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CORE TRAINING FOR DOCTORS

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Investigating *Clostridium difficile*

Clostridium difficile is one of the leading health-care-associated infections in the UK and has a significant clinical and economic impact. Optimal laboratory testing for this pathogen is controversial and interpretation of results can lead to confusion. In the context of the clinical syndrome of *C. difficile* infection, this article reviews disease presentation, the diagnostic tests available, and their translation into information that can assist clinical management at the bedside.

Introduction

Antimicrobial-associated diarrhoea is a frequent occurrence, with 12% of patients treated with antimicrobials developing loose stool (Wiström et al, 2001). There does remain a differential diagnosis in antimicrobial-associated diarrhoea (*Figure 1*), but one of the more frequently encountered causes is *C. difficile* infection, defined as one episode of diarrhoea that occurs at the same time as a positive toxin assay or endoscopic evidence of pseudomembranous colitis and that is not attributable to any other discernible cause (Department of Health, 2012).

C. difficile is a Gram-positive, anaerobic, sporing bacteria first identified in 1935 as part of the intestinal flora of infants. Its role as a human pathogen was not described until Larson et al (1978) confirmed it as an agent responsible for cases of pseudomembranous colitis. Clinical disease is caused by those strains of *C. difficile* which produce two exotoxins – toxin A and toxin B, both of which act to inactivate Rho-GTPases which have a role as regulatory proteins of the eukaryotic actin cell cytoskeleton. This leads to disorganization of the cell cytoskeleton, cell death and the characteristic mucosal damage of plaque-like lesions which can lead to the formation of a pseudomembrane.

C. difficile is now one of the leading causes of health-care-associated infections in the UK, with an eightfold rise in deaths caused by *C. difficile* infection between 1999 and 2007 when the rate peaked at over 4000 deaths per year. Following strategic changes to health policy in antimicrobial stewardship, infection prevention and clinical management in the UK, this has since fallen and in 2011, 2000 deaths were reported (Office of National Statistics, 2012).

C. difficile spores can survive in the environment for months and effective cleaning of the environment has been demonstrated to reduce the incidence of *C. difficile* infection in health-care settings. Neither alcohol nor common detergents used in health care eradicate *C. difficile* spores, so assiduous cleaning and use of sporicidal

Figure 1. Differential diagnosis of antimicrobial-associated diarrhoea.

Seventy to eighty per cent of cases of antimicrobial-associated diarrhoea have no discernible cause and may be a result of failure of the faecal flora to catabolize carbohydrates resulting in an osmotic diarrhoea (Young and Schmidt, 2004). In those cases that do have an identifiable cause, the most frequently encountered causes include:

Clostridium difficile

Other infectious agents (*Staphylococcus aureus*, enterotoxin-producing strains of *Clostridium perfringens*, Salmonella spp., Campylobacter spp.)

Drug-related including: laxatives, biguanides, colchicine, cytotoxics, dipyridamole, iron preparations, laxatives, magnesium preparations (including antacids), metoclopramide, non-steroidal anti-inflammatory drugs, orlistat, proton pump inhibitors (Lee, 2006)

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agents are important in reducing transmission. There are also substantial financial implications for health-care providers from *C. difficile* infection – a systematic review by Wiegand et al (2012) found that, per case, *C. difficile* infection costs a health-care provider approximately £2917 in Finland, £4577 in Ireland, £6986 in the UK and £8843 in Germany.

Clinical features

C. difficile asymptomatically colonizes the gastrointestinal tract in 2–3% of the adult population, and at much higher rates in the elderly residing in long-term care facilities and in under 2-year-olds. When symptoms do arise, clinical manifestations range from self-limiting mild diarrhoea through to pseudomembranous colitis and toxic megacolon.

Assessing the severity of *C. difficile* infection is key to appropriate management, with several scoring systems in existence. A prospective observational study looked at the accuracy of eight severity scores and found the 'Hines VA index' was best able to predict more severe forms of *C. difficile* infection (Fujitani et al, 2011). Public Health England (2013) guidance cites the need for further validation of severity scores before widespread adoption, and until such time as this is available, a simple algorithm should be used to discern severe *C. difficile* infection that may benefit from second-line treatments (*Figure 2*). Assessment of the severity of colitis can be aided by imaging or flexible sigmoidoscopy to specifically look for evidence of pseudomembranes and obtain biopsies to look for alternative diagnoses where appropriate.

Figure 2. Clinical indicators of severity in Clostridium difficile infection. From Public Health England (2013).

Current UK guidance recommends evidence of any one of the following indicates severe *Clostridium difficile* infection and so use of oral vancomycin or fidaxomicin in preference to metronidazole:

White cell count >15x10^9/litre

Acutely rising blood creatinine (e.g. >50% increase above baseline)

Temperature >38.5°C

Evidence of severe colitis (abdominal signs, radiology)

Testing

The choice of laboratory test for *C. difficile* infection is contentious, with one of the main issues revolving around the sub-optimal sensitivity and specificity of available tests. Without a clear assessment of pre-test probability before entering into the diagnostic pathway, this can translate into unhelpful positive and negative predictive values which are difficult to interpret at the bedside (Eastwood et al, 2009). In order to optimize interpretation of results, several caveats must be accepted:

- Only stool samples from patients with liquid or loose stools should be tested (Bristol stool chart 5–7)
- Retesting may be required for *C. difficile* toxin-negative cases where there is a strong clinical suspicion of *C. difficile* infection
- Retesting should be avoided for *C. difficile* toxin-positive cases within a period of 28 days, as should the routine use of test for cure
- In suspected cases of 'silent' *C. difficile* infection, such as ileus, other diagnostic procedures, such as colonoscopy or axial imaging, are likely to be more useful than stool testing
- It is generally not advisable to test children younger than 2 years of age because of the high rate of asymptomatic carriage.

There are several options available for diagnosing *C. difficile* infection based upon testing patient faecal samples:

Glutamate dehydrogenase enzyme immuno-assay

This enzyme is prevalent in most pro- and eukaryotic cells, but a particular glutamate dehydrogenase subunit is present in all *C. difficile* and of limited prevalence in other organisms (with the exception of *Clostridium sporogenes*, *Peptostreptococcus anaerobius* and some strains of *Staphylococcus aureus*).

This *C. difficile* common antigen has been used in enzyme immuno-assay form to detect the presence of these bacteria and can be processed in a few hours. However, it does not determine whether the *C. difficile* strain is actively producing toxin. Sensitivity is in the region of 90% with specificity of 93%. In a *C. difficile* high prevalence setting of 10% (i.e. where pre-test stratification of which samples to test has occurred) this translates to a positive predictive value of 59% but a negative predictive value of 99%. In a *C. difficile* low prevalence setting of 2% (i.e. where no pre-test stratification has occurred) the positive predictive value drops off markedly to 21%.

Toxin A/B enzyme immuno-assay

Direct detection in patient faecal samples of the toxin A/B through an enzyme immuno-assay (or less frequently through a lateral flow assay) is one of the most common clinical laboratory tests for *C. difficile* infection and delivers a result in a few hours. The accuracy of these tests varies markedly, but sensitivity has been cited from 43% to 92% and specificity from 91% to 99%. It is therefore the positive predictive value from this specific test which is clinically relevant and in a *C. difficile* high prevalence setting this can be up to 88%.

Toxin A/B polymerase chain reaction

This rapid test takes less than an hour and looks for *C. difficile* that has the genetic potential to produce toxin A/B. Initially thought to offer high sensitivity, recently concerns have been raised around clinical false positives from this test, where positive polymerase chain reaction results do not correlate with a clinical syndrome of *C. difficile* infection. It is suggested that this is the result of detection of asymptomatic carriage of *C. difficile* strains that, although encoding toxin genes, are not actively producing toxin.

Sensitivity for this test has been cited as up to 92% with specificity of 94%. In a *C. difficile* high prevalence setting this translates to a positive predictive value of 63% but a negative predictive value of 99%. In a low prevalence setting the positive predictive value drops off markedly to 23%.

Cytotoxigenic culture

This is one of two tests considered as gold standard for *C. difficile* diagnosis and involves the anaerobic subculture of stool samples (*Figure 3*), then subsequent detection of toxin production in vitro through detecting cytotoxic effect on cell monolayers. The turnaround time for a result of 4–5 days makes this test impractical for routine clinical use and therefore this is rarely now carried out. A positive result

indicates that a patient is potentially infectious, but there have been arguments that the potential of an isolate to produce toxin after laboratory culture does not necessarily equate to toxin production in vivo.

Cytotoxicity assay

This is the second of the two tests considered as gold standard for *C. difficile* diagnosis. This assay detects the presence of *C. difficile* toxin directly from the patient's stool through the faecal filtrate's cytopathic effect on cell culture at 24 and 48 hours. The requirement for cell culture precludes the commonplace use of this test in most routine clinical laboratories.

Ribotyping

Polymerase chain reaction-based ribotyping can be applied to *C. difficile* through partial sequencing of 16S and 23S rRNA. This typing can aid cross-transmission investigations and also help delineate those patients at greater risk of severe disease associated with specific ribotypes (predominantly 001, 017 and 027).

Current testing practice

Both the UK and the USA have now advocated two-stage testing algorithms reflecting the lack of a single fast, accurate test from those currently available (Wilcox et al, 2010). This has led to some confusion in interpretation of test results, further compounded by variations in reporting and interpretative comments from individual clinical laboratories. *Figure 4* illustrates the possible outcomes from the testing algorithms commonly used in the UK.

Figure 3.* Clostridium difficile *subculture on blood agar.

Management

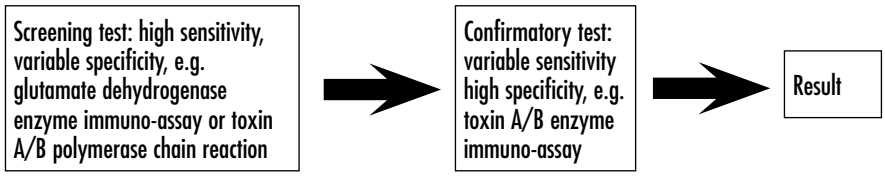
Patients being treated as having *C. difficile* infection (either confirmed toxin enzyme immuno-assay positive, or glutamate dehydrogenase enzyme immuno-assay/toxin polymerase chain reaction positive but toxin enzyme immuno-assay negative, in whom there is clinical suspicion of *C. difficile* infection) should have a regular clinical review including cardiovascular status and fluid balance. A review of concomitant medications should be undertaken, with a particular view to stopping, where possible, likely causative antimicrobials and any antimotility agents, laxatives or proton pump inhibitors. In the majority of symptomatic cases of *C. difficile* infection specific antimicrobials are indicated and four are licensed in the UK – metronidazole, oral vancomycin, oral teicoplanin and fidaxomicin.

A systematic review of available *C. difficile* infection treatment strategies by Drekonja et al (2011) concluded that no study comparing two agents has demonstrated a statistically significant difference for initial cure. However, Public Health England (2013) guidance does support the preferential use of oral vancomycin or fidaxomicin over metronidazole in severe *C. difficile* infection (*Figure 2*), and notes the evidence of reduced frequency of *C. difficile* infection relapse with fidaxomicin. Other potential options for *C. difficile* infection management that need assessment on a case-specific basis include: per-rectal vancomycin administration, faecal transplant, rifamixin, immunoglobulin therapy and probiotics (Gilchrist et al, 2012).

Conclusions

The optimal test for *C. difficile* infection remains controversial, and the plurality of laboratory techniques, all with variable sensitivities and specificities, contributes to uncertainty in the diagnostic pathway. The

Figure 4. Two-stage algorithm for* Clostridium difficile *testing advocated in the UK. From Department of Health (2012).



Glutamate dehydrogenase enzyme immuno-assay (or toxin polymerase chain reaction) positive and toxin enzyme immuno-assay positive (positive predictive value 91.4%) = *Clostridium difficile* infection is likely

Glutamate dehydrogenase enzyme immuno-assay (or toxin polymerase chain reaction) positive and toxin enzyme immuno-assay negative = *C. difficile* could be present, i.e. potential *C. difficile* excretors

Glutamate dehydrogenase enzyme immuno-assay (or toxin polymerase chain reaction) negative and toxin enzyme immuno-assay negative (negative predictive value 98.9%) = *C. difficile* infection is very unlikely to be present

KEY POINTS

- Understanding the pre-test probability is key to interpreting laboratory results from *Clostridium difficile* testing.
- Current UK and USA guidelines advocate a two-stage testing algorithm for *Clostridium difficile* infection.
- No test or combination of tests is infallible and the clinical condition of the patient should always be taken into consideration when making management decisions.
- Where there is non-concordance between the test outcomes or a strong clinical suspicion of disease with a negative laboratory test(s), repeat testing may be indicated.
- Routine repeat testing in patients known to be laboratory confirmed positive within 28 days, and tests of cure, should be avoided.
- Severity assessment is important and ensures the patient receives appropriate initial management; always refer to your local organization's guidance.

detection of *C. difficile* glutamate dehydrogenase by enzyme immuno-assay or toxin A/B by polymerase chain reaction, both with reasonable sensitivity, combined with a second stage toxin A/B specific enzyme immuno-assay is likely to be the optimal currently available option. Current UK guidance suggests that all cases of potentially infective diarrhoea are managed with SIGHT:

S Suspect that a case may be infective where there is no clear alternative cause for diarrhoea

I Isolate the patient and consult with the infection control team while determining the cause of the diarrhoea (this should be immediate, and certainly within 2 hours of identification to limit the risk of spread of infection)

G Gloves and aprons must be used for all contacts with the patient and his/her environment

H Hand washing with soap and water should be carried out before and after each contact with the patient and the patient's environment

T Test the stool for toxin, by sending a specimen immediately. **BJHM**

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TOP TIPS

- To reduce the risk of *Clostridium difficile* infection, always evaluate whether ongoing antimicrobial prescriptions are indicated, and whether the spectrum and duration are appropriate.
- Isolate patients who have diarrhoea that may be infective as soon as they are identified.
- Discuss cases of infective diarrhoea with infection specialists early, particularly where *Clostridium difficile* is a differential diagnosis.
- Rapidly refer to infection specialists those patients with *Clostridium difficile* infection who are not improving to enable escalations in therapy.