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# NATURAL ALTERNATIVE REMEDIES IN THE BACKGROUND OF UPDATED RECOMMENDATIONS FOR THE PROPHYLACTIC AND THERAPEUTIC APPROACH OF *CLOSTRIDIUM DIFFICILE* INFECTIONS

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## Abstract

*Clostridium difficile* infection (CDI) remains one of the greatest concerns in current worldwide medical practice. Its incidence, severity and patient susceptibility range have known a steady growth. The antibiotics of choice are associated with increasing recurrence rates, decreased susceptibility for some strains, the selection of other antibiotic-resistant enteric pathogens and high cost of treatment, difficult to be supported by the healthcare system. Even if there is a general agreement over the first choice CDI treatment options, the most important international guidelines are not consistent regarding alternative treatment options, such as probiotics, intravenous immunoglobulins (IVIG), monoclonal antibodies, novel antibiotics (fidaxomicin), most of which remaining controversial. Upon consulting international guidelines, numerous discordant recommendations and unsolved issues are revealed. These differences may be due to the constant evolution of prevention and management strategies, in the background of emergence of new scientific and novel treatment suggestions. Many efforts are targeting the discovery of an ideal CDI treatment approach, which would not only eliminate the bacteria and reduce its toxin production, but also achieve restoration of beneficial intestinal microflora and assist the patient immune system. Therefore, many off-label, experimental, novel, natural or combination solutions have been addressed in the latest published research on this matter. In order to assist the potential guidance of future research in this field, this study reviews the recent literature findings on alternative CDI treatment and prophylaxis options.

## Rezumat

Infecția cu *Clostridium difficile* rămâne o problemă dificilă a practicii medicale curente pe plan mondial. Incidența și severitatea bolii, precum și susceptibilitatea pacienților au cunoscut o creștere semnificativă. Antibioterapia este asociată cu o creștere a ratei de recurență post-terapie, cu o scădere a sensibilității pentru anumite tulpini, cu selecția altor patogeni intestinali antibio-rezistenți, dar și cu un cost ridicat, dificil de suportat de către sistemele de sănătate. Deși există un oarecare consens în ceea ce privește terapia de primă linie, cele mai importante ghiduri internaționale nu sunt concordante în ceea ce privește alternativele terapeutice cum ar fi folosirea probioticelor, a imunoglobulinelor administrare intravenos, a anticorpilor monoclonali, a noilor antibiotice (fidaxomicina), cele mai multe dintre ele fiind încă subiect de controversă. Studiul ghidurilor internaționale a relevat multiple discordanțe și aspecte nerezolvate. Aceste diferențe pot fi explicate prin evoluția constantă a strategiilor de prevenire și tratament, pe fondul progreselor științifice și a apariției a unor noi metode terapeutice. Eforturile sunt îndreptate spre găsirea unui tratament optim al infecției cu *Clostridium difficile* care să asigure nu numai eliminarea bacteriilor și reducerea producției de toxină, ci și refacerea microflorei intestinale și echilibrarea sistemului imun al pacientului. Astfel, în literatura de specialitate au fost descrise numeroase alternative terapeutice *off-label*, experimentale, de fitoterapie. Pentru a contribui la o posibilă orientare a recomandărilor viitoare în acest domeniu, acest studiu trece în revistă atât recomandările actuale, cât și ultimele cercetări publicate având ca subiect alternativele de tratament și profilaxie a infecției cu *C. difficile*.

**Keywords**: *Clostridium difficile* infection (CDI), CDI treatment, CDI prophylaxis, adjunctive/alternative treatments, natural remedies

## Introduction

*Clostridium difficile* infection (CDI) is presently one of the most increasingly diagnosed re-emerging health-care associated problem and vastly gaining in importance in worldwide medical practice, as driven by higher incidence and severity over the last decades. To respond to this constant trend and current emergence

of BI/NAP1/027, PCR ribotype 078 [17] and other hipervirulent *C. difficile* strains, the rising shift in asymptomatic carriage, significant rates of recurrence and continuous extending of susceptible population profile, treatment and prevention options are steadily developed.

**Current guidelines for CDI treatment**

*IDSA-SHEA*

Before 2018, according to IDSA and SHEA recommendations (the Infectious Diseases Society of America and Society for Healthcare Epidemiology of America, respectively), metronidazole used to be considered the drug of choice for the treatment of initial mild to moderate CDI episode, usually administered orally in 500 mg doses given 3 times *per* day for 10 - 14 days, whereas vancomycin was primarily elected for initial severe CDI episode (125 mg given orally 4 times *per* day, for 10 - 14 days). Since the publication in February 2018 of the updated recommendations [39], metronidazole has ceased to be indicated as first therapeutic option in adults, with IDSA-SHEA recommendation of vancomycin or fidaxomicin regard-less of CDI severity. Before 2018, in complicated cases (ileus) metronidazole could be associated with vancomycin (either orally or *per* rectum). Anyway, given its cumulative neurotoxicity potential, metronidazole was only recommended for the first CDI recurrence and not for long term therapy. However, even the new guidelines stress the possibility to use metronidazole when vancomycin or fidaxomicin treatment is not feasible.

Probiotics were not recommended for the prevention of CDI, as numerous drawbacks are still associated with studies which support the effectiveness of probiotics in CDI prevention, such as the diversity of probiotic products, lack of standardization, variation in bacterial counts during storage, small number of patients in trials, exclusion of patients receiving high risk antibiotics from trials etc. Nevertheless, IDSA-SHEA always suggested larger trials before use of probiotics in CDI prevention can be recommended. Even the new guidelines stress that lack of evidence for efficiency prevents recommendations for probiotics use.

*ACG - American College of Gastroenterology*

The American College of Gastroenterology recommends the same treatment options as IDSA-SHEA before 2018, in the same dosages for moderate to severe CDI as the IDSA-SHEA [65]. However, due to its toxicity, ACG recommends that metronidazole is interrupted after 5 - 7 of failure to therapy. In addition to the two antibiotics, ACG is making conditional recommendation for faecal microbiota transplant (FMT), in case of multiple recurrences. Also, the ACG maintains that, despite moderate indications of *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii* ability to decrease the incidence of antibiotic associated diarrhoea, insufficient evidence is yet available in support of probiotics for prevention of infection with *C. difficile*. Nevertheless, ACG only moderately recommends the use of probiotics to decrease new episodes in patients with recurrent *Clostridium difficile* infection (RCDI). An additional option according to ACG could be intravenous immune globulin (IVIG), but only for patients with hypo-gammaglobulinaemia.

*The ESCMID – the European Society of Clinical Microbiology and Infectious diseases*

The ESCMID also still recommends oral metronidazole and oral vancomycin as commonly used antibiotics for CDI treatment [16]. In addition to IDSA-SHEA and ACG guidelines, the ESCMID brings to attention emerging problems related to both antibiotics: vancomycin may be associated with high risk of vancomycin resistant enterococci (VRE) selection and transmission, while metronidazole is increasingly associated with evidence of reduced susceptibility in some *C. difficile* strains, eventhough larger study groups are required to prove this. Therefore, the ESCMID recommends use of two additional anti-biotics, fidaxomicin and teicoplanin, with similar or superior efficiency against CDI, as vancomycin, but less likelihood of reported VRE associations. Teicoplanin is known as an antimicrobial glycopeptide acting against Gram-positive anaerobes, some authors also considering its activity against *C. difficile* just as efficient as that of vancomycin or metronidazole [54]. Its limitations to severe repeated recurrences of CDI are related to the high cost.

Regarding alternative treatment regimens, the ESCMID makes IVIG recommendations similar to those of ACG, stating that IVIG therapy could be useful for the subgroup of patients with CDI associated with hypo-gammaglobulinaemia following solid organ transplant. Regarding the insufficient evidence for potential pro-biotic efficiency in CDI prophylaxis, the ESCMID is calling for caution in probiotics use, citing evidence of invasive disease associated with use of *Saccharomyces boulardii*, and reports of increased probiotic associated increased mortality in randomised control acute pancreatitis trial. The ESCMID recommends for FMT following antibiotic treatment in case of multiple RCDI. The ESCMID concludes for insufficient evidence in support of resort to probiotics, toxin-binding resins and polymers, or monoclonal antibodies to alternatively treat CDI.

*The WSES (World Society of Emergency Surgery)*

The WSES makes the same recommendations for metronidazole and vancomycin, as ACG and the ESCMID, while fidaxomicin is recommended for patients with higher recurrence, as an alternative agent [57]. Unlike the other cited guidelines which plead

for insufficient evidence supporting probiotics use in CDI treatment or even call for caution due to reported systemic disease and high mortality, WSES recommends probiotics to be considered for immunocompetent patients with RCDI. Similarly to the ESCMID and the ACG, the WSES recommends FMT and additionally, intestinal microbiota transplantation (IMT) as adjunctive treatment to antibiotics for RCDI treatment, or for immunocompromised patients or patients with solid organ transplants. WSES recommends intravenous immunoglobulin (IVIG) for multiple RCDI or fulminant CDI. Unlike THE ESCMID, which invokes insufficient evidence for monoclonal antibodies recommendation of use, WSES states that infusion with monoclonal antibodies could be used in RCDI, especially in cases with hypervirulent *C. difficile* strains such as 027. WSES makes no recommendations for probiotics use in CDI treatment.

*The ASID (Australasian Society for Infectious Diseases)*

The ASID still maintain metronidazole and vancomycin as first choice anti-biotherapy, with similar recommendations as the other cited guidelines [66]. Fidaxomicin is strongly recommended as newer therapy, with certain benefits in addition to existing treatment, however not recommended as first therapeutic option because of uncertain cost effectiveness relative to metronidazole and vancomycin. The ASID also recommends FMT only for recurrent or refractory disease. In addition to teicoplanin, also mentioned by the ESCMID, the ASID also recommends fusidic acid and tigecycline, but not as first-line therapy. ASID also mentions rifaximin as alternative option, but not as first-line therapy. Monoclonal antibodies, also recommended by the WSES, are indicated by the ASID only as adjunctive therapy for RCDI prevention. Due to promising *in vitro* or animal studies, the ASID mentions several new possible agents to be considered for CDI treatment, but does not make recommendations for use because of insufficient or no published clinical trials: cadazolid (an oxazolidinone-type antibiotic), auranofin (2,3,4,6-tetra-*o*-acetyl-l-thio-β-D-glycopyranp-sato-S-(triethyl-phosphine)-gold (i.e. a gold based product for rheumatoid arthritis therapy), ramoplanin (a glycolipodepsipeptide with vancomycin-like action on bacterial cell walls), surotomycin (a cyclicliopeptide), thuricin CD (a new class of post-translationally modified bacteriocin).

*The Sub-commission of Microbial Resistance Control, the Public Health Division, the Romanian Ministry of Health (RMoH)*

The recommendations of the Sub-commission of Microbial Resistance Control, the Public Health Division, the Romanian Ministry of Health (RMoH) are different from those of the IDSA-SHEA but are similar to those of the ACG, the ESCMID and the WSES, stating that metronidazole should be the first-choice option for mild CDI, whereas vancomycin should be administered in severe cases, or as the first option for second or higher recurrences [49]. The RMoH recommends the use of fidaxomicin only for CDI induced by ribotypes other than 027 and does not recommend its use in Romania, since most CDI cases in Romania are caused by 027 ribotype. The RMoH provides additional reasons for not recommending fidaxomicin use in Romania, such as lack of timely possibility of 027 ribotype exclusion from CDI cases in most clinics, insufficient evidence for safety of use in severe complications (e.g., toxic megacolon) and the cost of treatment. The RMoH is against probiotics use in acute CDI, because of reported risk of bacterial or fungal systemic disease.

Eventhough there is an agreement upon the commonly used first-choice treatment antibiotics, the six cited guidelines are not consistent throughout the recommendations regarding the adjuvant or alternative treatment options. The indications considering the use of prophylactic probiotics are significantly different among guidelines, varying from recommended in certain groups of patients with RCDI only for prevention of recurrence (the WSES), to caution in use (the ESCMID), not recommended due to insufficient evidence (the IDSA-SHEA, the ACG) or even against use in acute CDI (the RMoH). Such contradictions concern other alternative treatment options, such as IVIG, monoclonal antibodies and even fidaxomicin. As previously described, fidaxomicin acceptance remains highly controversial, ranging from strong recommendation by most of the international guidelines (the ESCMID, the ASID, the WSES), to advice against recommendation (the RMoH). Fidaxomicin is recommended due to a number of efficiency benefits and is considered equally effective or even superior to vancomycin in certain aspects. There are reports indicating that fidaxomicin may result in lower environmental *Clostridium difficile* contamination, also bearing highly desired positive impact on in-hospital spread of spores [9]. The RMoH advises against use of fidaxomicin in Romania, due to several reasons among which cost of treatment, or insufficient evidence for safety of use in severe complications. Most new agents reported as promising solutions, by the six sources, could not be included in the guidelines recommendations because of insufficient or lack of clinical trial data or insignificance of data related to the study group size.

Recent publications compared and summarized the content of the five international guidelines discussed above, except for the Romanian guide, revealing discordant recommendations, numerous controversies and unsolved issues on numerous topics regarding CDI management [19]. These differences mainly derive from constant development of prevention and treatment strategies and emergence of further scientific evidence and innovative suggestions for treatment.

**Alternative, off-label and emerging CDI therapeutic options**

*Other antibiotic compounds*. Rifamycins, especially rifamixin, commonly used as an antituberculosis agent, have been proposed as chaser therapy for *C. difficile* [4]; however, prior exposure may reasonably pose the risk of rifamixin-resistant *C. difficile* emergence.
Recent publications indicate a number of novel antimicrobial substances for CDI treatment, currently undergoing clinical trial, among which: ramoplanin, surotomycin, a new cadazolid (oxazolidinone-fluoroquinolone), ridinilazole [54]. However, even if efficacy is shown to be similar to first choice antibiotics, the phase II or III clinical trials conducted so far have failed to prove ramoplanin or surotomycin non-inferiority [54].
*Intestinal microbiota transplantation (IMT)* is also cited in current international guidelines as adjunctive treatment option for advanced CDI [61], but important limitations have been reported, such as reluctance of both patients and physicians, the risk of spreading other infections (HIV and hepatitis) and the risk of dramatic microbiota change, which may increase susceptibility to obesity or autoimmune conditions [4].
*DNA vaccination*. The immunogenicity of candidate antigens in animals is currently tested for high-level antibody responses, which will allow producing of sub-unit based recombinant toxin proteins, as vaccines. Monoclonal antibodies, also mentioned by current guidelines, but not recommended because of insufficient clinical trial evidence of effectiveness, can be produced by animal or human volunteers who received terminal regions of *C. difficile* toxins. Several receptor-binding regions of *C. difficile* A and B toxins (N-terminal or C-terminal regions) are considered candidates for protective antibody responses [4]. Considering significant recent progress in toxin specific monoclonal antibodies technology, researchers report opportunities for further studying and optimization of immunization approaches for CDI treatment and prophylaxis [4].
*Bacteriophages*. There are studies indicating that the ability of several specific *C. difficile* bacteriophages to significantly decrease *C. difficile* count and reduce toxin production both in remedial and prophylactic treatment [54]. Nevertheless, there are indications that bacteriophage-resistant colonies may emerge after initial reduction of bacterial load [54].
*Bacteriocins*. GE2270, nisin, lacticin 3147, Thuricin CD, actagardine, LFF571 and diffocin antimicrobial activity has been brought to our attention by recent studies which highlight the efficacy of such bacteriocins in preclinical, as well as clinical studies [54].
*Toxin binding agents*. Several compounds have been discussed in relation to CDI treatment or prophylaxis. Cholestyramine, one of the first agents to be tested for effectiveness against recurrent CDI, is an ion exchange resin, proposed as CDI prophylaxys in patients on systemic antibiotic therapy [51]. Tolevamer, a polymer with toxin-binding activity, of lower efficacy against *C. difficile* than first choice antibiotics, is nevertheless helpful in lowering the recurrence rates of CDI, more than vancomycin or metronidazole [20]. An additional toxin-binding compound proved effective in sequestering *C. difficile* toxins is calcium aluminosilicate anti-diarrheal (CASAD), a naturally occurring clay known for its cation exchange absorbent related ability [64]. Although deemed a promising adjuvant agent in CDI therapy, no proof could be obtained in clinical trials, because of unsuccessful enrolment.
*Bile Acids Therapy*. The intestinal composition of bile acid plays a notable role in prevention of *C. difficile* spore germination and growth and certain bile acids, such as ursodeoxycholic acid, have accordingly been proven effective [70], in their inhibition. A further compound, CamSA (a taurocholate analogue) was shown to inhibit *C. difficile* germination [28] and also to efficiently achieve dose-dependent CDI prophylaxis in preclinical studies [27]. However, recent reviews of research activity in the field indicate that it remains unclear whether bile acids and their analogues could be considered for CDI prevention or adjunct treatment [20].
*Premature toxin activators*. Ivarsson ME *et al* [31] have proposed an inositol hexakisphosphate (IPS) analogue (INS-5010) as premature toxin activator agent in a preclinical study on *C. difficile* infected mice, proving improvement of histological signs compared to control subjects. This compound was developed from IP6, a natural substance found in all eukaryotic cells, considered the main phosphorous storage source in plant seeds, constituting 2% of the dry weight of common grains and FDA-granted Generally Recognized as Safe (GRAS) compound status [30]. Nevertheless, Inositec AG, the company which develops INS-5010 is still seeking to partner certain drug candidate and discovery assets, not having moved on to clinical trials so far [30].
*Intestinal Antibiotic Inactivators*. One of the major risks, unanimously recognised as intimately associated with CDI development, is systemic antibiotic induced disrupter of intestinal microbiota balance. Therefore, protecting this balance by eliminating antibiotic therapy effect means eliminating one of the major CDI risks.
There are some products that literature indicates to inactivate systemic antibiotics that reach the colon, through various mechanisms:
*(1) DAV132 - a product based on activated-charcoal*, provided with enteric coating and able to absorb antibiotics (e.g., amoxicillin or moxifloxacin) from the proximal colon [15]. DAV132 efficiently prevented moxifloxacin-induced CDI in hamsters [40].
*(2) SYN-004* is a recombinant beta-lactamase product, inactivating parenteral beta-lactam antibiotics on reach into the intestine. A recent study [34] proved effective ceftriaxone inactivation by SYN-004 in dog intestine,

with antibiotic serum levels remaining unchanged. This product also proved valuable after clinical trial testing [53].

*(3) Cephalosporinase-producing Bacteroides thetaiotaomicron* was used in a study for the inactivation of parenteral administered ceftriaxone which reaches the intestine [63]. The study reveals successful protection of normal intestine microbiota and protection against CDI and also vancomycin-resistant *Enterococcus spp.* in mice model.

*Marine Mammal Microbiota able to produce novel antibiotic agents effective against C. difficile*. In a study of intestinal microorganisms in five marine mammals, Ochoa JL *et al* [45] have identified a *Micromonospora* strain which proved selective activity against various Gram-positive pathogens without possessing any human cytotoxicity. The authors isolated phocoenamicin, a novel complex glycosylated polyketide with anti *C. difficile* antimicrobial activity. Using fluorescence imaging and flow cytometry, Ochoa JL *et al* [45] confirmed that phocoenamicin can shift the membrane potential leaving membrane integrity unaltered.

*Insect peptide coprisine*. An antibacterial peptide isolated from *Copristripartitus*, a Korean dung beetle, coprisine, includes a nine amino-acid peptide with antimicrobial activity in its helical region (LLCIA LRKK) [29]. In a study on a mouse model with experimental induced CDI, Jin Ku Kang *et al* [32] examined the possibility for a coprisin analogue (a disulphide dimer of the nine peptides) to prevent *C. difficile* infection induced inflammation and mucosal damage. The authors revealed that coprisine analogue treatment is associated with improved survival rate, decreased inflammation response and mucosal damage. A further positive aspect communicated by the same researchers is the lack of antibiotic activity of coprisine analogue, on commensal bacteria such as *Lactobacillus* and *Bifidobacterium* [32]. These findings suggest that the coprisine analogue should be considered as a good candidate for alternative treatment and prophylaxis of CDI.

## Natural products as promising alternative and adjunctive options

Disputed recommendations of current international guidelines on CDI treatment derived from controversies and unsolved issues in the context of constant increase of incidence and severity of CDI cases, call for intensive research for the development of alternative treatment options. Currently, the commonly accepted and recommended conventional antibiotic treatment choices for CDI, are very limited. Even though the excessive and widespread use of antibiotics is being condemned worldwide, it remains a common practice which is still linked with the development of anti-biotics resistance in many pathogens. This problem is especially dangerous for infections such as CDI, for which the available treatment choices are limited from the beginning [62]. Natural remedies have been used since ancient times as cures for diseases and are currently the sources of a significant part of modern drugs. Therefore, natural-, plant- or animal-derived remedies, used as alternative or adjunctive treatment or prophylaxis become desirable options to prevent and contain *C. difficile* resistance and provide safe and effective therapy.

### Virgin coconut oil

Shilling M. [59] has recently published a study evaluating the lipidic components of virgin coconut oil in terms of antibacterial activity against *C. difficile*. The authors have assessed the *in vitro* antimicrobial effect of whole virgin coconut oil, specifically the effect against *C. difficile* of its most important individual fatty acids, reporting that lauric acid (C12) revealed the most intensive inhibitory action, by reduction of CFU/mL. Other fatty acids, such as capric and caprylic acids (C10 and C8, respectively) exhibited lower inhibitory activity, whereas whole coconut oil only exhibited antimicrobial action against *C. difficile,* when lipolyzed and used in 0.15 - 1.2% concentration. Furthermore, using electron transmission microscopy, the authors reported that disruption of cell membrane and cytoplasm of *C. difficile* strains is the mechanism by which medium-chain fatty acids from virgin coconut oil (lauric acid 2 mg/mL), exert their antibacterial effect. The results of this study are intensively cited and positively appreciated by other researchers [3, 4]. Coconut oil was also reported to exhibit *in vitro* anti-fungal activity comparable with chlorhexidine and ketoconazole, in a study using *C. albicans* isolated from tooth surfaces of children with early childhood caries [60].

The antibacterial effect of coconut oil has been extensively studied and is nowadays acknowledged against pathogens such as *L. monocytogenes* [36], *Streptococcus mutans* [47], *Streptococcus pyogenes* [43].

In more recent studies, other authors confirm the inhibitory effect of virgin coconut oil (VCO) free fatty acids (FFA) against pathogens like *Bacillus subtilis* (ATCC 11774), *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076) and *Staphylococcus aureus* (ATCC 25923) [44]. The authors used *Candida rugosa* lipase (CRL) to obtain the coconut oil free fatty acids and performed hydrolysis at 1:5 w/w VCO to buffer ratio, 1.5% w/w oil CRL, pH = 7 and 40°C. However, the authors revealed that only FFA exhibited inhibition against the tested pathogens, while the residual hydrolysed virgin coconut oil and the whole VCO did not present any antibacterial activity. Similar conclusions were drawn by Nagase S. [43], who demonstrated that eventhough whole

virgin coconut oil has antimicrobial effect against *Streptococcus spp*., no activity was noticed against *S. aureus* or Gram-negative bacteria. The same authors proved that lauric acid (50% of virgin coconut oil fatty acids) has different antimicrobial spectrum, exhibiting antimicrobial activity against *S. aureus*, towards which virgin coconut oil has no effect. Furthermore, the authors reveal stronger antimicrobial effect of lauric acid against several *Streptococcus* species, in comparison with coconut oil. Based on such results, the need becomes apparent to test coconut oil antimicrobial activity on separate fatty acids to achieve targeted antibacterial action. This approach could be useful for an efficient antibacterial evaluation of coconut oil components against *Clostridium spp*.

**Pomegranate extracts (*Punica granatum* L.)**

The antimicrobial activity of pomegranate extract was reported against several pathogens such as *Propionibacterium granulosum*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [35], as well as against *Salmonella enteritidis*, *Listeria monocytogenes*, *E. coli* and *Yersinia enterocolitica*, due to its peel high content (262.5 mg/g) in active inhibitors (phenolics, flavonoids) [5].

In a very recent publication [18], the authors report the possibility to attribute pomegranate antimicrobial activity to ellagitannins (ETs), a class of hydrolysable tannins (HT) including over 500 different compounds. The authors indicate that ETs are known to be found only in dycotyledonous angiosperms and they are commonly consumed along with some of their hydrolysis products (such as ellagic acid), in fruits, nuts and their processed products. Pomegranate includes two main types of ETs, namely punicalagins and punicalins. This study indicates that 250 mL pomegranate juice could provide 0.05% w/w punicalagins in the colon. By *in vitro* exposure of intestinal *Clostridium spp*. bacteria to punicalagins and punicalins, it was reported that the inhibition ranges from 26% to 100%, depending on the *Clostridium* species. The authors also mark that ETs from pomegranate exhibit inhibition activity towards pathogenic bacteria, without adverse effects on the beneficial bacteria and furthermore, even favouring *Lactobacillus spp*. bacteria growth [18]. Similar results are communicated by Finegold S.M. *et al* [20], which revealed that pomegranate extract exhibits MICs ranging from 12.5 to 25 mg/mL gallic acid equivalent range, against toxigenic *C. difficile* strains.

***Angelica archangelica* L. (*Apiaceae*)**

A study by a group of Italian researchers on the chemical composition and supposed antimicrobial activity of *Angelica archangelica* L. (*Apiaceae*) root essential oil has revealed significant activity against *C. difficile*, *C. perfringens*, *E. faecalis*, *Eubacterium limosum*, *Peptostreptococcus anaerobius* and *Candida albicans* (MIC values of 0.25%, 0.25%, 0.13%, 0.25%, 2.25% and 0.50% v/v, respectively) [22]. Effectiveness against these pathogens is even more significant since the antimicrobial activity against the useful microflora is very week (MIC values > 4.0% v/v against bifidobacteria and lactobacilli) [22].

***Nigella sativa* and *Commiphora myrrha* (Myrrh)**

In a study by Khalid M. Aljarallah [33], seeds of black cumin (*Nigella sativa*) and *Commiphora myrrha* (Myrrh) were studied as potential inhibitors of *C. difficile* growth. Their antibacterial activity was studies under oil (*Nigella sativa*) and water extract (*Nigella sativa* and Myrrh) forms. According to study results, Myrrh water extract and black seed oil (2%) are similarly effective natural antibacterial agents for inhibition of *C. difficile* growth, in a wide range of environment pH (4.5 - 7). Khalid M. Aljarallah [33] suggests that *Nigella sativa* seeds and *Commiphora myrrha* (Myrrh) should be considered as potential alternative and adjuvant therapy options for the treatment of human CDI. Black cumin seeds have been extensively used as spice and valued for a wide range of pharmacological actions, among which immunity modulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic etc. [2, 14, 24]. Many of *Nigella sativa* therapeutic properties have been attributed to thymoquinone, considered the most significant bioactive component of the essential oil [18]. The antimicrobial effects of *Nigella sativa* have extensively been studied *in vitro* [7, 68] and *in vivo* [52] against various microorganisms such as *Enterobacteriaceae*, *Staphylococcus*, *Streptococcus*, *Salmonella*, *Listeria*, *Helicobacter*, *Pseudomonas*, *Klebsiella*, *Proteus* etc. Therefore, including *Nigela sativa* bioactive compounds in clinical trials for testing the efficiency against CDI would be of interest.

**Manuka honey**

Manuka honey is known for its *in vitro* antimicrobial effect against numerous pathogenic bacteria [23, 37, 58]. Manuka honey stands out among conventional honeys due to its non-peroxide antimicrobial activity, attributed to certain 1,2-dicarbonyl compounds, and especially to methylglyoxal (MGO) [23].

A study by Hammond and Donkor [26], revealed Manuka honey *in vitro* bactericidal concentrations of 6.25% (v/v) for *C. difficile*. A more recent study confirms the 6.25% (v/v) MIC and MBC of Manuka honey activity against various *C. difficile* (PCR) ribotypes (RTs) (e.g., RT017, RT023, RT027 and RT046), also showing its ability to effectively inhibit *C. difficile* strains-induced biofilm formation [48]. The study of Piotrowski *et al* [48] suggests the possibility to successfully use Manuka honey as *C. difficile* spread prevention agent, whereas it's possible use for CDI

prophylaxis, as well as its efficacy on *C. difficile* spores remain to be investigated further. It is even more important to admit the need to perform clinical trials on Manuka honey to test its efficacy in CDI prevention, especially when honey supplementation in CDI clinical trials has already been tried very recently on patient groups at higher *C. difficile* infection risk. In a cross-sectional study by Mohsen Saleh El Alfy *et al* [41], on paediatric patients with malignancy, it is revealed that honey supplementation improved the occurrence rate of gastrointestinal complications associated with chemotherapy, reducing the rate of CDI.

### *Humulus lupulus*

A recent study by Cermak P *et al* [10] tested the antimicrobial activity against *B. fragilis*, *C. perfringens* and *C. difficile* of hop (*Humulus lupulus* L.) constituents' humulone, lupulone and xanthohumol. The authors revealed that xanthohumol showed the most relevant antimicrobial effects, with MIC and MBC values of 15 - 107 μg/mL, close to those of conventional antibiotics. Cermak P *et al* [10] suggest that hop purified compounds may thus be potential alternatives for the treatment of CDI.

### Traditional Chinese herbal formulas

For centuries, Chinese herbal medicines have been used for the treatment of various disorders, including gastrointestinal disease. The effectiveness of Chinese herbal medicines on intestinal bowel disease (IBD) symptoms improvement has been proved in clinical trials conducted two decades ago [8]. The diversity of medicinal plants found around the world is fairly unlimited, but very few have been investigated for their bioactive compounds and far less than that have been actually included in clinical trials [46]. However, recent articles reveal that Chinese medicines such as berberine, can successfully inhibit *C. difficile* and help regulate the intestinal microflora, when prescribed after initial vancomycin treatment [38]. Another example of Chinese medicine tested for efficacy in *C. difficile* associated diarrhoea (CDAD) prevention, is *QPYF* (consisted of *Rhodiola rosea, Poria cocos*, *Codonopsis pilosula*, *Atractylodes macrocephala*, *Radix puerariae*, *Rhizoma zingiberis* and *Glycyrrhiza glabra*). When administered a week prior to *C. difficile* experimental infection in a mouse model, traditional Chinese medicine QPYF showed effective protection for CDAD, by significantly lowering histopathological scores and toxin production [25].

### Various other raw natural compounds and pure compound extracts

The investigation of the antimicrobial activity against *Clostridium difficile in vitro* targeted various other natural compounds such as garlic juice, indicated by Roshan N. *et al* [55], as the most active raw natural products. Moreover, the same study suggested four pure compounds (i.e., trans-cinnamaldehyde, allicin, menthol and zingerone) as the most active processed products against *Clostridium difficile*. Roshan *et al* [56] also reveals that, as determined by the fractional inhibitory concentration index and the conventional checkerboard titration method, the combined effect of antibiotics and natural compounds should be considered for future complementary CDI treatments.

Alongside carvacrol (a compound extracted from oregano oil), an additional *C. difficile* related study also mentioned trans-cinnamaldehyde, the most important cinnamon component as a substance able to reduce production of the *C. difficile* toxin and toxin-mediated cell-toxicity [1, 42]. The same study revealed inhibition of toxin production by a mechanism of down-regulation of toxin production genes (global repressor CodY).

Another important aspect is that these compounds do not interfere with intestinal microbiota. Toxin production being a *C. difficile* major virulence factor, such natural plant compounds are promising agents for future development of alternative CDI treatment solutions [1].

In a more recent study, certain commercially available supplements, such as ginger, peppermint oil and aloe vera are indicated as remedies for the symptoms of non-specific inflammatory bowel disease (IBD) [54]. The authors indicate that for the peppermint oil, the improvement of symptoms in IBD patients was proved in placebo-controlled clinical trials. Therefore, further investigation of the antimicrobial properties of peppermint oil against *C. difficile* may be relevant.

## Discussion

The first-choice therapy options for CDI recommended by all international guidelines remain metronidazole and vancomycin. However, numerous drawbacks have been reported, among which the lack of efficiency for some *C. difficile* strains, the increasing recurrence rates, the selection of additional resistant enteric pathogens, the difficult management of complications in patients with associated diseases and other risk category patients. Fidaxomicin, suggested for higher efficiency and compatibility with beneficial microflora survival, is severely restricted by the high cost of treatment. The few other novel antibiotic choices either failed to prove the non-inferiority to first choice option drugs or are under clinical trials. In the last years, insufficient new antibiotics have received approval from clinical trials, despite the significant demand due to increased resistance. The most important 6 international guide-lines are

inconsistent regarding alternative treatment options. Recommendations for probiotics, FMT, IVIG, monoclonal antibodies and even fidaxomicin, may range from strong recommendation, to advice against use. On the background of such unresolved issues and controversies, research efforts are aiming the ideal treatment option for CDI, which would not only reduce the infectious and toxin burden given by *C. difficile*, but also restore intestinal microbiota and assist patient immune system. Increasing attention is lately awarded to traditional medicine plants, natural vegetal or animal extracts, natural animal derived products, which proved significant efficiency *in vitro* or in animal model studies, but also considerable efficiency in combinational antibiotic therapies in clinical trials [50]. Among the most popular alternative suggestions for CDI treatment and prevention the latest publications address bacteriophage, toxin-binding agents, premature toxin activators, intestinal antibiotic inactivators, bile acid therapy, animal micro-biota compounds, insect extracted compounds, vegetal extracts (roots, leaves, fruits, seeds), animal made products, herbal combinations in traditional Chinese formulas. Many of these natural alternatives achieve selective antimicrobial activity against *C. difficile*, improve clinical signs, reduce complications, protect and restore the beneficial microbiota or achieve effective protection against contamination.

## Conclusions

*C. difficile* infection is continuing to be a major problem impacting present medical practice, with increasing incidence and severity. Standard treatment recommendations are considered suitable for most CDI cases, yet recurrence rates, complications, treatment options in risk patients and increased prevalence in intensive care units, create difficulties in CDI management and prevention. International guidelines are inconsistent in alternative options recommendations, while continuous research is currently addressing strategies and solutions for treatment and prophylaxis. Many off-label, experimental, novel, natural or combination alternatives are approached in the recent literature. Promising results related to natural compounds and extracts should be considered for future research of combinational antibiotic therapies. Many of these options need further investigation of toxicological profiles, of possible interactions and clinical trial confirmation.

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