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REVIEW

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Clostridium difficile infection: new approaches to prevention, non-antimicrobial treatment, and stewardship

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ABSTRACT

Introduction: Despite the large amount of scientific publications exploring the epidemiology and the clinical management of *Clostridium difficile* (CD) infection, some issues remain unsolved or need further studies. The aim of this review is to give an update on the hot topics on CD prevention, including stewardship programs, and on the non-microbiological treatment of CD infection.

Areas covered: This article will review the importance of minimizing the CD spore shedding in the healthcare environment for potentially reducing CD transmission. Moreover, antimicrobial stewardship programs aimed to reduce CD incidence will be reviewed. Finally, new strategies for reducing CD infection recurrence will be described.

Expert commentary: Besides the basic infection control and prevention practices, including hand hygiene, contact isolation and environmental cleaning, in the prevention of CD infection other issues should be addressed including minimizing the spread of CD in the healthcare setting, and implementing the best strategy for reducing CD infection occurrence, including tailored antimicrobial stewardship programs. Regarding new advancements in treatment and management of CDI episodes, non-antimicrobial approaches seem to be promising in reducing and managing recurrent CD infection.

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1. Introduction

The gram-positive anaerobic bacterium *Clostridium difficile* (CD) represents the commonest cause of nosocomial diarrhea worldwide and is responsible for increased morbidity, mortality, and prolonged hospital stay [1,2]. The picture of the current CD burden is alarming with a total of 15–25% of all cases of antibiotic-associated diarrhea resulting from CD infection (CDI), a mortality rate ranging between 3% and 15%, a recurrence rate of around 20% and an excess cost of more than 4 billion dollars/year in US acute care facilities [1,3].

Despite the large amount of scientific publications exploring the epidemiology and the clinical management of CDI, there is still a huge need for studies that could clarify some important aspects of this complex disease.

First, more efforts are needed in order to reduce the spread of CDI, especially among hospitalized patients. The intestinal burden of CD has a significant impact on its hospital spread; indeed, the persistent shedding of CD after the completion of anti-CDI antimicrobial therapy and even after the resolution of diarrhea contributes significantly to CD transmission [4,5].

The second hot issue on CD prevention concerns the important role of antimicrobial stewardship programs (ASPs). Since decreasing antibiotic use has been shown to result in lower CDI incidence, implementation of ASPs is one of the strongly recommended interventions aimed at reducing CD spread. However, several issues affect the design and the application of ASPs in daily clinical practice that deserve a specific discussion.

A further critical issue in the management of CD regard the high recurrence rate observed with the currently available CDI therapy. Metronidazole and vancomycin, the mainstay for antimicrobial CDI treatment, even when timely and correctly used, are not as much effective in assuring sustained and bacteriological cure and are affected by recurrence rates up to 20–30% after the treatment of an initial CDI episode [1]. This risk increases following each subsequent recurrence up to 50–65% [3]. Fidaxomicin, a more recent anti-CDI antimicrobial, has been proven superior to vancomycin in achieving a sustained clinical response after treatment [6–8].

The alarming rates of CD recurrence have prompted the search for different therapeutic approaches. Importantly, in the last few years, many advances in the field of pathogenesis of CDI and on the role of intestinal microbiota have been made and new strategies for the treatment and the prevention of CDI are being studied.

The aim of this review is to give an update on the hot topics on CD prevention, including stewardship programs, and on the nonmicrobiological treatment of CDI. Published articles reporting data on these topics were identified through computerized literature searches using MEDLINE (National Library of Medicine Bethesda MD) and by reviewing the references of retrieved articles.

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2. Hot topics in the prevention and control of CD

2.1. The effect of anti-CD antimicrobials on CD spore shedding

The most important cause of concern in controlling CD hospital spreading is the environmental contamination and the cross-transmission due to spore shedding from CD patients.

It has been reported that fidaxomicin was more effective than vancomycin for achieving symptomatic cure and was associated with lower recurrence rates [7–10]. Interestingly, an *in vitro* study comparing the effect of fidaxomicin and its metabolite, OP-1118, on CD sporulation kinetics versus that of vancomycin, metronidazole, and rifaximin, demonstrated that spore production stopped completely following the addition of fidaxomicin or OP-1118 to stationary-phase cells of CD strains. On the contrary, the other drug comparators were not able to halt the spore formation [5].

Fidaxomicin inhibits spore formation by blocking the synthesis of bacterial mRNAs associated to sporulation when compared to vancomycin and metronidazole [5]. A previous study had already demonstrated that CD spore counts were significantly lower posttreatment in patients receiving fidaxomicin as compared to patients receiving vancomycin [11]. Moreover, this study reported that reappearance of CD toxin B was more likely following vancomycin treatment [11].

Since spores have a major role in the transmission of CD in the healthcare setting, the use of fidaxomicin might have an important ecological impact, along with its therapeutic role.

Interestingly, Biswas et al. found significant lower environmental contamination rates in the rooms of CDI patients treated with fidaxomicin as compared to CDI patients treated with metronidazole and/or vancomycin [12]. Importantly, PCR ribotyping revealed that CD isolated from patient were indistinguishable from environmental isolates [12].

2.2. The impact of ASPs on CD occurrence in healthcare settings

Numerous studies have demonstrated that a prior antimicrobial exposure is a strong risk factor for developing CDI, with fluoroquinolones, third-generation cephalosporins and clindamycin as the most frequently implicated antibiotics. Patients in hospitals have a high probability of receiving an antibiotic and up to 50% of all antibiotic administration in hospitals can be inappropriate [13]. Patients unnecessarily exposed to antibiotics are placed at risk for serious adverse events with no clinical benefit.

In the last decade, ASPs have been widely implemented with the aim to achieve optimal clinical outcomes, to minimize toxicity and other adverse events including CDI, to limit the selection for antimicrobial-resistant strains, and to ensure a cost-effective use of antimicrobials thus reducing, as a side effect, healthcare costs.

In 2014, a meta-analysis was performed to assess the effect of ASPs on the risk for CDI in hospitalized adult patients [14]. This meta-analysis included experimental, quasi-experimental, and observational studies reporting the incidence of CDI before and after the introduction of interventions of policy changes and programs that altered or restricted the use of antibiotics for adult inpatients [14]. Sixteen studies were included, eight of them described the effect of restrictive antibiotic stewardship whereas five reported the application of persuasive policies, and three studies did not clearly stated the methodology used. Overall, the implementation of ASP was associated with a significant CDI reduction (52% risk reduction); however, the heterogeneity between studies was huge. Studies were then stratified according to the setting, type of intervention, quality, drug classes, ASP duration. The majority of subgroup analysis confirmed the beneficial effect of ASPs on CDI rate; importantly, it has been demonstrated that the greatest benefit was observed in geriatric wards and that restrictive policies were more effective than persuasive interventions in reducing CDI incidence [14].

In 2016, two systematic reviews and meta-analyses were published, that considered CDI rate among the clinical outcomes for the evaluation of the beneficial effect of ASPs [15,16]. However, both reviews included only three studies reporting CDI rate and, therefore, were unable to demonstrate an effect of ASP on the risk for CDI [15,16].

More recently, in 2017, two systematic reviews assessed the effect of ASP on CDI rates. The first is a Cochrane review assessing the effectiveness and safety of interventions to improve antibiotic prescribing to hospital inpatients and to investigate the effect of restriction and enablement interventions [17]. Among secondary outcomes, the review assessed CDI rates following the interventions, and reported that ASPs were associated with a significant reduction of 49% in risk for CDI [17].

The second one is a systematic review and meta-analysis on the effect of ASP on the incidence of infection and colonization with antibiotic-resistant bacteria and CDI [18]. A primary outcome of this meta-analysis was the change in the incidence of CDI in hospital inpatients after implementation of antibiotic stewardship [18]. This meta-analysis included eleven studies (mostly before–after, also included cohort study, interrupted time-series and a point-prevalence survey). The most frequent antibiotic stewardship interventions evaluated in these studies were antibiotic restriction, guideline implementations, and audits [18]. The results showed that ASP implementation was associated with significant reductions in the incidence of CDI, with an estimated protective effect of 32% ($p = 0.0029$) [18]. Of importance, the work by Baur et al. included eight studies that were not analyzed in the previous review by Feazel et al.

A limited number of studies of the literature failed to demonstrate a significant effect of ASPs on the incidence of CDI [19–28]. These studies present some limitations, including too small sample sizes or an inadequate number of observed CDI cases [19–21]. A too short follow-up period was adopted in some studies [19,21,26,28]. Two studies failed in obtaining satisfying ASP implementation and appropriate targeted antimicrobial restriction [23,24]. One study reported a huge heterogeneity in size between nonintervention and intervention facilities [26]. Failure to deal with confounders was observed in three studies [25,27,28].

In 2017, Dingle et al. [29] used the whole-genome sequencing analysis to retrospectively clarify which of the control measures was responsible for the decline in CDI rate in the UK [30–32]. The authors identified a significant decline in

transmitted cases caused by fluoroquinolone-resistant isolates after the adoption of a fluoroquinolone restriction protocol [29]. On the contrary, there was no reduction in the incidence of CDI caused by fluoroquinolone-susceptible strains. Therefore Dingle et al. highlighted the importance of fluoroquinolone restriction over other antimicrobial classes [29]. However, besides fluoroquinolones, the authors did not exclude that the CDI decline may be attributable also to clindamycin, cephalosporin, and extended-spectrum penicillins' restriction [29].

As shown in Table 1, in the literature, only 3 [52,54,58] out of 66 articles on ASP and antimicrobial restriction strategies aimed at reducing CDI rate evaluated the effect of restricting only fluoroquinolone [19–21,23–28,33–59].

In 2014, a before–after study by Wenish et al. evaluated the effect of the introduction of a moxifloxacin formulary restriction (along the distribution to the hospital personnel of a bundle of information on CDI) in reducing the number of CDI cases in a large tertiary care community hospital in Austria [52]. Moxifloxacin use was significantly reduced and fluoroquinolone use decreased by about 37% after the ASP implementation. The mean numbers of CDI dropped from 59 to 32 cases per month, respectively, with a 46% reduction ($P = 0.0044$) [52].

In 2015, Sarma et al. published an interrupted time-series analysis on the effects of fluoroquinolone restriction on CDI rate over a 5-year period (from 2007 to 2012) in two large acute hospitals in the UK [54]. Measures implemented in this study included enhanced terminal cleaning of isolation rooms, daily cleaning of environment, review of patients on proton pump inhibitors, and restriction of fluoroquinolone use, particularly levofloxacin and ciprofloxacin [54]. At the end of the antimicrobial restriction intervention, a decrease in CDI rate of around 60% [Relative Risk (RR) = 0.394; 95% Confidence Intervals (CI) 0.199–0.781) was observed [54]. However, the authors stated that before fluoroquinolone restriction, there was a decreasing trend in CDI rate, likely due to infection control measures' implementation. Since the fluoroquinolone restriction was included into a multifaceted approach, it is not possible to exclude that decrease of CDI rate was due to several interventions [54].

In 2017, Shea et al. performed a multicenter, quasi-experimental study to assess the impact of an education program on fluoroquinolone use followed by a respiratory fluoroquinolone restriction program on fluoroquinolone utilization, appropriateness of quinolone-based therapy, and CDI rates [58]. After the full ASP implementation, the hospitals experienced an average reduction in moxifloxacin utilization by 88% [58]. ASP resulted to be effective in reducing CDI rate ($P = 0.044$) with a decrease in the mean monthly CDI cases by roughly 50% (from 4.0 to 2.2 CDI cases/10,000 patient days pre-intervention and at the end of the study, respectively) [58]. In particular, after the education phase, the restriction phase led to a decrease from 3.43 cases to 2.2 cases/10,000 patient days [58].

3. Non-antimicrobial approaches to CD infection

In the last years, important developments in the knowledge of the role of intestinal microbiota has led to the conception of new non-antimicrobial strategies against CDI.

3.1. The human gut microbiota

Microbiota is a complex community of microbes that live in association with the host. The human gut microbiota is home to around 100 trillion bacterial cells, with a density of 10^{11} to 10^{12} cells per gram in the colon [60,61]. The genome size of microbiota is at least 100-fold greater than human and a large number of species are present, many of them not yet cultured [60,61]. In the last decade, special attention has been paid to explore the functions of the microbiota structure, in particular its metabolic activity and the association with several diseases. It has been shown that commensal bacteria have specific spatial geography in the human gut, and that the balance between different species depends on both host and external factors [62].

Host factors that shape the intestinal microbial niche are represented by nutrients (affecting the mucus, the surface carbohydrates), luminal microenvironment (modifications in oxygen, pH, ions levels), immune effectors (mammalian antimicrobial peptides, IgA), and non-immune factors (bile salts, digestive enzymes) [62–64].

Mammalian antimicrobial peptides are made by white blood cells and epithelial cells that line mucosal surface [65]. These peptides have broad-spectrum antibiotic activity by generating holes in bacterial cell membranes. Defensin and cathelicidin are the major mammalian antimicrobial peptides [62,63]. Paneth cell defensins regulate the gastrointestinal microbiota and intestinal homeostasis [62,63].

Among external factors, a research area of particular interest is the study of the effects of antibiotics on microbiota composition and metabolic activity.

A recent crucial discovery was the observation that microbiota in patients with CDI and with recurrent infection is altered, or dysbiotic [66,67]. Gut microbiota in recurrent CDI possesses a peculiar bacterial composition, but also has a decreased range of bacterial diversity when compared with controls or with patients with single CDI episodes [66,67]. Moreover, dysbiosis observed in CDI cases is associated with an alteration of the fecal metabolite composition in the gut [68]. The changes in the gut microbiota described during the shift from a healthy state to disease supported the idea that modulation of the microbiota may treat CDI and prevent its recurrence [69,70].

Colonization resistance is the ability of the intestinal microbiota to protect itself against exogenous pathogens. Administration of antibiotics may lead to a loss of colonization resistance.

Antibiotics may modify the metabolic activity of the microbiota facilitating the growth of enteric pathogens [70,71]. The majority of the gut microbiota in healthy humans is represented by two dominant phyla, *Firmicutes* and *Bacterioidetes*. *Firmicutes* are able to degrade polysaccharides and to ferment aminoacids whereas *Bacterioidetes* can break down host glycans and non-digestible carbohydrates. In antibiotic-treated animals, there is an increase in *Proteobacteria* and a decrease in the *Firmicutes* and *Bacterioidetes* populations [70,71]. Human studies on the gut microbiota have demonstrated that in the fecal microbiota of patients with CDI, there is a decrease in bacterial diversity and species' richness. Another

Table 1. ASP and CDI: studies evaluating the effect of antimicrobial restriction on CDI occurrence.

Author/year/ reference	Country	Study design	Study period/Setting	Antimicrobials restricted	Effect of ASP on CDI decrease (p value)
McNulty et al., 1997 [33]	UK	Before–after	1994–1995 A elderly care unit of four wards	Cephalosporins	Yes $p = 0.002$ From 37 to 16 CDI cases
Jones et al., 1997 [34]	UK	Cohort	1996 Acute general hospital	Cephalosporins	Yes 40% reduction of CDI cases
Stone et al., 1998 [35]	UK	Before–after	1994–1996 Three acute medical wards for elderly people (one of 18 beds, two of 24 beds)	Cephalosporins	Yes $p < 0.05$ From 36 CDI cases out of 1075 admissions (3.35 cases per hundred admissions) to 27 out of 1392 admissions (1.94 cases per 100 admissions)
Ludlam et al., 1999 [36]	UK	Before–after	1996–1997 A 900-bed teaching hospital	Cephalosporins	Yes $p < 0.001$ From 98 CDI cases out of 2.157 admissions to 45 out of 2.037 admissions
Thomas C, 2002 [37]	Australia	Interrupted time series	1993–2000 A 560-bed urban public teaching hospital	Cephalosporins	Yes $P < 0$ .0001 From 2.09 to 0.87 CDI cases per 1.000 discharges
O'Connor et al., 2004 [38]	Ireland	Retrospective analysis	1992–2002 Acute geriatric unit with a total of 25 beds	Ceftriaxone	Yes $p = 0.03$ From 13 to 4 CDI cases Relative risk of developing CDI with the old policy compared to the new antibiotic policy: 3.24 (95% CI: 1.07–9.84)
Valiquette et al., 2007 [39]	Canada	Interrupted time-series	2003–2006 A secondary/tertiary-care university hospital with a total of 683 beds	Fluoroquinolone, 2nd and 3rd generation Cephalosporins, Clindamycin, Macrolides	Yes $p = 0.007$ From 2.03 to 0.82 CDI cases per 1.000 patient days
Fowler et al., 2007 [40]	UK	Interrupted time series	1999–2003 Three acute medical wards for elderly people in a teaching hospital, with a total of 78 beds	Amoxicillin/Clavulanate and Cephalosporins	Yes $p = 0.009$ Reduction in CDI, Incidence rate ratio: 0.35 (95% CI: 0.17, 0.73)
Bouza et al., 2007 [41]	Spain	Prospective randomized	2003–2005 A medical-intensive care unit, a resuscitation service and a cardiac surgery-intensive care unit with a total of 42 beds	All antimicrobials	Yes $p < 0.01$ 3 (1.8%) CDI cases in the intervention group vs. 8 (9.6%) CDI cases in the control group
Starks et al., 2008 [42]	UK	Case–control	2003–2007 Hip Fracture Unit of a university hospital	Cefuroxime	Yes $p = 0.009$ 38 (4.2%) CDI cases in the initial group vs. 14 (1.6%) CDI cases in the intervention group
Kaier et al., 2009 [43]	Germany	Interrupted time series	2003–2007 University hospital	Fluoroquinolone, Cephalosporins, Macrolides	Yes
Gulihar et al., 2009 [44]	UK	Interrupted time series	2003–2007 University hospital	Fluoroquinolone, Cephalosporins	Yes $p < 0.001$ Reduced incidence of CDI from 7.1% (52 CDI cases out of 731 admissions) to 1.5% (11 CDI cases out of 760 admissions)
Price et al., 2010 [45]	UK	Interrupted time series	2007–2009 820-bed university hospital	Fluoroquinolone, Cephalosporins	Yes $p = 0.03$ From 353 CDI cases out of 82.887 admissions to 258 CDI cases out of 117.358 admissions
Chan et al., 2011 [25]	Taiwan	Before–after	2003–2009 3500-bed medical center	Fluoroquinolone, 3rd and 4th generation Cephalosporins, Aminoglycosides, Carbapenems, Monobactams, Penicillins, Glycopeptides, Oxazolidinones	No
Schön et al., 2011 [23]	Sweden	Point prevalence survey	2007–2008 3 different County hospitals	Fluoroquinolone, Cephalosporins	No 12.6% (182 CDI cases/1438) vs. 13.0% (191 CDI cases/1466)
Talpaert et al., 2011 [46]	UK	Interrupted time series	2005–2007 An acute general hospital	Fluoroquinolone, Cephalosporins, Clindamycin, Penicillins	Yes $p = 0.0001$ decrease in CDI, incidence rate ratio of 0.34 (95% CI: 0.20–0.58)

(*Continued*)

Table 1. (Continued).

Author/year/ reference	Country	Study design	Study period/Setting	Antimicrobials restricted	Effect of ASP on CDI decrease (p value)
Leung et al., 2011 [21]	Canada	Before–after	2009–2010 12-bed combined medical and surgical intensive care unit	Fluoroquinolone, Gentamicin, Meropenem, Piperacillin/Tazobactam, Tigecycline	No 0 CDI cases pre intervention and 1 CDI cases post intervention
Storey et al., 2012 [27]	USA	Before–after	2009–2010 A medical-surgical service of a 100-bed community hospital	Fluoroquinolone, Clindamycin, Daptomycin, Linezolid, Sulfamethoxazole/Trimethoprim	No From 3.7 to 9.2 mean monthly CDI incidence rate
Pate et al., 2012 [28]	USA	Before–after	2009–2011 An urban, 60-bed long-term acute care hospital	Fluoroquinolone, Cefazolin, Cefepime Clindamycin, Daptomycin, Ertapenem Fluconazole, Linezolid, Metronidazole, Piperacillin/Tazobactam, Sulfamethoxazole/Trimethoprim, Vancomycin	No
Elligsen et al., 2012 [47]	Canada	Interrupted time series	2008–2010 Single tertiary care center with 3 intensive care units.	Fluoroquinolone, Ceftriaxone, Ceftazidime), Piperacillin/Tazobactam, Meropenem, Ertapenem, Vancomycin	Yes $p = 0.04$ 31% reduction in the number of monthly CDI, from 16 to 11 CDI cases.
Jump et al., 2012 [48]	USA	Before–after	2006–2010 160-bed long-term care Facility	Fluoroquinolone, Carbapenems, Piperacillin/Tazobactam, Beta-lactams, 3rd generation Cephalosporins, Sulfamethoxazole/Trimethoprim	Yes $p = 0.04$
Dubrovskaya et al., 2012 [20]	USA	Before–after	2009–2011 General surgery service	Fluoroquinolone, Ampicillin/Sulbactam	No
Malani et al., 2013 [49]	USA	Retrospective	observational cohort	2008–2010 A 535 bed non-university-affiliated community teaching hospital	Aztreonam, Daptomycin, Ertapenem, Linezolid, Meropenem, Tigecycline
Yes $p < 0.01$ From 46 (12.4%) to 20 (5.8%) CDI cases					
Dancer et al., 2013 [50]	UK	Before–after	2007–2009 A 450-bed general hospital	Fluoroquinolone, Ceftriaxone	Yes $P = 0.09$ 45.22% reduction in the rate of CDI
Cruz-Rodriguez et al., 2014 [51]	Mexico	Before–after	2013–2015 An orthopedic ward unit	Clindamycin	Yes $p = 0.056$ From 1.07 to 0.12 CDI cases for 1.000 patients days
Palmay et al., 2014 [24]	USA	Stepped-Wedge Randomized Trial	2010–2011 Six inpatient services within one Health Sciences Center	Fluoroquinolone, Ertapenem, Meropenem, Piperacillin/Tazobactam, Ceftazidime, Ceftriaxone, Vancomycin	No
Wenisch et al., 2014 [52]	Austria	Before–after	2013–2014 A large tertiary care community hospital with 1,081 beds and 357,892 patient days in 2013	Fluoroquinolone	Yes $p = 0.0044$ From 59 to 32 CDI cases per month
Ostrowsky et al., 2014 [26]	USA	Before–after	2010–2012 Ten medical centers (6 intervention – 4 non-intervention)	Fluoroquinolone, Piperacillin/Tazobactam	No
Cook et al., 2015 [53]	USA	Cohort	2001–2013 904-bed, tertiary care teaching hospital	Fluoroquinolone, Clindamycin, Colistin, Daptomycin, Fidaxomicin, Linezolid, Tigecycline, Voriconazole	Yes $p = 0.005$ CDI rates cases per 10.000 patient days decrease of 42.6%
Borde et al., 2015 [19]	Germany	Interrupted time series	2013–2014 200-bed community hospital	Fluoroquinolone, Cephalosporins	No From 0.26 CDI cases per 1,000 patient days to 0.18 CDI cases per 1,000 patient days
Sarma et al., 2015 [54]	UK	Interrupted time series	2007–2012 Nine hospitals and a number of long-term care facilities	Fluoroquinolone	Yes Decrease in CDI rate of 60% RR: 0.394 (95% CI: 0.199–0.781) From >280 cases per year in 2007–2008 to 72 cases in 2011–2012
Tedeschi et al., 2017 [55]	Italy	Before–after	2011–2014 150-bed rehabilitation hospital dedicated to patients with spinal-cord injuries	Fluoroquinolone, 3rd generation Cephalosporins, Carbapenems	Yes $p = 0.001$ Decrease from 22 to 7 CDI cases, with a decline in the CDI incidence from 3.6 to 1.2 cases per 10.000 patient days
Färber et al., 2017 [56]	Germany	Cohort	2014–2016 Department of orthopedic surgery that comprises three independent wards	Fluoroquinolone, Clindamycin, 3rd generation Cephalosporins	Yes $p < 0.05$ Mean CDI incidence decreased from 2.13 CDI per 1.000 hospital bed days to no CDI case

(*Continued*)

Table 1. (Continued).

Author/year/ reference	Country	Study design	Study period/Setting	Antimicrobials restricted	Effect of ASP on CDI decrease (p value)
Lawes et al., 2017 [57]	UK	Nonlinear time series	1997–2012 Health board serving 11% of the Scottish population	Amoxicillin/Clavulanate, Clindamycin, 3rd generation Cephalosporins	Yes $p = 0.0077$ Hospital-onset CDI prevalence density was reduced by 68% (mean reduction of 1,01 CDI cases per 1000 occupied bed-days) Community-onset CDI prevalence density was reduced by 45% (0.083 CDI cases per 100.000 inhabitant-days)
Shea et al., 2017 [58]	USA	Before–after	2013–2014 Four hospitals	Fluoroquinolone	Yes $p = 0.044$ From 4.0 to 2.2 monthly CDI cases per 10.000 patient days
Libertin et al., 2017 [59]	USA	Before–after	2013–2015 One hospital	Amikacin, Aztreonam, Cefepime, Ceftaroline, Daptomycin, Doripenem, Ertapenem, Fosfomycin, Imipenem, Linezolid, Meropenem, Tigecycline	Yes $p < 0.001$ From 3.35 to 1.35 cases per 1.000 occupied bed days

ASP: antimicrobial stewardship program; CDI: *Clostridium difficile* infection; CI: confidence interval.

study reported an association between loss of *Bacterioides, Lachnospiraceae*, and *Ruminococcaceae* and development of CDI [72].

Moreover, one of the effects of antibiotic treatment includes an alteration of anaerobic fermentation of carbohydrates in the gut that in turn causes a decrease in short-chain fatty acids production [73]. The expression of CD toxin genes is influenced by the levels of glucose and other nutrients, and it has been shown that a higher production of short-chain fatty acids, especially butyrate, is correlated with the inhibition of CD colonization [74,75]

Studies performed in murine model have shown that in the gastrointestinal tract, after antibiotic treatment, there is an increase in primary bile acids, aminoacids, and carbohydrates supporting CD germination and growth [71]. Therefore, alterations in the gut metabolome after antibiotics allow CD spore germination and outgrowth of vegetative cells.

3.2. The role of bile acid

Among fecal metabolites, recent research has been mostly directed on sialic and bile acids.

Sialic acids are fecal metabolites identified as having a role in CDI development. Commensal bacterial strains can introduce sialic acids into the gut lumen, and they represent a potential energy source for invading pathogens [75]. External factors, such as antibiotic administration, can increase sialic acid levels, enhancing CD growth [75].

Bile acids have been identified as one of the key factor having a role in shaping the microbiota and in controlling CD proliferation and CDI development [76]. Bile acids are important mediators of lipid absorption, and they also act as hormone-like regulators and as antimicrobial molecules [77]. Bacteria that are normally part of the gut microbiota directly confer resistance to CD colonization through competition for nutrients, production of inhibitory molecule and through bile salt metabolism [76].

Over the last decade, research on the role of bile acid in CD pathogenesis and occurrence has increased, leading to the proposal of a general rule in which primary bile acids, such as cholate, promote germination of CD spores while secondary bile acids such as lithocholate inhibit CD vegetative growth [65,78,79], even if there are relevant exceptions to this rule, such as the opposite effect of the primary acid chenodeoxycholate and the secondary acid deoxycholate [78].

A first step toward a better definition of the mechanism mediating bile salt protective or detrimental effect was achieved by Weingarden et al. when they tested the effect of different combinations of bile acids, representative of those found in the feces of patients with recurrent CDI prior to fecal microbiota transplantation (FMT), on spore germination and on vegetative growth of CD. They showed that changes in colonic bile acid composition associated with FMT could inhibit CDI recurrence [79].

Subsequently, in 2013, Francis et al. found that a CD-encoded bile acid receptor is required for spore germination and for the establishment of the infection [80]; in 2014, Taur and Pamer described a secondary bile salt-mediated inhibition of CD growth [81].

Afterwards, in 2015, Buffie et al. had the intuition to relate different duration of susceptibility to CDI with the administration of different antibiotic and correlated microbiota components with CDI resistance. The authors identified microbial taxa associated with CD inhibition in murine and human lower gastrointestinal tracts and found evidences for a protection against CDI mediated by four commensal bacterial species: *B. intestihominis, Blautia hansenii, Pseudoflavoni fractorcapillosus*, and *Clostridium scindens* [82].

Interestingly, *C. scindens* is one of the few bacterial species able to convert primary bile salts into secondary bile salts [83]. In fact, *C. scindens* mediates one of the most important primary bile acid transformations, the 7a-dehydroxylation, thus generating secondary bile acids, including deoxycholic acid and lithocholic acid [77,84]. Therefore, Buffie et al. hypothesized a bile acid-dependent, microbiota-mediated CD

inhibition model [82]. According to this model, a microbial network in the gut provides resistance against CDI.

Microbiota-mediated modification of bile acids contributes to host resistance to intestinal pathogens such as CD. Specifically, C. *scindens* has a pivotal role in this process, having the ability to convert primary bile salts into secondary bile salts thus promoting inhibition of CD vegetative growth.

In this view, after a broad-spectrum antibiotic treatment, the patient intestinal microflora gets disrupted, including a reduction in C. *scindens* population. This imbalance between CD and *C. scindens* gut colonization prevents the metabolism of bile acids increasing the ratio between primary and secondary bile acids. This in turn facilitates CD germination and overgrowth. In fact, since C. *scindens* is no longer in the intestine, even after adequate antibiotic therapy for CDI, recurrence of CDI are observed because the remaining CD spores germinate, favored by the relative increase of primary bile salts and the absence of secondary bile salts [85].

In the light of these considerations, bile salts compounds could be useful either as prophylaxis or as therapy for relapsing CDI [78]. Bile salts compounds may be used in association with traditional antimicrobial treatment to provide a stable inhibition of CD spore germination during the damage caused by antibiotics on bacterial flora [78]. Weingarden et al. [86] reported a case of recurrent CDI successfully treated with oral administration of the bile acid ursodeoxycholate.

New studies are needed to demonstrate the efficacy and feasibility of treatment based on bile acids administration for CDI.

3.3. Fecal microbiota transplantation (FMT)

FMT is a relatively new therapeutic approach for recurrent CDI [87]. Over the years, the initial idea that restoration of the gut microbiota is a key factor for preventing CDI recurrence has been developed to obtain the actual FMT procedures. The original intuition finally proved to be victorious, being supported by the 90% rate of sustained cure achieved by FMT [88].

Nevertheless, FMT approach still presents unsolved questions and logistical challenges. First of all, the exact mechanism of how FMT works has not been fully elucidated [89].

So far, it is known that successful FMT performed in patients with recurrent CDI is associated with a normalization of the microbial community structure as early as 24 h after the procedure with an increase in the overall microbial diversity, an increase of *Bacteroidetes* and *Firmicutes* phyla and a decrease in *Proteobacteria* in fecal microbiota [66]

Importantly, it has been demonstrated that fecal microbiota of recurrent CDI patients turn back to a normal composition after FMT [88,90], in particular, there is a functional restoration of secondary bile acid metabolism [90] and it has been hypothesized that intra-colonic bile acids play a key role in FMT success [79,91].

Second, the potential risk of infections transmitted via FMT is also a current issue, and several donor-screening panels have been proposed [92].

In order to mitigate these potential risk, meticulous donor screening has been adopted so far, including the check for the absence of viruses and other pathogens in the donor microbiota [88,93,94].

On this issue, the use of lyophilized and frozen-and-thawed stool preparations for FMT represents a promising future direction to increase the feasibility and safety of the procedure [95–97]. A small non-blinded, nonrandomized study by Youngster et al. demonstrated the efficacy of oral administration of capsules containing frozen FMT in achieving clinical resolution of recurrent CDI [96]. Afterwards, Lee et al. demonstrated, in a double-blind randomized clinical trial, that freeze-thawed feces was as effective as fresh feces for patients with recurrent CDI [97]. These preparations offer the advantage to freeze the collected stool sample until donor screening results are available [96] and are well tolerated [95].

Finally, long-term outcomes of patients undergoing FMT should be tracked [89] and clinical trials are urged to define FMT-related adverse events [92]. There is actually a need for evidence-based guidelines to drive FMT practices [93,94].

3.4. Live biotherapeutic preparations

More recently, live microbiota preparations have been developed to obtain the beneficial effect of FMT with a more controlled and regulated product. Two clinical trials on this method have been released, respectively, a phase II trial on a microbiota suspension prepared from donated human stool [98] and a phase II trial on *Firmicutes* spores fractionated from healthy donors stool specimens and then treated with ethanol to reduce the risk of transmissible infectious agent contamination [99]. These trials reported favorable results on CDI recurrence prevention, even though further studies are needed to confirm the efficacy of these products.

3.5. Probiotics

According with the International Scientific Association for Probiotics and Prebiotics, probiotics are live nonpathogenic bacteria that are capable of colonizing the gut and, if administered in adequate amounts, can confer a health benefit to the patient [100]. Most common microorganisms contained in probiotics include lactobacilli, bifidobacteria, and *Saccharomyces boulardii* [100].

In the last years, probiotics have been increasingly given to patients, particularly to those receiving antibiotics with the aim of reducing antibiotic-associated diarrhea and CDI.

However, the results of studies exploring the role of probiotics for the prevention of CDI are controversial. Moreover, it is noteworthy that the use of probiotics has been associated with the occurrence of bacteremia and fungemia [101]; in particular, various cases of Lactobacillus endocarditis and bacteremia have been reported, especially in elderly people or immunocompromised patients [101]. Consequently, beside clinical awareness on probiotics' potential beneficial effect, also awareness on the risks associated with their use should increase, especially for patients with specific comorbidities or underlying disease.

A number of hypotheses support the assumption that probiotics can be useful in the management of CDI. After the gastrointestinal colonization by probiotics, several

mechanisms have been proposed that may mediate a protective effect against CDI. Different bacterial species found in the composition of the probiotics products may possess different mechanism of action. In particular, for *S. boulardii* the secretion of proteases degrading CD toxins and the inhibition of CD-binding receptors along with the brush borders of the intestinal lining have been described [102,103]; for *Lactobacillus rhamnosus*, a stimulatory effect on intestinal immunity and a reduction in intestinal permeability defects have been advocated [104]; for *Lactobacillus lactis,* a production of a peptide with lytic activity against CD has been observed [105].

However, a Cochrane review assessed the incidence of CD-associated diarrhea (CDAD) in patients taking antibiotics [106]. It is important to underline that CDAD is a heterogeneous condition based on the definitions reported by authors in the included studies. The overall pooled results showed that the incidence of CDAD was significantly lower in the group of patients taking probiotics during antibiotic treatment (2.0%) as compared to patients taking placebo or no treatment (5.5%; RR 0.36; 95% CI 0.26 to 0.51) [106]. However, when authors analyzed only those studies that reported CDI incidence, the overall pooled results did not show a statistically significant reduction (incidence 12.6 versus 12.7% in the probiotics and control group, respectively; RR 0.89; 95% CI 0.64 to 1.24) [106].

The authors of the Cochrane review concluded that the short-term use of probiotics appears to be safe and effective when used along with antibiotics in patients who are not immunocompromised or severely debilitated; the quality of evidence was moderate. Authors also suggested that probiotics might be effective in preventing symptoms of infection or in limiting the extent of infection rather than inhibiting the colonization and infection itself.

In a meta-analysis including randomized studies assessing primary prevention of CDI with probiotics, Johnson et al. analyzed studies according to the formulation of probiotic used. The meta-analysis of three studies assessing the use of formulation of *L. acidophilus* and *L. casei* showed an overall protective effect against CDI; the meta-analysis of four studies assessing the use of formulation of *S. boulardii* showed no significant difference in CDI rates [103].

Regarding the secondary CDI prevention, a meta-analysis included four trials that had the primary outcome to assess the efficacy of probiotics for the prevention of CDI recurrence. The trials tested, in the treatment group, the administration of S. *boulardii* or *L. rhamnosus GG*. Patients in the treatment group also received antibiotic additional treatment for CDI [107]. The meta-analysis results on probiotics *versus* controls showed that, when trials were pooled by similar types of probiotic species, neither *S. boulardii* nor *L. rhamnosus* GG were significantly efficacious for secondary CDI prevention [107].

Probiotics are frequently used in addition with standard antibiotic treatment in patients with CDI; however, the evidence supporting this use is scanty. The Society of Healthcare Epidemiology of America (SHEA)/Infectious Diseases Society of America's (IDSA) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines do not recommend probiotics administration for the prevention or the treatment of CDI [6,108].

3.6. Nontoxigenic strains

Among nonantibiotic strategies against CDI, a recently introduced preparation of nontoxigenic *C. difficile* deserves to be mentioned [109]. The product appears to be different from other 'traditional' probiotics, providing a nonpathological strain of *C. difficile* and probably acting by direct displacement of *C. difficile* pathological strains colonizing the patient gut [109]. A recent study on this approach observed a relatively low percentage of colonization by the nontoxigenic strain after its administration (69%), and a good efficacy in prevention of recurrences in colonized patients (2%) [109]. Further analysis will be needed to confirm these findings.

4. CD and the host immune response: implications for prevention and treatment

4.1. Humoral immunity

It is well known that the CD virulence is mediated by toxins A and B, and in the last decades several studies evaluated the role of humoral immunity against CD toxins.

Some studies suggest that patients with an anamnestic systemic immune response to CD toxins are less likely to develop CDI symptoms and to have recurrent CDI, and serum IgG levels against toxin A have been reported to be lower in patients with relapsing CDI [110].

However, it should be emphasized that, even if both toxin A and toxin B share the ability to inactivate Rho GTPases expressed in host cells, causing direct and indirect cytopathic effects, toxin B appears to be the most important toxin from a pathogenetic point of view [111]. CD exotoxins have been identified as a possible target for preventing CDI recurrence.

4.1.1. Monoclonal antibodies targeting CD exotoxins

Monoclonal antibodies (mAb) targeting CD exotoxins, namely actoxumab and bezlotoxumab, are currently being introduced into the clinical practice. Bezlotoxumab and actoxumab have the ability to bind and neutralize CD toxin B and toxin A, respectively.

Bezlotoxumab was approved by the US FDA with the indication of reducing CDI recurrence in adult patients who are at high risk for recurrence and who are receiving antibiotic treatment for CDI. A phase II, randomized placebo-controlled trial that included patients with both first episode and recurrent CDI, evaluated the efficacy of bezlotoxumab with actoxumab in combination with anti-CDI antibiotics for the prevention of CDI recurrence [112]. Authors reported that the rate of CDI recurrence was significantly lower in patients treated with mAb compared to the placebo group [112].

A phase III placebo-controlled trial (MODIFY I and MODIFY II) evaluated the efficacy of mAb added to standard anti-CDI antibiotics in patients with primary or recurrent CDI. The primary end point was the proportion of patients with recurrent CDI during the 12 weeks of follow up in the modified intention-to-treat analysis. Three groups of patients were evaluated: population following administration of bezlotoxumab alone, actoxumab alone (MODIFY I only), bezlotoxumab-actoxumab, or placebo. According to the results of both MODIFY 1 and 2 trials

and of the analysis of pooled data from the two trials, the rate of recurrent CDI at week 12 was significantly lower in patients treated with bezlotoxumab alone compared with those treated with placebo. The authors reported that treatment with the combination of bezlotoxumab-actoxumab did not result in higher efficacy compared with bezlotoxumab alone; treatment with the sole actoxumab was not effective [113].

No explanation for this finding has been provided so far, but probably recent literature made some progress to solve the puzzle. In regard, Hernandez et al. characterized the mechanism of action of actoxumab against toxin A showing that this antibody binds at two distinct sites. Therefore, the antibody neutralizes toxin activity by preventing its binding to host cells [114]. Bezlotoxumab acts against toxin B in a similar fashion, even if differences in the binding sites of the tridimensional molecular structure of the two toxins have been found when comparing bezlotoxumab and actoxumab [114]. In particular, the peculiar tridimensional conformation of the binding sites on toxin B recognized by bezlotoxumab seems to allow the formation of larger immunocomplexes in comparison with toxin A-actoxumab [115].

The peculiar mechanism of action of human mAb anti-CD toxin is characterized by appealing features: preventing toxin binding to the host cells, these components halt an early step in the pathogenesis of CDI. The protection against toxin-mediated damage on the gut mucosa also prevents toxins migration from the gastrointestinal tract to the systemic circulation. Moreover, differently from anti-CD antimicrobials, mAb do not cause disruption of the gut flora and do not cause antibiotic resistance [116].

4.1.2. New vaccines for CD

The development of a vaccine against CD may be a promising option to reduce the rate of recurrent CDI [93]. Most of the vaccines currently under clinical evaluation focus on humoral immunity.

Currently, there are at least three different intramuscular vaccines targeting CD toxin that have been evaluated in phase II trials [93,117–119].

The administration of these vaccines containing toxoid A or B or peptidic segments of A or B toxin should induce the production of antitoxin IgG.

Recently, Hong et al. studied a new kind of oral vaccine that consists of spores of *Bacillus subtilis* engineered for the presentation of toxin A cell-binding domain on their surface [120]. This vaccine appears capable to stimulate not only IgG antitoxin production, but also secretory IgA production at the level of the gut mucosa [120,121]. In animal studies, this new vaccine allowed stable neutralization of both toxin A and B, inducing the production of IgG and secretory IgA against toxin A, toxin B, and also a number of other proteins present in vegetative cells or spores of CD [121]. Importantly, this vaccine not only showed to ensure protection against primary CDI, but also resistance against CD colonization [121]. The mechanism for colonization resistance offered by this vaccine seems due to a secretory IgA-mediated reduction in vegetative CD cells adhesion to mucus-producing cells of the gut lining [121].

4.2. Innate immunity

More recently, beside adaptive immunity, innate immunity is also gaining attention as a key determinant for the disease outcome and progression [110].

Innate immunity against CD includes the mucosal immune system, constituted by the mucosal barrier and also by epithelial cells, eosinophils, and macrophages. CD exotoxins at the luminal gut may lead to an inflammatory response mediated by proinflammatory cytokines such as Interleukin (IL)-12, IL-18, interferon gamma, IL-1beta, tumor necrosis factor-alpha [122]. Moreover, CD exotoxins, as well as CD flagellin, lead to the activation of innate immune receptors on the macrophage surface such as Toll-like receptor 4 and 5 and the activation of the IL-1beta inflammasome [123]. The secretion of proinflammatory cytokines successively causes neutrophil activation and mast cell degranulation [124–127]. The colonic epithelium and submucosa of patients with severe CDI are highly infiltrated by activated neutrophils [124–127].

The innate immune response may lead to increased damage of the gut mucosal layer, but also increased defense against the infection [124–127].

The advancements in our understanding of the immune response toward CD and the role of innate immunity against the infection are contributing to the development of promising new approaches for the management of CDI.

4.3. The role of albumin

Hypoalbuminemia is one of the known risk factors associated with CDAD [128] and of severe and recurrent CDI [128,129]. Hypoalbuminemia may also worsen the clinical picture causing intravascular volume depletion and dehydration [129].

Kumarappa et al. hypothesized that the role of hypoalbuminemia in CDI is determined by a decreased colloid osmotic pressure that may lead to intestinal mucosal edema and impairment of the gut mucosal barrier, favoring toxin-mediated damage and disease progression [129].

More recently, from the observation that CDI pathogenesis is strongly related to the harmful effects of bacterial toxins, it has been postulated that albumin may also exert a protective effect by direct binding toxin B [130].

To test this hypothesis, Di Bella et al. performed an *in vitro* study exposing human epithelial colorectal adenocarcinoma cells to toxin A or toxin B and observing the effect of the presence of human serum albumin. They were able to demonstrate that human serum albumin may protect the colorectal cells exposed to toxin B from the cytotoxic effect of Toxin B [130,131].

5. Expert commentary

CDI represents a challenging disease in which a multifaceted approach is advisable. Besides the basic infection control and prevention practices, including hand hygiene, contact isolation, and environmental cleaning, other issues should be addressed including minimizing the spread of CD in the healthcare setting, and implementing the best strategy for reducing recurrence. Regarding new advancements in treatment and management

of CDI episodes, non-antimicrobial approaches seem to be promising in reducing and managing recurrent CDI.

The findings reported in our review regarding the impact of some anti-CDI drugs, such as fidaxomicin, on the intestinal spore shedding need to be further evaluated in larger studies with adequate design and accurate control of confounding. It is important to emphasize that available evidence does not automatically allow to infer that fidaxomicin may reduce CDI incidence in the healthcare setting.

Nowadays, the body of evidence supporting the role of ASPs in reducing the incidence of CDI should not be ignored. However, published studies still have some shortcomings and importantly the long-term applicability and effectiveness of ASP programs in the 'real life' need to be evaluated so far, and the best way for improving and reducing the unnecessary antibiotic use should be better defined.

Importantly, the published meta-analyses assessing the impact of ASP on the incidence of CDI showed a significant beneficial effect. However, in some meta-analyses, the average quality of the studies was low, most of included studies having an observational or quasi-experimental design. Moreover, the high heterogeneity between studies represents an important limitation of some meta-analyses.

In spite of the shortcomings of published studies, it is undeniable that special efforts should be done in all healthcare settings in order to improve antibiotic prescription practices for reducing the incidence of CDI; moreover, it should never be neglected the importance of the marriage between ASP and infection control to achieve a synergistic result.

We do believe that a global approach to the problem of antibiotic use should be applied. Isolated initiatives in single hospitals or in confined regions cannot lead to a solution to a problem that is widespread and therefore needs to be tackled globally. Only the active involvement of scientific societies and an official stance by governments might achieve a significant improvement of antibiotic use worldwide and a consequent reduction of the alarming CDI increased rates.

Along with the attempt of reducing the incidence of CDI, we should get into our stride also in improving the outcome of patients with CDI. Recurrence of symptomatic CDI following initial resolution of symptoms represents one of the most troublesome aspects of CDI.

Even if pathogenesis of the first CDI episode is well defined, risk factors and determinants of a recurrence are still poor known. Nowadays, it is known that important modifications in the metabolic activities of intestinal microbiota are involved in the risk for occurrence and recurrence of CDI. The disruption caused by broad-spectrum antibiotics alters the metabolism of cholate allowing CD germination and overgrowth. In addition, disruption of the overall diversity of the colonic microbiota by antibiotic treatment gives CD more space for colonization. This allows CD to flourish and to recur. In spite of the knowledge of these mechanisms, we paradoxically continue to treat CDI with antibiotics that can cause damage of the 'protective' intestinal microbiota.

Fortunately, the research on CD is passing through a phase of 'positive rage,' giving us a number of innovative, promising therapeutic approaches for CDI coming from the important developments in the knowledge of the role of intestinal microbiota and of immunity.

FMT is now recommended for the treatment of recurrent CDI and can be considered for refractory CDI. Currently, there is no evidence for the use of FMT in primary CDI, even if there are some reports of its use for this indication. An assessment of the role of FMT for primary CDI would be useful and potentially fill the gap of the current therapy with antimicrobials that damage the intestinal microbiota and might interrupt the 'vicious circle of recurrence.'

Bile salts compounds could be useful both as prophylaxis and as therapy for recurrent CDI but further studies are needed to demonstrate their efficacy and treatment feasibility.

The role of probiotics for prevention of recurrent CDI is not supported by evidences and, in our opinion, does not deserve further research, being most convenient to explore alternative strategies.

Since CD exotoxins were identified as a possible target for preventing CDI progression, a successful new strategy consists of the administration of Mab targeting CD toxin B.

Surely the results of the MODIFY trial on bezlotoxumab are exciting; however, in the future a comparison with alternative strategies (i.e. vaccine, new therapies) for the treatment and prevention of CDI would be advisable. An important issue is represented by the cost-effectiveness of this strategy in particular in comparison with the other available therapies. Finally, we have only currently data coming from trials in which, by definition, severe patients are excluded; further data on the effectiveness of mAb among severely ill patients and among patients with recurrent CDI and with a need for concurrent antibiotic therapy are sought.

Additionally, the vaccine approach appears promising in generating protection against occurrence of symptomatic infection, but no effect on CD colonization is expected. Finally, harnessing innate immunity to prevent and treat CDI represents a modern and promising challenge for both reducing the burden of CDI and better managing recurrences that represent one of the most troublesome aspects of CDI.

6. Five-year view

In the next years, there will be more efforts to elucidate CD pathogenic mechanisms that in the recent past have been hampered by several problems, including lack of good molecular tools, animal models, and difficulty of genetic investigation on clinical CD isolates. Revealing the mechanisms of persistence of CD spores in the intestinal mucosa and its role in recurrent disease represents the most important challenge to better understand and manage CD spore–host interactions. Studies are ongoing on the host innate and adaptive response to CD attachment.

Finally, whereas toxin binding on the gut epithelial cells represents the main target for the current research, the study of nontoxin virulence factors of CD is the new frontier for the management of this troublesome infection. Active and passive vaccination targeting CD toxins will be soon available, but also vaccines targeting CD nontoxin molecules, i.e. Cwp84 protease, PS II, are in development.

Moreover, in the next 5 years, bacterio-therapy will include not only FMT, that has several shortcomings, but also microbe suspensions and complex spore formulations. Finally, ASPs

aimed to reduce CDI occurrence in the healthcare setting are constantly evolving and they are becoming more and more tailored to the characteristics of the patient, i.e. immunocompromised, hematologic disorders, aged, transplanted, etc.

Key issues

- One of the most important cause of concern in controlling CD hospital spreading is the environmental contamination due to spore shedding from patients. Fidaxomicin has demonstrated to be more effective than traditional anti-CD antimicrobial therapy in halting spore production, and the use of fidaxomicin might have an important ecological impact. Available evidence does not automatically allow to infer that fidaxomicin may reduce CDI incidence in the healthcare setting.
- The most recent meta-analyses evaluating the effect of antimicrobial stewardship programs on the incidence of CDI showed a significant beneficial effect. Special efforts should be done in all healthcare settings in order to improve antibiotic prescription practices, along with infection control measures, for reducing the incidence of CDI.
- Microbiota in patients with CDI is altered, with a decrease in bacterial diversity and species richness. Recent developments in our knowledge of the role of intestinal microbiota has led to the conception of new non-antimicrobial strategies against CDI, including bile salts compounds, fecal microbiota transplantation and live microbiota preparations.
- Since CD exotoxins were identified as a possible target for preventing CDI progression, a successful new strategy consists of the administration of Mab targeting CD toxin B. Monoclonal antibodies halt an early step in the pathogenesis of CDI and, importantly, do not cause disruption of the gut flora.
- The development of a vaccine against CD may be a promising option to reduce the rate of recurrent CDI. A new kind of oral vaccine consists of spores of engineered *Bacillus subtilis*; this vaccine not only showed to ensure protection against primary CDI, but also resistance against CD colonization.
- Recently, beside adaptive immunity, the advancements in our understanding of the role of innate immunity against the infection are contributing to the development of promising new approaches for the management of CDI.

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