# Clostridioides difficile

Subjects: Microbiology

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*Clostridioides difficile* is an increasingly common pathogen both within and outside the hospital and is responsible for a large clinical spectrum from asymptomatic carriage to complicated infection associated with a high mortality. While diagnostic methods have considerably progressed over the years, the optimal diagnostic algorithm is still debated and there is no single diagnostic test that can be used as a standalone test. More importantly, the heterogeneity in diagnostic practices between centers along with the lack of robust surveillance systems in all countries and an important degree of underdiagnosis due to lack of clinical suspicion in the community, hinder a more accurate evaluation of the burden of disease. Our improved understanding of the physiopathology of CDI has allowed some significant progress in the treatment of CDI, including a broader use of fidaxomicine, the use of fecal microbiota transplantation for multiples recurrences and newer approaches including antibodies, vaccines and new molecules, already developed or in the pipeline.

Keywords: clostridium difficile ; Clostridioides difficile ; epidemiology ; diagnosis ; treatment ; severity ; carriage ; prevention

## 1. Introduction

*Clostridioides* (formerly *Clostridium*) *difficile* is a major cause of healthcare-associated diarrhea, and is increasingly present in the community. A lot has changed in our understanding of the physiopathology of this frequent pathogen as well as in the diagnosis and treatment of *Clostridioides difficile* infection (CDI). However, several questions remain unanswered. Diagnostic approaches and surveillance systems vary considerably between regions hindering an accurate estimation of the global burden of CDI. Furthermore, diagnosis remains suboptimal, especially in certain settings, such as the community. The management of recurrences, severe and complicated disease, and the optimal use of new therapeutic molecules to target the right population remain challenging. Finally, areas of uncertainty persist regarding the significance of asymptomatic carriage, the optimal clinical endpoints and the long-term follow-up and outcomes of these patients. We selected a number of publications by searching into Pubmed to review the epidemiology, clinical presentation, outcomes and management of CDI. We discuss current diagnostic approaches and treatment options, with a special focus on areas of uncertainty and recent advances in the field.

## 2. Epidemiology

The incidence of *Clostridioides difficile* infection markedly increased worldwide in the 2000s [1][2][3], in part due to the emergence and rapid spread throughout North America and Europe of the virulent, epidemic ribotype 027 strain (North-American Pulsefield type 1, NAP1/027), which was associated with increased severity of disease and mortality [4][5][6]. At the same time, the introduction of more sensitive diagnostic assays, such as nucleic acid amplification assays (NAAT), seems to have contributed to a substantial increase in the reported CDI incidence [7][8]. Further adding to an already high burden, recurrences after diagnosis of CDI are frequent, with 10–30% of patients developing at least one recurrence and the risk increasing with each successive episode [9][10]. To be able to accurately evaluate the burden of CDI, there is a need for standardization of diagnostic algorithms and a robust surveillance system, and this need is not entirely nor universally met.

In the US, an estimated 453,000 cases of *C. difficile* infection occurred in 2011 based on data from active population- and laboratory-based surveillance across different geographic areas, resulting in approximately 29,000 deaths [3]. On a more positive note, according to a more recent study by Guh et al., the estimated burden of CDI decreased in the US between 2011 and 2017, probably as a result of improved infection control measures and a concomitant overall decline of healthcare-associated infections (HAIs) [11].

Epidemiological data are scarcer in Europe. The lack of a standardization of diagnostic procedures in hospitals, as well as the heterogeneity in the presence and the methodology of national surveillance and the availability of molecular typing, hinder a more accurate overview of the burden of CDI. In a study conducted in 34 European countries in 2008, the incidence and distribution of causative ribotypes varied greatly between countries, with an overall incidence of 4.1 per 10,000 patient-days per hospital [1]. *C. difficile* was the sixth most frequent pathogen responsible for healthcare-associated infections in a European point prevalence study conducted in 2016–2017, with an annual estimated number of cases of 189,256 [12].

Continued molecular typing is also important for a better understanding of the current epidemiology as well as in order to timely detect emerging *C. difficile* strains. For instance, even though the prevalence of 027 ribotype is decreasing in Europe [1][13], the emergence of a virulent strain ribotype 078 has been reported in the Netherlands, with an increasing prevalence between 2005 and 2008 and a severity similar to that reported with ribotype 027 [14]. The unmet needs in epidemiology are summarized in Box 1.

**Box 1.** Unmet Needs in Epidemiology.

Despite the important burden of CDI, diagnostic methods and surveillance vary across regions and countries in Europe, hampering a global and more precise overview of the burden of CDI. There is an urgent need to obtain European data on CDI burden in hospitals and in the community.

## 3. Diagnosis and Microbiology

Despite the great progress in diagnostic methods and the availability of international guidelines [14][15], the ideal diagnostic algorithm is still a matter of debate. The large clinical spectrum from asymptomatic carriage of a toxigenic strain to complicated disease and variability of outcomes, highly contribute to the confusion.

During the last decades, major changes occurred in diagnostic methods for the detection of toxigenic CDI. Previously, the main laboratory methods included toxigenic culture (TC) and cell cytotoxicity assays (CCA) and while these methods are still considered to be the reference methods for the diagnosis of CDI, they are no longer routinely used in conventional diagnostic laboratories. These methods present several limitations including the slow time to results (turnaround time of two to four days) and require high workloads performed by expert microbiologists rendering them not suitable to process large sample volumes with a high throughput [16]. These reference methods have been thus replaced by easier-to-use, rapid tests (around 30 min to 4 h) with little hands-on time such as direct toxin A/B enzyme immunoassay (EIA) EIA, lateral flow immunoassays (LFA) and NAAT [17].

EIA and rapid tests such as LFA (15–30 min) present high sensitivity and specificity for the detection of CD antigen glutamate dehydrogenase (GDH). However, a high specificity (97–100%) but a low sensitivity (29–86%) is observed for toxin A/B EIA or LFA detection, depending on the test and the patient population tested [17]. This low sensitivity of LFA for toxin detection excludes its use as a standalone approach and underlines the need for additional different tests to exclude the presence of toxigenic CD with a high negative predictive value (NPV). Before the introduction of NAAT in routine diagnostic facilities, GDH positive and toxin A/B negative EIA/LFA tests required additional analysis by usually performing selective culture methods for CD isolation and enrichment to repeat toxin A/B LFA or EIA assays to achieve higher sensitivity. Nowadays, this culture approach, requiring several days, has been replaced mostly by rapid NAAT assays for the detection of toxigenic *C. difficile* strains, following GDH positive EIA/LFA assays.

Most NAAT assays detect only the toxin A/B encoding genes *tcdA* and/or *tcdB*, which are usually sufficient for the diagnosis. Some NAAT assays (eg., Verigene, Xpert *C.difficile*) include additional important clinical and epidemiological gene targets by combining the detection of the toxin A/B encoding genes *tcdA*/*tcdB* with the detection of the binary toxin genes (*cdt*) and a deletion at nucleotide position 117 on the regulatory *tcdC* gene present in CD ribotype 27 strains and other related isolates [16]. The combined detection of the toxin B and the binary toxin is a prognostic factor of severe CDI [18]. The detection of CD ribotype 027 is important since the mutation at nucleotide position 117 of the regulatory *tcdC* gene can be associated with an increased toxin production (hypervirulence) and enhanced spore formation. Enhanced spore formation is associated with increased environmental and healthcare persistence favoring the emergence of epidemiological outbreaks [18]. Thus, NAAT assays offer the possibility of rapid detection of hypervirulent CDI ribotype 027, allowing a more stringent healthcare surveillance system. The rapid CDI diagnosis provided by EIA/LFA and NAAT compared to slow time to results of TC and CCA has significantly improved infection control management to prevent CDI transmission in healthcare facilities. Moreover, the use of NAAT allows rapid detection of CDI from symptomatic patients and with a high sensitivity that is essential to take rapid preventive measures.

### 3.1. Why Are We Not Using NAAT as the Ultimate Tool for CDI Diagnosis?

Current commercialized NAAT assays used in routine diagnostic laboratories are only qualitative (positive or negative) and cannot characterize the bacterial load and the viability of CD (viable or dead bacteria) in stool samples. NAAT, as a standalone test, is not appropriate to provide an adequate clinical positive predictive value (PPV) with low CDI prevalence [17]. Due to their high sensitivity, positive NAAT assays require a thorough and sometimes difficult clinical evaluation to discriminate CDI from (1) asymptomatic carriage of live toxigenic CD; (2) DNA from dead bacteria; and (3) long-lasting bacterial shedding following treated CDI [17][19]. However, these highly sensitive assays do not usually require further testing to exclude CDI with a high negative predictive value (NPV).

The recommended two- to three-step algorithms combining EIA/LFA, NAAT and TC proposed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guideline certainly improved PPV but are still insufficient to cover, with high PPV and NPV, the complex spectrum of CDI clinical presentations and transmission [17]. The cornerstone of the proposed two- to three-step algorithms is the detection of free toxins by EIA/LFA techniques, either directly from stools or following TC. However, although presenting high clinical specificity, EIA/LFA present reduced sensitivity compared to NAAT, implying that negative EIA/LFA results with positive NAAT assays cannot exclude with a high NPV active disease but with low toxin concentrations. Clinical evaluation of all cases presenting with NAAT positive and toxin A/B EIA (NAAT+/EIA-) negative results showed that 46.4% of the patients were colonized and 53.6% presented active disease [20]. Polage et al. reported that among NAAT+/EIA-, 38% were toxin positive by the reference method CCA [21]. However, many studies clearly demonstrate that NAAT+/EIA+ results are usually associated with higher CD bacterial loads, more severe symptoms and higher mortality rate than NAAT+/EIA- [21][22][23][24].

### 3.2. Could Quantitative NAAT Be Used to Predict Clinical Outcome?

Since a toxin A/B EIA/LFA positive assay is associated with higher bacterial loads, quantitative NAAT could have the potential to provide toxin quantification corresponding to positive toxin EIA/LFA and CCA, that could be used as a marker of infection severity and clinical outcomes. Davies et al. investigated the potential utility of toxin gene quantitative NAAT by determining the predictive value of low cycle threshold (Ct) for toxin positivity, CDI severity, mortality and CDI recurrence [25]. Unfortunately, the authors only observed a limited specificity and sensitivity of quantitative NAAT for these clinical parameters excluding its use as a standalone test. These preliminary study results demonstrate that accurate CDI diagnosis will likely require the use of combined direct (toxin detection and quantification) and indirect (infection and inflammation markers) assays to obtain optimal PPV and NPV. Moreover, the shedding of CD in stools is not constant and a variation in bacterial/toxins loads in stools collected from the same patients during a short period of time has been observed, limiting the utility of quantitative approaches as a prognostic marker.

### 3.3. Why Do We Not Routinely Perform Antibiotic Susceptibility Testing (AST) for CDI?

Antibiotic susceptibility testing (AST) is not routinely performed for CDI due to the absence of interpretation criteria from EUCAST and/or method uncertainty for currently used treatments, namely metronidazole, vancomycin and fidaxomicin. For vancomycin and metronidazole, the breakpoints are based on epidemiological cut-off values (ECOFFs) and applied to oral treatment. As stated by EUCAST, there is no conclusive data regarding the relation between MICs and outcome for these two antibiotics. For fidaxomicin, no breakpoints and ECOFF have been set by EUCAST since major variations in MIC distribution between studies have been observed (The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020).

### 3.4. Are We over- or Underdiagnosing CDI?

Fully comprehending the burden of disease requires a profound understanding of the diagnostic methods used and their respective weaknesses and merits. A growing body of evidence suggests that the detection of free toxin in stools by EIAs correlates with clinical symptoms and outcomes while the mere presence of a positive NAAT could lead to overdiagnosis and an overestimation of the real incidence of CDI [21][23][24][26]. On the other hand, despite the higher specificity of EIAs for toxins, detection of free toxins may lack sensitivity and EIAs can be negative in the early stages (due to the smaller bacterial burden) and in patients with complicated disease [27].

Several studies suggest a considerable degree of underdiagnosis in Europe. In a prospective point prevalence study conducted in 482 European centers on two sampling days, 23% of all positive CDI samples, as determined by the reference national laboratory, were not diagnosed by participating hospitals due to a lack of clinical suspicion and suboptimal laboratory methods. As a result, an estimated 40,000 inpatients are potentially undiagnosed per year [28]. In

another Spanish study where 807 specimens were addressed to a reference laboratory from 118 participating centers, two out of every three episodes of CDI were not diagnosed due to lack of clinical suspicion or non-sensitive techniques [29].

This “underdiagnosis” seems to be even more relevant in the community, where the main problem is the lack of clinical suspicion and limited awareness among physicians. While CDI is identified as the leading cause of hospital-acquired diarrhea and easily suspected in this setting, its role in the community is less clear. More than a quarter of all CDI cases are attributed to community acquisition and occur frequently in the absence of traditional risk factors, such as advanced age or prior antibiotic exposure [3][30][31]. In a large US study, an estimated of 345,000 cases occurred outside of the hospital with almost half of them considered purely community-associated (CA) (and so by definition with no healthcare exposure in the previous 12 weeks) [3]. However, another study using the same surveillance program but data from earlier years showed that some kind of exposure, such as visit in an outpatient healthcare setting, was present in 80% of CA cases [32]. More studies from Europe further highlight the role of *C. difficile* as a community pathogen. In a population-based cohort a significant proportion of CDI cases were CA (41% of 385) and occurred in younger patients who presented less severe disease [30]. In a nationwide population-based study in Finland, one third of all CDIs were CA and again patients were younger and mortality was lower in comparison to hospital-acquired CDI (3.2% vs 13.3%, $p < 0.001$) [31].

### 3.5. So Where Exactly Is the Problem?

According to one Dutch study, when CDI testing was performed in all unformed stool samples submitted by general practitioners (GPs) in search for any enteric pathogen, 1.5% were positive for *C. difficile* (out of 12,714 samples). This rate was comparable to other classically community-acquired pathogens, such as *Salmonella* spp. Interestingly, CDI testing was requested by GPs for only 7% of all samples, which would lead to potential missed diagnosis in 60% of all CDIs [33]. This rate of positive CDI testing is similar to other studies performed in a comparable setting [34][35]. Similarly, when stool samples were tested irrespectively of GPs’ request in 15 different laboratories in France, the incidence of toxigenic *C. difficile* as detected by toxigenic culture was 3.27% and 1.81% by a positive cytotoxicity assay. In this study, *C. difficile* was the second more frequent pathogen after *Campylobacter* spp. GPs requested *C. difficile* testing in only 13% of all stool samples thereby detecting only half of all potential CDI cases. It is worth mentioning that among patients with positive CDI testing, more than half had not been hospitalized within 12 weeks (CA-CDI) [36]. These data highlight an urgent need to raise awareness among physicians regarding CDI in the community, which can present with a less severe disease and affect younger patients, with a lower comorbidity load and in the total absence of healthcare exposure. The unmet needs in diagnosis are summarized in Box 2.

**Box 2.** Unmet Needs in Diagnosis.

- Accurate CDI diagnosis cannot be achieved with a single assay.
- NAAT, as a standalone test, is not appropriate to provide an adequate clinical PPV with low CDI prevalence, although some controversy remains.
- The recommended two- to three-step algorithms combining EIA/LFA, NAAT and TC proposed by the ESCMID guideline is a diagnostic improvement but is insufficient to cover with high PPV and NPV the complex spectrum of CDI clinical presentations and transmission. Nevertheless, EIA/LFA toxin assays should be avoided due to relatively low sensitivity.
- Even though the role of *C. difficile* is increasingly being recognized in the community, this diagnosis is not systematically suspected in the community setting and in the absence of traditional risk factors.
- More population-based studies are required to better appreciate the true burden of disease in the community.
- There is a need to provide specific recommendations for testing for *C. difficile* in patients with diarrhea outside the hospital and an increase in physicians’ awareness.

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