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Laboratory diagnosis of *Clostridium difficile*-associated disease in the Republic of Ireland: a survey of Irish microbiology laboratories

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Summary The Health Protection Surveillance Centre (HPSC) established a group to produce national guidelines for *Clostridium difficile* in Ireland in 2006. A laboratory questionnaire was distributed to determine current *C. difficile* diagnostic practices. Twenty-nine out of 44 laboratories providing *C. difficile* diagnostic services to 34 hospitals responded. Twenty-five out of 29 (86%) laboratories processed specimens for *C. difficile* and four (13.8%) forwarded specimens to another laboratory. Sixteen laboratories (64%) processed specimens for other healthcare facilities. None routinely examined stool for *C. difficile*, seven (28%) examined specimens only when requested to do so and 18 (72%) used specific selection criteria, including testing all liquid stools (39%), all nosocomial diarrhoea (44%), specific clinical criteria (28%) and history of antibiotic therapy (22%). All tested stool

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directly for *C. difficile* toxin with a variety of enzyme immunoassays, with 24 (96%) detecting both toxin A and B and one detecting toxin A only. Three (12%) laboratories used cytotoxicity assays; none used polymerase chain reaction and six (24%) laboratories performed *C. difficile* culture but only under specific circumstances. Seven (28%) laboratories had isolates typed during outbreaks, but none had the facilities to do so on-site. The HPSC group will produce national recommendations for laboratory diagnosis, surveillance and management of *C. difficile* infection. Since there are marked differences in diagnostic practices throughout the country and no national reference laboratory, the implementation of these recommendations will have cost implications that will need to be addressed.

Introduction

Clostridium difficile is responsible for a spectrum of infection ranging from asymptomatic colonisation to diarrhoea of varying severity, including life-threatening colitis. In the Republic of Ireland, the Health Protection Surveillance Centre (HPSC) is responsible for the collation and analysis of weekly notifications of infectious diseases.[1] Unlike many infectious diseases, *C. difficile* is not notifiable; therefore the extent of *C. difficile* infection in the country is unclear. The only source of national data is that from the third Hospital Infection Society (HIS) prevalence study of healthcare-associated infections in acute hospitals in the UK and Ireland conducted in 2006.[2] Forty-four acute hospitals in the Republic of Ireland participated in this study, which surveyed 7541 patients. The number of patients with current *C. difficile* diarrhoea (defined as a patient with diarrhoea which was positive for *C. difficile* toxin) was recorded for each patient. Thirty-six patients (0.5% prevalence) were reported as having *C. difficile* infection. The majority, 25/36 (69%) were aged >75 years.

Unlike sporadic cases of *C. difficile* infection, outbreaks of infectious diseases have been notifiable in Ireland since 1 January 2004.[1] Between January 2004 and September 2007, eight outbreaks of *C. difficile* infection were reported to the HPSC, five in acute hospital settings and three in residential institutions.[3] However, unlike other countries the number of patients involved ranged from three to 18 patients, and with the exception of a hospital-wide outbreak reported in the 1990s, there have been no reported large-scale outbreaks of *C. difficile* in the Republic of Ireland.[4–9]

As *C. difficile*-associated disease (CDAD) and particularly that associated with ribotype 027 has high epidemic potential, the European Centre for Disease Prevention and Control (ECDC) has expressed a need for individual member states to develop early-warning mechanisms and to implement a patient-based surveillance system.[8] While neighbouring countries such as the UK have introduced various systems of mandatory and voluntary surveillance, the Republic of Ireland has no national information on the incidence of CDAD.

In view of the paucity of information and the clear need to establish ongoing national surveillance to guide future health policies and to provide a benchmark for future interventions, the scientific advisory committee of the HPSC established a group to produce national guidelines on the surveillance, diagnosis and management of *C. difficile* in Ireland. In order to produce recommendations for standardised national surveillance of CDAD, it is essential that laboratories use similar testing protocols for *C. difficile*. As part of its work, the group undertook a laboratory survey to determine the current laboratory diagnostic practices for *C. difficile* in the Republic of Ireland.

Methods

A questionnaire was designed by the group to evaluate all aspects of diagnostic testing and specimen processing for *C. difficile* (Figure 1). It was divided into five main sections focusing on routine laboratory diagnostic methods, use of *C. difficile* culture, typing of strains, specimen selection and strategies for repeat *C. difficile* testing. Not all hospitals in Ireland have a microbiology

1. Diagnostic method routinely used for *C. difficile* in your laboratory

Does your laboratory

a. Process faecal specimens for *C. difficile*? Yes / No / Yes but done elsewhere

If tested elsewhere, please state where:

b. Process faecal specimens for *C. difficile* for other hospitals? Yes / No

If yes, please list the hospitals:

c. Have an SOP for processing faecal specimens for *C. difficile*? Yes / No

d. Test for toxin directly from stools? Yes / No

Cytotoxicity assay Yes / No

ELISA Yes / No

Toxin A only Yes / No

Toxin A and B Yes / No

Please specify

PCR Yes / No

Other *(please provide details)* Yes / No

2. Culture of *C. difficile* strains

Does your laboratory culture *C. difficile*? Yes / No / Yes but done elsewhere

– Selective agar Yes / No
– *Please specify the selective medium used:*
– Alcohol shock Yes / No
– Alcohol shock and selective agar Yes / No

Does your laboratory confirm toxin detection on *C. difficile* isolates? Yes / No

– Toxin detection from strains Yes / No
– Cytotoxicity assay Yes / No
– ELISA Yes / No

Please specify:

– PCR Yes / No

3. Typing of *C. difficile* strains

Does your laboratory type *C. difficile* isolates? Yes / No / Yes but done elsewhere

Please specify method and laboratory:

4. Strategy for *C. difficile* testing (SPECIMEN SELECTION)

[ ] Only when specifically requested

[ ] Systematically based on the following criteria:

[ ] On all stool cultures sent to the laboratory

[ ] On stools from certain departments *(if so, please specify below)*

[ ] On all liquid stools

[ ] If antibiotic treatment is stated

[ ] In cases of suspected nosocomial diarrhoea

[ ] Only on patients over a certain age *(age cut-off……)*

[ ] On outpatient community specimens

[ ] Other criteria, please specify:

5. Strategy for REPEAT *C. difficile* testing

Does your laboratory have a policy on repeat testing for patients previously positive for *C. difficile* toxin? Yes / No

[ ] Once a week

[ ] All repeat specimens tested

[ ] Specimens from previously positive patients are not retested for four weeks

Figure 1 Questionnaire on diagnosis of *C. difficile disease* in Irish laboratories. SOP, standard operating policy; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

laboratory, therefore questionnaires were sent only to those hospitals with such a facility. In November 2006, questionnaires were sent to 44 acute hospital laboratories. Reminders were sent to laboratories by e-mail and followed up by phone call if responses were not received. The results of the survey were collated at the HPSC and analysed with Microsoft Access.

Results

Questionnaires were returned from 29/44 laboratories (66% response) providing *C. difficile* diagnostic services to 34 hospitals. Responses were received from 10 regional/tertiary hospital laboratories (representing all regional/tertiary hospitals), 14 general or private hospital laboratories and five single specialist hospitals. Non-responders were from general or private hospital laboratories.

Specimen selection

Twenty-five out of 29 (86%) laboratories processed specimens for *C. difficile*. Four (13.8%) laboratories did not perform *C. difficile* diagnosis on-site but forwarded specimens to an outside laboratory for processing. Sixteen (64%) laboratories processed specimens for other healthcare facilities including nursing homes, general practitioners and other hospitals.

Seven (28%) laboratories examined specimens for *C. difficile* only when requested to do so and 18 (72%) used specific selection criteria for examining specimens. These criteria included the following:

- stool consistency (seven laboratories tested all liquid stools)
- patient age (one laboratory tested all specimens when patients were aged >1 year)
- patient location (two laboratories tested all stools from specific departments such as oncology and high-dependency care units)
- antibiotic therapy (four laboratories tested stools if the request form indicated that the patient was on antibiotics),
- clinical criteria (five laboratories)
- nosocomial diarrhoea suspected (eight laboratories).

Policies for *C. difficile* testing

Of the 25 laboratories that tested specimens for *C. difficile*, four (16%) did not have a standard operating policy for *C. difficile* testing. Twenty (80%) had a policy for repeat testing, although these policies varied greatly: this included testing repeat specimens weekly (two laboratories), testing all repeat specimens (five laboratories) or not retesting specimens from previously positive patients for four weeks after the last positive specimen (four laboratories). The remaining nine laboratories retested specimens after two weeks, 10 days or decided either on an individual basis or after discussion with the consultant microbiologist.

Routine *C. difficile* diagnostic methods

Twenty-five laboratories that tested specimens for *C. difficile* tested for *C. difficile* toxin (Table I). Twenty-four (96%) hospitals used enzyme immunoassays (EIAs) that detect both toxin A and B. Just one hospital used an assay that detected toxin A only. Twenty-three laboratories provided details of the EIA used to detect *C. difficile* toxin (Table I). In addition, three laboratories used a cytotoxicity assay and none used polymerase chain reaction testing for *C. difficile* toxin.

None of the laboratories routinely cultured all specimens for *C. difficile*. Six (24%) cultured specimens in specific circumstances such as during outbreaks. Three laboratories used selective agar and three cultured onto blood agar following faecal alcohol shock.

C. difficile typing

Seven (28%) laboratories typed strains in the case of an outbreak. These isolates were typed either in the UK (two laboratories) or at University College Dublin (three laboratories). The location of typing was unknown for two laboratories.

Discussion

This is the first time that a survey of laboratory methods for *C. difficile* diagnosis has been performed in the Republic of Ireland. The majority

Table I Routine *C. difficile* diagnosis in 25 Irish laboratories

Diagnostic methods	No. of laboratories
Toxin detection by enzyme immunoassays	25
No details	2
Meridian Premier Toxin A + B	12
Meridian Premier Immunocard A + B	4
Techlab *Clostridium difficile* Tox A/B II™	3
Combination of Meridian Premier Toxin A + B & Meridian Immunocard A + B	2
Remel Xpect® *Clostridium difficile* Toxin A/B Test Kit	1
VIDAS[R] *C. difficile* Toxin A II (CDA 2) assay (bioMérieux, Inc.)	1
Cytotoxicity assay	3
Polymerase chain reaction	0
Culture	
Routine	0
Only in specific circumstances	6

of completed questionnaires were received from laboratories with a consultant microbiologist and an infection control nurse either on-site or with a sessional commitment and represented all regional/tertiary hospital laboratories and many of the larger general hospitals. The major finding of this survey is that there are marked differences in *C. difficile* testing strategies and methodologies between Irish laboratories. This is similar to other countries where other surveys have been performed, and underlines the need for agreed national guidelines.[10–12]

Twenty-five (86%) Irish laboratories performed *C. difficile* diagnosis on-site and 16 (64%) processed specimens for other hospitals. The rest forwarded specimens to an outside laboratory for processing. None of the laboratories routinely examined stool specimens for *C. difficile*, seven (28%) examined specimens only when requested to do so and 18 (72%) used specific selection criteria. These criteria included testing all liquid stools (39%), all stools from nosocomial diarrhoea (44%), specific clinical criteria (28%) and history of antibiotic therapy (22%). Notably, while 16% of laboratories did not have a written standard operating policy for testing stool specimens for *C. difficile*, the majority (80%) had a policy for repeat testing; however, there were marked variations in repeat testing strategies between laboratories. While the numbers in this survey are smaller, the findings are similar to those from a 2002 European survey of diagnostic methods and testing protocols for *C. difficile* among 212 hospitals in eight countries.[10] In that survey, marked differences were found among laboratories with respect to the methods and strategies used for diagnosing CDAD. While 88% of laboratories performed *C. difficile* diagnosis with 40% testing all liquid specimens, a higher proportion of laboratories in that survey tested specimens if there was a history of antibiotic therapy (45%) or nosocomial diarrhoea (57%).[10]

While the issue of specimen selection is of importance in the day-to-day management of patients, there is surprisingly little in the literature on this topic. UK recommendations are based on the assumption that the presence of *C. difficile* toxin is only of clinical relevance in patients with diarrhoea and that CDAD occurs rarely in children aged <2 years. Hence the recommendation to restrict testing to diarrhoeal stools only; a diarrhoeal stool being defined as one that takes up the shape of its container. In addition, testing of children aged <2 years is not advised.[13] A recent study evaluated this approach and supported the recommendation that testing should only be performed on stools that take up the shape of their container. In this study, restricting testing to liquid stools only (as opposed to 'soft' samples — 'soft' being defined as diarrhoeal according to the definition above, but not liquid) would have missed at least 54.9% of clinically significant results. Refusing to test samples that did not take up the shape of their container, however, did not seem to cause the diagnosis of CDAD to be delayed or missed.[14] Other authors also recommend that tests for *C. difficile* or its toxins be done only on diarrhoeal (unformed) stool specimens unless ileus is present.[15,16] With regard to which patients to test, in one study prior antibiotic therapy, significant diarrhoea (defined as new onset of more than three partially formed or watery stools per 24 h period) and abdominal pain were independent predictors of a positive cytotoxin assay result. A decision rule (defined as positive if prior antibiotic use and either significant diarrhoea or abdominal pain are present) that was applied to specimens before testing demonstrated sensitivity and specificity of 86 and 45%, leading the authors to conclude that patients without prior antibiotic use and either significant diarrhoea or abdominal pain may not routinely require cytotoxin testing.[17] One of the main disadvantages of this approach is the reliance on accurate clinical data being recorded on sample submission to the laboratory, which in practice may be an unattainable goal. Furthermore, recent studies have described severe cases of CDAD in patients without traditional risk factors for CDAD including prior hospitalisation and previous exposure to antimicrobials. Restricting laboratory diagnosis to patients with more than three days hospitalisation and a history of antibiotic exposure could underestimate CDAD cases.[18]

Regarding the methods used for *C. difficile* diagnosis, all laboratories used EIAs to test stool directly for *C. difficile* toxin, with the majority (96%) testing for both toxin A and B. A large variety of EIAs were used by laboratories. In addition three (12%) laboratories used a cytotoxicity assay. Only six (24%) laboratories performed *C. difficile* culture and only in specific circumstances such as during an outbreak. Although seven (28%) laboratories typed strains during outbreaks, none had the facilities to do so on-site. These findings differ from the 2002 European study where 55% of laboratories were capable of culturing for *C. difficile*.[10] Wide variations existed among countries that participated in this study, with culture performed in more than 90% of the laboratories in Denmark and Belgium, but only in 28% of Spanish and 20% of UK laboratories. Culture enables typing and antimicrobial susceptibility testing of *C. difficile*

strains that are important from an epidemiological perspective; typing allows clonal strains to be traced and recognition of the emergence of specific virulent clones and an effective *C. difficile* surveillance programme requires that susceptibility testing be performed on isolates so that resistance rates and trends can be monitored to track the emergence of drug resistance.[19]

Current UK guidelines recommend testing for *C. difficile* toxin by either immunoassay or cell cytotoxic assay.[13] The reason why adjunctive culture is not recommended is probably linked to a combination of increased cost and a requirement for specific technical expertise. However, these guidelines were implemented before the increasing incidence of *C. difficile* 027 and many of the current EIAs in use in Irish laboratories have been demonstrated to have poor sensitivity. Two recent studies demonstrated sensitivity rates of 64% and 59% respectively with toxin AB EIAs.[20,21] These poor sensitivity rates may reflect the sample population being analysed as both hospitals processed samples for other healthcare facilities and samples may not have been stored optimally before transportation to the testing laboratories. Other studies have demonstrated that suboptimal storage of faecal samples may have a detrimental effect on toxin titres.[22]

Some authors have shown that increased yields of positive results can be obtained by using culture in combination with toxin assays.[20,23,24] This strategy, which is currently recommended in Denmark and Belgium, has recently been demonstrated to produce high sensitivity (>90%) and specificity (>98%) when used to detect for CDAD.[25] A survey performed in the UK by the Healthcare Commission and Health Protection Agency among 118 National Health Service Trusts in 2005 revealed that little had changed in the UK with respect to the number of laboratories performing *C. difficile* culture from their previous survey in 2002 (25% laboratories); however, a further 20% laboratories were considering introducing culture.[26] In addition, there was considerable variation in the use of culture strategies between different trusts. The extra resources required for culture (cost and expertise) are considered drawbacks to the introduction of *C. difficile* culture in many laboratories. The introduction of a repeat testing strategy where toxin-positive patients were not retested for two weeks would save a significant part of the *C. difficile* budget. In addition to cost and expertise issues, the low percentage of Irish laboratories that culture or send strains for *C. difficile* typing may also be due to the lack of a *C. difficile* reference laboratory in the Republic of Ireland. Laboratories that wish to type strains either have to send isolates to another country (usually to the UK) or send them to University College Dublin where typing is carried out as part of a research project; this is not a routine diagnostic service.

In summary, similar to other national surveys of *C. difficile* diagnosis, our survey has revealed marked differences in testing strategies and diagnostic methodologies in laboratories in the Republic of Ireland. Recently, the first case of *C. difficile* 027 in Ireland was reported from a patient transferred from a UK hospital.[27] This report also described two clusters of *C. difficile* ribotype 027 in two Irish hospitals. Since there is no national *C. difficile* surveillance programme or reference facility in Ireland and isolates are not routinely cultured or typed, the extent of *C. difficile* infection in the country is unclear. Our group will produce national recommendations for laboratory diagnosis and typing and surveillance of *C. difficile* infection, including standardization of *C. difficile* diagnostics. As the survey has shown marked differences in practice throughout the country, the implementation of these recommendations will have cost implications that will need to be addressed.

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