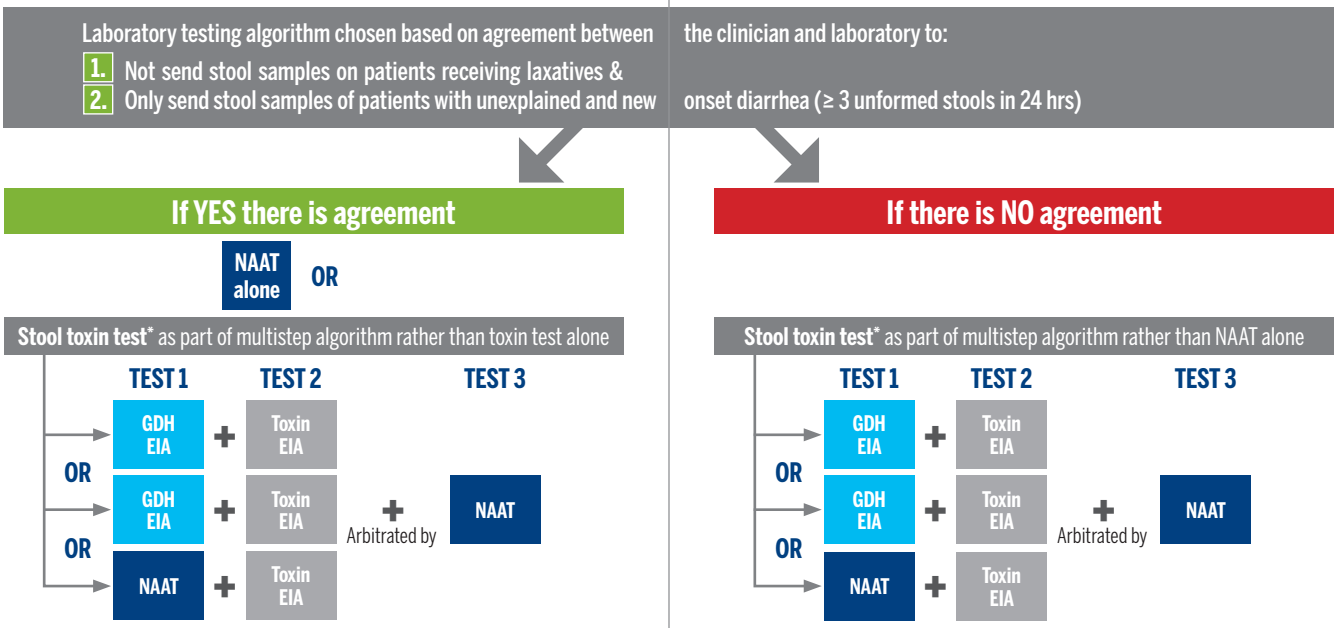
Consequently, the latest **IDSA/SHEA CDI guidelines** have advocated the use of **the stool toxin test** as part of a **multistep algorithm (Figure 7)**. *[McDonald et al, 2018]*

This **two-stage testing recommendation** is consistent with **ESCMID CDI diagnostic guidelines** *[Crobach et al, 2016]* **(Figure 8)**.

Such **two- or three-stage algorithms** provide an optimal **balance between sensitivity, specificity, time-to-result and cost**.

Figure 7: IDSA/SHEA Update for Clinical Practice Guidelines for CDI

Adapted from McDonald et al, *Clin Infect Dis.* 2018;66:987-994



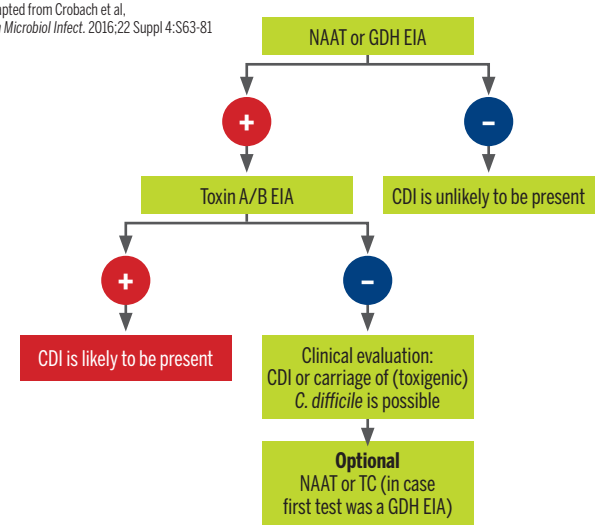
* Approved stool EIA toxin tests vary widely in sensitivity. Laboratories should choose a toxin test with sensitivity in the upper range of sensitivity as reported in the literature [146-149, 156].

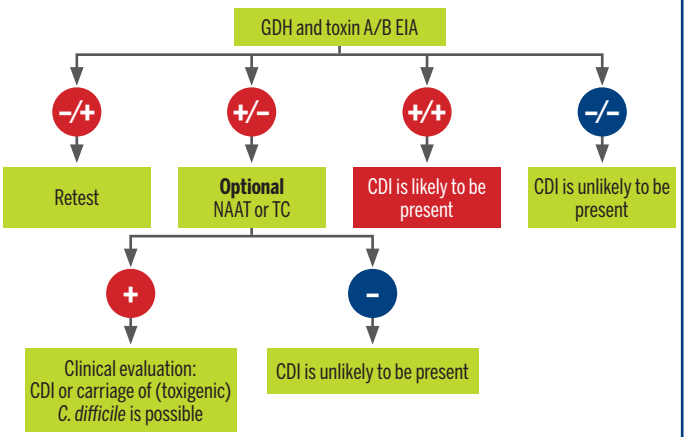
The **IDSA/SHEA guidelines** (**Figure 7**) do still offer the possibility of using a NAAT alone for all specimens if there are pre-agreed institutional criteria for patient stool submission, i.e. frequent diarrhea and absence of other factors such as laxatives. This requirement aims to **increase the predictive value of NAAT-alone testing, although over-diagnosis of true CDI remains a clear risk** here *[Planche et al, 2013; Longtin et al, 2013; Polage et al, 2015; Marra et al, 2017]*.

Given the high chance that *C. difficile* strains (including toxigenic strains) can be carried asymptomatically in infants, the guidelines make a strong recommendation that **testing for CDI should never be routinely recommended for neonates or infants ≤12 months of age with diarrhea** *[McDonald et al, 2018]*.

Figure 8: ESCMID upate of Diagnostic Guidance for CDI

Adapted from Crobach et al, *Clin Microbiol Infect.* 2016;22 Suppl 4:S63-81



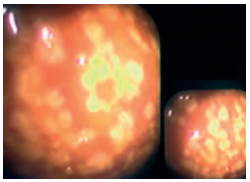


EIA: enzyme immunoassay • GDH: glutamate dehydrogenase • NAAT: nucleic acid amplication test • TC: toxigenic culture

What other CDI diagnostic methods are available?

Endoscopy

Colonoscopy or sigmoidoscopy are invasive investigations used mainly to **confirm cases of pseudomembranous colitis (PMC)**. These are now rarely performed in relation to CDI and have largely been replaced by imaging techniques (e.g. computerized tomography).



Pseudomembranous colitis, endoscopy / BSIP, Cavallini James

Fecal leukocytes, lactoferrin and calprotectin

Detection of fecal leukocytes by **methylene blue staining** can help distinguish between **inflammatory and non-inflammatory causes** of diarrhea, but is not commonly performed. The analysis should be performed rapidly after specimen collection to prevent leukocyte degradation. However, the presence of leukocytes is **not specific for CDI** and can occur with other infections (e.g. *Shigella* infection) or inflammatory bowel disease (e.g. Crohn's Disease, ulcerative colitis).

Fecal lactoferrin or **calprotectin** measurement (available via commercial assays) have been examined in several CDI studies. These biomarkers increase in the presence of infection/inflammation, but there is insufficient evidence to currently recommend these tests for routine use *[McDonald et al, 2018]*.

Screening for *C. difficile*

The different patient groups that may act as a source of *C. difficile* are outlined on page 6. A key point here is that, at present, there are no proven interventions either to decolonize patients identified as carriers or to reduce the risk of *C. difficile* germination in such individuals (although there are experimental preventative approaches, including some in clinical trials). Therefore, any studies to investigate the **potential utility of screening for *C. difficile*** are currently **primarily infection prevention and control based** i.e. to reduce the risk of transmission of (toxigenic) *C. difficile* strains from asymptomatic carriers, and so lower the chance of CDI in contacts.

The latest **IDSA/SHEA CDI guidelines reviewed the evidence to support the effectiveness of screening for *C. difficile***, i.e. to detect asymptomatic carriers of toxigenic strains *[McDonald et al, 2018]*. Although some studies have examined the effectiveness of screening for *C. difficile* as a way of reducing transmission risk, the conclusion was that there are **insufficient data to recommend screening for asymptomatic carriage and placing asymptomatic carriers on infection control (contact) precautions**. Nevertheless, it is possible that future *C. difficile* control strategies could incorporate screening and isolation of asymptomatic carriers

4 TREATMENT

Protocols for the treatment of CDI are well defined in European and US guidelines *[Debast et al, ESCMID, 2014; McDonald et al, SHEA/IDSA, 2018]*. However, the management of CDI recurrence is more variable. In particular, fecal microbiota transplantation (FMT) utilization varies markedly across and between countries. Current recommendations/evidence are summarized in **Tables 1 and 2**.

The use of **probiotics** to treat *C. difficile* carriers and CDI patients remains controversial and is currently not recommended *[McDonald et al, SHEA/IDSA, 2018]*.

Table 1: Clinical CDI Definitions and Treatment Recommendations

Adapted from Debast et al, ESCMID, 2014; McDonald et al, SHEA/IDSA, 2018

CLINICAL CDI DEFINITION	TREATMENT RECOMMENDATIONS
NON-SEVERE	Vancomycin 125 mg given 4 times daily by mouth for 10 days OR Fidaxomicin 200 mg given twice daily for 10 days* **Metronidazole is no longer a preferred treatment option.**
SEVERE	Vancomycin, 125 mg 4 times per day by mouth for 10 days OR Fidaxomicin 200 mg given twice daily for 10 days*
CDI WITH INCREASED RISK OF RECURRENCE	Extended duration fidaxomicin was associated with a very low rate of recurrent CDI *[Guery et al, 2018]*. OR Bezlotoxumab is a recently approved monoclonal antitoxin B antibody that reduces the risk of recurrence in patients at increased risk of recurrence/poor outcome (particularly those with multiple risk factors: age ≥65 years, history of CDI, compromized immunity, severe CDI, and ribotype 027/078/244) *[Wilcox et al, 2017; Gerding et al, 2018]*.
FULMINANT	Vancomycin, 500 mg 4 times per day by mouth or by nasogastric tube. If ileus, consider adding rectal instillation of vancomycin. Intravenously administered metronidazole (500 mg every 8 hours) should be administered together with oral or rectal vancomycin, particularly if ileus is present.

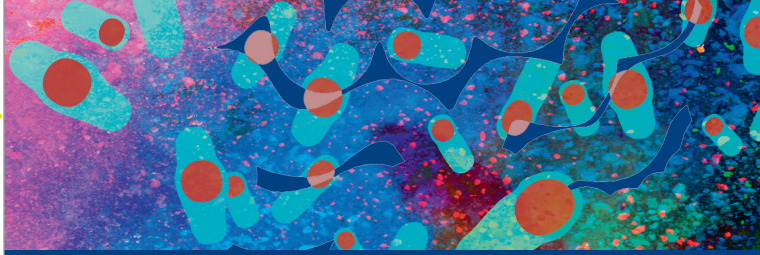
*Fidaxomicin is associated with a lower risk of recurrent CDI. Patients at increased risk of recurrence should be considered for fidaxomicin.

Table 2: Clinical Definitions of Recurrent CDI and Treatment Recommendations

Adapted from Debast et al, ESCMID, 2014; McDonald et al, SHEA/IDSA, 2018

CLINICAL CDI DEFINITION	TREATMENT RECOMMENDATIONS
1ST RECURRENCE (SYMPTOMATIC RECURRENCE SHOULD BE CONFIRMED AS CDI BY LABORATORY TESTING)	Vancomycin 125 mg given 4 times daily for 10 days if metronidazole was used for the initial episode OR Use a prolonged tapered and pulsed vancomycin regimen if a standard regimen was used for the initial episode (e.g. 125 mg 4 times per day for 10–14 days, 2 times per day for a week, once per day for a week, and then every 2 or 3 days for 2–8 weeks). OR Fidaxomicin 200 mg given twice daily for 10 days if vancomycin was used for the initial episode
2ND RECURRENCE (SYMPTOMATIC RECURRENCE SHOULD BE CONFIRMED AS CDI BY LABORATORY TESTING)	Vancomycin in a tapered and pulsed regimen (e.g. 6 week regimen: Vancomycin 125 mg 4 times per day by mouth for 1 week, 125 mg 3 times per day for 1 week, 125 mg 2 times per day for 1 week, 125 mg once daily for 1 week, then 125 mg every alternate day for 1 week, and finally 125 mg every 3rd day for 1 week). OR Vancomycin, 125 mg 4 times per day by mouth for 10 days followed by rifaximin 400 mg 3 times daily for 20 days OR Fidaxomicin 200 mg given twice daily for 10 days OR Fecal microbiota transplantation (FMT)*

** The long-term safety of FMT has not been established, and so this option is not recommended unless pharmacological alternatives have been tried for prior recurrent CDI. IDSA/SHEA recommend that FMT is not used prior to a 2nd recurrence episode.*



5 PREVENTION AND CONTROL OF CDI

C. difficile is **highly transmissible**, particularly as its spores survive for long periods outside in the body. It was formerly believed that most hospital-associated CDI cases represented **case to case transmission**.

Evidence from **whole genome sequencing based studies** shows that this is not the case, as in a non-outbreak setting *[Eyre et al, 2013]*, *C. difficile* **colonized patients** (whether **symptomatic or asymptomatic**) contribute to transmission, but CDI cases are more often linked to other infected patients *[Mawer et al, 2017; Kong et al, 2018]*.

While case to case transmission explains only a minority of acquisitions of CDI in the sporadic/endemic setting, cases resulting from such transmission have significantly **worse outcomes** *[Martin et al, 2018]*.

Prevention of transmission of *C. difficile* remains a key part of CDI control programs (**Table 3**), in particular:

- **early diagnosis and treatment**
- **prompt patient isolation**
- **hand and environmental hygiene measures**

Detailed CDI infection prevention and control guidelines are available from **ESCMID and IDSA/SHEA** *[Tschudin-Sutter et al, 2018; McDonald et al, 2018]*.

Antimicrobial Stewardship

The importance of **antimicrobial stewardship** in reducing the risk of CDI has increasingly been recognized, and is now accepted as a major control intervention *[Davey et al, 2017; Dingle et al, 2017; Tschudin-Sutter et al, 2018; McDonald et al, 2018]*.

A SUCCESSFUL ANTIMICROBIAL STEWARDSHIP POLICY SHOULD AIM TO:

- **reduce the frequency and duration** of antibiotic therapy
- **limit the number** of antimicrobial agents prescribed
- **reduce the use** of antibiotics that are associated with a higher CDI risk (cephalosporins, clindamycin, fluoroquinolones)
- **select antibiotics** associated with a lower risk of CDI whenever possible
- **implement an antimicrobial stewardship program** based on local epidemiology and the *C. difficile* strains present in the healthcare facility
- **educate and raise awareness** of the risks of CDI following the use of a specific class of antibiotic

Table 3: Mnemonic protocol (SIGHT) for managing suspected potentially infectious diarrhea

Adapted from *Clostridium difficile* infection: How to deal with the problem, Health Protection Agency and Department of Health, UK, 2008

S	Suspect that a case may be infective where there is no clear alternative cause for diarrhoea
I	Isolate the patient and consult with the Infection Prevention and Control Team while determining the cause of the diarrhoea
G	Gloves and aprons must be used for all contacts with the patient and their environment
H	Hand washing with soap and water should be carried out before and after each contact with the patient and the patient's environment
T	Test the stool for toxin/ investigation by sending a specimen immediately

6 CDI AS A HEALTHCARE QUALITY INDICATOR

Healthcare systems and institutions have recognized over the past decade that CDI rates can be used as a marker of the quality of healthcare delivery. As a result, multiple **surveillance** and **performance improvement** schemes have been developed to highlight the **patient and healthcare burden** imposed by CDI, and to **control rates of infection**, notably through multifaceted **Antimicrobial Stewardship Programs (ASPs)** *[Libertin et al, 2017; Patton et al, 2018]*.

For example, the CDC has a **CDI Targeted Assessment for Prevention** program (https://www.cdc.gov/hai/prevent/tap/cdiff.html) that focuses on:

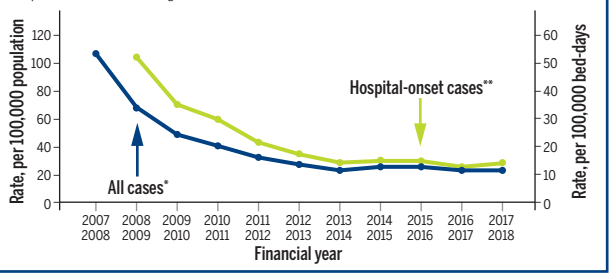
- General Infrastructure, Capacity, and Processes
- Antibiotic Stewardship
- Early Detection and Isolation, Appropriate Testing
- Contact Precautions/Hand Hygiene
- Environmental Cleaning
- Laboratory Practices

Similarly, there are comprehensive CDI control programs in the UK e.g. by Public Health England (https://www.gov.uk/government/collections/clostridium-difficile-guidance-data-and-analysis).

IN THE UK, the incidence of CDI has decreased markedly in response to such quality improvement programs. For example, in England there has been a 75% decrease in CDI rates from the peak incidence in 2007/08 (**Figure 9**).

Figure 9: Trends in the rate of *C. difficile* infection in England

Adapted from Public Health England, 2018



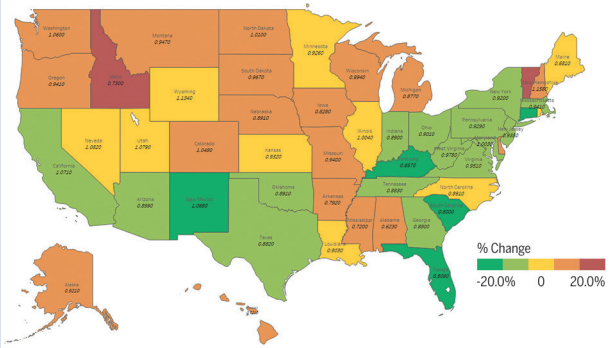
This decrease has been attributed to several factors:

- **introduction of enhanced surveillance** (e.g. in the UK, mandatory tesing for *C.difficile* for all hospital inpatients over the age of 65 with diarrhea).
- **sensitization and enhancing responsibility of hospital administrators** regarding CDI rates; recently supplemented by fines for institutions not meeting their annual CDI targets.
- **reinforced implementation** of infection prevention and control measures.
- **centrally funded access** to *C. difficile* strain ribotyping and enhanced DNA fingerprinting.
- **more prudent antibiotic use** ("antibiotic stewardship" programs).
- **improved diagnostic algorithms**.

IN THE US, some states have recorded significant decreases in CDI incidence in recent years (**Figure 10**). Decreases of between **14 and 20%** were observed in Connecticut, Florida, Kentucky, New Mexico and South Carolina.

Figure 10: Hospital-onset CDIs in all reporting acute care hospitals - changes in state-specific standardized infection ratios (SIRs) between 2015 and 2016

Adapted from Figure 10g. 2016 National and State HAI Progress Report – Acute Care Hospitals, CDC / National Healthcare Safety Network (NHSN)

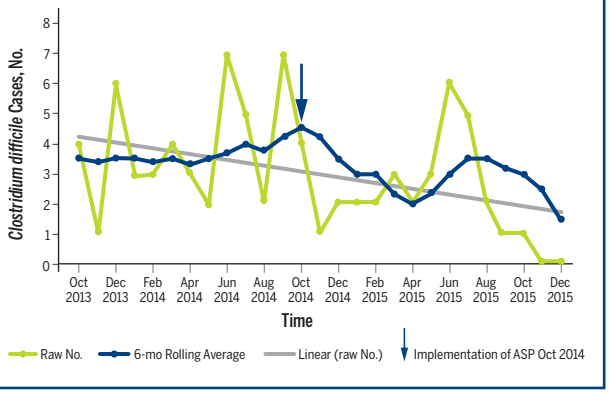


Another US study involving the implementation of a new ASP in a rural community hospital with no prior ASP improved a patient safety metric and significantly decreased costs (**Figure 11**):

- *C. difficile* infections decreased from 3.35 cases per 1,000 occupied bed days (OBDs) in 2013 to **1.35 cases per 1,000 OBDs** in 2015
- total targeted **antimicrobial costs decreased 50%** from $16.93 per patient day in 2013 to $8.44 per patient day in 2015
- overall **antimicrobial use decreased 10%** from before the ASP initiative to 1 year after implementation
- **annualized savings were $280,000 in 1 year**, based on drug savings only.

Figure 11: Decrease in inpatient CDI rate following implementation of an Antimicrobial Stewardship Program (ASP)

Adapted from Libertin C, et al. *Am J Infect Control* 2017;45:979-982



7 CONCLUSIONS AND FUTURE PERSPECTIVES

There have been considerable advances in our knowledge of *C. difficile* and CDI, particularly in the last decade as the epidemiology, clinical presentation, treatment and consequences of CDI have changed so dramatically. In the same timeframe, **the optimal CDI diagnostic approaches have evolved**, as a result of some pivotal clinical trials.

The future holds the prospect of **improved screening approaches** to reduce the risk of CDI and *C. difficile* acquisition, as well as optimized prevention and treament options through vaccines or oral therapies for those receiving higher risk antibiotics.

Prevention of CDI

Two different CDI prophylactic approaches are currently being pursued for patients treated with antibiotics:

- **DAV132** is an uncoated formulated activated charcoal (FAC) product that absorbs some antibiotics in the large intestine, thereby reducing their negative impact on the colonic microbiome (and so theoretically CDI) *[de Gunzburg et al, 2018]*.
- **Ribaxamase** is a β-lactamase that when given orally can degrade β-lactams present in the colon. In a phase 2 clinical trial, ribaxamase compared with placebo recipients had a 71.4% relative risk reduction for CDI in subjects treated with ceftriaxone for pneumonia (p=0.045) *[Synthetic Biologics, 2016]*.

Several ***C. difficile* vaccines** (based primarily on toxins as antigens) have advanced to clinical trial stage. A recent phase 3 clinical trial (NCT01887912) was terminated following an interim analysis that suggested there was little prospect that the study would demonstrate a significant reduction in CDI among recipients of the active *vs* placebo vaccine. A different vaccine is currently well advanced in a phase 3 clinical trial (NCT03090191).

A key issue to consider for all CDI preventative approaches is WHO BEST TO TARGET.

Assuming that effective preventative therapies will become available, their use will ultimately depend on:

- the degree of protection afforded
- the number of subjects needed to treat to prevent cases
- and how long before the CDI at-risk period the therapy needs to be administered

Possible links to the food chain

Emerging evidence indicates that there are **two distinct patterns of *C. difficile* ribotype spread**; these are consistent with either predominantly **healthcare-associated acquisition** of *C. difficile* or **wide dissemination via other routes/ sources**, i.e. possibly the food chain *[Eyre et al, 2018]*.

Epidemic *C. difficile* ribotype 078 was initially prevalent in the Netherlands (~2005-2008), where it was recovered from both humans (third most common type found in community-onset disease) and several animal species (calves, pigs, horses) *[Goorhuis et al., 2008; Jhung et al, 2008]*. CDI due to ribotype 078 was associated with similar severity compared with cases caused by ribotype 027, but tended to affect younger and more community-based individuals. Ribotype 078 has also been reported in hospitalized patients in many other countries, e.g. England, Germany, Switzerland and France *[Rupnik et al., 2008; Wilcox et al., 2012]*.

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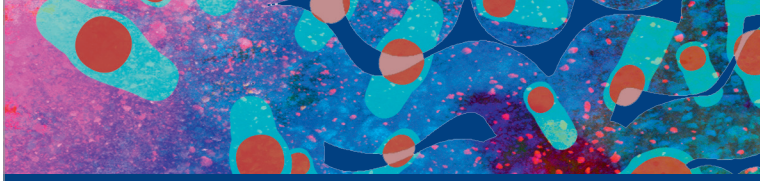

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bioMérieux S.A. • 69280 Marcy l'Étoile • France
Tel.: + 33 (0)4 78 87 20 00 • Fax: +33 (0)4 78 87 20 90
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