Review

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Clostridium difficile. A review on an emerging infection

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Abstract

Clostridium difficile causes antibiotic-associated diarrhoea and pseudomembranous colitis. The main virulence factors of *C. difficile* are the toxins A (TcdA) and B (TcdB). A third toxin, binary toxin (CDT), which pathogenetic role had been remained largely overlooked until few years ago, nowadays have been detected in 5%-23% of strains. *C. difficile* has spread around world. *Clostridium difficile* infection (CDI) is one of the most common health-care associated infections and a significant cause of morbidity and mortality among older adult hospitalized patients. Diagnosis of CDI is often difficult and has a substantial impact on the management of patients with disease. It is usually based on a clinical history of recent antimicrobial usage and diarrhoea in combination with laboratory tests. Although the conventional methods are crucial for the diagnosis and the subsequent treatment of CDI, a timely laboratory diagnosis is essential for the detection of toxigenic strains providing either to an effective and immediately treatment or to the prevention of further disease transmission.

In this review we provide general recommendations for testing of samples collected from patients with suspected CDI. ***Clin Ter 2019; 170(1):e41-47. doi: 10.7417/CT.2019.2106***

Key words: *Clostridium difficile* infection, diagnostic methods, therapy

Background

This work is a review of the strategies that may be used for laboratory diagnosis of *Clostridium difficile* infection (CDI). Worldwide, CDI represents an important public health problem. European and American studies report an increasing of CDI in terms of incidence, prevalence, morbidity and mortality (1). CDI causes a wide spectrum of disease, ranging from mild to severe diarrhoea (2-3) due to cytotoxic power of their enzymes (*C. difficile* toxin A: TcdA and *C. difficile* toxin B: TcdB). Both the toxins are potent monoglucosyltransferases, active on small GTP-binding proteins (Rho, Rac and Cdc42) involved either in the regulation or in the formation of the cytoskeletal actin in the intestinal epithelium (4-5). In addition, some *C. difficile* strains produce a third toxin (Binary toxin, CDT), composed of an enzymatic component (CDTa) and a binding component (CDTb). CDTb binds to a cell receptor leading to the internalization of CDTa into the cytosol catalysing the ADP-ribosylation of momomeric actin and the resultant disruption of the actin cytoskeletal (6-7). The majority of *C. difficile* strains harbouring the binary toxin genes are also A+ B+ (A+ B+ CDT+). Several studies have reported that the production of CDT in addition to TcdA and TcdB by *C. difficile* is associated with severe disease, higher mortality and an elevated risk of recurrence in humans, suggesting that CDT might play an important role in disease pathogenesis (8). Among A+ B+ CDT+ strains, the most prevalent type is the epidemic PCR ribotype 027/ toxinotype III, also known as 027/B1/NAP1 (9). This ribotype has an 18-base pair deletion at nt117 of TcdC, a negative regulator of expression either of TcdA or TcdB, therefore there is an iper-production of TcdA and TcdB (10). In the last decade, this strain has increased in Europe, USA, Canada and Asia (11). In Italy, it actually represents around the 9% of the toxigenic strains isolated in hospitalized patients (12).

Recently, some studies have highlighted an increase of *C. difficile* strains producing the binary toxin, despite the lack of cdtA gene (A- B+ CDT+) (13-14). This strain is the epidemic PCR ribotype 036/ toxinotype X (15). Interestingly, Geric et al. and Eckert et al. have described also some strains (about 2%) producing the binary toxin but negative for cdtA and cdtB genes (A- B- CDT+) (16-17). Among A- B- CDT+ strains, the most prevalent type is the epidemic PCR ribotype 033/toxinotype XIa/b (18-19). In the last ten years, the incidence of diseases associated with the new emerging ribotypes has increased either in Europe or in other Countries (USA, Canada and Asia), therefore, the timely laboratory diagnosis is crucial for the treatment of CDI (11, 20-21).

The diagnosis of CDI should be based on a combination of clinical and laboratory findings.

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CDI should be suspected in patients with acute diarrhoea (>3 loose stools in 24 hours), particularly in the setting of relevant risk factors (including recent antibiotic use, hospitalization and advanced age) (22). Although the conventional methods are crucial for the diagnosis and the subsequent treatment of CDI caused by TcdA and TcdB producing strains, a timely laboratory diagnosis is essential for the detection of hypervirulent strains providing either to an effective treatment immediately or to the prevention of further disease transmission (1).

The aim of this review is to briefly provide general recommendations for testing of samples taking in account the most accepted guidelines for diagnosis, treatment and prevention of CDI (23).

Conventional methods

Previously, the stool cytotoxicity (CTA) and the toxigenic culture (TC) methods had been used in the diagnosis of CDI (24-26). The CTA is sensitive and specific but it is relatively slow, time-consuming and expensive for the necessity of maintaining cell lines. For regards the TC, it is either very slow (48 to 72 h) or laborious. Moreover, the test is not able to identify the non-toxigenic strains. Therefore both conventional methods are unlikely to be adopted by a clinical laboratory as the standard methods for *C. difficile* testing. As of today, most laboratories have adopted enzyme immunoassays (EIAs) for toxins A and B as the routine method of testing (27).

Multistep algorithm for the laboratory diagnosis of CDI

According to the The European Society of Clinical Microbiology and Infectious Diseases (ESCMID), the diagnosis of CDI recommends the use of a two or three-step algorithm. The first step consists of either a glutamate dehydrogenase enzyme immunoassay (GDH-EIA) or the nucleic acid amplification test (NAATs) as screening test. Samples resulting negative from the first step can be reported as negative CDI, but those with positive results should be confirmed (toxins-EIA). Subsequently, the samples confirmed by this second step can be reported as positive CDI (28).

A number of laboratory stool tests are available alone or in combination as part of a diagnostic algorithm:

– GDH-EIA
– toxins EIA
– NAATs

GDH-EIA

The *C. difficile* produces and secretes GDH (highly conserved enzyme), which allows to the bacterium to limit the oxidative stress derived from inactivating hydrogen peroxide through the production of ketoglutarate (29). For this assay, several studies have shown a sensitivity of 85-95% and a specificity of 89-99%, underlining in particular a high negative predictive value, making it useful for a rapid screening (30-31) (Tab.1). However, its value is limited because it is not able to discriminate between toxigenic and non-toxigenic strains (32).

Table 1. Results in terms of sensitivity and specificity from different assays.

Assays	Sensitivity %	Specificity %	References
GDH-EIA	85-95%	89-99%	[32]Carman RJ et al., 2012
Toxin EIA	60,00%	99,00%	[40]Alcala L et al., 2008
Illumigene™	77-97%	93-100%	[55]Coyle K et al., 2010
Cepheid Xpert™	90-100%	92.9-98.6%	[49]Huang H et al., 2009

Toxin- EIA

Although some strains produce only toxin B, most *C. difficile* strains produce both toxins A and B (33-37). No CDI due to strains producing toxin A alone has been reported.

In the last two decades, the toxins EIA has been among the most widely used for diagnosing CDI for their rapid and inexpensive performance despite their poor sensitivity (38). In fact, this assay shows a sensitivity of around 60% and a better specificity (up to 99%), though several studies report the presence of false positives values associated to assay (39-41) (Tab.1). Moreover, it is important to note that *C. difficile* toxin degrades at room temperature and might be undetectable within two hours after collection; therefore, specimens should be kept at 4ºC. To overcome these limits, the NAATs represent the best method to detect the toxigenic strains.

NAATs

NAATs detect one or more specific genes of toxigenic strains; the critical gene is *tcdB*, which encodes for toxin B. NAATs are highly sensitive (42-44) compared to EIA (38, 45-46).

NAATs are specific for toxigenic strains but do not test for active toxin protein production and are capable of detecting asymptomatic carriers of *C. difficile* leading to either an overdiagnosis of CDI or an antibiotic treatment of patients who may not require such therapy with subsequently overestimation of hospital CDI rates (47). In fact, Polage et al. in their study on more than 1400 patients with suspected CDI have demonstrated that patients whose stool were positive by NAAT but negative by immunoassay had a lower toxin load and less diarrhoea than patients for whom both assays were positive (48). For circumstances in which initial testing consisted of NAAT (with positive result) is appropriate subsequent testing with the toxins EIA to bolster the clinical specificity.

Currently, real-time PCR (RT-PCR) assays have been commercially developed in order to overcome the lack of sensitivity of EIA. The RT-PCR designed to detect the conservative region of *tcdB* within the locus of pathogenicity (PaLoc) are: Quidel Lyra Direct *C. difficile*™ assay [Quidel], ProdGastro™ Cd [Prodesse], BD GeneOhm Cdiff™ [Becton

Dickinson], and Xpert™ *C. difficile* [Cepheid]. They were cleared by the Food and Drug Administration (FDA) for U.S. laboratory use. In Europe, the Xpert *C. difficile* assay targets *tcdB* in combination with binary toxin and deletion of *tcdC* for the presumptive identification of the 027 clone. This assay does not have FDA clearance for distribution in the United State, whereas it is commercially available in Europe. Several clinical studies have shown that these assays exhibit the best concordance with EIA and therefore could represent a promising alternative for the diagnosis of CDI (38, 49-54) (Tab.1).

Another amplification assay (*illumi*gene™ *C. difficile*, Meridian Bioscience, Cincinnati, OH) is based on the original loop-mediated isothermal amplification (LAMP) technology. This assay detects the PaLoc by targeting a DNA fragment in the 5' region of the *tcdA*, which is intact in all strains, including those with a large deletion in the *tcdA* gene. Coyle et al. recently shew that *Illumi*gene *C. difficile* was positive in stools spiked with $A^- B^+$ strains (55). Many clinical trials have recently evaluated the performance of the real-time PCR-based methods currently available on the market. Their sensitivity and specificity range from 77.3% to 97.1% and 93% to 100%, respectively (38, 49-54, 56) . The performance characteristics of these *Illumi*gene *C. difficile* assays are in agreement with those data, with sensitivity and specificity of 91.8% and 99.1%, respectively (Tab.1). Moreover the technology is isothermal contrary to the RT-PCR, therefore requires no costly capital equipment.

More recently, there are a variety of multiplex syndromic platforms (Luminex xTAG GPP, Luminex Molecular Diagnostics and BioFire FilmArray GI Panel, BioFire Diagnostics) able to detect the main causative agents of diarrhoea among bacteria, viruses and protozoa. These assays have been performed on a multiplex reverse transcriptase PCR technology (57-58).

Treatment

The choice of antibiotic therapy should be tailored to the severity of disease presentation. The common antibiotics used for non severe disease are either oral metronidazole or vancomycin. Among their, metronidazole is usually recommended for treatment of mild-moderate disease, whereas oral vancomycin is generally preferred (24, 59-60). The vancomycin is poorly absorbed from the gastrointestinal tract, therefore luminal drug levels are high leading to either a more rapid suppression of *C. difficile* or a faster resolution of diarrhoea, contrary to metronidazole (61-62). However, both oral metronidazole and oral vancomycin have associated with the persistent overgrowth of vancomycin resistant enterococci (VRE) in stool samples obtained from colonized patients during CDI treatment, increasing the risk of transmission (63). Moreover, they cause a significant destruction of the commensal microbiota, predisposing to intestinal colonization of VRE and *Candida spp.*

An alternative treatment of mild/moderate disease is the use of Fidaxomicin, which appears to cause either less disruption of the microbiota or a lower risk of colonization by VRE (64). For regards the severe disease, a recent study have suggested an anti-IP-10 antibody, BMS-936557, as a potentially effective therapy (65).

According to ESCMID guidelines, the use of high doses of vancomycin (500 mg four times daily) is recommended for the management of severe complicated CDI, while the patients with fulminant CDI require surgical intervention (colectomy). In fact, several studies suggest that earlier colectomy is associated with improved survival (66). Recently, an alternative surgical approach involves the laparoscopic creation of a loop ileostomy and colonic lavage. This procedure involves creating a loop ileostomy, with intra-operative colonic infusion and lavage with warmed poly-ethilene glycol solution and post-operative instillation of vancomycin flushes antegradely (67).

Recurrent CDI presents when the return of symptoms occurs within 8 weeks of the previous episode. About 10-20% of CDI recur after an initial episode of *C. difficile*, but when a patient has had one recurrence, rates of further recurrences increase to 40-65% (68). For the treatment of the first CDI recurrence, ESCMID recommends the same therapeutic drug used in the initial episode, while for the multiple recurrent CDI unresponsive to repeated antibiotic treatment, faecal transplantion in combination with oral antibiotic treatment is strongly recommended (69).

Emerging therapy for *C. difficile* hypervirulent strains: Studies performed since 2000 in Europe, Canada and the United States have shown an association with fluoroquinolones exposure and infection with hypervirulent strains (70-74). On basis of the antibiotic associations, these studies have somewhat different prevalences about strains, but a meta-analysis supports the fluoroquinolones use as a risk factor for infection with PCR ribotype 027. This is occurring because the fluoroquinolones are a class of antibiotics with a wide spectrum of activity used extensively and inappropriately to treat a great variety of infections (75-76). These findings have obvious implications for antibiotic stewardship interventions, but further studies in settings where this strain predominates are needed.

Nowadays, CRS3123 is becoming a promising therapy for the treatment of emerging ribotypes (77). CRS3123 is a therapeutic agent that selectively inhibits the growth, spore and toxin production of *C. difficile*. CRS3123 has not demonstrated any cross resistance to existing antibiotics remaining active against all *C. difficile* strains, above all against the epidemic fluoroquinolones-resistant NAP1/BI/027 strains.

Conclusion

C. difficile has a worldwide distribution and its toxigenic strains are responsible of clinically relevant infections. In the last decade, despite the knowledge on risk factors responsible of CDI, in many countries there was however an increase in terms of prevalence (78-82).

Nowadays, CDI is one of the most common healthcare-associated infections and it is responsible of morbidity and mortality increased among adult hospitalized patients. Among risks factors, the most important is represented by inappropriate antibiotics usage, especially broad-spectrum antibiotics, that destroy the intestinal microbiota exacerbating the disease.

One of the challenging in the management of CDI is the early diagnosis. To date, the most widely used tests in clinical microbiology laboratories for detection of CDI have not adequate sensitivity as stand-alone tests (83). For this reason, a multistep testing which includes NAATs is recommended for a better diagnosis combining high sensitivity with a short turnaround time (3). Moreover, a timely and accurate laboratory diagnosis for CDI is important also to limit the nosocomial spread of *C. difficile* in healthcare settings. In fact, the main objective of laboratory tests is to identify cases of CDI excluding those not due to CDI, since diarrhoea is the most frequent symptom, caused by infectious and non-infectious agents, in hospitalized and long-term care patients. Therefore, a proper diagnosis of CDI reduces transmission and prevents either inadequate or unnecessary treatments.

Some limitations in this review must be accounted. Firstly, it is a narrative review, thus an evaluation of the methodological quality of the cited studies by a standardized scoring tool has not performed and also the articles selection could be potentially biased. Secondly, among all the most frequently diagnostic methods reported in literature, just a few have been described. An accurate selection of the diagnostic tests must be tailored to each laboratory needs in order to optimize sensitivity, specificity and turnaround time.

Finally, it is important to highlight that the provided laboratory results must be interpreted with caution because they must be associated with clinical data and accurate evaluation of risk factors of CDI in order to exclude the presence of a non pathological *C. difficile* colonization. Further studies will be needed to improve the knowledge of the pathogenesis of CDI providing to the clinicians new strategies to overcome the actual limits of the laboratory diagnosis.

Conflict of interest: The authors declare that they have no conflict of interest to disclose.

References

1. Del Prete R, Ronga L, Addati G, et al. Prevalence of Clostridium difficile and ribotype 027 infection in patients with nosocomial diarrhoea in Southern Italy. New Microbiol 2017; 40:264-8
2. Napolitano LM, Edmiston CE Jr. Clostridium difficile disease: Diagnosis, pathogenesis, and treatment update. Surgery. 2017; 162:325-348. http://dx.doi.org/10.1016/j.surg.2017.01.018
3. Martínez-Meléndez A, Camacho-Ortiz A, Morfin-Otero R, et al. Current knowledge on the laboratory diagnosis of Clostridium difficile infection. World J Gastroenterol. 2017; 23:1552-1567. http://dx.doi.org/10.3748/wjg.v23.i9.1552
4. Just I, Selzer J, Wilm M, et al. Glucosylation of Rho proteins by Clostridium difficile toxin B. Nature. 1995; 375:500-503. http://dx.doi.org/10.1038/375500a0
5. Just I, Wilm M, Selzer J, et al. The enterotoxin from Clostridium difficile (ToxA) monoglucosylates the Rho proteins. J Biol Chem. 1995; 270:13932-13936
6. Aktories K and A Wegner. Mechanisms of the cytopathic action of actin-ADP-ribosylating toxins. Mol Microbiol. 1992; 6:2905-18
7. Simpson LL, Zepeda H and Ohishi I. Partial characterization of the enzymatic activity associated with the binary toxin (type C2) produced by Clostridium botulinum. Infect. Immun. 1988; 56:24-7
8. Stubbs S, Rupnik M, Gibert M, et al. Production of actin-specific ADP-ribosyltransferase (binary toxin) by strains of Clostridium difficile. FEMS Microbiol Lett. 2000; 186(2):307-12
9. Warny M, Pepin J, Fang A, et al. Toxin production by an emerging strain of Clostridium difficile associated with outbreaks of severe disease in North America and Europe. Lancet. 2005; 366:1079-1084. http://dx.doi.org/10.1016/S0140-6736(06)68417-1
10. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. N Engl J Med. 2005; 353:2442-9. http://dx.doi.org/10.1016/S0084-3873(08)70387-0
11. Kuijper EJ, Coignard B, Tull P. Emergence of Clostridium difficile-associated disease in North America and Europe. Clin Microbiol Infect. 2006; 12:2-18. http://dx.doi.org/10-1111/j.1469-0691.2006.01580.x
12. Bartlett JG. Narrative review: the new epidemic of Clostridium difficile-associated enteric disease. Ann Intern Med. 2006; 145:758-64
13. Kato H, Kato N, Watanabe K, et al. Identification of toxin A-negative, toxin B-positive Clostridium difficile by PCR. J Clin Microbiol. 1998; 36:2178–82
14. Alfa MJ, Kabani A, Lyerly D, et al. Characterization of a toxin A-negative, toxin B-positive strain of Clostridium difficile responsible for a nosocomial outbreak of Clostridium difficile-associated diarrhea. J Clin Microbiol. 2000; 38:2706–14
15. Gerding DN, Johnson S, Rupnik M, et al. Clostridium difficile binary toxin CDT: mechanism, epidemiology, and potential clinical importance.Gut Microbes. 2014; 5:15-27. Review. http://dx.doi.org/10.4161/gmic.26854
16. Geric B, Johnson S, Gerding DN, et al. Frequency of binary toxin genes among Clostridium difficile strains that do not produce large clostridial toxins. J Clin Microbiol. 2003; 41:5227-32
17. Eckert C, Emirian A, Le Monnier A, et al. Prevalence and pathogenicity of binary toxin-positive Clostridium difficile strains that do not produce toxins A and B. New Microbes New Infect. 2014;3:12-7. http://dx.doi.org/10.1016/j.nmni.2014.10.003
18. Rupnik M. Heterogeneity of large clostridial toxins: importance of Clostridium difficile toxinotypes. FEMS Microbiol Rev. 2008; 32:541–555. http://dx.doi.org/10.1111/j.1574-6976.2008.00110.x
19. Rupnik M, Brazier JS, Duerden BI, et al. Comparison of toxinotyping and PCR ribotyping of Clostridium difficile strains and description of novel toxinotypes. Microbiology. 2001; 147: 439–447. http://dx.doi.org/10.1099/00221287-147-2-439
20. Pituch H, Brazier JS, Obuch-Woszczatynski P, et al. Prevalence and association of PCR ribotypes of Clostridium difficile isolated from symptomatic patients from Warsaw with macrolide-lincosamidestreptogramin B (MLSB) type resistance. J Med Microbiol. 2006; 55:207-13
21. McDonald LC, Owings M, Jernigan DB. Clostridium difficile infection in patients discharged from US short-stay hospitals, 1996-2003. Emerg Infect Dis. 2006; 12:409-415. http://dx.doi.org/10.3201/eid1205.051064
22. Aschbacher R, Indra A, Wiedermann CJ, et al. Predominan-

ce of Clostridium difficile 027 during a five-year period in Bolzano, Northern Italy. Infez Med. 2017; 25:13-20
23. La Torre G, Backhaus I, Mannocci A. Rating for narrative reviews: concept and development of the International Narrative Systematic Assessment tool. Senses Sci. 2015; 2:31-35. http://dx.doi.org/:10.14616/sands-2015-1-3135
24. Cohen SH, et al. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control Hosp Epidemiol. 2010; 31:431–455. http://dx.doi.org/10.1086/651706
25. Crobach MJ, Dekkers OM, Wilcox MH, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID):data review and recommendations for diagnosing Clostridium difficile-infection (CDI). Clin Microbiol Infect. 2009; 15:1053–1066. http://dx.doi.org/10.1016/j.cmi.2016.03.010
26. Delmee M, Van Broeck J, Simon A, et al. Laboratory diagnosis of Clostridium difficile-associated diarrhoea: a plea for culture. J Med Microbiol. 2005; 54:187–191. http://dx.doi.org/10.1099/jmm.0.45844-0
27. Barbut F, et al. Delmée M, Brazier JS, et al. A European survey of diagnostic methods and testing protocols for Clostridium difficile. Clin Microbiol Infect. 2003; 9:989–96
28. Novak-Weekley SM, Marlowe EM, Miller JM, et al. Clostridium difficile testing in the clinical laboratory by use of multiple testing algorithms. J Clin Microbiol. 2010; 48:889. http://dx.doi.org/10.1128/JCM.01801-09
29. Girinathan BP, Braun SE, Govind R. Clostridium difficile glutamate dehydrogenase is a secreted enzyme that confers resistance to H2O2. Microbiology. 2014; 160:47–55. http://dx.doi.org/10.1099/mic.0.071365-0
30. Snell H, Ramos M, Longo S, et al. Performance of the TechLab C. DIFF CHEK-60 enzyme immunoassay (EIA) in combination with the C. difficile Tox A/B II EIA kit, the Triage C. difficile panel immunoassay, and a cytotoxin assay for diagnosis of Clostridium difficile–associated diarrhea. J Clin Microbiol. 2004; 42:4863–4865. http://dx.doi.org/10.1128/JCM.42.10.4863-4865.2004
31. Zheng L, Keller SF, Lyerly DM, et al. Multicenter evaluation of a new screening test that detects Clostridium difficile in fecal specimens. J Clin Microbiol. 2004; 42:3837–3840. http://dx.doi.org/10.1128/JCM.42.8.3837-3840.2004
32. Carman RJ, Wickham KN, Chen L, et al. Glutamate dehydrogenase is highly conserved among Clostridium difficile ribotypes. J Clin Microbiol. 2012; 50:1425–6
33. Limaye AP, Turgeon DK, Cookson BT, et al. Pseudomembranous colitis caused by a toxin A(-) B(+) strain of Clostridium difficile. J Clin Microbiol. 2000; 38:1696
34. Barbut F, Lalande V, Burghoffer B, et al. Prevalence and genetic characterization of toxin A variant strains of Clostridium difficile among adults and children with diarrhea in France. J Clin Microbiol. 2002; 40:2079
35. Johnson S, Kent SA, O'Leary KJ, et al. Fatal pseudomembranous colitis associated with a variant clostridium difficile strain not detected by toxin A immunoassay. Ann Intern Med. 2001; 135:434
36. Knapp CC, Sandin RL, Hall GS, et al. Comparison of vidas Clostridium difficile toxin-A assay and premier C. difficile toxin-A assay to cytotoxin-B tissue culture assay for the detection of toxins of C. difficile. Diagn Microbiol Infect Dis. 1993; 17:7. https://doi.org/10.1016/0732-8893-(93)90062-C
37. Lyras D, O'Connor JR, Howarth PM, et al. Toxin B is essential for virulence of Clostridium difficile. Nature. 2009; 458:1176. http://dx.doi.org/10.1038/nature07822.
38. Eastwood K, Else P, Charlett A, et al. Comparison of nine commercially available Clostridium difficile toxin detection assays, a real-time PCR assay for C. difficile tcdB, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. J Clin Microbiol. 2009; 47:3211–7
39. Sloan LM, Duresko BJ, Gustafson DR, et al. Comparison of real-time PCR for detection of the tcdC gene with for toxin immunoassay and culture in diagnosis of Clostridium difficile infection. J Clin Microbiol. 2008; 46:1996-2001. https://doi.org/10.1128/JCM.00032-08
40. Alcala L, Sanchez-Cambronero L, Catalan MP, et al. Comparison of three commercial methods for rapid detection of Clostridium difficile toxins A and B from fecal specimens. J Clin Microbiol. 2008; 46:3833-5
41. Brecher SM, O'Brien C, Strymish J, et al. Clostridium difficile EIA and PCR testing of EIA high positive, low positive and high negative stool samples. In: 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 12-15, 2009
42. Luo RF, Banaei N. Is repeat PCR needed for diagnosis of Clostridium difficile infection? J Clin Microbiol. 2010; 48:3738. https://doi.org/10.1128/JCM.00722-10
43. van den Berg RJ, Bruijnesteijn van Coppenraet LS, Gerritsen HJ, et al. Prospective multicenter evaluation of a new immunoassay and real-time PCR for rapid diagnosis of Clostridium difficile-associated diarrhea in hospitalized patients. J Clin Microbiol. 2005; 43:5338. https://doi.org/10.1128/JCM.43.10.5338-5340.2005
44. Longtin Y, Trottier S, Brochu G, et al. Impact of the type of diagnostic assay on Clostridium difficile infection and complication rates in a mandatory reporting program.Clin Infect Dis. 2013; 56:67. https://doi.org/10.1093/cid/cis840.
45. Kvach EJ, Ferguson D, Riska PF,et al. Comparison of BD GeneOhm Cdiff real-time PCR assay with a two-step algorithm and a toxin A/B enzyme-linked immunosorbent assay for diagnosis of toxigenic Clostridium difficile infection. J Clin Microbiol. 2010; 48:109
46. van den Berg RJ, Vaessen N, Endtz HP, et al. Evaluation of real-time PCR and conventional diagnostic methods for the detection of Clostridium difficile-associated diarrhoea in a prospective multicentre study. J Med Microbiol. 2007; 56:36. https://doi.org/ 10.1099/jmm.0.46680-0
47. Kociolek LK, Bovee M, Carter D, et al. Impact of a healthcare provider educational intervention on frequency of Clostridium difficile polymerase chain reaction testing in children: a segmented regression analysis. J Pediatr Infect Dis Sc. 2017; 6:142–8
48. Polage CR, Gyorke CE, Kennedy MA, et al. Overdiagnosis of Clostridium difficile Infection in the Molecular Test Era JAMA Intern Med. 2015; 175:1792. https://doi.org/10.1001/jamainternmed.2015.4114
49. Huang H, Weintraub A, Fang H, et al. Comparison of a commercial multiplex real-time PCR to the cell cytotoxicity neutralization assay for diagnosis of Clostridium difficile infections. J. Clin. Microbiol. 2009; 47:3729–31
50. Kvach EJ, Ferguson D, Riska P F, et al. Comparison of BD GeneOhm Cdiff real-time PCR assay with a two-step algorithm and a toxin A/B enzyme-linked immunosorbent assay for diagnosis of toxigenic Clostridium difficile infection. J Clin Microbiol. 2010; 48:109–114. https://doi.org/10.1128/

JCM.01630-09
51. Stamper PD, Alcabasa R, Aird D, et al. Comparison of a commercial real-time PCR assay for tcdB detection to a cell culture cytotoxicity assay and toxigenic culture for direct detection of toxin-producing Clostridium difficile in clinical samples. J Clin Microbiol. 2009; 47:373–378. https://doi.org/10.1128/JCM.01613-08
52. Stamper PD, Babiker W, Alcabasa R, et al. Evaluation of a new commercial TaqMan PCR assay for direct detection of the Clostridium difficile toxin B gene in clinical stool specimens. J Clin Microbiol. 2009; 47:3846–50
53. Tenover FC, Novak-Weekley S, Woods CW, et al. Impact of strain type on detection of toxigenic Clostridium difficile: comparison of molecular diagnostic and enzyme immunoassay approaches. J Clin Microbiol. 2010; 48:3719–3724. https://doi.org/10.1128/JCM.00427-10
54. Terhes G, Urban E, Soki J, et al. Comparison of a rapid molecular method, the BD GeneOhm Cdiff assay, to the most frequently used laboratory tests for detection of toxin-producing Clostridium difficile in diarrheal feces. J Clin Microbiol. 2009; 47:3478–3481. https://doi.org/10.1128/JCM.01133-09
55. Coyle K, Elagin S, Kraft J, et al. Reactivity of Clostridium difficile toxinotypes with the Illumigene C. difficile molecular assay, 2010 abstr. C-1107. Abstr. 110th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC
56. Barbut F, Braun M, Burghoffer B, et al. Rapid detection of toxigenic strains of Clostridium difficile in diarrheal stools by real-time PCR. J Clin Microbiol. 2009; 47:1276–1277. https://doi.org/10.1128/JCM.00309-09
57. Binnicker MJ. Multiplex Molecular Panels for Diagnosis of Gastrointestinal Infection: Performance, Result Interpretation, and Cost-Effectiveness. J Clin Microbiol. 2015; 53:3723–3728. https://doi.org/ 10.1128/JCM.02103-15
58. Beckmann C, Heininger U, Marti H, et al. Gastrointestinal pathogens detected by multiplex nucleic acid amplification testing in stools of pediatric patients and patients returning from the tropics. Infection. 2014; 42:961–970. https://doi.org/10.1007/s15010-014-0656-7
59. Surawicz CM, Brandt LJ, Binion DG et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol. 2013; 108:478–498. https://doi.org/10.1038/ajg.2013.4
60. Cheng AC, Ferguson JK, Richards MJ et al. Australasian Society for Infectious Diseases guidelines for the diagnosis and treatment of Clostridium difficile infection. Med J Aust. 2011; 194:353–8
61. Nassir AWN, Sethi AK, Nerandzic MM, et al. Comparison of clinical and microbiological response to treatment of Clostridium difficile-associated disease with metronidazole and vancomycin. Clin Infect Dis. 2008; 47:56–62. https://doi.org/10.1086/588293
62. Wilcox MH, Howe R. Diarrhoea caused by Clostridium difficile: response time for treatment with metronidazole and vancomycin. J Antimicrob Chemother. 1995; 36:673–9
63. Al-Nassir WN, Sethi AK, Li Y, et al. Both oral metronidazole and oral vancomycin promote persistent overgrowth of vancomycin-resistant enterococci during treatment of Clostridium difficile-associated disease. Antimicrob Agents Chemother. 2008; 24:2403–2406. https://doi.org/10.1128/AAC.00090-08
64. Louie TJ, Cannon K, Byrne B et al. Fidaxomicin preserves the intestinal microbiome during and after treatment of Clostridium difficile infection (CDI) and reduces both toxin reexpression and recurrence of CDI. Clin Infect Dis. 2012; 55:S132–S142. https://doi.org/10.1093/cid/cis338
65. Ragusa F. Th1 chemokines in ulcerative colitis. Clin Ter. 2015; 166:e126-31. https://doi.org/10.7417/CT.2015.1835
66. Koss K, Clark MA, Sanders DSA, et al. The outcome of surgery in fulminant Clostridium difficile colitis. Colorectal Dis. 2006; 8:149–154. https://doi.org/10.1111/j.1463-1318.2005.00876.x.
67. Brown CJ, Boutros M, Morris A, et al. CAGS/ACS Evidence Based Reviews in Surgery Group. CAGS and ACS evidence based reviews in surgery. Is a diverting loop ileostomy and colonic lavage an alternative to colectomy for the treatment of severe Clostridium difficile-associated disease? Can J Surg. 2014; 57:214–216. https://doi.org/10.1503/cjs.005014
68. McFarland LV, Elmer GW, Surawicz CM. Breaking the cycle:treatment strategies for 163 cases of recurrent Clostridium difficile disease. Am J Gastroenterol. 2002; 97:1769–1775. https://doi.org/10.1111/j.1572-0241.2002.05839.x
69. Debast SB, Bauer MP, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases. European Society of Clinical Microbiology and Infectious Diseases:update of the treatment guidance document for Clostridium difficile infection. Clin Microbiol Infect. 2014; 20:1–26. https://doi.org/10.1111/1469-0691.12418
70. Goorhuis A, Van der Kooi T, Vaessen N, et al. Spread and epidemiology of Clostridium difficile polymerase chain reaction ribotype027/toxinotype III in The Netherlands. Clin Infect Dis. 2007; 45:695–703. https://doi.org/10.1086/520984
71. Barbut F, Mastrantonio P, Delmee M, et al. European Study Group on Clostridium difficile. Prospective study of Clostridium difficile infections in Europe with phenotypic and genotypic characterisation of the isolates. Clin Microbiol Infect. 2007; 13:1048–1057. https://doi.org/10.1111/j.1469-0691.2007.01824.x
72. Fenner L, Frei R, Gregory M, et al. Epidemiology of Clostridium difficile-associated disease at University Hospital Basel including molecular characterisation of the isolates 2006-2007. Eur J Clin Microbiol Infect Dis. 2008; 27:1201–1207. https://doi.org/10.1007/s10096-008-0564-9
73. Labbe AC, Poirier L, Maccannell D, et al. Clostridium difficile infections in a Canadian tertiary care hospital before and during a regional epidemic associated with the BI/NAP1/027 strain. Antimicrob Agents Chemother. 2008; 52:3180–3187. https://doi.org/10.1128/AAC.00146-08
74. Goorhuis A, Debast SB, Dutilh JC, et al. Type-specific risk factors and outcome in an outbreak with 2 different Clostridium difficile types simultaneously in 1 hospital. Clin Infect Dis. 2011; 53:860–869. https://doi.org/10.1093/cid/cir549
75. Hooper DC. Emerging mechanisms of fluoroquinolone resistance. Emerg Infect Dis. 2001; 7:337-344. https://doi.org/10.3201/eid0702.700337
76. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J.Antimicrob Chemother. 2003; 51:1109-1117. https://doi.org/10.1093/jac/dkg222
77. Jarrad AM, Karoli T, Blaskovich MAT, et al. Clostridium difficile drug pipeline:challenges in discovery and development of new agents. J Med Chem. 2015; 58:5164–5185. https://doi.org/10.1021/jm5016846
78. Desai K, Gupta SB, Dubberke ER, et al. Epidemiological and economic burden of Clostridium difficile in the United States: estimates from a modeling approach. BMC Infect Dis. 2016; 16:303. https://doi.org/10.1186/s12879-016-1610-3

79. Bouza E. Consequences of Clostridium difficile infection: understanding the healthcare burden. Clin Microbiol Infect. 2012; 18:5–12. https://doi.org/10.1111/1469-0691.12064
80. Yasunaga H, Horiguchi H, Hashimoto H, et al. The burden of Clostridium difficile-associated disease following digestive tract surgery in Japan. J Hosp Infect. 2012; 82:175–180. https://doi.org/10.1016/j.jhin.2012.07.023
81. Choi HY, Park SY, Kim YA, et al. The epidemiology and economic burden of Clostridium difficile infection in Korea. Biomed Res Int. 2015; 2015:510386. https://doi.org/10.1155/2015/510386
82. Slimings C, Armstrong P, Beckingham WD, et al. Increasing incidence of Clostridium difficile infection, Australia, 2011-2012. Med J Aust. 2014; 200:272–6
83. Estwood K, Else P, Charlett A, et al. Comparison of nine commercially available Clostridium difficile toxin detection assays, a real time PCR assay for C. difficile tcdB, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. J. Clin Microbiol 2009; 47:3213217