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This paper was presented at the V[th]European Conference on Bloodstream Infections, November 2012, Limassol, Cyprus.

ABSTRACT

Clostridium difficile infection (CDI) is the primary cause of nosocomial diarrhea in industrialized countries, usually occurring as a complication of antibiotic therapy in elderly patients. Landmark events contributed to boost the interest on CDI over the last 10 years, including the emergence of unusually severe and recurrent CDI due to the NAP1/BI/027 strain, and reports suggesting that CDI is also significantly encountered in patients previously considered at no risk, such as community-acquired CDI in patients with no recent antibiotic use, or CDI during pregnancy. Despite this growing interest from the medical community, we don't know the real dimensions of the disease for the following reasons: i) despite comprehensive guidelines have been published in Europe and in America, most laboratories still use diagnostic tests with sub-optimal sensitivity as a 'rule-out' test. Hence a significant proportion of CDI remain undiagnosed; ii) the use of PCR as a stand-alone test by others, will probably overestimate the real incidence of CDI, and jeopardize any comparison between institutions with different diagnostic procedures; iii) transversal studies, with optimal design and diagnostic tests, are rapidly outdated, due to the dramatic changes in CDI epidemiology that may occur from one year to the other. To get an accurate picture of the real dimensions of CDI issue, we need more systematic use of adequate and homogenous diagnostic strategy on the field, and the implementation of continuous monitoring of CDI incidence through surveillance programs.

Keywords:Clostridium difficile; diagnostic tests, enzyme immunoassay, toxin B, glutamate dehydrogenase, polymerase chain reaction

1. Introduction

Clostridium difficile was first isolated in 1935 by Hall and O'Toole, from the stools of healthy neonates, but it was considered as nonpathogenic until 1978, when Bartlett et al. identified *C. difficile* as the principal source of cytotoxin in the stools of patients with pseudomembranous colitis, and the primary pathogen for antibiotic-associated colitis (1). *C. difficile* infection (CDI) disproportionately affects elderly patients (age > 65 years), patients with comorbidities, and patients with recent antibiotic use, due to disruption of normal flora (2). Although clindamycin was initially considered as the main risk factor for CDI, more recent study demonstrated that fluoroquinolones, and wide-spectrum beta-lactam agents (mostly third-generation cephalosporins, and combinations with betalactamase inhibitors) are now ahead of the list. CDI has long been considered as almost 100% healthcare-related, whether by antibiotic use, or by cross-transmission between at-risk patients in the hospitals.

Landmark events contributed to boost the interest on CDI from the medical community over the last 10 years, including: i) hospital outbreaks of unusually severe and recurrent CDI, beginning in the second half of 2002 and extending through 2006 in Québec, Canada(3); ii) dissemination of the strain responsible for this outbreak, now commonly designated 'NAP1/BI/027', in the USA and in Europe(4, 5); iii) emerging reports suggesting that CDI is also significantly encountered in patients who would be erroneously considered without risk, such as community-acquired CDI in patients with no recent antibiotic use(6-8), or CDI during pregnancy(9); iv) the development and the diversification of diagnostic tests, in-house or commercial, much faster than the pace of recommendations available to guide their use in routine practice(10, 11).

In this ever-changing context, knowing the real dimensions of the CDI problem, although challenging, would be most desirable: it would pave the way for renewed efforts on the area most at need (e.g. diagnostic tests ?), and for targeted prevention on the populations most affected. In addition, the uncertainty of the current dimensions will jeopardize the evaluation of any intervention currently implemented, such as progress on antimicrobials stewardship, reinforcement of infection control measures, or the development of new drugs for the treatment of CDI.

2. Do we really know how to diagnose *C. difficile* infections ?

To have an accurate picture of the real dimensions of CDI, we need to rely on accurate diagnostic tests. This issue has been the subject of much controversies with the development of rapid tests, aiming at replacing the standard reference tests, i.e. cell cytotoxicity assay (CCA), and toxigenic culture (TC), both being time-consuming, and requiring specific laboratory facilities and technical expertise (11, 12). International guidelines published by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) in 2009 (13), and by the Society for Healthcare Epidemiology of America (SHEA), and the Infectious Diseases Society of America (IDSA) in 2010 (14), have been most useful to clarify the yield of the different types of tests, and how they should be performed and interpreted. However, several issues, such as the yield of polymerase chain reaction (PCR) tests for toxin A and/or B, remain unresolved.

2.1 Standard reference tests

Cytotoxicity assay (CCA). Detection of neutralizable toxin activity in stools from patients with pseudo-membranous colitis was the initial observation that led to the discovery of CDI. Stool filtrates are inoculated onto a monolayer of a cell culture which is

then observed for a toxin B-induced cytopathic effect (rounding of the cells), after 24 and 48 h. The specificity of the cytopathic effect is confirmed by neutralization with an antiserum. The sensitivity of the CCA as a single test for the diagnosis of CDI is reported to range from 67% to 100%(14).

Toxigenic culture (TC). Along with CCA, TC has been the mainstay in the laboratory diagnosis of CDI and is essential for the epidemiologic study of isolates, and the surveillance of antimicrobial resistance. Stool samples are inoculated on selective media in an anaerobic atmosphere for at least 48 h. *C. difficile*produces flat, yellow, ground-glass appearing colonies with a surrounding yellow halo in the medium, and a typical odor. To determine the toxigenicity of the isolated strain, toxin detection may be performed by enzyme immunoassay (EIA), CCA, or PCR.

2.2 Rapid tests

Enzyme immunoassays (EIA). EIAs are rapid and easy-to-perform assays designed to detect *C. difficile* toxins or the enzyme glutamate dehydrogenase (GDH), which is an universal antigen in *C. difficile*, produced by both toxigenic and non-toxigenic strains. Of note, most strains of *C. difficile* produce either no toxin, or toxins A and B. Several commercial EIA tests have been introduced that either detect toxin(s), or GDH. Compared with diagnostic criteria that included a clinical definition of diarrhea and laboratory testing by TC, the sensitivity, and the specificity of EIA assays for toxin(s) detection have been estimated at, respectively, 63%-94%, and 75%-100%. The sensitivity, and the specificity of EIA assays for GDH detection have been estimated at, respectively, 85%-95%, and 89%-99% (13, 14).

Polymerase chain reaction (PCR). Several in-house or commercial tests targeting toxin A genes (*tcd*A) and/or toxin B genes (*tcd*B) are currently used, and may be more sensitive

and more specific than the other rapid tests. Although the use of PCR tests will most likely increase the number of positive samples, recent data suggests that the 'additional' cases diagnosed with PCR tests may be merely patients colonized with toxigenic *C. difficile*, rather than real CDI: Y. Loingtin et al. performed a one year prospective monocentric study (2010-2011), of patients for whom a *C. difficile* test was ordered (15). All specimens (n=1321) were tested in parallel by a commercial PCR assay targeting tcdB, and a 3-step algorithm detecting GDH and toxins A and B by EIA and CCA. They found that PCR diagnosed 50% more cases than the 3-step algorithm (estimated incidence, 8.9 per 10,000 patients-days with PCR versus 5.8 per 10,000 patients-days with the 3-step algorithm, P=.01). However, cases detected by PCR only were much less likely to develop any complication (3% versus 39%, P<.001) (15). Another study demonstrated that PCR use dramatically increases the number of patients diagnosed with CDI, and that these 'additional diagnosis' are significantly different than classical cases of CDI: JL Leslie et al. tested 107 PCR-positive samples for the presence of toxins by EIAs and CCA (16). Forty-free (40.2%) were toxin-negative by both tests. These PCR-positive and toxin-negative samples had 10^1-10^4 fewer DNA copies than toxin-positive samples, as measured by a FDA-validated real time PCR. They concluded that fecal *C. difficile* concentration is a major determinant of toxin detection, and that studies are needed to determine the significance of low concentrations of *C. difficile* in the absence of detectable toxins (16).

2.3 European and American guidelines for CDI diagnosis(13, 14)

Both guidelines were published a few months apart, were based on experts opinions and literature review, and both provide the basics to improve the use of diagnostic tests for CDI worldwide. As expected, they agree on most points, including: i) empirical treatment of CDI without diagnostic testing is inappropriate, because even in an epidemic

environment, only approximately 30% of hospitalized patients who have antibiotic-associated diarrhea will have CDI; ii) testing for *C. difficile* or its toxins should be performed only on diarrheal (unformed) stool, unless ileus due to *C. difficile* suspected; iii) a 2-step approach is encouraged, with the use of a test with a high negative predictive value as a first, 'rule-out' test (e.g. EIA for GDH detection), followed by the use of a confirmatory test in samples positive with the first test (e.g. CCA or EIA for toxin detection). Figure 1 represents the algorithm proposed by the ESCMID in 2009 (13).

Two significant differences must be outlined: i) the American guidelines discouraged the use of toxin detection by EIA as a first test, because of the unacceptably low sensitivity observed in some studies with these tests; ii) Although the use of real time PCR targeting *tcd*B was included in the European guidelines in 2009, the 2010 American guidelines stated that 'more data on their utility are necessary before this method can be recommended for routine testing' (14).

2.4 How do we diagnose CDI in the real world ?

Despite the quality of these international guidelines, diagnosis of CDI is probably not performed appropriately in most laboratories, even in 2012. The potential reasons for the misuse of diagnostic tests for CDI are many, and may include cost issues, the ease of use of EIAs for toxins detection, and/or unawareness of the guidelines. In addition, the use of more sensitive diagnostic tests in an institution, will inevitably lead to an increase in the estimated incidence of CDI diagnosis. In some countries, mandatory and public reporting of CDI rates may place institutions under pressure to report the lowest rates as possible. In 2010, more than 90% of laboratories in the USA used toxin detection by EIAs as a 'rule-out test', despite its suboptimal sensitivity (14).

L. Alcala et al. recently performed an instructive study on the 'underdiagnosis' of *C. difficile* in Spain (17): They performed *C. difficile* cultures on 807 unformed stool samples sent to a network of laboratories covering 75% of the Spanish population, irrespective of the type of request. They identified *C. difficile* in 63 stool samples (7.8%), of whom 45 (5.6%) had toxins detected by CCA. More importantly, two out of three episodes of CDI have not been diagnosed at the local laboratory, either because the test was not ordered (47.6%), or a non-sensitive diagnostic test was used (19.0%) (17).

3. The dynamics of *C. difficile* infections epidemiology

Despite the sub-optimal use of diagnostic tests for CDI in most laboratories, many important epidemiological studies have been performed over the last years, with the use of standard reference tests for CDI diagnosis (i.e. CCA, TC, or a 2-step algorithm) (18)(9, 19, 20). These studies provide a clear picture of what were the real dimensions of CDI in different settings, and different populations, by the time these studies were conducted. However, as dramatically illustrated by the outbreak of NAP1/BI/O27 CDI, the epidemiology of CDI is subject to very significant changes, from one year to another: In the Estrie region of Quebec, the incidence of CDI, stable from 1991 to 2002 (22.2 and 25.2 per 100,000 population, respectively), quadrupled in 2003 (92.2 per 100,000) (1, 3). It took years, for the international medical community, to get a comprehensive picture of what happened in Quebec. Earlier awareness of the extent of the problem may have attenuated the dissemination of this strain in the USA and Europe. However, this can only be secured through i) systematic use of adequate diagnostic strategy in all laboratories, as recommended by the guidelines; ii) continuous monitoring of CDI incidence through surveillance programs – which cannot effectively operate if diagnostic tests performed on site are not adequate.

Fortunately, the dynamics of CDI epidemiology is not always driving us to the worse: As illustrated by the last report of the Health Protection Agency, in the United Kingdom, the incidence of CDI in the general population has gradually decreased from 111.3 per 100,000 population in 2007-2008, to 35.4 per 100,000 population in 2011-2012 (figure 2) (21). This dramatic decrease is definitely not an 'artefact', as it was paralleled by a 70% decrease in the annual number of death certificates mentioning *C. difficile* between 2007 and 2010 in this country, from 7916 to 2335(21). In addition, similar favorable trends have been observed in Northern France with the fall of the NAP1/BI/027 strain (figure 3) (22), and in the Netherlands(23). Taken together, these dramatic increases or decreases in CDI epidemiology over time demonstrate that transversal studies, even with the best design and diagnostic methods, may still provide an inaccurate picture of CDI epidemiology, because significant changes occur faster than the time it takes for a paper to be published.

4. Conclusions

Two main factors concur to our suboptimal awareness of the real dimensions of CDI. First, despite the publication of international guidelines, from Europe and America (13, 14), which agree on most recommendations, the diagnostic strategy remains suboptimal in most settings. Hence, a significant proportion of CDI remains undiagnosed in the field (17), and will not come to our attention. Second, the epidemiology of CDI evolves at a fast pace, and may decrease (21), or increase (3), very significantly from one year to the other. Because of this, even the best transversal surveillance studies may already be out of date by the time they are published, due to the time elapsed between the conduct of the study, and its communication.

To better know the real dimensions of CDI, we should move toward a more systematic use of adequate diagnostic strategy in all laboratories, as recommended by the guidelines, and implement continuous monitoring of CDI incidence through surveillance programs.

Fig. 1. Recommended algorithm to diagnose *C. difficile* infections (CDI), ESCMID 2009 (13)

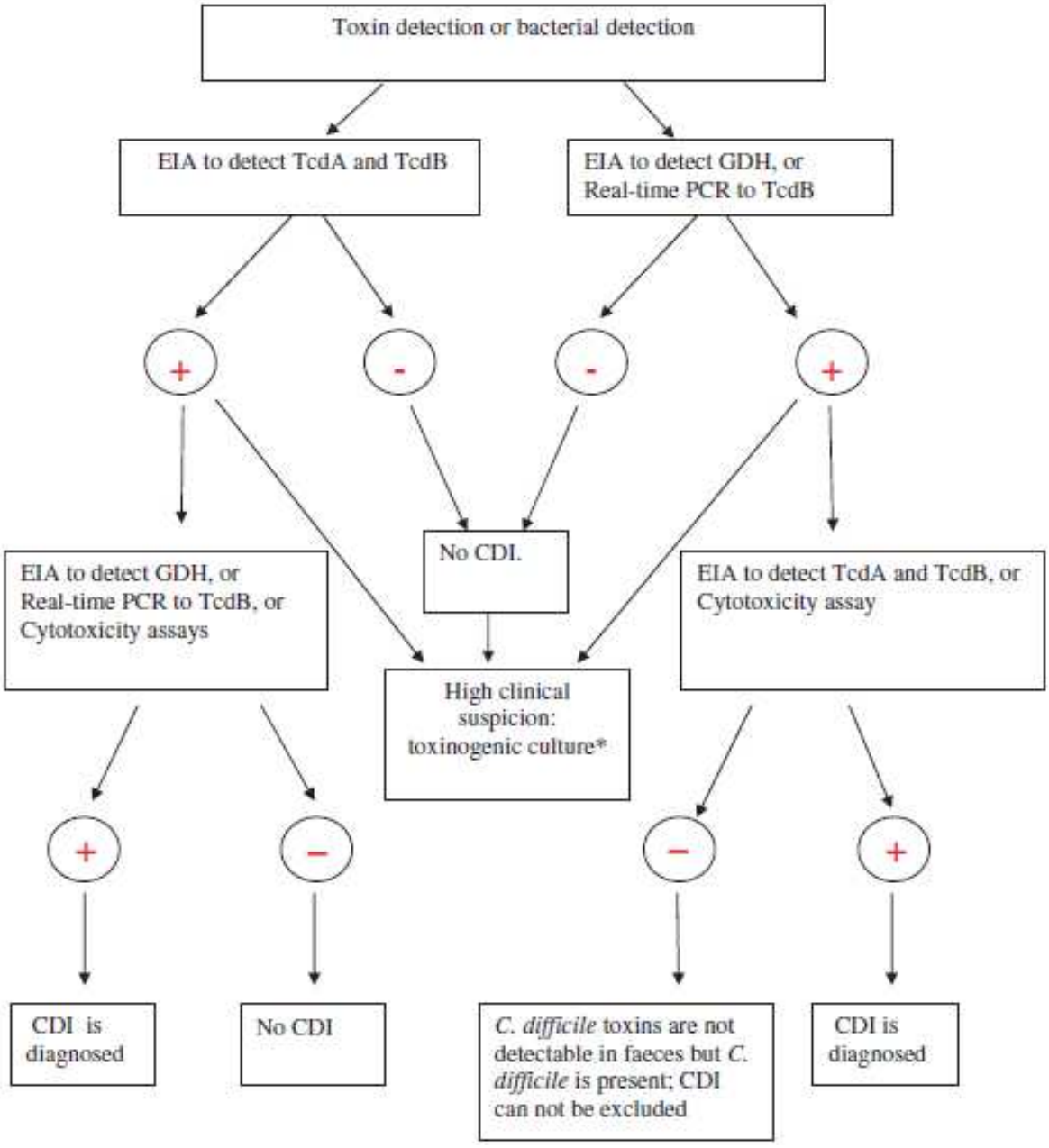


Fig. 2. Rates of *C. difficile* infections in the United Kingdom (National Health Service, 2012) (21)

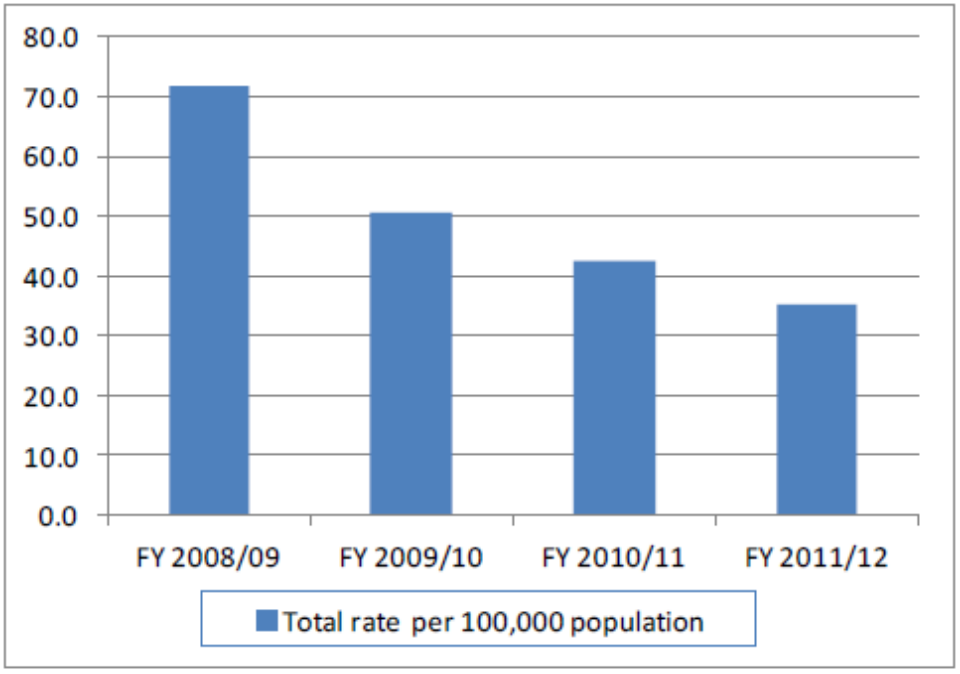
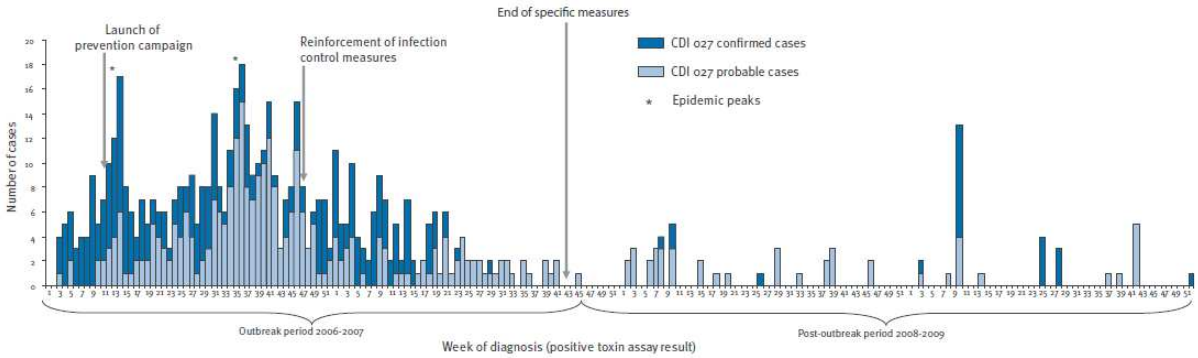


Fig. 3.*C. difficile* infection with NAP1/BI/027 in Northern France, 2006-2009 (n=602) (22)



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