

A European survey of diagnostic methods and testing protocols for *Clostridium difficile*

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Objective To conduct a survey of the methods used in clinical microbiology laboratories in Europe to diagnose infection with *Clostridium difficile*.

Methods A questionnaire was devised and sent to a co-ordinating member of the Study Group in each of eight European countries. This co-ordinator was in charge of forwarding the questionnaire to hospital laboratories arbitrarily selected. The number of laboratories in each country was determined on the basis of one laboratory for 10 000 beds of hospitalization. This questionnaire covered different aspects pertaining to *Clostridium difficile* associated to diarrhea (CDAD) diagnosis such as circumstances of request, criteria used for undertaking *C. difficile* investigations, methods used for the diagnosis, etc.

Results A total of 212 questionnaires were completed and submitted for analysis: 87.7% of laboratories reported routinely performing *C. difficile* diagnostic tests. Methods used included toxin detection (93%), culture (55%), and glutamate dehydrogenase (GDH) detection (5.9%). Among the laboratories detecting toxins, different enzyme immunoassays (EIA) and cytotoxicity assays were used in 79% and 17.3% of cases, respectively. Among the different strategies reported, 4.8% were considered suboptimal for the diagnosis of *C. difficile* infections, but marked discrepancies could be observed between countries. The overall incidence (median) of CDAD was estimated at 1.1 for 1000 patient admissions.

Conclusion The results of this study suggest marked discrepancies between laboratories and also between countries regarding the criteria by which *C. difficile* is investigated for, and the methods and the strategies that are used for the diagnosis of *C. difficile*. These discrepancies could be explained by the lack of clear guidelines for *C. difficile* diagnosis in each country, and by the importance that physicians attach to *C. difficile*. Precise guidelines for *C. difficile* diagnosis would be the first step to make possible accurate comparison of the incidence and the epidemiology of CDAD from one hospital to another or from one country to another.

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INTRODUCTION

Since the recognition of *Clostridium difficile* as the main cause of pseudo-membranous colitis (PMC) in 1978, this anaerobic spore-forming bacterium has emerged as an important enteropathogen [1,2]. *C. difficile* is currently responsible for virtually all cases of PMC and for 10–25% of cases of antibiotic-associated diarrhea [3,4]. *C. difficile* is also the leading cause of nosocomial diarrhoea in adults from industrialized countries and many hospital outbreaks throughout the world have been described [5–7]. In a French multicenter point prevalence study, *C. difficile* has been isolated from 11.5% of diarrhoeal stool cultures sent to microbiology laboratories [8]. A large number of tests for the diagnosis of *C. difficile*-associated diarrhea (CDAD) are now commercially available [9,10]. These tests include faecal culture on selective media, detection of a nonspecific antigen (glutamate dehydrogenase, GDH) and direct detection of toxins A and/or B from stools. This latter method can be performed either by the cytotoxicity assay or by a direct enzyme immunoassay (EIA) [11–22]. The cytotoxicity assay is considered by many authors as the standard because of its great sensitivity for the detection of toxin B [23]. The variety of tests available and the lack of guidelines in Europe can make the choice of testing protocols difficult. Under the auspices of the ESCMID Study Group on *C. difficile* (ESGCD), a survey of diagnostic methods used in European laboratories was performed to get an overview of the current practices in bacteriology laboratories. The second goal of this survey was to estimate the incidence of CDAD in European hospitals.

MATERIALS AND METHODS

A standardized questionnaire about diagnostic methods for *C. difficile* was sent to a co-ordinator in each of the eight countries that agreed to participate in this survey, namely Belgium, Denmark, France, Germany, United Kingdom, Italy, Netherlands and Spain. This questionnaire

covered a range of relevant aspects including circumstances of request, criteria and methods used for *C. difficile* diagnosis. The questionnaire also focused on the ability of each laboratory to perform antimicrobial susceptibility testing and typing of *C. difficile* isolates. Data on the number of *C. difficile* cases recorded in year 2000 and the total number of admissions were also collected in order to estimate the incidence of CDAD in the different hospital settings.

Each co-ordinator was in charge of forwarding the questionnaire to hospital laboratories arbitrarily selected: the number of laboratories in each country was determined on the basis of one laboratory for every 10 000 beds of hospitalization. The questionnaires were sent back to each co-ordinator, then to the main investigator. For data acquisition and analysis, Epi-Info software was used (version 6, Centers for Diseases Control and Prevention, Atlanta, GA, USA). The Chi-squared test was used to assess differences between hospitals.

RESULTS

A total of 214 questionnaires were returned and of these, 212 were used in the analysis. This represented 91% of the expected number of questionnaires and covered more than 170 607 beds of short-stay hospitalization and 19 075 beds of long-stay hospitalization, making a grand total of 189 682 beds. The returns from each country was as follows: 67 from Germany, 52 from France, 23 from Italy, 20 from the United Kingdom, 18 from Spain, 16 from Belgium, 10 from the Netherlands, and 6 from Denmark. This was in accordance with the mode of selection of laboratories. Almost half of the questionnaires (47.6%) came from university-affiliated hospitals and approximately one-third (34.9%) from smaller hospitals with less than 500 beds.

Only 12.3% of laboratories reported being unable to perform *C. difficile* diagnosis and routinely forwarded stool samples requesting *C. difficile* investigations to an outside laboratory. This frequency is statistically higher for small hospitals

(32.4% vs. 1.4%, respectively, $P < 0.001$) or for non-university-affiliated hospitals (21.6% vs. 2%, respectively, $P < 0.001$). In addition, 3.3% of laboratories reported that they never or very rarely received requests for *C. difficile* testing. For laboratories performing *C. difficile* diagnosis, a stool investigation was carried out the same day in 90.7% of laboratories, and the result was issued on the same day in 82.9%.

Criteria for investigation for *C. difficile*

In 58% of the cases, laboratories undertook *C. difficile* investigations only if it was specifically requested by physicians, and in 40.7%, routinely on the presence of specific criteria. Nevertheless, marked discrepancies could be observed between countries (Figure 1). For example, in almost all laboratories from the United Kingdom, investigations for *C. difficile* were routinely performed on specific criteria determined by the microbiologist whereas this strategy was unusual in Italy, Spain and Belgium.

The three most common criteria for undertaking *C. difficile* testing included all the macroscopically loose or watery stools (40.3%), all the stools from patients having a past history of antibiotic therapy (45.5%), and all the stools from nosocomial diarrhoea (57.1%) (Figure 2). Twenty-three percents of laboratories also systematically performed *C. difficile* testing on stools from specific departments such as oncology, hematology, intensive care unit, or gastroenterology.

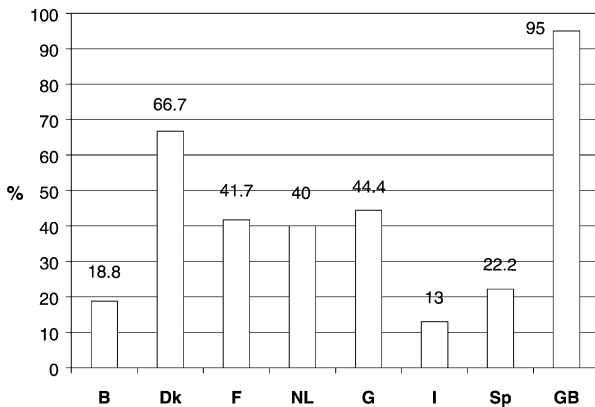


Figure 1 Use of specific criteria (such as antibiotic intake, nosocomial diarrhoea or liquid stools) determined by the microbiologist for testing for *C. difficile* (B: Belgium, Dk: Denmark; F: France, G: Germany, GB: the United Kingdom I: Italy, NL: the Netherlands, Sp: Spain).

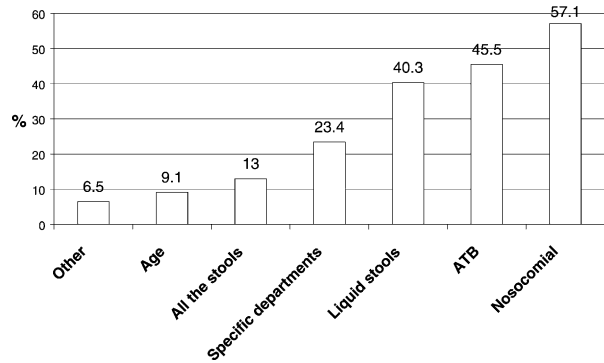


Figure 2 Criteria used for undertaking *C. difficile* investigations on stools. ATB, antibiotic intake.

Methods

Regarding the methods used for diagnosis, 93% of the laboratories reported that they assayed directly for *C. difficile* toxins in the stools. The rate was similar for all countries except, markedly so, from laboratories in Denmark (Figure 3), whose strategy consists in culturing *C. difficile* only. In this latter country, some of laboratories, then detect toxin production from the colonies. Among laboratories detecting toxins, enzyme immunoassays (EIA) and cytotoxicity assay were used in 79% and 17.3% of cases, respectively. Cytotoxicity assay was more often used by laboratories in the United Kingdom, the Netherlands, France and Belgium than in Germany, Italy and Spain where the rate was less than 10% (Figure 4). In 3.5% of the laboratories, both methods were reported to be available in order to detect possible toxA- toxB+ strains or to provide an emergency result whenever it is deemed essential.

The commercially produced (EIA) kits used were different in each country. Rapid immunochromatographic kits designed for individual use (i.e. ColorPAC™, Becton Dickinson, New Jersey,

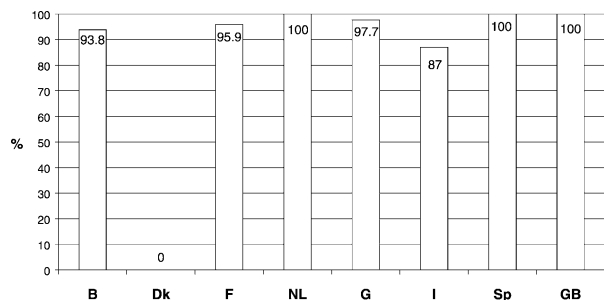


Figure 3 Percentage of laboratories performing toxin detection on stools by country.

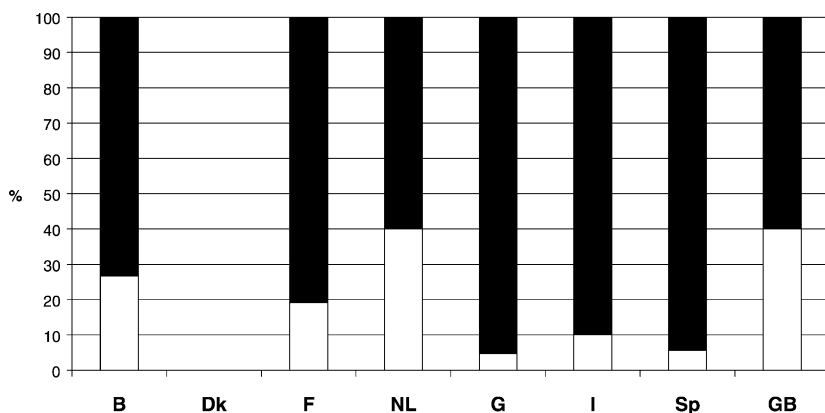


Figure 4 Methods used for toxin detection (white bars represent the cytotoxicity assay and black bars the enzyme immunoassays).

USA; TriageTM, Biosite, San Diego, CA, USA), although only on the market for a few years, represented 35% of all the EIA tests used. They were much more commonly employed in France, Belgium, Italy and Netherlands than Germany, Spain or the United Kingdom (Figure 5). Conversely, the EIA detecting both toxins A and B (i.e. CytocloneTM and Tox AB TestTM) were less frequently used (20%), except in Germany (53%) and the United Kingdom (34%).

Among laboratories performing toxin detection, an overall 58% detect only toxin A.

Fifty-five percent of laboratories reported performing culture but there were significant differences between countries (Figure 6). Culture was uncommon in the United Kingdom and Spain, but was often undertaken in Belgium, Denmark and to a lesser extent, in France. No significant difference was observed between university and nonuniver-

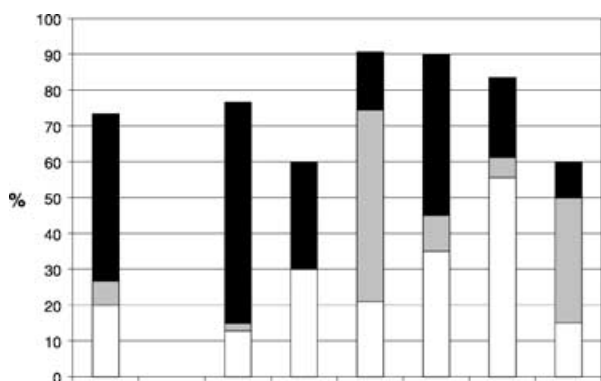


Figure 5 Types of enzyme immunoassay (EIA) used for toxin detection. The black bars represent rapid individual devices (i.e. ColorPACTM, TriageTM), the grey bars represent classic EIA in microtiter plates that detect both toxins A and B (i.e. CytocloneTM and ToxAB TestTM) and the white bars represent classic EIA in microtiter plates that detect either toxin A or B.

sity hospitals (60.6% vs. 48.8%, $P = 0.14$) and between small and large hospitals (57.1% vs. 54.4%, $P = 0.87$).

Among laboratories performing culture, commercially available CCA (cycloserine, cefoxitin agar) or variations were predominant (68.6%). In 32.3% of cases an enrichment step, most commonly alcohol shock (69.7%), was performed before inoculation of stools. This enrichment step was performed by more than half of the laboratories from Italy (54.5%), Denmark (50%), the Netherlands (50%) but only by 5% in the United Kingdom. Plates were more frequently incubated in jars than in anaerobic chambers (70.6% vs. 29.4%, respectively) but chambers were very common in Spain and Denmark (100%). The length of incubation was 48 h in 82% of cases.

Identification of *C. difficile* relied only on morphological criteria and characteristic odour in 20% of cases. More than half of the laboratories (57.9%) employed a commercial kit or gallery and 18% used latex agglutination of somatic antigen to confirm identification. This latter method was specific to the United Kingdom, Italy, and the

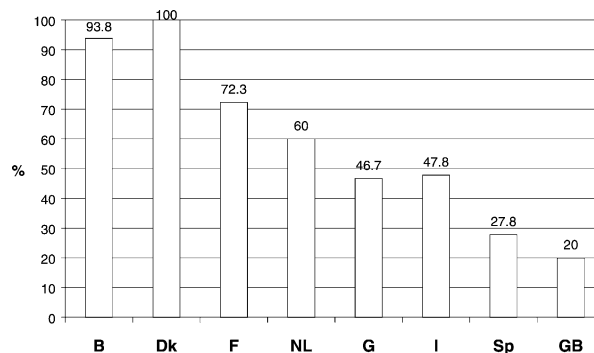


Figure 6 Percentage of laboratories performing culture for *C. difficile*.

Table 1 Classification of strategies for *C. difficile* diagnosis

Strategy	Tests
Minimal strategy	Laboratories performing culture only or antigen detection
Standard strategy	Laboratories detecting only toxins (A or B or both)
Optimal strategy	Laboratories performing simultaneously culture and toxin detection or toxigenic culture
Other strategy	i.e. laboratories performing culture only when toxin detection or glutamate dehydrogenase detection are positive

Netherlands. Other methods, i.e. the detection of proline aminopeptidase (6.9%), UV fluorescence of colonies (8.7%) or gas chromatography (2.9%) were less frequently performed.

A GDH detection was reported in 5.9% of the laboratories and polymerase chain reaction (PCR) methods were used by only 1.9% of the laboratories.

Strategies

Different strategies were used for the diagnosis of *C. difficile* infections. We arbitrarily classified these strategies into four groups (Table 1): minimal, standard, optimal, and others. The proportion of the different strategies are reported for each country in Figure 7. Denmark was unique in that most laboratories only performed culture. More than

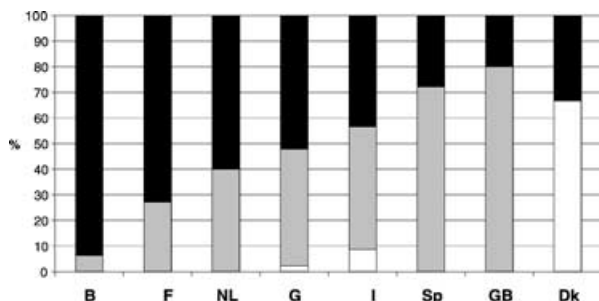


Figure 7 Strategies used in different countries for *C. difficile* diagnosis (white bars, minimal strategy; grey bars, standard strategy; hatched bars, optimal strategies and black bars, other strategies).

50% of the laboratories from Belgium, France and the Netherlands employed an optimal strategy whereas a standard strategy was predominant in Spain and the United Kingdom.

Susceptibility testing and typing methods

Overall, 18.3% of the laboratories routinely tested for *C. difficile* susceptibility, however France (40%) and the Netherlands (50%) performed these tests much more frequently than Italy (11%) and Spain (17%) whereas no laboratories from Denmark, the United Kingdom and Belgium reported as performing these investigations.

Large hospitals and university hospitals performed susceptibility testing more frequently than smaller and non-university hospitals, respectively (22.1% vs. 8%, $P = 0.04$ and 24.2% vs. 11.5%, $P = 0.039$, respectively).

Only 10.7% of laboratories reported experiences in *C. difficile* typing; there were significant differences between small and large hospitals (13.6% vs. 2.3%, $P = 0.04$) and between nonuniversity and university hospitals (16.1% vs. 4.0%, $P = 0.022$).

Estimation of the incidence of CDAD

Yearly rates of detection of *C. difficile* were calculated using a variety of different denominators (Table 2).

The incidence of CDAD for 1000 patient admissions was estimated from comprehensive data given by 112 laboratories. The overall median

Table 2 Annual rate of CDAD, positive culture for *C. difficile* or positive toxin for *C. difficile*

	Incidence of CDAD for 1000 patient admissions ($n = 112$ labs)	Frequency of positive culture for 100 stool samples ($n = 72$ labs)	Frequency of positive toxins for 100 stool samples ($n = 136$ labs)
Median	1.08	8.3	8.2
Min - Max	0.042-12.09	0-20.0	0-32.4
Percentile 25	0.44	4.7	5.0
Percentile 75	2.17	12.5	12.9

incidence of CDAD in hospitalized patients across eight European countries was 1.1 for every 1000 patient admissions. The incidence was higher in large and university-affiliated hospitals, but the difference was not statistically significant. The frequency of stools with positive toxin and those with positive culture were established from a database of 136 and 72 questionnaires, respectively. The rate of positive toxin assays was influenced neither by the size and the type of hospitals, nor by the methods or the criteria used for diagnosis. No significant difference in incidence was observed between the eight participating countries. The rate of positive culture did not vary significantly with or without the enrichment step.

DISCUSSION

This study is, to our knowledge, the first European survey of diagnostic methods for *C. difficile*.

The results that have been obtained however, could be biased by the mode of selection of laboratories. First, there was no procedure for controlling the laboratories selection and one could suspect that some of them were probably selected on the basis of having a contact likely to respond to the questionnaire. Secondly, we can hypothesize that laboratories performing *C. difficile* diagnosis probably sent back the questionnaire more readily than laboratories that did not perform the diagnosis. Finally, the number of laboratories was proportional to the number of hospitalization beds and, as a consequence, the data from countries such as France and Germany was heavily weighted.

In addition, incidence was estimated from rough data without eliminating repeat results and we took into account all the departments including paediatrics or oncology where the incidence is known to be higher.

Despite these limitations, the major finding is that 87.7% of European laboratories are able to perform the *C. difficile* diagnosis. The remaining laboratories either forwarded the stool samples to reference laboratories or never received *C. difficile* requests: the major reason mentioned for this was that physicians did not perceive CDAD as either a potential or a real problem. There are no previous European-wide studies with which to compare these results, but when compared to a study performed in France in 1995, the number of labora-

tories performing the *C. difficile* testing has increased from 77% in 1995 to 92.3% in 2001 (unpublished personal data). For 50% of the laboratories, investigation for *C. difficile* is routinely performed only in the presence of specific criteria. Nosocomial diarrhoea is commonly characterized by an interval of more than three days between the date of admission and the onset of symptoms. This strategy is in accordance with the guidelines from the American Society for Microbiology which recommend that stool cultures from adult in-patients be examined for *C. difficile* after day 3 of hospitalization [24].

The variety of methods and strategies used is an indication of the controversy that exists regarding the diagnosis of *C. difficile* and underlines the need for guidelines. The strategies were arbitrarily classified in the present study into four groups (minimal, standard, optimal and other) based on previous reports and North American guidelines [25]. The second major finding of this survey is that 93% of the laboratories undertake direct detection of toxins in stools and 80% use a commercial EIA. Analysis of strategies suggests marked discrepancies between countries that may result in a significant under-reporting of CDAD. For example, approximately 58% of the laboratories detecting toxins from stools are unable to identify toxin A-negative toxin B-positive strains because they only look for toxin A. The prevalence of these toxin A-negative toxin B-positive strains varies from 1.5% to 3% in Europe [26,27]. In addition, 4.8% of laboratories still use a minimal strategy, i.e. performing stool culture only. This method cannot predict the toxigenic status of the isolate and, from a clinical point of view, only toxigenic strains are considered pathogen [28]. Conversely, 41.6% of the laboratories use both culture and toxin detection; this strategy has been described in the literature as the optimal method for *C. difficile* diagnosis [10,12,29,30]. Culture and in vitro toxin production of isolates may increase up to 10% the diagnosis of *C. difficile*-associated diseases compared to toxin detection from stools [30].

The data from this survey have enabled us to make an estimate of the average incidence of CDAD across the participating European countries of 1.1 per 1000 patient admissions. This incidence is similar to the incidence of 0.5 per 1000 admissions in Australia as reported by Riley et al. [31] during a survey performed in 14 Australian hospitals in 1993. Data from the US studies

showed that the incidence of CDAD among hospitalized patients varies widely, ranging from one to 20 for every 1000 admissions, the highest rate being observed for bone marrow transplant recipients and patients who had undergone cardiothoracic surgery [32–34].

The determination of the yearly incidence, as well as the estimation of the annual rate of positive toxins per 100 samples processed for *C. difficile* are indicators that could be used to identify the outliers, i.e. laboratories who have rates out of the percentiles 25 and 75.

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