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CLOSTRIDIUM DIFFICILE INFECTIONS

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PREFACE

Clostridium difficile emerged in the first decade of this millennium from a pathogen considered mainly as a nuisance to a position of notoriety. This transformation was likely driven by three 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, notably those with epidemic potential;
- and thirdly, **confusion about when, where and how best to test** for evidence of *C. difficile* infection has contributed to under-ascertainment of cases and so fuelled the spread of this opportunistic pathogen.

Given that a high proportion of hospitalised patients receive antibiotics, this means that there are large numbers of potentially susceptible hosts who may acquire, be colonised by, transmit and/or become infected by *C. difficile*. In short, *C. difficile* is a nosocomial pathogen that has found and exploited 'weaknesses' in 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, and improved use of detection and diagnosis methods.

Professor Mark Wilcox

Consultant Medical Microbiologist, Leeds Teaching Hospitals,
Professor of Medical Microbiology, University of Leeds, Leeds, UK.

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

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

This booklet has been written with the kind collaboration and thorough reviewing of :

- **Prof. Mark Wilcox**
  Consultant Medical Microbiologist, Leeds Teaching Hospitals,
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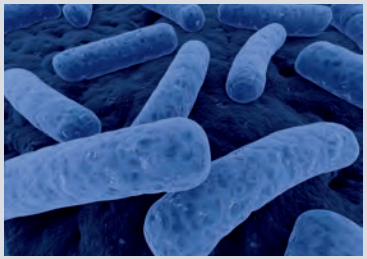
What is *Clostridium difficile*?

Clostridium difficile is a naturally-occurring species of **Gram-positive bacteria** of the genus *Clostridium*. It is commonly referred to as "*C. difficile*" or "*C. diff*".

- Clostridia are **motile, anaerobic, spore-forming rods** (bacilli), which are ubiquitous and especially prevalent in soil.
- Under the microscope, clostridia appear as long, irregularly (often "**drumstick**" or "**spindle**") shaped cells with a bulge at one end.
- When stressed, the bacteria produce **spores that are resistant to extreme conditions** of heat, drying, and a wide range of chemicals, including some disinfectants).
- *C. difficile* may be present in the human intestine of 1-3% of healthy adults and the majority of healthy infants (but who normally only remain colonised for 1-2 years at most).

Clostridium difficile may cause diarrhea and other intestinal disease (colitis, pseudomembraneous colitis, toxic megacolon) when commensal bacteria of the gut flora have been altered by antibiotics or other situations.



How does *C. difficile* induce disease?

Clostridium difficile proliferates in the human bowel when there is a **modification of the normal balance** of bacterial intestinal flora (e.g. during or after antibiotic therapy).

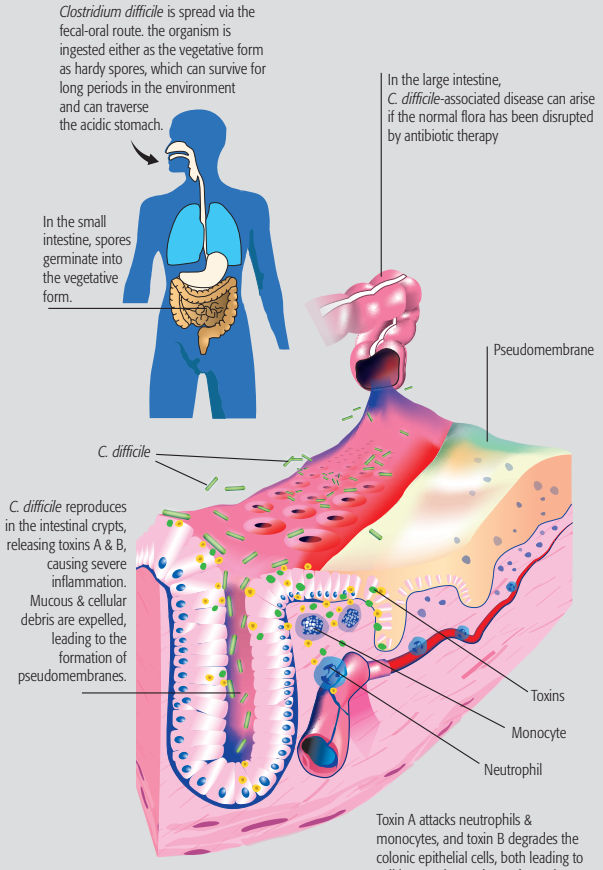
Only **pathogenic strains** of *C. difficile* cause disease, due to the production of one or two distinct toxins, A and B. Strains or types of *C. difficile* not expressing either toxin do not cause clinical illness.

Toxinogenic strains of *C. difficile* cause disease by damaging the intestinal cells of the colon (large bowel), causing cell breakdown and an inflammatory response.

Another toxin, **binary toxin** (CDT) is also expressed in some virulent strain groups but its role in pathogenicity is not yet fully understood *(Barth et al, 2004; Cartman et al, 2010)*.

Host response should also be taken into account as people can acquire/be colonized with toxinogenic strains and yet remain asymptomatic (*Planche et al., 2013*).

Figure 1: Pathogenesis of *C. difficile*-associated disease



Adapted from illustration by David Schumick (Sunenshine RH and McDonald LC, Cleveland Clinic Journal of Medicine, 2006)

How is *C. difficile* infection (CDI) transmitted?

C. difficile is transmitted from person to person by the **fecal-oral route**.

The organism forms large numbers of **heat-resistant spores**, that are not killed by alcohol-based hand cleansers or routine cleaning of surfaces, and can persist in the environment for months to years. These spores can be killed by some high-level disinfectants (i.e. high concentrations of bleach providing there is sufficient contact time) and with sterilization techniques.

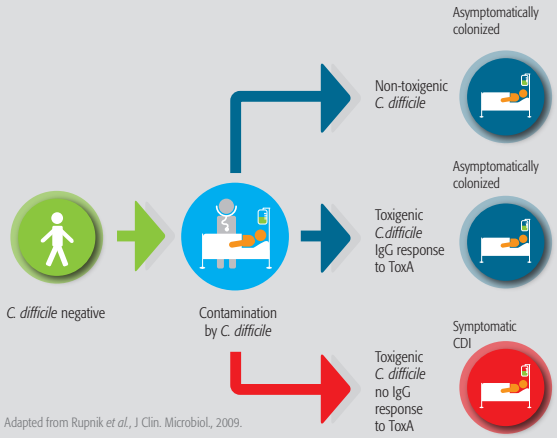
When spores are ingested by a patient, they pass into the intestine where they multiply. In healthy people, the normal flora present in the intestine controls the proliferation of *C. difficile*. However, when the **normal balance of bacterial flora is disturbed,** (e.g. by antibiotics), *C. difficile* can rapidly multiply and produce toxins which cause illness.

Infected patients excrete large numbers of bacteria/spores in their liquid feces. Therefore, in the healthcare setting, **spores can be cross-transmitted** to other patients through contact with:

- infected patients
- healthcare staff (who may inadvertently spread the bacteria typically via hands)
- contaminated medical equipment
- contaminated surfaces.

The rate of acquisition of CDI increases linearly with length of hospital stay, and can reach 40% after 4 weeks of hospitalization *(Clabots et al., 1992)*.

Figure 2: Acquisition of *Clostridium difficile* infection (CDI)



Adapted from Rupnik *et al.*, J Clin. Microbiol., 2009.

How important is CDI recurrence?

One of the major issues with CDI is the high recurrence rate. Recurrences usually occur within 4 weeks after ending treatment for CDI. In people suffering a recurrence, there is also a risk of sequential multiple recurrences, particularly in the elderly (>65 years of age).

Following treatment with metronidazole or vancomycin, recurrence of CDI occurs in approximately **20% of first-time cases**, increasing to **40% to 60% after subsequent recurrences** *(Kelly and LaMont, 2008)*.

Recurrence may occur due to:

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

What are the risk factors for CDI recurrence?

There are a number of risk factors for recurrence of *C. difficile* infection *(Eyre et al., 2012; Bauer et al.(ESCMID) 2009)*:

- advanced age (>65 years)
- severe underlying disease
- concomitant antibiotic use
- a decreased antibody response against *C. difficile* toxins A and B
- immunodeficiency
- strain type

Can CDI recurrence be predicted?

Several studies have aimed to develop **scoring systems to identify patients at high risk of CDI recurrence**, in order to predict recurrence and better target patients likely to benefit from enhanced initial treatment.

The score proposed by Eyre et al. includes **important risk factors for recurrence** that should be present in electronic patient records (age, emergency admission, admission with CDI, stool frequency, C-reactive protein, past healthcare exposure, antibiotic selection…). The 4-month absolute recurrence risk was found to increase by approximately 5% for every 1-point increase in this score *(Eyre et al., 2012)*.

A smaller study developed a score for prediction of CDI recurrence (incorporating age >65 years, severe underlying disease and concomitant antibiotics) and had a 72% positive predictive value in a validation case cohort *(Hu et al., 2009)*.

How to treat recurrent CDI?

For recommendations on treatment of recurrent CDI, see page 21.

EPIDEMIOLOGY

How frequent is CDI?

C.difficile accounts for 15-25% of cases of healthcare-associated diarrhea and is the **primary cause of antibiotic-associated colitis** (Bartlett JG, 2002).

In Europe, the incidence is approximately **4-5.5/10,000 patient days** *(Bauer et al., 2011)*.

Figure 3: Epidemiology of CDI in Europe (2008)

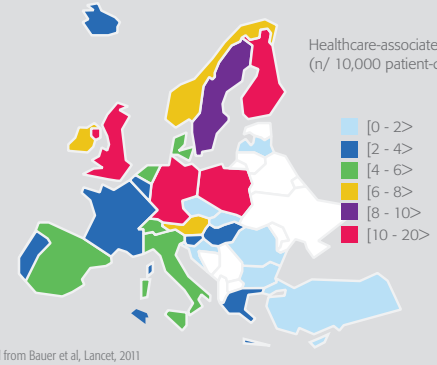
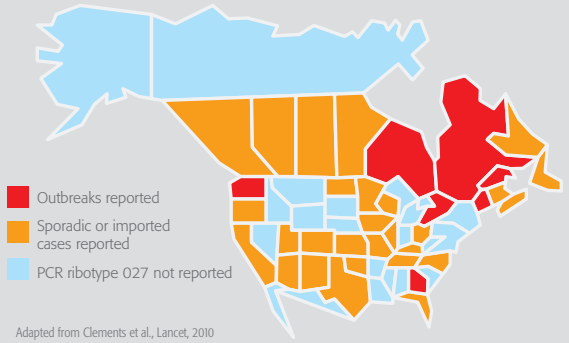


Figure 4: Epidemiology of 027 strain in US



In the United States, the incidence is approximately **7.5-12/10,000 patient days** with distinct geographic variation *(Freeman et al., 2010)*.

How is the incidence of CDI evolving?

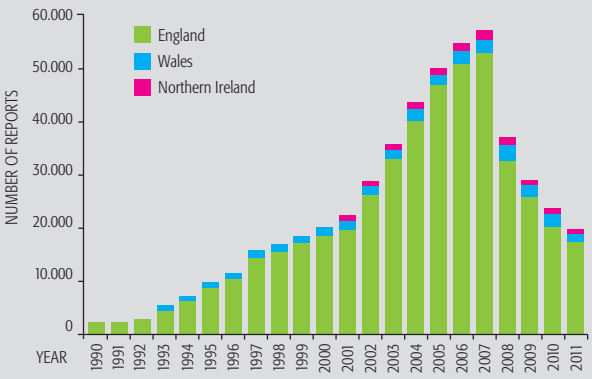
In the US, CDI rates have been increasing steadily over the past decade and CDI may now be the **most commonly identified bacterial cause of acute diarrhea** in the US. (*DuPont et al., 2011*). In 2008, an estimated **1 million cases of CDI** may have occurred in the US *(Dubberke et al., 2012)*.

- In 2010, a study showed that, for the first time, **healthcare-associated CDI exceeded the rate of methicillin-resistant *Staphylococcus aureus* (MRSA) infection; rates of CDI were 25% higher than for MRSA** in 28 community hospitals in several states (*Miller et al., 2011).*
- CDI also surpasses the incidence of many other healthcare-associated infections such as catheter-associated intravascular infections, vancomycin-resistant enterococcal infections and ventilator-associated pneumonia *(Miller et al., 2011)*.
- However, a recent CDC report showed a **promising 20% reduction** in CDI rates in less than two years in 71 hospitals that **followed infection control recommendations** *(CDC Vital signs 2012)*.
- In many countries (USA, Canada, UK, the Netherlands), outbreaks of CDI and the increased overall incidence have been attributed to a **hypervirulent strain** referred to as **027/NAP1/BI**.
- At the present time, **CDI is not a mandatory reportable disease in the United States** and in many other countries. Mandatory reporting exists in certain Canadian provinces and some European countries.

On a European level, an ECDC incidence survey in 34 European countries in 2008 showed that CDI incidence **was generally higher than documented in 2005**, but varied widely across hospitals and countries *(Bauer et al., 2011)*.

- **In the UK**, where **reporting** of all CDI cases has been **mandatory** since 2004, **incidence of CDI increased significantly** from less than 1000 cases/year in the early 1990s to approximately 60 000 cases in 2007/2008 *(AR HAI program 2009, Wilcox et al., 2012)*.
- However, since 2007, CDI incidence in the U.K. has **decreased by up to 61%** in parallel with the **successful control of the prevalence of ribotype 027** *(Wilcox et al., 2012, Freeman et al., 2010)*.

Figure 5: Voluntary laboratory reports of *C. difficile* positive faecal specimens: England, Wales and Northern Ireland 1990 - 2011



Adapted from: Voluntary surveillance of *Clostridium difficile* in England, Wales and Northern Ireland, 2011 Health Protection Report Vol 6 No. 7 - 17 February 2012

In Australia, after a high incidence of CDI in the 1980s, a significant decrease was observed in the late 1990s and early 2000s, which was attributed to a **decreased use of broad-spectrum cephalosporins** *(Thomas et al., 2002)*. The first case of ribotype 027 detected in Australia was reported in 2009. *(Riley et al., 2009)*

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*).

In other regions (Latin America, Africa), few or no data are available.

Why is the incidence of CDI decreasing in some countries?

In at least one country (the U.K.), the incidence of CDI has started to decrease in recent years.

This decrease has been attributed to several factors:

- **introduction of enhanced surveillance** (e.g. in UK, mandatory screening of all hospital inpatients over the age of 65 with diarrhea for *C. difficile*)
- **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 ribotyping and enhanced DNA fingerprinting
- **more prudent antibiotic use** ("antimicrobial stewardship" programs)
- **improved diagnostic algorithms**

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

CDI is now increasing 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 ascertainment of **community-associated CDI (CA-CDI)**.

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*). An increase of >20% has been reported in the UK between 1994 and 2004 *(Dial et al., 2005)* and in Canada, CA-CDI cases more than doubled between 1998 and 2004. (*Dial et al., 2008*).

Pediatric CA-CDI is also increasing, 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*).

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 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*).

Great care needs to be taken when interpreting such data given the possibility of ascertainment bias, due to **high colonization rates** and **different institutional 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*).

How is the virulence of *C. difficile* strains evolving ?

The severity of *C. difficile* infections has been increasing in recent years due to the emergence of hyper-virulent strains. The most well-known virulent strain is the 027 strain, but other epidemic strain types which also require reinforced detection and active surveillance, include 078, 017, 001, 014, 020.

Strains 027, 078 and 017 are currently the main hyper-virulent strains involved in hospital outbreaks.

↳ *Clostridium difficile* 027

Severe outbreaks of CDI associated with high mortality rates have been reported **in Canada** and **many states in the US** since 2002, and **in the UK** since 2006.

The most common strain isolated during these outbreaks has been characterized as **North American ribotype 027 ("027"), PFGE type 1 ("NAP1"), and REA type BI ("BI"),** now widely known as the **"hypervirulent" strain 027/NAP1/BI**.

CDI caused by the 027 strain is associated with the use of antimicrobials, especially **extended-spectrum cephalosporins**. Isolates have also been found to be resistant to fluoroquinolones, which may have provided a selection pressure for these strains to spread *(O'Connor et al., 2009; He et al., 2012)*.

This strain has now disseminated in all Canadian provinces, at least 40 states in the US *(O'Connor et al. 2009)* and at least 16 European countries. *(Kuijper et al., 2008)* Elsewhere, isolated cases have been reported in Korea, Hong Kong, and Australia, however, no epidemics in these areas have been documented *(Gerding et al. 2010)*.

↳ *Clostridium difficile* 078

Another emerging *C. difficile* ribotype is 078. This ribotype has become much more **prevalent in the Netherlands**, where it has been recovered from both **humans** (third most common type found in community-onset disease) and **several animal species** (calves, pigs, horses) *(Goorhuis et al., 2008)*.

Type 078 has also been found in hospitalized patients in England, Germany, Switzerland and France *(Rupnik et al., 2008; Wilcox et al., 2012)*.

Currently there have been **no proven cases of animal-to-human transmission**, and no definitive evidence to link food sources and human *C. difficile* infection *(Clostridium difficile Ribotyping Network for England and Northern Ireland 2008/09 report)*.

↳ *Clostridium difficile* 017

Severe hospital outbreaks of CDI due to another toxin-variant strain of *C. difficile*, ribotype 017, which produces toxin B but not toxin A (A-,B+), have been reported **mainly in Asia** (China, South Korea, and Japan) *(Gerding et al., 2010)*. Clindamycin resistance, mediated via the erm(B) gene, is a common feature found in 017 strains.

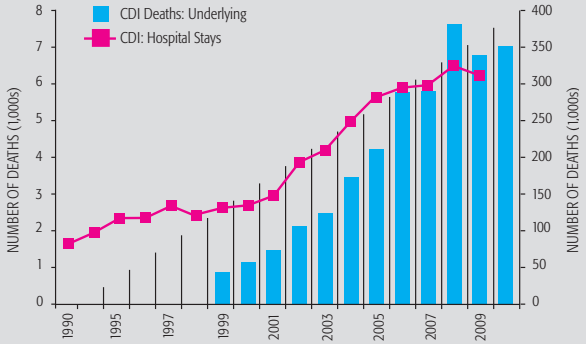
What is the mortality/morbidity associated with CDI?

Significant increases in severity of infection and mortality due to the disease have been observed over the past decade.

In the US, *C. difficile* infections are linked to **14,000 deaths per year**.

- Between 2000 and 2007, deaths related to *C. difficile* increased 400%, partly due to the increasing spread of the more virulent strain 027.
- Over **90% of deaths** related to CDI occur in **patients aged 65 and older** *(CDC Vital Signs. March 2012)*.

Figure 6: CDI Cases and Mortality in US



* Adapted from Healthcare Cost and Utilization Project: CDC NVSR Reports

- In **Europe and North America** a recent review found **all-cause mortality at 30 days** to be high, varying from **9–38%**, with over 15 studies reporting a **mortality rate of 15% or more** *(Mitchell et al., 2012)*.

Key risk factors associated with mortality due to CDI include:
- increasing age
- concomitant antibiotics
- higher white cell count and creatinine levels at time of CDI diagnosis
- lower albumin levels.

These factors could be potentially interesting for assessing risk of mortality in CDI through scoring systems (*Bloomfield et al., 2012*).

Recently, one such scoring system for predicting treatment course and CDI-related mortality has been reported. Known as the **ATLAS Score**, it takes into account age, temperature, leukocytosis, albumin, creatinine and concomitant antibiotics *(Miller MA et al., 2013)*.

What is the economic impact of CDI?

In the US, the annual economic burden of CDI on the U.S. healthcare system is estimated to be as high as **$4.8 billion** in **excess costs in acute-care facilities** alone *(Dubberke et al., 2012)*.

Most costs have been shown to be incurred during a primary episode of CDI, with costs as high as **$12,607 per case** *(McGlone et al., 2012)*.

In Europe, three studies in Ireland *(Al-Eidan et al., 2000)*, the UK *(Wilcox et al., 1996)* and Germany *(Vonberg et al., 2008)* have shown **estimated incremental costs per CDI case** ranging from **£4,577 to £6,986 and £8,843** respectively, when adjusted to 2010 GBP *(Wiegand et al., 2012)*.

Such high costs are largely due to the need for patient isolation, costly treatment, and increased length of hospital stay.

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 yet to be studied. Furthermore, the burden of disease may rise significantly if CDI becomes increasingly common in the community.

Innovative infection control strategies, accurate diagnosis, proactive surveillance, vaccine development or new therapies may potentially contribute to cost-savings since they aim to reduce the incidence, duration, severity and transmission of CDI.

CLINICAL DIAGNOSIS

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

What are the clinical signs and symptoms of CDI?

The usual symptoms are often common to other gastro-intestinal infections, making clinical diagnosis more challenging. They may include any or all of the following:
- watery diarrhea
- fever
- lower abdominal cramps
- nausea
- abdominal bloating

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

Who is most at risk of CDI?

People in good health are usually not infected by *C. difficile* since the healthy intestinal flora keep the bacterium in check.

Populations most at risk of a CDI include:
- people who take antibiotics
- prolonged stay in healthcare facility
- the elderly (>65 yrs)
- those with a serious underlying illness
- the immunocompromized

How long after initiation of antibiotic therapy can CDI occur?

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

Which antibiotics are associated with an increased risk of CDI?

Historically, **clindamycin, ampicillin, amoxicillin, cephalosporins and fluoroquinolones** have been most commonly associated with an increased risk of CDI. Further studies have shown that **other penicillins, sulfonamides, trimethoprim, cotrimoxazole, macrolides and aminoglycosides** can also be associated with CDI *(Bouza et al., 2006, Loo et al., 2005)*.

LABORATORY DIAGNOSIS

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 24 hour period or less; others recommend testing after a single unexplained diarrhoeal stool) *(ESCMID 2009, SHEA/ISDA 2010, HPA 2008)*.

Figure 7: Bristol Stool Form Scale

Type	Description	Image
Type 1	Separate hard lumps, like nuts	
Type 2	Sausage-shaped but lumpy	
Type 3	Like a sausage or snake but with cracks on its surface	
Type 4	Like a sausage or snake, smooth and soft	
Type 5	Soft blobs with clear-cut edges	
Type 6	Fluffy pieces with ragged edges, a mushy stool	
Type 7	Watery, no solid pieces	

Adapted from Lewis SJ, Heaton KW. Scand J Gastroenterol 1997

- **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 (*DR/HAI 2012*)
- **Repeat testing** during the same episode of diarrhea is of limited value and is not recommended if a reliable laboratory test for CDI is utilized *(SHEA/IDSA 2010)*.
- 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)*.

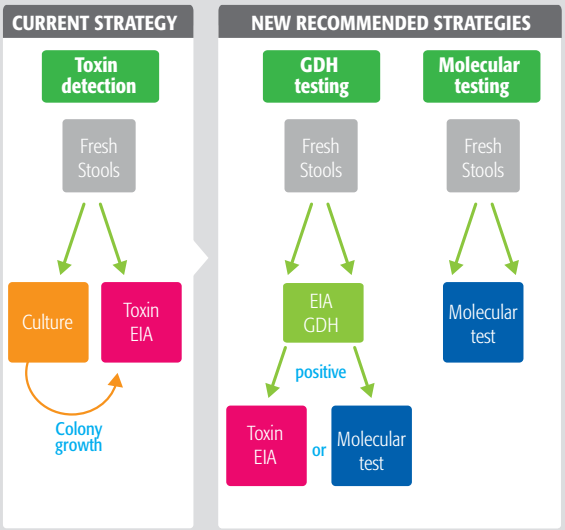
What are the different laboratory techniques available?

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

- 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 coding 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 *(ESCMID 2009, SHEA/ISDA 2010, DR/HAI 2012)*.

Figure 8: Routine Laboratory Diagnosis of CDI



Identification, susceptibility testing and strain typing are not routinely performed, but are important for **epidemiological studies** and in the event of **outbreaks** to determine the presence of specific strains.

Detection of *C. difficile* bacteria in stools

Glutamase dehydrogenase (GDH) immunoassay

- The enzyme GDH is produced in large quantities by *C.difficile*. Its presence therefore 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) *(Eckert et al., 2011)*.
- 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 or Cycloserine-Cefoxitin-Fructose Agar [CCFA] medium) in an anaerobic environment at 37°C.
- *C. difficile* strains have a characteristic "candle-wax" appearance, a typical "horse-dung" smell and a yellow-green fluorescence under UV light.
- Specific agar plates supplemented with blood and certain antibiotics are also used for highly selective culture of *C. difficile*.
- Pre-treatment of stool with heat or alcohol shock can be used to decrease normal feces flora and select bacterial spores prior to culture, especially if using non-selective media *(Eckert et al., 2011)*.

Detection of *C. difficile* toxins in stools

Enzyme Immunoassay (EIA)

- ***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 sensitivity of available EIA assays varies considerably *(Eastwood et al., 2009)*.
- Due to the presence of toxin A-negative, toxin B-positive pathogenic strains of *C. difficile*, **an EIA for detection of toxin B or both toxins** is recommended and the use of an assay for toxin A only is highly discouraged.

Cell culture cytotoxicity assay (CTA)

- Traditionally, one of the gold standard techniques to which most methods have been compared.
- **CTA detects toxins directly in stool specimens**, using a cytopathic effect in cell culture; confirmation is done by neutralizing this effect by adding antibodies to *C. difficile* toxins (*Planche et al., 2013*).

Toxigenic culture

- Another of the gold standard techniques for the diagnosis of CDI (*Planche et al., 2013*).
- **Two-step technique: culture followed by detection of toxins** produced by the isolated strain using CTA or EIA technique.
- This method can be useful in cases where patients have negative toxin stool results, but present with clinical symptoms suggestive of CDI.
- However, this method cannot differentiate 'colonization' from 'infection' by a toxigenic strain.

Table 1: Main features of *C. difficile* laboratory techniques

	Detection of Bacteria		Detection of Toxins		Detection of Toxin Genes
METHOD	**GDH**	**Culture**	**EIA**	**CTA**	**Toxigenic culture**	**NAAT**
Use	GDH enzyme detection	Strain isolation Susceptibility testing Typing	Toxin A&B detection	Toxin B detection	Strain isolation Toxin detection	Toxin B gene detection Typing
Time-to-result	15 min - 2 hrs	2-4 days	15 min - 2 hrs	1-2 days	1-2 days	< 2 hrs
Main features	• Sensitive • Manual • Automated • Rapid	• Sensitive • Manual • Low price • Excellent NPV*	• Specific • Standardized • Manual • Automated • Rapid	• Sensitive • Not standardized • Time-consuming • Technical expertise required	• Sensitive • Gold standard • Time-consuming	• Sensitive • Rapid • High cost

Adapted from Eckert et al., Journal des anti-infectieux, 2011 **NPV: Negative Predictive Value*

Detection of *C. difficile* toxin genes in stools

- **Nucleic Acid Amplification Techniques (NAAT)**
  - Molecular testing is based on toxin B gene detection and performed directly on a liquid stool sample.
  - It is the only technique recommended as a stand-alone test in some guidelines because of its high sensitivity.
  - It is specific for the presence of toxigenic *C. difficile* but cannot differentiate 'colonization' from 'infection' by a toxigenic strain.

What are the new trends in laboratory diagnostic strategies for CDI ?

Although cell culture cytotoxicity assay (CTA) and toxigenic culture are traditionally recognized as the gold standard laboratory techniques for diagnosis of CDI, more recent guidelines issued by both American and European societies are now advocating a **shift in diagnostic strategies**.

The main guidelines published recently recommend either **two- or three-step algorithms** to obtain an optimal **balance between sensitivity, specificity, time-to-result and cost**.

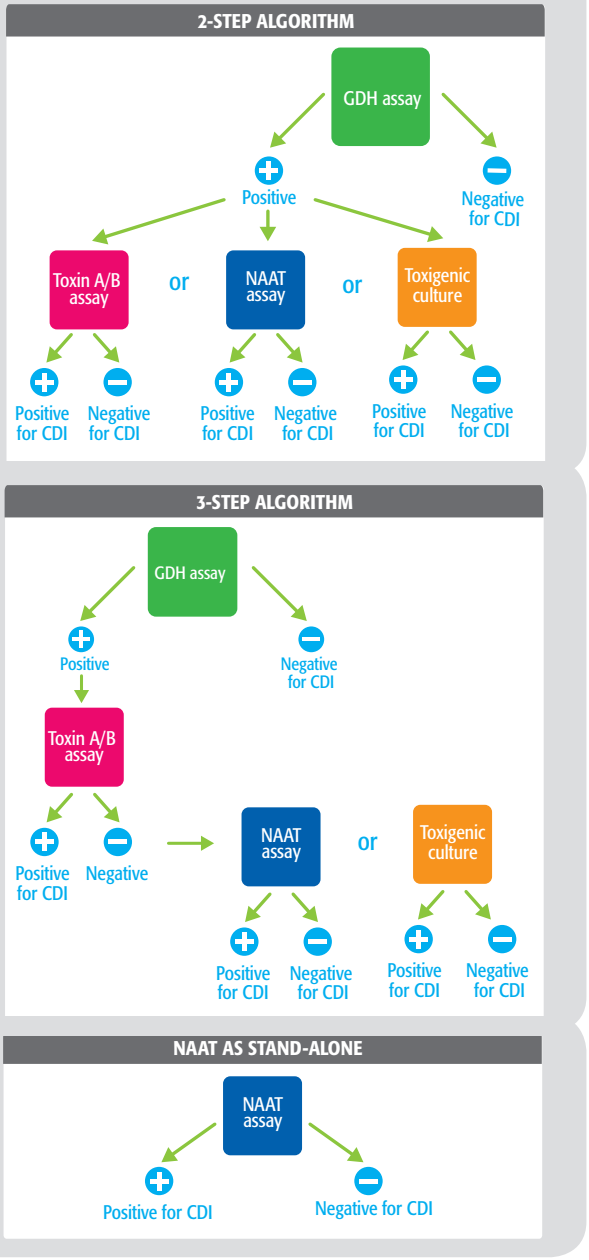
Molecular testing directly on stools could be used as a stand-alone test, but is a costly strategy. Molecular testing cannot distinguish infection from colonization and so patient/sample selection is important to minimize over-diagnosis of CDI.

Several algorithms are recommended as there is **currently no standardized approach**. The different methods and strategies used for diagnosing CDI often depend on **regional incidence rates, local laboratory capacities, technical expertise** and **budget constraints**.

Figure 9 is adapted from the main European, Australasian and US guidelines (ESCMID, ASID, SHEA / IDSA / ASM).

For list of guidelines, see page 28.

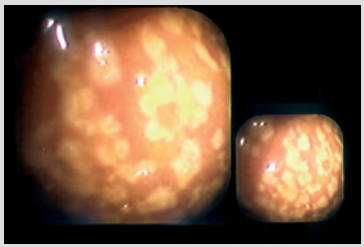
Figure 9: Recommended Algorithms for Laboratory Diagnosis of CDI



What other diagnostic methods are available?

Endoscopy

Invasive investigation used mainly to confirm cases of pseudomembranous colitis (PMC).



Pseudomembranous colitis, endoscopy / BSIP, Cavallini James

Fecal leukocytes and lactoferrin

Detection of fecal leukocytes by **methylene blue staining** can help distinguish between **inflammatory and non-inflammatory causes** of diarrhea. 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).

TREATMENT

Protocols for the treatment of CDI are well defined in European and American guidelines *(Bauer et al. ESCMID, 2009 Cohen et al.; SHEA/IDSA,2010)*. However, the management of CDI recurrence remains an issue.

Who should receive treatment ?

- **In mild cases of CDI**, clearly induced by antibiotic therapy, stopping the inciting antibiotic may be sufficient for the patient to recover within 2-3 days. However, patients should be closely monitored and treated, if the clinical condition deteriorates *(Bauer et al. ESCMID, 2009)*.
- **For all other cases of suspected CDI**, initiation of empirical treatment is recommended without delay *(Cohen et al. SHEA/IDSA, 2010)*.

What is the treatment of choice for an initial episode of CDI ?

- **Metronidazole** is the first-line antibiotic treatment for **initial, non-severe episodes of CDI**.
- **Vancomycin** is the preferred treatment for **initial episodes of severe or complicated CDI** (with or without intravenous metronidazole). Vancomycin can also be used in **second intention** for non-severe episodes when patients do not respond to/are intolerant to metronidazole.
- **Oral fidaxomicin**, a recently-approved CDI therapy associated with a decreased recurrence rate, may be indicated as first-line treatment for individuals at **high risk of recurrent disease** (e.g. extreme elderly, immunocompromised, patients who have recurrent CDI, patients on concomitant antibiotics) *(Crook et al., 2012)*.
- **Colectomy** should be considered for **severely ill patients** (perforated colon, toxic megacolon, severe ileus, deterioration despite maximal appropriate therapy, or rising serum lactate).

Table 2: CDI Treatment Guidelines

Type of therapy	Antibiotic	Dose	Frequency	Duration
Oral (if possible)				
- non-severe	Metronidazole	400 or 500 mg	tid	10-14 days
- severe	Vancomycin	125 mg	qid	10-14 days
- life-threatening	Vancomycin	500 mg	qid	
IV (if oral not possible)				
- non-severe	Metronidazole	500 mg	tid	10-14 days
- severe	Metronidazole + Vancomycin (intracolonic) and/or Vancomycin by nasogastric tube	500 mg 500 mg (in 100 mL of normal saline) 500 mg	tid Every 4-12 hrs qid	10-14 days

Adapted from Bauer *et al.* Clin. Microbiol. Infect. 2009 tid = Three times a day - qid = Four times a day

How to treat recurrent CDI ?

- **For a first recurrence of CDI**, follow recommendations for treatment of an initial episode of CDI. It is recommended **not to use metronidazole beyond the first recurrence** due to potential cumulative neurotoxicity *(Cohen et al. SHEA / IDSA, 2010)*.
- **In the event of second and subsequent recurrences**, the treatment of choice is **vancomycin** using a **tapered and/or pulse regimen**: *(ESCMID, 2009, SHEA/IDSA, 2010)*.
- **For patients at high risk of multiple recurrences** (e.g. extreme elderly, immunocompromised, patients who have recurrent CDI), **fidaxomicin** may be the preferred treatment *(Crook et al., 2012)*.

Are there alternative treatments ?

Several promising treatment options are currently being investigated, and may be of particular interest for recurrent disease:

- **Fecal microbiota transplantation (FMT)** or **fecal bacteriotherapy** has shown promising results. Experience in Europe and the US has been successful in **breaking the relapsing pattern of CDI** by restoring normal intestinal flora. A systematic review has shown fecal bacteriotherapy to be **successful in 92% of cases** *(Gough et al., 2011, van Nood et al., 2013)*.
- The use of **probiotics** to treat C difficile carriers and CDI patients remains controversial *(Hsu et al., 2010, Miller et al., 2009)*.

How to assess clinical recovery?

- **Positive response to treatment:**
  - stool frequency/consistency and abdominal pain improves within 3 days
  - no new signs of colitis, sepsis or ileus; decreasing blood white cell count.

Once clinical symptoms have improved or ceased, there is no need to perform further diagnostic tests to assess patient recovery. **Repeat stool testing for CDI is not warranted unless a post-treatment recurrence is suspected**. This is because, even in patients who have a good symptomatic response, *C. difficile* tests may still be positive.

- **Recurrence of symptoms, after initial treatment response and cessation of therapy:**
  - stool frequency increases for 2 consecutive days, or stools become looser
  - new signs of colitis develop
  - toxin-producing *C. difficile* is found in stools, without evidence of another cause of diarrhea.

In the event of symptom recurrence after initial treatment response and cessation of therapy, refer to treatment guidance for recurrent CDI above.

OUTBREAK PREVENTION AND CONTROL

Spread through oral-fecal transmission, *C. difficile* is highly transmissible. Prevention of cross-infection requires rapid implementation of a **multifaceted approach** involving **patient isolation, hygiene measures and environmental cleaning**.

On a more long-term basis, **antimicrobial stewardship programs and antibiotic use restrictions** are also likely to reduce CDI rates.

How does transmission of healthcare-associated *C. difficile* occur ?

In a hospital setting, patients may be exposed to *C. difficile* through:

- contact with a healthcare worker with contaminated hands,
- contact with a contaminated environment (toilet, bed-rails, door handles, medical equipment, etc.),
- direct contact with a patient with CDI.

The following recommendations are largely based on SHEA/IDSA guidelines (2010).

How to manage patients with CDI?

- Patients diagnosed with CDI should be treated promptly, if necessary, and **immediately isolated** from other hospitalized patients.
- In the event of an outbreak, an **alert mechanism** should be in place in the healthcare facility.
- Private rooms with **full barrier precautions** should be implemented for all patients with CDI. If single rooms are not available, symptomatic patients should be cohorted, with a personal commode for each patient.
- Dedicated healthcare workers for infected patients.
- Patients should also be instructed on **optimum hygiene measures**, such as good hand hygiene, and flushing the toilet with the lid closed to avoid aerosol release.

How to manage the spread of contamination in the healthcare setting ?

Contamination of the environment and healthcare workers' hands are usually closely related. Therefore, implementing **multiple infection control measures** is recommended to contain the spread of the bacteria.

Barrier methods

Strict contact precautions with hand hygiene measures have been reported to reduce CDI incidence by up to 80%. *(Riddle et al., 2009, Muto et al., 2007)*

↳ Contact precautions / hand hygiene

- Healthcare workers and visitors should wear gloves and gowns when entering the room of a patient with CDI. Wearing of gloves has been shown to be the **most effective single measure** for preventing CDI transmission. *(Dubberke et al., 2012)*
- **Hand-washing** after caring for or being in contact with CDI patients is essential, preferably with (antimicrobial) soap and water, as alcohol-based hand rubs are not as effective against spore-forming bacteria.
- **Contact precautions** should be maintained at least for the duration of the diarrhea. Recent evidence supports extending isolation measures for **up to 2 days after diarrhea resolves**, as contamination in the environment persists. The optimal duration of contact precautions is unknown and controversial *(Dubberke et al., 2008)*.
- Routine identification of asymptomatic carriers is currently not recommended for infection control purposes.

Simple tips for better hygiene rule compliance

Implementing simple actions can help increase healthcare workers' and visitors' adhesion to hygiene rules:

- **easy access** to hand-washing facilities,
- use of cleaning agents that **protect** rather than irritate skin,
- hospital-wide **educational programs** (including cleaning staff, nurses, physicians and other support staff),
- **posters** as a reminder of basic hygiene rules

↳ Environmental cleaning

- **Disinfection** should be performed using a **hypochlorite-based solution** (1000-5000 ppm available chlorine), or other sporicidal cleaning agents, as *C. difficile* spores are resistant to standard cleaning measures *(SHEA/IDSA, 2010, Dubberke et al., 2008)*.
- Disinfection should be performed thoroughly **at least twice a day**, and special attention given to items such as bedrails, bedside commodes, toilets and floors which are likely to be contaminated with feces or spores.
- Use of **disposable thermometers** can significantly reduce the incidence of CDI.
- **Vaporized hydrogen peroxide** has also been demonstrated as being efficient for room decontamination, but the need for specialized equipment and cost may limit this approach.
- Routine environmental screening for *C. difficile* is not recommended, but could be useful in case of persistent outbreaks.

Antibiotic use restrictions and antimicrobial stewardship

A direct link has been clearly established between extensive use of antibiotics and CDI, as well as between restricted use of antibiotics and reduced incidence of CDI *(Jump et al., 2012; Dubberke et al., 2012)*.

Multiple (either sequential or simultaneous) and prolonged antibiotics are a risk factor for CDI.

Most patients with CDI have been shown to have **prior and recent exposure to antibiotic therapy**. In a recent study, up to 85% received antibiotics within 28 days of onset of symptoms (*Chang et al., 2007*)

Restriction of antibiotic use is therefore a promising approach in reducing CDI rates, and has been shown to be particularly successful in the case of high-risk antibiotics for CDI, such as cephalosporins, clindamycin and possibly fluoroquinolones.

A successful restrictive antibiotic 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.

Recommendations For Clinicians: 6 Steps to Prevention of CDI

1. **Prescribe and use antibiotics carefully**. About 50% of all antibiotics given are not needed, unnecessarily raising the risk of *C. difficile* infections.
2. **Test for *C. difficile*** when patients have diarrhea while on antibiotics or within two months of taking them.
3. **Isolate patients** with *C. difficile* immediately.
4. **Wear gloves and gowns** when treating patients with *C. difficile*, even during short visits. Alcohol-based hand sanitizer does not kill *C. difficile*, and hand washing with soap and water is preferred.
5. **Clean room surfaces** with bleach or another EPA*-approved, spore-killing disinfectant after a patient with *C. difficile* has been treated there.
6. **When a patient transfers**, notify the new facility if the patient has a *C. difficile* infection.

*EPA – Environmental Protection Agency - Source: http://www.cdc.gov/hai/organisms/cdiff/Cdiff_clinicians.html

WHAT DOES THE FUTURE HOLD?

Is transmission through food possible?

Several studies have identified ***C. difficile* contamination in retail meat**, including pork, beef, turkey and chicken, with a predominance of ribotypes 027 and 078 strains. *(Rodriguez-Palacios et al., 2009; Songer et al., 2009; Weese et al., 2009)*.

Contamination of meat with *C. difficile* strains implicated in human infections raises concerns about food as a source of CDI. The main concern is that spores are known to survive the cooking process.

However, the relevance of food contamination is not yet clear, and **no definitive evidence exists** to link food sources and human CDI *(Weese et al., 2010)*.

Is animal to human transmission possible?

Animal reservoirs have been recognized in several studies:

In the Netherlands, *C. difficile* ribotype **078** has been found in both humans and several animal species (calves, pigs, horses) and the emergence of this ribotype in humans is epidemiologically linked to its presence in animals. *(Goorhuis et al., 2008; Hesgens et al., 2012)*

In Slovenia, *C. difficile* has been shown to be present in pigs and calves in both large and small farms *(Avbersek et al., 2009)*.

In Australia, a recent study isolated six different ribotypes of *C. difficile* from diarrheal horses, with a predominance of ribotype **012**. Interestingly however, ribotype 078, which is common elsewhere in the world, was not found in any of the isolates *(Thean et al., 2011)*.

However, direct animal-to-human transmission of CDI has not yet been proven, and there is little evidence that PCR ribotypes such as 01, 014 and 027 have a zoonotic source *(Hensgens et al., 2012)*.

Can CDI be prevented by vaccination?

The **host immune response** plays a fundamental role that can explain the large disparities in the clinical manifestation of CDI, which range from asymptomatic colonization to mild diarrhea to fulminant colitis and death *(Madan et al., 2012)*.

Increased antibody concentrations against toxins have been correlated with favourable outcome. The presence of antibodies directed against toxins is associated with a reduced risk of CDI and may also reduce the risk of recurrence *(Kelly et al., 2011; Wullt et al,. 2012)*.

Therefore, patients suffering from a deficient immune response could benefit in the future from treatment through **parenteral administration of concentrated anti-toxin immunoglobulins**, or **prevention through vaccination**. These two approaches are currently under clinical evaluation *(Loo et al., 2011; Tschudin-Sutter et al., 2012)*.

OFFICIAL GUIDELINES

US / CANADA		
Society for Healthcare Epidemiology of America (SHEA) / Infectious Diseases Society of America (IDSA)	2010	Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by SHEA / IDSA Infect. Control Hosp. Epidemiol. 2010;31(5): 25 pages.
American Society for Microbiology (ASM)	2010	A Practical Guidance Document for the Laboratory Detection of Toxigenic *Clostridium difficile*. 2010 http://www.asm.org//images/pdf/Clinical/clostridiumdifficile9-21.pdf
Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)	2013	A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases. Clin Infect Dis. 2013;57: e22-e121
Association for Professionals in Infection Control and Epidemiology (APIC)	2013	Guide to Preventing *Clostridium difficile* Infections. http://apic.org/Professional-Practice/Implementation-guides
American Academy of Pediatrics (AAP)	2013	Policy Statement : *Clostridium difficile* Infection in Infants and Children. Pediatrics 2013;131:196 -200
EUROPE		
European Society of Clinical Microbiology and Infectious Diseases (ESCMID)	2009	ESCMID: Data review and recommendations for diagnosing *Clostridium difficile*-infection (CDI). Clin. Microbiol. Infect. 2009;15:1053-1066
	2009	ESCMID: Treatment guidance document for *Clostridium difficile* infection (CDI). Clin. Microbiol. Infect. 2009;15:1067-1079
Department of Health (DH/ARHAI)	2012	Updated DH/ARHAI Guidance on the Diagnosis and Reporting of *Clostridium difficile*. http://www.dh.gov.uk/health/2012/03/clostridium-difficile-6-march-2012/
AUSTRALASIA		
Australasian Society for Infectious Diseases (ASID)	2011	Australasian Society for Infectious Diseases guidelines for the diagnosis and treatment of *Clostridium difficile* infection. Medical Journal of Australia 2011; 194: 353–358

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