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Chapter Title: European practice for CDI treatment.

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Abstract

Clostridium difficile infection (CDI) remains a significant cause of morbidity and mortality worldwide. Historically, two antibiotics (metronidazole and vancomycin) and a recent third (fidaxomicin) have been used routinely for CDI treatment; convincing data are now available showing that metronidazole is the least efficacious agent. The European Society of Clinical Microbiology and Infectious Diseases CDI treatment guidelines outline the treatment options for a variety of CDI clinical scenarios, including use of the more traditional anti-CDI therapies (e.g., metronidazole, vancomycin), the role of newer anti-CDI agents (e.g., fidaxomicin), indications for surgical intervention and for non-antimicrobial management (e.g., faecal microbiota transplantation, FMT). A 2017 survey of 20 European countries found that while the majority (n=14) have national CDI guidelines that provide a variety of recommendations for CDI treatment, only five have audited guideline implementation. A variety of restrictions are in place in 13 (65%) countries prior to use of new anti-CDI treatments, including committee/infection specialist approval or economic review/restrictions. Novel anti-CDI agents are being evaluated in Phase III trials; it is not yet clear what will be the roles of these agents. Prophylaxis is an optimum approach to reduce the impact of CDI especially in high-risk populations; monoclonal antibodies, antibiotic blocking approaches and multiple vaccines are currently in advanced clinical trials. The treatment of recurrent CDI is particularly troublesome, and several different live bio therapeutics are being developed, in addition to FMT.

Keywords

C. difficile treatment, Anti-CDI agents, CDI guidelines, Novel C. difficile agents, C. difficile prophylaxis

Introduction

The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) first published guidelines for Clostridium difficile infection (CDI) treatment in 2009, which were revised in 2014. (Debast et al., 2014) These evidence-based guidelines outline the treatment options for a variety of CDI clinical scenarios, including recommendations for use of the more traditional anti-CDI therapies (e.g., metronidazole, vancomycin), the role of newer anti-CDI agents (e.g., fidaxomicin), indications for surgical intervention and for non-antimicrobial management (e.g., faecal microbiota transplantation, FMT). Many European countries have published their own national CDI treatment guidelines, which are broadly similar to the ESCMID guidelines, though contextualised to the local setting. (ECDC, 2017)

When discussing European practice for CDI treatment, variability between countries is inevitable for a number of reasons. Treatment of patients with CDI begins with making the diagnosis, specifically having a high index of clinical suspicion if a patient has a combination of signs and symptoms and/or CDI risk factors and thereafter confirmation by microbiological testing or colonoscopic/ histopathological findings. Clinician awareness of CDI as part of the differential diagnosis and access to timely laboratory diagnostics is therefore crucial for appropriate patient management. However, there remains considerable variability across countries with an estimated 40 000 inpatients potentially undiagnosed annually in European hospitals. (Davies et al., 2014) Mnemonic checklists can be useful tools to reduce clinician error and promote awareness. (Chew et al., 2016) Albeit potentially more useful when English is the commonly spoken language, the SIGHT mnemonic is a useful aide memoire for clinicians when managing patients with suspected potentially infectious diarrhoea (**Figure 1**). (Public Health England, 2013)

Once CDI is diagnosed, variability in anti-CDI treatment practices may be due to individual judgement and/or knowledge, individualised patient factors and national regulatory or economic issues, e.g., the availability of newer (more expensive) anti-CDI agents. Lastly, the ESCMID (and national) guidelines recommend a number of potential treatment options for similar CDI clinical scenarios, so individual clinician preference will likely be a potential cause of variability. This variability in anti-CDI treatment preferences has previously been described in Ireland.(Prior et al., 2017) In the United States (US) almost half of patients with severe CDI were treated with metronidazole, despite vancomycin being recommended in national guidelines at that time.(Stevens et al., 2017)

In this chapter, we firstly review the ESCMID CDI guideline recommendations and include an update as relevant of subsequent publications, present the findings of a 2017 survey of European CDI national experts regarding CDI guidelines and their implementation and lastly look to the future as we summarise promising new therapies for CDI treatment.

1. ESCMID guidelines for CDI treatment

The ESCMID guidelines provide a number of definitions to guide clinical management of patients with CDI, including diagnosis, treatment response, severity and recurrence.(Debast et al., 2014) A number of CDI scenarios are considered including the initial management of CDI in addition to the management of recurrent and severe CDI. (**Table 1**) For all scenarios the timely implementation of appropriate infection prevention and control measures to prevent further cross-infection is highlighted, in addition to the discontinuation of antimicrobial therapy (if clinically indicated), fluid and electrolyte replacement, review of proton pump inhibitor use and avoidance of anti-motility medications.

1.1 Non-severe CDI:

Three potential options are recommended for treatment of non-severe CDI, namely metronidazole, vancomycin or fidaxomicin. Metronidazole, which is a relatively safe and inexpensive antimicrobial is the treatment of choice (grade A-I), once there is no contraindications for its use. However, adverse effects such as metallic taste and nausea may limit its use/compliance in certain patient populations. Another recommended option for treatment of non-severe CDI is fidaxomicin (Grade B-I). Fidaxomicin is also recommended later in the guidelines for treatment of severe/complicated and first recurrent CDI (Grade B-I) and multiple recurrent CDI (Grade B-II). The non-inferiority of fidaxomicin to vancomycin for treatment of CDI with lower recurrences rate and superior sustained clinical response has been reported, though patients with severe CDI were not evaluated.(Louie et al., 2011, Cornely et al., 2012) Subsequently, superiority of fidaxomicin to vancomycin in patients with non-NAP1/BI/027 strains was reported.(Crook et al., 2012) However, in patients infected with the NAP1/B1/027 strain, there was no significant difference in recurrence rates between the two drugs. What implication this particular finding has for clinical practice in Europe will depend on the current prevalence rate of this strain in a country. However fidaxomicin is considerably more expensive than metronidazole or vancomycin, therefore economic factors may come into play in European countries regarding its availability and use.(Nelson et al., 2017)

Since publication of the ESCMID guidelines, the superiority of vancomycin over metronidazole for treatment of mild-to-moderate primary or recurrent CDI has been reported. (Johnson et al., 2014) and numerous publications have examined the benefits of fidaxomicin in a number of patient populations. A recent Cochrane review evaluated anti-CDI treatment options and reported that vancomycin is superior to metronidazole and fidaxomicin is superior to vancomycin for achieving symptomatic cure.(Nelson et al., 2017) The authors noted that the lack of any 'no treatment' control studies does not allow for any conclusions regarding the need for specific anti-CDI treatment in patients with mild CDI and pointed to the economic advantage of metronidazole.

1.2 Definition and Treatment of Severe CDI:

Classification of CDI by severity can be problematic, as patients with severe ileus may not have diarrhoea. In practice, the clinical spectrum of severe CDI varies considerably and the diagnosis is usually reached using a combination of findings. The ESCMID guidelines summarise the range of patient, laboratory, endoscopic and radiological factors associated with severity of CDI colitis and recommend three unfavourable prognostic factors, namely raised leukocyte count >15 x 10^9/L, decreased albumin < 30g/L and rise in serum creatinine level (>1.5 times the premorbid level or >133uM).(Debast et al., 2014) A recently validated clinical prediction rule to identify patients at risk of severe outcomes (age ≥ 60 years, peak serum creatinine ≥1.5 mg/dL and peak leukocyte count of ≥20,000 cells/μL) may be useful for clinicians to identify high-risk patients likely to benefit from more aggressive therapy (e.g., early administration of oral vancomycin).(Na et al., 2015)

The recommended treatment of choice for severe CDI in the ESCMID guidelines is oral vancomycin (Grade A-I) which achieves high intracolonic concentrations with minimal systemic adverse effects.(Debast et al., 2014) Intravenous metronidazole combined with vancomycin retention enema or oral/NG vancomycin at the higher 500mg dose is provided as an alternative. (Grade B-III). A recent retrospective study comparing vancomycin and metronidazole reported superiority of vancomycin for severe CDI, though no difference in CDI recurrence rates.(Stevens et al., 2017) At the time of publication of ESCMID guidelines, it was noted that there was insufficient data available for fidaxomicin. While there have been subsequent reports of fidaxomicin use in critical care patients with CDI and case reports of salvage use after failure of standard therapy, (Penziner et al., 2015, Arends et

al., 2017) , as most studies exclude patients with severe CDI the role of fidaxomicin in these patients has yet to be fully elucidated.(Nelson et al., 2017)

The precise role of surgical management in severe CDI is a topic of debate.(Fitzpatrick, 2008) There are no clear guidelines or protocols to guide the timing of surgical intervention. Certainly, the decision that surgical management is required for CDI should be taken by the multidisciplinary team, surgeons consulted at an 'early' stage (though there is no clear definition as to when this is) and an interdisciplinary risk/benefit analysis of surgery individualised for that patient. The ESCMID guidelines recommend total colectomy, 'before colitis becomes very severe', if colonic perforation or if there is systemic inflammation and the patient's condition has deteriorated and is not responding to anti-CDI therapy (**Table 1**).(Debast et al., 2014) Because of the morbidity (and mortality) associated with colectomy in a systemically unwell patient, there is increased interest in evaluating options that avoid colon resection.(Kautza and Zuckerbraun, 2016, Sartelli et al., 2015) The potential role of FMT as an alternative to emergency bowel surgery has also been recently highlighted.(van Beurden et al., 2017)

1.3 Recurrent CDI:

Recurrent CDI itself is a significant risk factor with the risk of recurrence increasing significantly with each episode of recurrence. Predicting which patients will develop recurrent CDI would enable clinicians to minimise recurrence risk (e.g., avoid concomitant antimicrobials) and also by heightening awareness, facilitates prompt diagnosis and treatment of recurrences. (Hu et al., 2009) The ESCMID guidelines recommendation for the first recurrence of non-severe CDI is either vancomycin or fidaxomicin (both B-I recommendations). For subsequent recurrences, while a variety of strategies are recommended (**Table 1**), FMT is allocated an A-I recommendation.

Recent surveys have highlighted the interest of European clinicians in FMT as a therapeutic option for patients with CDI; though note its potential underutilisation.(Porter and Fogg, 2015, Prior et al., 2017) Since publication of the ESCMID guidelines, a recent two-centred randomized controlled trial of FMT via colonoscopy for recurrent CDI reported a 91% cure rate with donor FMT (63% with autologous FMT – though this varied significantly between the two centres at 43% and 90% cure rates respectively).(Kelly et al., 2016) Notably patients with recurrence after autologous FMT resolved after a subsequent donor FMT. Severe and

severe-complicated indication, inpatient status during FMT, and the number of previous CDI-related hospitalizations are strongly associated with early failure of a single FMT for CDI.(Fischer et al., 2016)

2. **Survey of European CDI experts on CDI Treatment**

Though European and National CDI guidelines exist and variability in practice for treatment of patients with CDI is likely as previously discussed, to our knowledge there has been no recent assessment of CDI treatment guideline recommendations and their implementation in European countries. We designed an interactive online survey in this regard using Demographix® software (57 Chestnut Road, London SE27 9EZ UK). The purpose was to describe the practice for CDI management and treatment in Europe. National experts from European countries were invited by email to complete the online survey, during the period 07thJune 2017 to 28th July 2017. Data was analysed using an Excel® database (Microsoft Corp., Redmond, WA, USA).

Eighty-three CDI experts from 35 European countries were invited to take the survey with 34 respondents, representing 20 (57%) countries. Respondents included experts in the fields of microbiology, public health and infection prevention and control, who were working in hospitals (n=10), laboratories (n=2), health protection, public health or infectious diseases agencies (n=4) or other organisations (n=4). To avoid study bias arising from multiple respondents from the same country, data from one respondent per country was included in the analysis.

National guidelines for managing patients with CDI were available in 14 (70%) countries with guideline revisions undertaken during the last five years (n=7), one year (n=2), or were presently under revision (n=1). Revisions had not been undertaken in four countries with these guidelines published in 2007, 2011 (n=2) and 2013. Of the six countries that did not have national guidelines, guidance was sought from the ESCMID CDI guidelines (n=5) or local guidelines (n=1). The recommendations provided in national guidelines varied by country, as outlined in **Table 2**. Of the options provided in the survey, the commonest recommendation was treatment of patients with CDI (93%; n=13) and the least common were CDI key performance indicators (KPIs) and audit of guideline implementation (21%; n=3). Other recommendations were provided in national guidelines of 36% (n=5) countries, including: essential elements of a CDI prevention programme, use of tools such as checklists,

C. difficile reference laboratory requirements, access to infection specialists in the non-acute sector, healthcare facility infrastructure requirements, environmental and equipment decontamination, epidemiology, clinical diagnosis of CDI, antimicrobial stewardship, FMT and defining roles and responsibilities to support the implementation of the guidance.

In total, 36% (n=5) of countries previously surveyed or audited some (but not all) aspects of the implementation of national CDI guidelines though the majority, 64% (n=9), had not. Of the five surveys/audits conducted:

- Two were conducted in the past five years and three more than five years ago. No surveys were conducted in the last year.
- CDI treatment was included in one national survey only.
- Facilities surveyed included hospitals only (n=4) or diagnostic microbiology laboratories only (n=1)

Of the six countries that did not have national guidelines, a previous survey or audit of some (but not all) aspects of local CDI guidelines was conducted for one and five (83%) did not previously conduct a survey or audit. For the survey that was conducted, CDI treatment was not included and facilities surveyed included hospitals only. Information on when the survey took place was not provided.

Severe CDI was defined as a variable combination of factors, as outlined in **Figure 2**. The commonest being leucocytosis of ≥15,000 cells per μL (n=17; 85%). A variety of anti-CDI regimens were recommended as summarised in **Table 3.** In addition, a number of other factors were reported to influence choice of the recommended anti-CDI therapy including:

- C. difficile ribotype.
- Patient factors:
    - Risk factors for recurrence.
    - Patient tolerance / ability to take oral medications /response to treatment.
- Fidaxomicin use:
    - Approval required from microbiology/ infectious diseases for use.
    - Economic considerations because of high cost.
    - Reservations about its use as lack of survival benefit.
- FMT:

    - Availability of facilities for a FMT service.
    - Use as an option for severe CDI when surgery is not possible.
- Immunoglobulin therapy recommended in case of severe protein loss.

Of the 20 countries, a variety of restrictions were in place in 13 (65%) countries before new anti-CDI therapies could be used including:

- Reimbursement restrictions (n=1).
- Health technology assessment (n=1).
- Pharmacoeconomic review (n=3).
- Committee approval either national (n=6) or local (n=4).
- Microbiology or infectious diseases approval (n=2).
- CEO/ financial director approval (n=2).
- Cost and access issues re monoclonal therapy (n=1).
- Antimicrobial resistance (n=1).

3. Clostridium difficile pipeline prophylactic and therapeutic agents

The four current approved therapeutic agents for CDI vary markedly in efficacy. Whilst metronidazole has historically been the most commonly used option for treating CDI, as previously discussed, it is now known that this antibiotic is inferior to vancomycin.(Johnson et al., 2014, Nelson et al., 2017) Concern regarding treatment failures with metronidazole remains. (Vardakas et al., 2012) Metronidazole achieves poor intra-luminal colonic concentrations, especially as mucosal inflammation subsides, such that the antibiotic may be undetectable as diarrhoea resolves. Also, some C. difficile isolates show reduced susceptibility to metronidazole, which may be relevant given the sub-optimal pharmacokinetics for this antibiotic in CDI. Laboratory detection of reduced metronidazole susceptibility is itself problematic with variations in methodology and MIC interpretation limiting analysis of trends and comparisons with published data.(Moura et al., 2013)

Fidaxomicin and bezlotoxumab, a monoclonal anti-toxin B antibody and the most recently approved therapeutic agent, have been shown to reduce the risk of recurrent CDI by 40-50% in comparison with vancomycin alone.(Wilcox et al., 2017, Cornely et al., 2012, Crook et al., 2012) High acquisition cost of fidaxomicin has inhibited uptake in some settings and was observed in our survey of European countries as outlined above. However, a recent real

world study suggested a reduction in mortality associated with fidaxomicin use and that this was therapy was cost-effective.(Goldenberg et al., 2016) In the phase 3 trials, bezlotoxumab was associated with a significant reduction in CDI readmissions.

The ideal antimicrobial agent for CDI should reduce vegetative C. difficile cells, toxins and spores in the host gut lumen without perturbation of the host microbiota, both to avoid creating an environment that is conducive to C. difficile expansion or to select for resistant potential pathogens (e.g. vancomycin resistant enterococci [VRE] or multi-resistant Gram-negative bacilli).(Chang et al., 2008) This is a very challenging profile for an antibiotic and indeed recent 'failures' of two antimicrobial agents in late-stage clinical trials emphasise how difficult it is to improve on current CDI therapies.

3.1 Surotomycin and Cadazolid:

Surotomycin, an oral lipopeptide derivative of daptomycin, was examined in two phase 3 trials (NCT01598311 and NCT01597505) but did not demonstrate non-inferiority compared with vancomycin.(Boix et al., 2017) Notably, surotomycin dosing caused an overgrowth of Gram-negative bacilli in both in mice and in a gut model of CDI that is highly predictive of human disease; recurrent CDI was also seen in the latter model.(Deshpande et al., 2016, Chilton et al., 2014b) Most recently, a press release announced that cadazolid (Actelion), which is a novel hybrid oxazolidinone-fluroquinolone antibiotic that inhibits C. difficile protein synthesis and, to a lesser extent, DNA synthesis, did not meet its primary endpoint in comparison with vancomycin in one of two phase 3 trials.(ActelionLtd., 2017, Gehin et al., 2015, Chilton et al., 2014a, Baldoni et al., 2014) It is too early to determine why this result was obtained, but may relate to activity of cadazolid on the gut microbiome in vivo, and/or persistence of C. difficile spores.(Chilton et al., 2014a)

3.2 Ridinilazole:

Ridinilazole (SMT19969) is a novel, non-absorbable, very narrow-spectrum antimicrobial with minimal activity against host gut microbiota.(Goldstein et al., 2013) While its mode of action has not been fully determined, it does not appear to act through classical antibiotic pathways, such as inhibition of cell wall, protein, lipid, RNA or DNA synthesis.(Vickers et al., 2016) Bassiere et al described the effects of ridinilazole on C. difficile cell morphology, as visualised by scanning electron microscopy and confocal microscopy.(Basseres et al., 2016) Following exposure to sub-lethal concentrations of ridinilazole, bacterial cell division

was halted and there was an absence of septum formation; this resulted in marked cell elongation. It has not been confirmed whether these observations are a direct effect of ridinilazole, or a downstream response to the antibiotic. Ridinilazole has good activity against some but not all clostridia; it is 7-17-fold more active in vitro than metronidazole and vancomycin and has similar potency to fidaxomicin against C. difficile.(Baines et al., 2015, Weiss et al., 2014, Sattar et al., 2015, Corbett et al., 2015) Notably, in vitro, in vivo and gut model data confirm that ridinilazole has little antimicrobial activity against indigenous gut microflora groups, except selected clostridia. (Freeman et al., 2015, Goldstein et al., 2013, Baines et al., 2015, Corbett et al., 2015, Chang et al., 2016b)

Safety and tolerability of ridinilazole was established in healthy subjects and in a recently reported phase II randomised double-blind trial (CoDIFy).(Vickers et al., 2015, Vickers et al., 2017) CoDIFy was designed as a non-inferiority study and compared 10 days therapy of either oral ridinilazole 200mg BD or oral vancomycin 125mg QDS. Sustained clinical response rates were 67% and 42%, respectively (n=69 mITT population); CDI recurrence occurred in 14% of ridinilazole recipients compared with 35% of vancomycin subjects; this difference meant that ridinilazole achieved a sustained response rate of 66.7% versus 42.4% for vancomycin, which met pre-set statistical superiority criteria.(Vickers et al., 2017) Microbiome analyses of faecal samples from subjects in this phase 2 study showed that vancomycin recipients had a marked loss of diversity and replacement of the predominant phyla of healthy stool (Bacteroides and Firmicutes) by Enterobacteriaceae. These disruptions were still present two weeks after the end of treatment, even in subjects who had not had a recurrence at that point. By contrast, ridinilazole, had a minimal effect on gut microbiota.(Chang et al., 2016a)

3.3 CDI prophylaxis:

3.3.1 Ribaxamase

Ribaxamase (SYN-004, synthetic biologics) is a recombinant beta-lactamase that has been formulated to be administered orally in patient receiving beta-lactam antibiotic therapy.(Kaleko et al., 2016, Connelly et al., 2015) Ribaxamase degrades unmetabolised antibiotic in the colon to reduce the deleterious effects on the gut microbiota.(Roberts et al., 2016) Animal studies have demonstrated safety, and notably no reduction in the systemic concentration of co-administered ceftriaxone.(Connelly et al., 2015) A phase 2 double-blind placebo-controlled study has examined the potential of ribaxamase to prevent CDI, antibiotic-

associated diarrhoea and the emergence of antimicrobial resistant potential pathogens in patients hospitalized with a lower respiratory tract infection treated with IV ceftriaxone.(Synthetic Biologics, 2017) Patients who received ribaxamase had a 71.4% relative risk reduction for CDI (p=0.045). There was also a significant reduction in new colonisation by VRE in ribaxamase versus placebo recipients (p=0.0002). Adverse events were similar in active and placebo patients.

3.3.2 DAV132

Another novel approach to CDI prophylaxis is DAV132 (DaVolterra), which is an activated charcoal based product that is administered as an enteric coated capsule. DAV132 irreversibly captures antibiotics in the intestine whilst avoiding interruption of antibiotic absorption. DAV132 has been examined in a proof-of-concept study involving 18 healthy subjects who had received DAV132, uncoated formulated activated charcoal (FAC) or water 16 and eight hours before, alongside the probe drugs, and eight hours thereafter. The AUC0-96 h of amoxicillin was reduced by more than 70% when it was taken with FAC, but was not adversely affected when taken with water or DAV132. By contrast, the AUC0-96 h of sulfapyridine was reduced by >90% when administered with either FAC or DAV132 in comparison with water. Hence, DAV132 can selectively adsorb drugs in the proximal colon, without interfering with their absorption.

A further healthy volunteer trial examined the efficacy of DAV132 to protects the gut microbiome and prevent CDI during moxifloxacin (MOX) treatment.(de Gunzburg et al., 2015) DAV132 decreased free faecal MOX concentration by >99% compared with MOX alone, but MOX plasma PK did not change significantly. Alterations of the faecal microbiome observed with MOX were prevented by co-administration of DAV132. In a human gut model DAV132 protected the microbiota and prevented C. difficile overgrowth and toxin production.(de Gunzburg et al., 2015) Hamsters were also fully protected by DAV132 against MOX-induced CDI.(de Gunzburg et al., 2015) Such results warrant further clinical development of DAV132 to protect the lower gut microbiota, and so prevent CDI associated with antibiotic administration.

3.4 Active C. difficile immunisation:

Vaccination to boost host antibody-mediated immunity is an attractive strategy to prevent CDI. The relative importance of C. difficile toxins A and B to human infection remains

controversial, but host immune response to these toxins likely influences the likelihood of infection, clinical severity and outcome of CDI.(Solomon et al., 2013, Kuehne et al., 2010) Higher serum IgG levels to toxin A have been shown in patients with asymptomatic colonisation compared with those with CDI, and recurrent infection is associated with poor IgG and IgM responses.(Kyne et al., 2000, Kyne et al., 2001) Interestingly, the effectiveness of the anti-toxin B monoclonal antibody bezlotoxumab at reducing the risk of CDI recurrence was not enhanced by the addition of an anti-toxin A monoclonal antibody, actoxumab; also, actoxumab alone was not efficacious at preventing recurrence. Nevertheless, it remains logical to design a vaccine around the augmentation of the host response to both toxins A and B.(Kuehne et al., 2010) Other C. difficile antigens may also be important, noting for example that antibodies to surface proteins are greater in colonised versus infected patients.(Pechine et al., 2005)

Three vaccines that use C. difficile toxin targets have progressed to phase 2 or 3 clinical development. The first to reach a phase 3 clinical trial is a formalin-inactivated toxoid-based vaccine developed by Sanofi Pasteur.(Foglia et al., 2012) Following vaccination, seroconversion to toxin A was more pronounced than to toxin B (but took up to 70 days) and notably was less common in elderly subjects; three vaccine doses were required to achieve an adequate neutralising-antibody response.(Foglia et al., 2012, Kotloff et al., 2001) A 100 µg dose (given with an AlOH adjuvant) was found to yield the best immunogenic response, and a phase 3 trial of this vaccine in the prevention of primary CDI in at-risk subjects aged >50 years commenced in 2013 (NCT01887912). Another formalin-inactivated toxoid based vaccine, but with alterations in both toxins A and B to reduce toxigenicity, has recently commenced a phase 3 primary CDI prevention trial (Pfizer; NCT03090191), also based on a three dose strategy.(Donald et al., 2013, Sheldon et al., 2016) A third C. difficile vaccine candidate (VLA84, Valneva) has completed a phase II trial with 500 subjects.(Valneva, 2016) VLA84 uses a different antigen approach to either of the two toxoid-based vaccines that are currently undergoing phase 3 evaluation. VLA84 is a single recombinant fusion protein consisting of portions of the C-terminal cell binding domains of toxins A and B. The developers claim that production and characterization of VLA84 could be simpler and less costly compared with toxoid-based vaccines. The phase 2 study of VLA84 met its primary endpoint in terms of identifying the dose and formulation with the highest seroconversion rate against both toxins A and B (subjects were followed up to day 210) and confirmed the

favourable safety profile that was seen in Phase I. A phase 3 programme for VLA84 is being planned.

3.5 Microbiome based therapeutics:

3.5.1 Faecal microbiota transplantation (FMT)

The evidence base concerning the effectiveness of FMT continues to grow, but it remains a non-regulated product, with many different versions reported. FMT comprises the administration of a complex live faeces-derived mixture of micro-organisms, including some of uncertain significance (some beneficial, others possibly harmful or neither) and so (particularly longer term) safety remains unproven. Of particular concern here is the increasing use of FMT when licensed CDI therapeutics has not been tried. Hence, different regulatory authorities have taken varied stances on FMT to safeguard patient interests. Requirements for consenting subjects, screening of donors and recipients, faecal material preparation and delivery via either rectal or nasogastro/duodenal routes, mean that there are intensive endeavours to develop alternatives to FMT that can still harness the restorative and protective effectiveness of specific components of the gut microbiota, but possibly with greater reassurance on safety. In the US, Openbiome is aiming to overcome some of the practical barriers to FMT, and safety concerns, by facilitating access to screened faecal transplant material and by collecting longer term follow up data. (http://www.openbiome.org/impact/).

The first randomised (sham procedure controlled) trial of FMT to treat recurrent CDI demonstrated an intention-to treat (ITT) efficacy rate of 81% to prevent further recurrences; notably, however, the study contained only 16 patients in the FMT arm.(van Nood et al., 2013) In a randomised but non-blinded clinical trial, 39 subjects with recurrent CDI were given FMT (preceded by vancomycin 125 mg QDS for 3 days), comprising at least one infusion of faeces via colonoscopy, or vancomycin 125 mg QDS for 10 days and then 125-500 mg/day every 2-3 days for at least three weeks. The primary end point was the resolution of diarrhoea related to CDI at week 10; surprisingly, a positive C. difficile test was not required to define recurrence post-study treatment.(Cammarota et al., 2015) The study was stopped after a one-year interim analysis, at which point 18/20 (90%) vs. 5/19 (26%) patients in the FMT vs. vancomycin treatment groups, respectively had resolution of C. difficile diarrhoea ($P < 0.0001$). There were no significant adverse events in either of the study groups.

Adults with recurrent or refractory CDI were enrolled in a randomised, double-blind, non-inferiority study in six Canadian centres of free-thawed (n = 114) vs. fresh (n = 118) FMT via enema. Clinical resolution without recurrence up to 13 weeks did not differ significantly in the per-protocol (83.5% vs. 85.1%) and mITT (75.0% vs. 70.3%) populations.(Lee et al., 2016) These results suggest that using freeze-thawed faecal material is a practicable alternative to fresh donor material. All patients received suppressive antibiotics for the most recent episode of CDI, and these were discontinued 24-48 hours before FMT; this probably explains why only 38% of the subjects were positive for toxin or toxin gene immediately prior to FMT administration. Notably, about one third of FMT recipients in both groups, who were ultimately, classified as resolved, required two FMTs, which is a relatively common observation. A non-blinded, non-randomised study of encapsulated (and freeze-thawed) faeces was performed in 20 subjects with at least three episodes of mild-to-moderate CDI and failure of a 6-8-weeks of vancomycin therapy, or $\geq$2 episodes of severe CDI requiring hospitalization.(Youngster et al., 2014) Diarrhoea resolution occurred in 14 patients (70%; 95% CI, 47%-85%) after a single capsule-based FMT; 4/6 re-treated non-responders had resolution of diarrhoea, giving an overall 90% (95% CI, 68%-98%) response rate. No serious adverse events were attributed to FMT.

The six randomised controlled trials of FMT have been recently reviewed; three that compared FMT to antibiotic management; the remainder compared FMT to various 'types' of FMT in terms of preparation, source and delivery.(Johnson and Gerding, 2017) It is important to note that, unlike prior uncontrolled studies that reported FMT efficacy rates of at least 90%, efficacy (for one FMT) in these RCTs was 44-91%, with four recording success rates of $\leq$65%. These include a randomized controlled trial of FMT versus a six-week vancomycin tapering regimen (VAN-TP).(Hota et al., 2017) VAN-TP was stopped early for futility; 56% of patients randomized to FMT by enema developed recurrent CDI, compared with 42% VAN-TP recipients.

There are many important factors for European clinicians to consider when establishing or using a FMT service. Factors that should be taken into account at an institutional level when commencing an FMT service are the national regulatory frameworks that FMT falls under (i.e. as a drug or biological material), donor selection and screening practices, stool preparation techniques and long term safety of microbiome manipulation in these patients.

Concerns regarding the long term safety of FMT are not unfounded, especially in patients with inflammatory bowel disease. Reports of peripheral neuropathy, Sjögren syndrome, idiopathic thrombocytopenic purpura, microscopic colitis, contact dermatitis, rheumatoid arthritis, obesity, bacteraemia, and ulcerative colitis flare after FMT.(Tariq et al., 2016, De Leon et al., 2013, Quera et al., 2014, Alang and Kelly, 2015) Institutions need to ensure they are working within their national and European frameworks and regulations. Where national regulations are absent, comparisons should be made to international standards to ensure the highest level of safety. In Europe, the regulation of FMT is currently at the discretion of the EU member states, though in many countries no such national regulation exists. Future planned EU regulation of FMT donor material may hinder its widespread use, depending on whether it is regulated as a drug or bodily tissue. A recent European Consensus paper provided recommendations on a number of areas pertinent to FMT implementation, including regulatory, administrative and laboratory guidelines.(Cammarota et al., 2017)

3.5.2 Live bio therapeutic microbiota preparations

3.5.2.1 RBX2660

RBX2660 is a live bio therapeutic microbiota suspension that aims to harness the effectiveness of FMT, but within a standardised, regulated product, for the treatment of recurrent CDI. It has been studied in three phase 2 clinical trials. PUNCH CD (NCT01925417) was a safety focussed, prospective multi-centre, open-label study; 34 subjects (with $\geq$2 recurrent CDI episodes or $\geq$2 severe episodes resulting in hospitalization) received at $\geq$1 dose of RBX2660 and 31 completed six months follow up.(Orenstein et al., 2016) Following a 10 -14 day course of anti-CDI antibiotics and a 24-48 hour washout period, RBX2660 was administered as a single dose via enema. Further recurrent CDI occurred in 48% of subjects after one dose of RBX2660, with 15/31 patients receiving a second enema; of these, 78.6% were considered to be treatment successes, contributing to an overall success rate of 27/31 (87.1%). No serious adverse events were related to RBX2660.

PUNCH CD 2 (NCT02299570) was a phase 2b multi-centre randomized double-blind, placebo-controlled trial with two year follow-up.(Dubberke et al., 2016) The primary efficacy objective was assessment of response (defined as no CDI recurrence) to RBX2660 versus placebo at eight weeks. A total of 127 patients formed the ITT population (enrolled at 21 sites in the U.S. and Canada); patients were randomized into three treatment arms: two doses of RBX2660 (Group A, n=41); two doses of placebo (Group B, n=44); or one dose of

RBX2660 and one dose of placebo (Group C, n=42) via enema with doses seven days apart. Efficacy for Group A was 61% vs. 45.5% for Group B, P= 0.152. Efficacy for Group C was 66.7% compared with Group B (45.5%), P=0.048; efficacy of Group A and C (63.9%) vs. B (45.5%), P= 0.046. For subjects who developed recurrent CDI after receipt of study drug, open-label treatment success was Group A (68.8%, 11/16); Group B (87.5%, 21/24); Group C (71.4%; 10/14) for an overall open label success rate of 77.8%. Adverse events at 56 days were primarily gastrointestinal, with no significant difference in the proportion of adverse or serious adverse events among the treatment groups. As the two doses of RBX2660 treatment arm was not superior to two doses of placebo, the primary efficacy endpoint was not met. The third phase 2 study, PUNCH Open Label (NCT02589847) had 31 active treatment sites and four control sites in the US and Canada. 132 RBX2660 and 110 historical control subjects were included; follow up results at eight weeks have been reported, although there is a two-year assessment point also.(Rebiotix Inc, 2017) RBX2660 met its primary efficacy endpoint at eight weeks, preventing CDI recurrence, with a success rate of 78.8% compared with 51.8% in historical controls treated with antibiotics alone ($p<0.0001$). No new safety concerns were identified. Analyses of faecal microbiomes shows that these became more diverse and aligned to a 'healthy' microbiome after treatment with RBX2660.(Blount et al., 2017, Ray et al., 2017) 16S rRNA sequencing was also performed on stool samples collected from 42 subjects treated with RBX2660 treatment arm and for 19 RBX2660 drug lots. The RBX2660 microbial profiles had similar taxonomic distributions, with a group mean that was highly divergent and significantly different from those of patients at baseline. However, after RBX2660 treatment, patients' microbiomes progressively resembled those of RBX2660.

3.5.2.2 SER-109

SER-109 (Seres) is also a live biotheraputic that comprises an encapsulated mixture of purified Firmicutes spores, obtained from the faeces of healthy humans, which were effective at preventing CDI in animal models. The resilience of the spores means that an ethanol based purification process can be applied to reduce the risk that transmissible infectious agents contaminate the therapeutic product. Also, resistance to gastric acid facilitates oral dosing. Two phase 2 studies of SER-109 have been completed. The first was a non-comparative study in patients with $\geq$3 CDI episodes during 12 months.(Khanna et al., 2016) Following standard of care CDI antibiotic treatment, patients received SER-109 either on two consecutive days (geometric mean dose, 1.7×10^9 spores), or on one day (geometric mean dose, 1.1×10^8 spores). The primary end point was absence of C. difficile-positive diarrhoea

during eight weeks of follow-up. In total, 26/30 patients (86.7%) across the two dosing groups met the primary efficacy end point. Three patients with early, self-limiting C. difficile-positive diarrhoea did not require antibiotic treatment, and were C. difficile-negative on re-testing at eight weeks; thus, 29/30 (96.7%) were considered to have achieved clinical resolution. Notably, gut microbiome analyses showed that baseline loss of microbiota diversity was rapidly reversed after receipt of SER-109, with persistence of Firmicutes spores. There were no safety concerns in the study.

A recently completed, phase 2 (ECOSPORE) study of SER-109 enrolled 89 subjects with ≥3 recurrences who were randomized (2:1 ratio) in a placebo-controlled, double-blind, 24-week trial. (Trucksis et al., 2017) SER-109 was administered orally as a single dose (1×10^8 bacterial spores), after CDI antibiotic treatment. Recurrence was defined as diarrhoea for ≥2 consecutive days, a positive CDI test, and the need for antibiotic treatment. The study's primary endpoint of reducing the relative risk of CDI recurrence at eight weeks was not achieved, despite a (non-significant) reduction in the relative risk of CDI recurrence. In the ITT population, recurrence occurred in 44% (26/59) vs 53% (16/30) of subjects who received SER-109 vs placebo, respectively. A pre-specified sub-group analysis showed that the lack of efficacy of SER-109 to prevent recurrence occurred in subjects aged <65 years old. However, in subjects aged ≥65 years old, CDI recurrence occurred in 45% of SER-109 (14 of 31) recipients, and in 80% of those who received placebo (12 of 15). A re-analysis showed that the disappointing results may be because cases were included and recurrences diagnosed without the most stringent requirement for free faecal toxin to be present. Also, while SER-109 was biologically active, a higher dose may be necessary. Further clinical trials are now in progress.

3.5.2.3 Non-toxigenic C. difficile

Non-toxigenic C difficile (NTCD) strains are avirulent. Theoretically, it may be possible to displace toxigenic strains in colonised (or infected) individuals. A randomized, double-blind, placebo-controlled, dose-ranging study examined the efficacy of a NTCD strain to prevent recurrent CDI in patients with either primary (>80%) or recurrent CDI who had completed treatment with metronidazole, vancomycin, or both.(Gerding et al., 2015) Approximately two thirds (69%) of recipients became colonised by NTCD. CDI recurrence rates were 2% in colonized subjects, compared with 31% (similar to placebo) in those not colonised ($p<0.001$), highlighting the correlation between engraftment and clinical efficacy. Interestingly, no

subjects who were colonised at week six remained so at week 26. It remains unclear whether this successful proof of concept phase 2 clinical trial will lead to commercial development of NTCD.

Summary

In summary, there are varied approaches in advanced clinical trials for the primary prevention, treatment and/or secondary prevention of CDI. Unfortunately, however, recent experience shows us that developing new management options for CDI is very challenging. Well-designed trials with clearly defined patient populations are key to delivering new therapeutic and preventative options.

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Table 1: **Overview of ESCMID recommendations for CDI treatment (Debast et al., 2014)**

Clinical scenario	**Oral antibiotic treatment**	**Oral treatment not possible**	**Non-antibiotic treatment**	**Not recommended**
Non-severe CDI	**Metronidazole 500 mg TDS (A-I)** Or Vancomycin 125 mg QDS (B-I) Or Fidaxomicin 200 mg BD (B-I) All 10 days	IV Metronidazole 500 mg TDS 10 days (A-II).	Stop inducing antibiotic (s) & 48 hours clinical observation (C-II).	Probiotics (D-I) Toxin binding (D-I)
First recurrence	Fidaxomicin 200 mg BD (B-I) Or Vancomycin 125 mg QDS (B-I) Or Metronidazole 500 mg TDS (C-I) All 10 days			
Multiple recurrences	Fidaxomicin 200 mg BD: 10 days (B-II) Or Vancomycin 125 mg QDS: 10 days followed by pulse or taper strategy (B-II)	**Faecal transplantation in combination with oral antibiotic treatment (A-I).**		Metronidazole 500 mg TDS (D-II) Probiotics (D-I) Passive immunotherapy with immune whey (D-I)
Severe CDI or complicated course	**Vancomycin 125 mg QDS (A-I)** Consider increasing to 500 mg QDS (B-III) Or Fidaxomicin 200 mg BD (B-I) All 10 days	**IV Metronidazole 500 mg TDS 10 days (A-II)** combined with either - Vancomycin retention enema (500 mg in 100	**Surgery:** Total colectomy & ileostomy	Metronidazole 500 mg TDS (D-I) Fidaxomicin (D-III)

		mL normal saline QDS intracolonic) - or Vancomycin 500 mg QDS by oral/nasogastric tube for 10 days (B-III).

PO: Oral, IV: Intravenous, BD: twice daily, TDS: three times daily, QDS: four times daily

Table 2: Recommendations for CDI management in 14 European countries with national CDI guidelines.

One country with local guidelines is not included as applicable data was not available for this country.

Recommendation	Included in guideline	Not included in guideline
	Number (n) and percentage (%) of countries	
Surveillance of CDI, n (%)	11 (79)	3 (21)
Laboratory diagnosis of CDI, n (%)	12 (86)	2 (14)
Treatment of patients with CDI, n (%)	13 (93)	1 (7)
Management of outbreaks and clusters of CDI, n (%)	11 (79)	3 (21)
CDI key performance indicators (KPIs), n (%)	3 (21)	11 (79)
Audit of guideline implementation, n (%)	3 (21)	11 (79)
Other recommendations, n (%)	5 (36)	9 (64)

Table 3: Recommendations for CDI treatment in 15 European countries with national (n=14) or local (n=1) CDI guidelines

	MTZ	Vancomycin	Fidaxomicin	Tapering vancomycin regimen	Immunoglobulin therapy	FMT
	Number (n) and percentage (%) of countries surveyed					
New CDI, n (%)	13 (87)	9 (60)	3 (20)	0 (0)	0 (0)	0 (0)
Recurrence (1st) , n (%)	4 (27)	13 (87)	6 (40)	1 (7)	0 (0)	1 (7)
Recurrence (2^{nd}), n (%)	0 (0)	8 (53)	7 (47)	6 (40)	1 (7)	3 (20)
Three or more recurrences, n (%)	0 (0)	6 (40)	4 (27)	9 (60)	3 (20)	12 (80)
Severe CDI, n (%)	3 (20)	11 (73)	4 (27)	1 (7)	1 (7)	1 (7)
Other , n (%)	3 (20)	2 (13)	1 (7)	0 (0)	1 (7)	1 (7)

MTZ: Metronidazole.

FMT: Faecal Microbiota Transplantation

Table 4: Anti-CDI agents in the pipeline agents that have completed at least a phase 2 clinical trial for treatment or prevention of CDI

Clinical trial phase	Drug/product (developer)	Indication Notes
Phase III	C. difficile vaccine (Sanofi Pasteur)	Primary prevention of CDI. NCT01887912: efficacy of vaccine (3 doses) containing toxin A & B toxoids.
	C. difficile vaccine (Pfizer)	Primary prevention of CDI. Vaccine containing toxoids of toxin A and B. 3 doses. NCT03090191: efficacy of vaccine (3 doses) containing toxin A & B toxoids.
	SER-109 (Seres)	Treatment of recurrent CDI. Oral microbiome therapeutic (mixture of bacterial spores) tested in a single-arm, open-label clinical trial. NCT03183128: Is SER-109 superior vs placebo to reduce recurrence of CDI?
Phase II	Ridinilazole (SMT 19969, Summit)	Treatment of CDI. Ridinilazole is a novel, small molecule, highly selective antibiotic. Successful phase 2 trial completed; phase 3 initiation expected 2018.
	RBX2660 (Rebiotix)	Treatment of recurrent CDI. Microbiota Suspension. 3 completed phase 2 trials. Expected to enter Phase 3 in 2017/18.

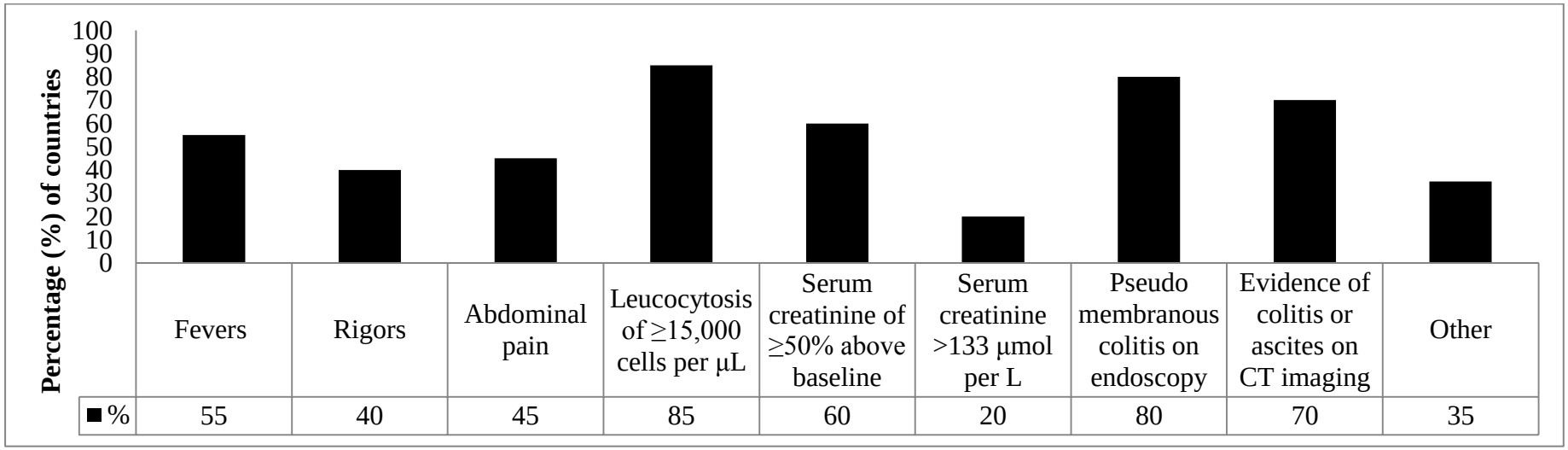
	SYN-004 (Synthetic Biologics)	Prevention of CDI. SYN-004 is a class A b-lactamase. Successful phase 2 trial completed; phase 3 initiation expected 2017/18.
	VLA84 (Valneva)	Primary prevention of CDI. Vaccine consisting of a fusion protein with portions of toxins A and B. Successful phase 2 trial completed in 2016.
	Non-toxigenic C. difficile (Viropharma)	Prevention of recurrent CDI. Biological therapy. Completed successful phase 2 trial in 2013.
	Ramoplanin (Nanotherapeutics)	Treatment of CDI. No new clinical efficacy data published since a phase 2 study was completed in 2004. Development plans/potential is therefore unclear. No clinical studies listed in clinicaltrials.gov

Figure 1: SIGHT Mnemonic protocol

S	Suspect that a case may be infective where there is no clear alternative cause for diarrhoea.
I	Isolate the patient/resident. Consult with the infection prevention and control team where available while determining the cause of the diarrhoea.
G	Gloves and aprons must be used for all contacts with the patient/resident and their environment.
H	Hand washing with soap and water should be carried out after each contact with the patient/resident and the patient/resident's environment.
T	Test the stool for C. difficile toxin, by sending a specimen immediately.

Adapted with permission from SIGHT Mnemonic UK protocol (DH. and HPA., 2008)

Figure 2 Definition of severe CDI in 20 European Countries as a percentage (%) of countries surveyed



*'Other' defining factors were included for 35% (n=7 countries), and were a combination of: toxic megacolon, ileus, colonic di*lation in CT scan >6cm, immunosuppression, shock, hypotension, admission to hospital for treatment of CDI acquired outside the hospital, admission to the ICU for treatment of CDI, colectomy due to CDI, mortality within 30 days of diagnosis of CDI, suspicion of pseudomembranous colitis, diarrhoea, positive stool test, hemodynamic instability, signs of septic shock, signs of peritonitis, decreased bowel sounds, vomiting, lack of bowel movements, left shift, hypoproteinemia, anaemia and increased serum lactate.

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