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Impact of Primary and Secondary Bile Acids on *Clostridioides difficile* Infection

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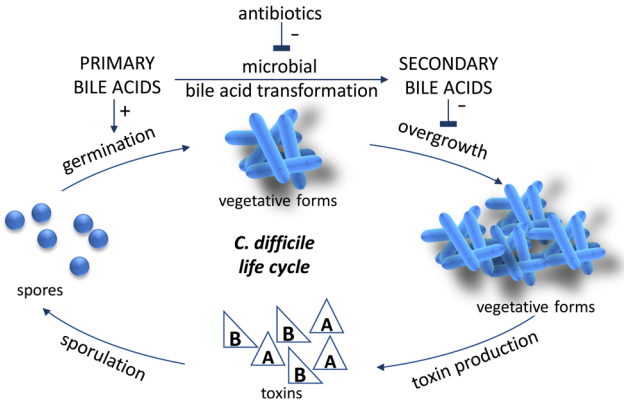
Abstract

Primary bile acids (BAs), synthesized from cholesterol in the liver, after their secretion with bile into the intestinal lumen, are transformed by gut microbiota to secondary BAs. As natural detergents, BAs play a key role in the digestion and absorption of lipids and liposoluble vitamins. However, they have also been recognized as important signaling molecules involved in numerous metabolic processes. The close bidirectional interactions between BAs and gut microbiota occur since BAs influence microbiota composition, whereas microbiota determines BA metabolism. In particular, it is well established that BAs modulate *Clostridioides difficile* life cycle *in vivo*. *C. difficile* is a cause of common nosocomial infections that have become a growing concern. The aim of this review is to summarize the current knowledge regarding the impact of BAs on the pathogenesis, prevention, and treatment of *C. difficile* infection.



K e y w o r d s: bile acids, *Clostridioides difficile* infection, ursodeoxycholic acid, obeticholic acid

Introduction

Bile acids (BAs) are cholesterol-derived products with amphipathic properties due to several hydrophilic hydroxyl groups, a polar carboxyl group, and a steroid nucleus (Fiorucci and Distrutti 2019). There are two types of BAs: primary and secondary. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are the main primary BAs produced by humans, while in mice, the predominant primary BAs are CA and muricholic acid (MCA) (Chiang and Ferrell 2019). Primary BAs are synthesized in the liver, and after conjugation with taurine or glycine, they are secreted into the biliary tracts. BAs constitute a main component of bile, which is accumulated in the gallbladder and, following the food intake, is secreted into the intestinal lumen (Ridlon et al. 2006). As natural detergents, BAs play a key role in lipid emulsification and absorption of lipids and liposoluble vitamins (Dawson and Karpen 2015).

Besides the participation in digestion, BAs are characterized by many other functions since they act as signaling molecules involved in regulating various metabolic processes (Fiorucci and Distrutti 2019). BAs are well-known ligands of widespread nuclear and membrane receptors. The nuclear receptors activated by BAs include farnesoid X receptor (FXR), pregnane X receptor (PXR), vitamin D receptor, liver X receptor (LXR), and glucocorticoid receptor (GR). Whereas G protein-coupled receptors (GPCR) on cell surface activated by BAs are Takeda G-protein receptor 5 (TGR5), sphingosine-1-phosphate receptor 2 (S1PR2), muscarinic receptors (M2 and M3), and formyl-peptide receptor (FPR) (Ticho et al. 2019).

The FXR activation modulates glucose, lipid, and protein metabolism, and energy expenditure. It also regulates various metabolic pathways in the liver, including hepatic fibrosis (Ferrebee and Dawson 2015). The FXR stimulation via BAs results in fibroblast

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growth factor 19 (FGF19) production in humans (FGF15 is the mouse ortholog). FGF19 regulates BA synthesis in the negative feedback loop by repressing cholesterol-7α-hydroxylase activity in hepatocytes, consequently leading to decreased hydroxylation of BAs and the reduction of BA synthesis (Babaknejad et al. 2018). Moreover, BA-mediated FXR stimulation regulates microbiota composition due to the enhanced expression of genes, the products of which, like nitric oxide, arrest bacterial overgrowth (Di Gregorio et al. 2021). Furthermore, FXR is involved in modulating intestinal innate inflammatory reactions through nuclear factor-kappa B (NF-κB) signaling. The BA binding to FXR also enhances mucosal integrity contributing to the protection against intestinal infections (Matsubara et al. 2013).

BA-mediated TGR5 activation influences glucose metabolism through enhanced secretion of incretins: glucose like peptide-1 and glucose like peptide-2 into the portal vein (Dawson and Karpen 2015). Moreover, it stimulates intestinal motility (Xie et al. 2021), induces gallbladder relaxation, reduces intrahepatic biliary pressure, and modulates BA pool composition (Bidault-Jourdainne et al. 2021). Additionally, the TGR5 activation restrains hepatic macrophage activity (Xie et al. 2021) and diminishes proinflammatory cytokines in monocytes, hence modulating inflammatory processes (Duboc et al. 2014). A summary of the main BA functions is presented in Fig. 1.

Due to the direct relationship between BAs and gut microbiota, and the impact of BAs on the regulation of various metabolic processes, especially modulation of the immune response, there is a growing attention directed toward the relationship between BAs and gastrointestinal infections, in particular *Clostridioides difficile*. It has already been well established that BAs modulate *C. difficile* life cycle (Abt et al. 2016). Moreover, several studies report BA alterations in the course of *C. difficile* infection (CDI) and during its treatment. The aim of this review is to summarize the current knowledge concerning the impact of BAs on the pathogenesis of CDI and the role of BAs in its prevention and treatment.

Two publication databases have been searched: PubMed and Scopus. Combinations of the following keywords were used ("bile acid" or "bile acids" or "ursodeoxycholic acid" or "ursodiol" or "obeticholic acid") and ("*Clostridioides difficile*" or "*Clostridioides difficile* infection" or "*Clostridium difficile*" or "*C. difficile*"). The search was limited to papers published between January 2005 and August 2021. Exclusion criteria were as follows: CDI only mentioned and non-English language.

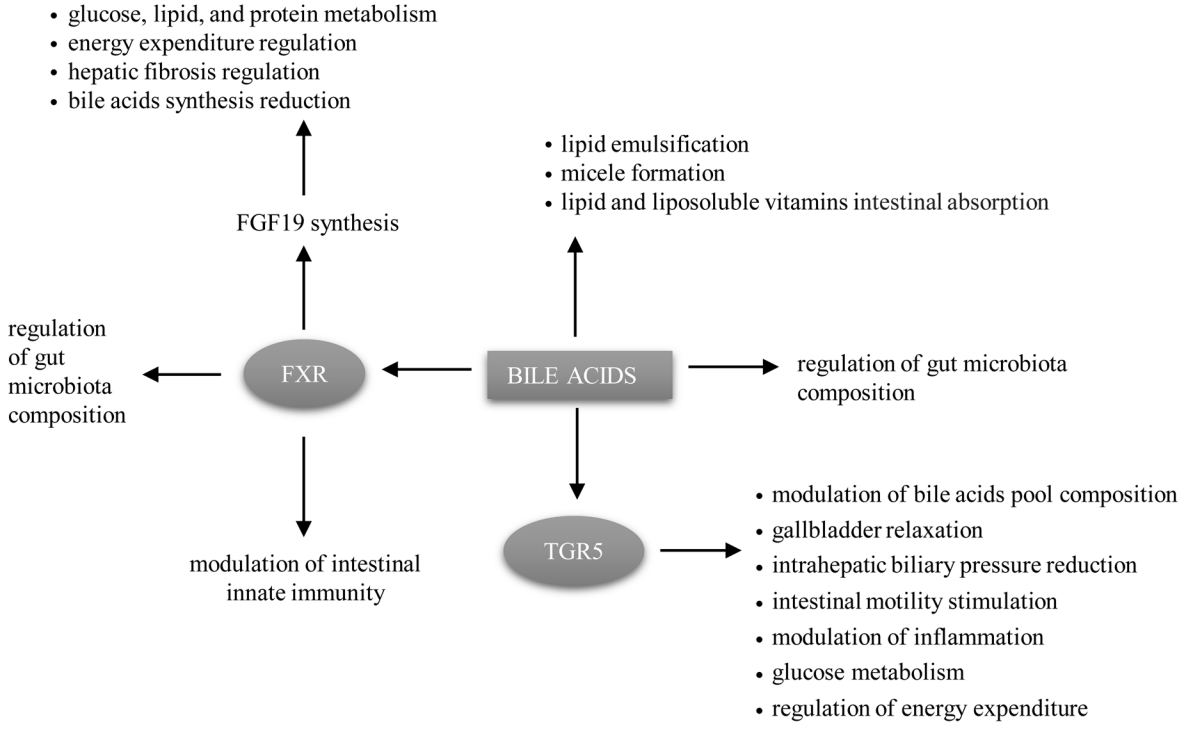

Fig. 1. Main functions of bile acids. Bile acids (BAs), as the main bile component, are responsible for lipid emulsification, micelle formation, and participation in the absorption of lipids and liposoluble vitamins. BAs also have an important impact on gut microbiota composition. BAs are farnesoid X receptor (FXR) ligands leading to fibroblast growth factor 19 (FGF19) synthesis. Moreover, FXR stimulation influences gut microbiota composition and intestinal innate immune response. BAs also activate Takeda G-protein receptor 5 (TGR5), stimulating intestinal motility, inducing gallbladder relaxation, reducing intrahepatic biliary pressure, modulating the composition of the BA pool, and affecting glucose metabolism. Additionally, the TGR5 activation regulates inflammatory processes and energy expenditure. Based on Dawson and Karpen (2015).

Crosstalk between bile acids and gut microbiota

In the small intestine, the gut bacteria metabolize BAs. The main transformation processes include deconjugation and oxidation of hydroxyl groups (Marin et al. 2015). Bile salt hydrolases (BSHs), the only enzymes responsible for the pivotal deconjugation reaction, allow BAs to become less toxic to the microbiota. BSHs cleave the conjugated glycine or taurine from BAs. The activity of BSHs serves as a gatekeeper to subsequent BA transformations (Foley et al. 2019). Approximately 95% of BAs are actively reabsorbed in the terminal ileum by the apical sodium-dependent bile acid transporter (ASBAT) to the portal vein from where they reach the liver and return into bile. It is called enterohepatic circulation (Ticho et al. 2019). The remaining 5% of unabsorbed BAs pass to the large intestine, where they undergo further microbial transformation (Marin et al. 2015). Only a narrow group of commensal bacteria can perform 7α-dehydroxylation, which is the crucial reaction in secondary BA origination. The *Lachnospiraceae* and *Ruminococcaceae* family members, including *Clostridium scindens*, *Clostridium hiranonis, Clostridium hylemonae*, and *Clostridium sordellii*, exert 7α-dehydroxylation activity (Wells and Hylemon 2000). 7α-Dehydratase releases hydroxyl group from BAs. As a result, primary BAs – CA, and CDCA are transformed into DCA and LCA, respectively. This reaction is possible due to prior deconjugation. Dihydroxylation and oxidation/epimerization of hydroxyl groups also occur in the large intestine. Epimerization of CDCA 7α-hydroxyl group to 7β-hydroxyl group provides the origination of ursodeoxycholic acid (UDCA) (Marin et al. 2015).

BA biotransformation results in modifying their properties, allowing for passive absorption of some BAs in the colon. Secondary BAs constitute an essential part of the BA metabolome. Partially BAs are also eliminated in the feces (Marin et al. 2015). Fig. 2 presents an overview of the BA circulation and biotransformation.

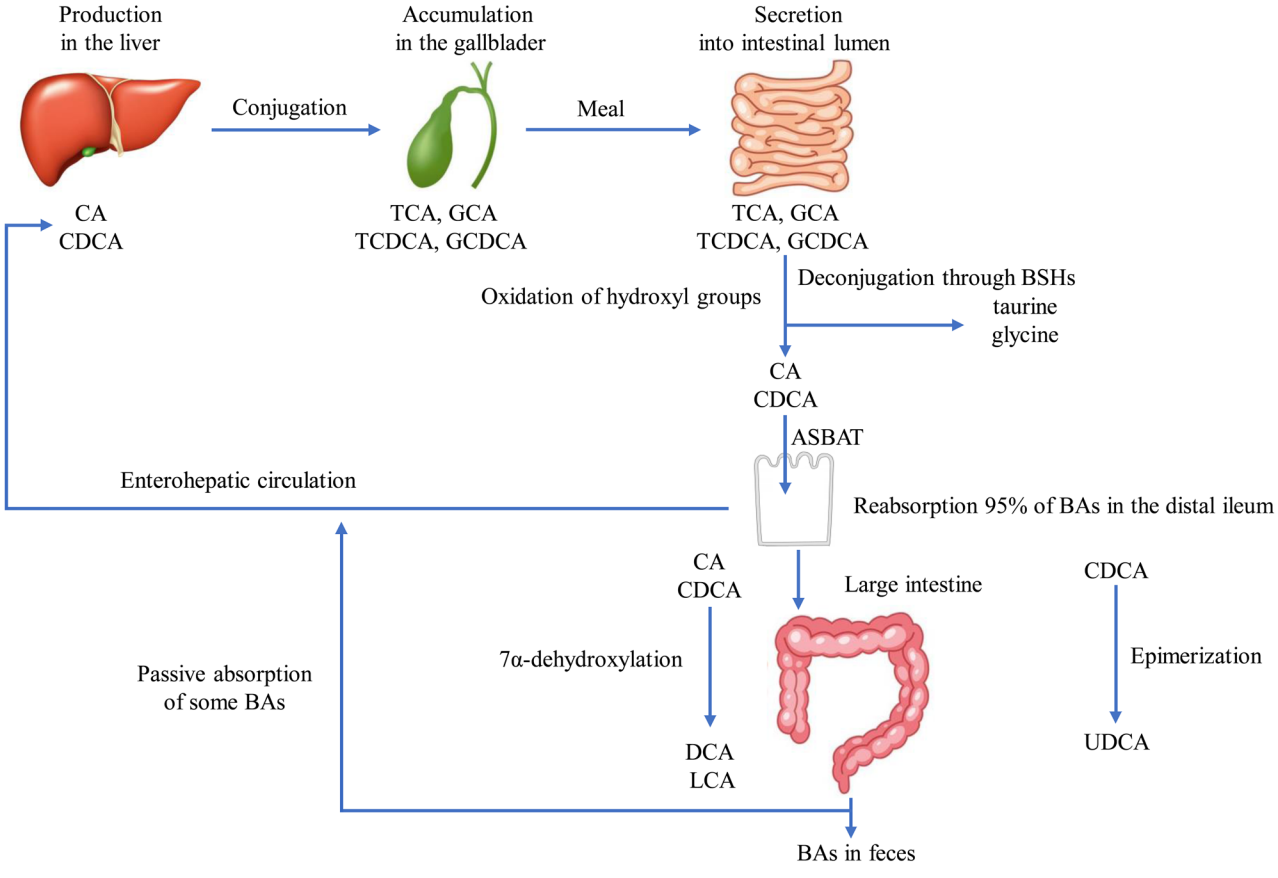

Fig. 2. Enterohepatic circulation and bile acid biotransformation. Primary bile acids (BAs) are produced in the liver and after conjugation with taurine or glycine are secreted into bile ducts. As a component of bile, they are accumulated in the gallbladder. After every meal, they are secreted into the intestinal lumen. In the small intestine, microbiota biotransformation starts. The deconjugation is performed by bile salt hydrolases (BSH), which constitute the key reaction enabling further transformations. Most BAs are absorbed in the distal ileum by the apical sodium-dependent bile acid transporter (ASBAT) to the portal vein from where they reach the liver and return into bile. The unabsorbed BAs pass to the large intestine, undergoing further microbial transformation. 7α-dehydroxylation is the crucial reaction in secondary BA origination. Some BAs are passively absorbed in the colon, and some are also eliminated in the feces. CA – cholic acid, CDCA – chenodeoxycholic acid, TCA – taurocholic acid, GCA – glycocholic acid, TCDCA – taurochenodeoxycholic acid, GCDCA – glycochenodeoxycholic acid, UDCA – ursodeoxycholic acid. Based on Fiorucci and Distrutti (2019).

Clostridioides difficile infection

CDI is the most common cause of antibiotic-associated diarrhea (Abt et al. 2016). It is related to a significant morbidity, mortality, and a substantial global burden to the healthcare system (Guh et al. 2020).

C. difficile is a Gram-positive bacillus with an ability to produce endospores, which are commonly found in the environment as well as in the intestinal tract of humans and animals (Czepiel et al. 2019). In the gastrointestinal tract, the spores may germinate and outgrow to pathogenetic vegetative forms, producing toxins A and B that evoke symptoms of CDI (Mahida 2019). Toxins A and B bind to specific surface receptors in the colon leading to intestinal epithelium necrosis and exfoliation, resulting in mucosal integrity disturbances. In consequence, immune cells and intoxicated epithelial cells release proinflammatory cytokines and chemokines such as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and IL-1β. Subsequently, an acute inflammatory reaction in the intestinal wall develops (Winston et al. 2020).

CDI may be manifested by an asymptomatic carriage, mild to moderate diarrhea, colitis, pseudomembranous colitis, or even fulminant colitis. Antibiotic therapy, abdominal surgery, hospitalization, age over 65, and severe concomitant diseases are the major risk factors of CDI (Napolitano and Edmiston 2017).

The gut microbiota composition has an ability to protect against invasion by pathogenic microorganisms. The mechanism is not fully known; however, it is proposed that microbiota-derived secondary BAs take an important part in this process (Winston and Theriot 2016).

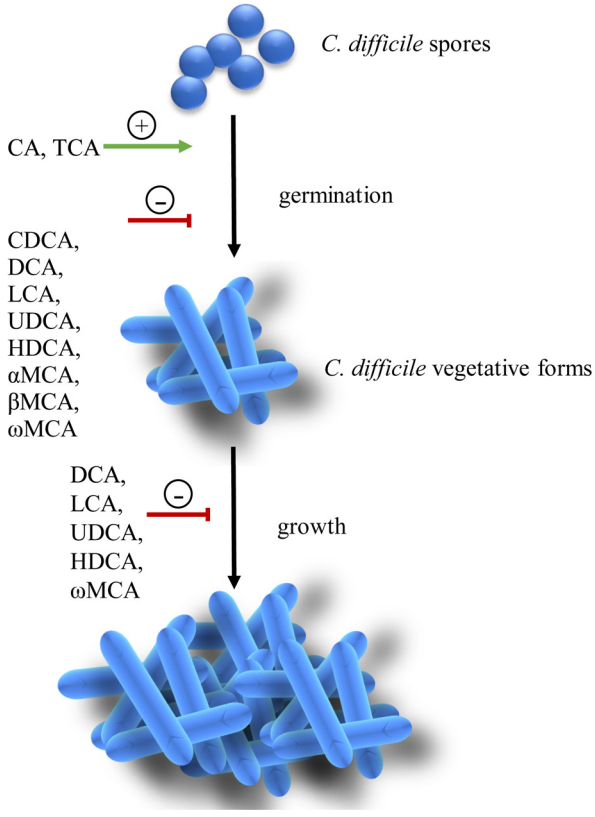

Fig. 3. Impact of bile acids on the life cycle of *Clostridioides difficile*. *C. difficile* is a Gram-positive bacillus, with an ability to produce endospores. Spores may germinate and outgrow in the gastrointestinal tract to produce pathogenic vegetative forms secreting toxins. Primary BAs such as cholic acid (CA) and taurocholic acid (TCA) are endogenous triggers to *C. difficile* spore germination. However, other primary BAs, including chenodeoxycholic acid (CDCA), α and β stereoisomers of muricholic acid (MCA), arrest *C. difficile* spore germination. Secondary BAs such as DCA, LCA, ursodeoxycholic acid (UDCA), hyodeoxycholic acid (HDCA), and ω-MCA inhibit *C. difficile* spore germination and the growth of *C. difficile* vegetative forms. Based on Studer et al. (2016).

Bile acid pool composition and *Clostridioides difficile* life cycle

Primary BAs such as CA and taurocholic acid (TCA), which are present in significant concentrations in the small intestine, promote *C. difficile* spore germination (Theriot et al. 2016). However, other primary BAs, including CDCA as well as α and β stereoisomers of MCA, prevent this process. Secondary BAs such as DCA, LCA, UDCA, hyodeoxycholate acid, and ω-MCA inhibit *C. difficile* spore germination as well as inhibit the growth of *C. difficile* vegetative forms (Studer et al. 2016) (Fig. 3). In the large intestine, secondary BAs are present in much higher concentrations than primary BAs. Therefore, *C. difficile* spores germinate to some extent in the small intestine, but the proper ratio of secondary BAs in the colon is pivotal for CDI prevention (Theriot et al. 2016). BAs influences *C. difficile* germination through the recently identified germinant receptor CspC (Weingarden et al. 2016b). They are capable of binding and inhibiting *C. difficile* toxin B as well. Secondary BAs are more potent in toxin inhibition than primary BAs (Mullish and Allegretti 2021). Interestingly, *C. scindens* and *C. sordellii* also produce tryptophan-derived antibiotics, which can inhibit the growth of *C. difficile*. DCA and LCA intensify the inhibitory activity of these antibiotics (Kang et al. 2019).

The healthy microbiota inhibits *C. difficile* spore germination and vegetative form growth through secondary BA production (Britton and Young 2014). Antibiotic therapy can lead to dysbiosis, disturbing secondary BA production and the BA pool composition (Studer et al. 2016). Consequently, increased primary BA level and decreased secondary BA level in the colon are observed (Theriot et al. 2016). It has been demonstrated that rats treated with antibiotics have higher primary BA concentration in stool than untreated rats (Hashimoto et al. 1996). In physiological conditions, secondary BAs constitute the majority of fecal BAs.

Disproportion in primary to secondary BA ratio after antibiotic therapy can be associated with loss of resistance to CDI (Keith et al. 2020).

Noteworthy, obesity constitutes CDI risk factor (Mulki et al. 2017). One potential mechanism underlying greater susceptibility of obese subjects to a more severe CDI course might be an obesity-related dysbiosis resulting in BA disturbances. In a study conducted by Jose et al. (2021), mice with diet-induced obesity had a significantly higher ratio of primary to secondary BAs in cecal content compared to mice on a regular chow diet. Primary BA production in the liver was enhanced in these obese mice, and consequently, *C. difficile* spore germination was promoted by cecal content contrary to non-obese mice. Obese mice developed severe and prolonged CDI (Jose et al. 2021).

In the study conducted by Wei et al. (2020), the number of *C. scindens* and *C. hylemonae* in feces was remarkably decreased in mice with diet-induced obesity contrary to non-obese mice (Wei et al. 2020). As aforementioned, *C. scindens* and *C. hylemonae* belong to a narrow group of bacteria, which can perform 7α-dehydroxylation, the crucial reaction in secondary BA origination (Wells and Hylemon 2000).

BA dysregulation has been reported in patients with CDI. Allegretti et al. (2016) have observed the shift in BA concentration in human stool with the primary BA predominance during CDI. Moreover, higher primary BA content in feces was noted during recurrent CDI compared to the first episode of CDI (Allegretti et al. 2016). On the other hand, *C. difficile* multiplication is associated with microbiota dysregulation contributing to the disturbances in BA metabolism. The perturbations of intestinal bacteria lead to *C. difficile* predominance and colonization (Czepiel et al. 2019). The presence of vegetative forms of *C. difficile* restrains from the colonization of other microorganisms. Numerous studies have shown a reduction in gut microbiota richness in patients with CDI compared to healthy controls, and in the first CDI episode compared to recurrent CDI (Theriot et al. 2011; 2014; Buffie et al. 2015). Lack of bacteria with 7α-dehydroxylation activity leads to decreased secondary BA production (Sehgal and Khanna 2021). It results in a a cause-and-effect relationship in which dysbiosis and BA pool alterations intensify and aggravate each other, leading inexorably to worsening of the situation, which could be described as a "vicious circle".

Therapeutic role of bile acids in *Clostridioides difficile* infection

Fecal microbiota transplantation. Antibiotics are the mainstay of CDI therapy. Vancomycin and fidaxomicin are recommended in the first CDI episode (Guh et al. 2020). However, these drugs can also lead to microbiota dysregulation. Even after successful treatment, 5–20% of patients with CDI may develop recurrent infection (Napolitano and Edmiston 2017). Relapses are treated with vancomycin for an extended time. However, while vancomycin eliminates *C. difficile* vegetative forms, it does not influence endospores (McDonald et al. 2018). Fecal microbiota transplantation (FMT) is the last choice option recommended in recurrent and refractory infections after the unsuccessful antibiotic treatment (Guh et al. 2020).

FMT is based on administering bacteria present in donor fecal filtrate to reestablish the resident microbiota. Apart from living bacteria, the products of their metabolism also significantly contribute to the FMT effectiveness. The microbial species with 7α-dehydroxylase activity present in the transplanted microbiota play a pivotal role in restoring resistance to *C. difficile* colonization, since 7α-dehydroxylase is responsible for the metabolism of primary BAs to secondary BAs in the colon (Mahida 2019).

Buffie et al. (2015) demonstrated that administration of *C. scindens*, a bacterial species with 7α-dehydroxylase activity, was associated with resistance to CDI in antibiotic-treated susceptible mice. Weingarden et al. (2016b) confirmed significant alterations in BA profile before and after FMT in patients with recurrent CDI. They have also demonstrated that the content of primary BAs in pre-FMT stool samples induces *C. difficile* spores germination and vegetative form growth, while post-FMT stool samples abounding in secondary BAs inhibit the life cycle of *C. difficile* (Weingarden et al. 2016b). Therefore, FMT contributes to the inversion of fecal BA profile from the predominance of primary BAs to the predominance of secondary BAs (Seekatz et al. 2018).

Ursodeoxycholic acid (UDCA). Given the relevant impact of BAs on the *C. difficile* life cycle, it has been proposed that secondary BAs can be possible therapeutic targets in preventing and treating CDI. UDCA, also known as ursodiol, is one of the human secondary BAs. UDCA has hydrophilic properties and is profusely reabsorbed in the distal ileum. Oral administration of UDCA results in its increased concentration in bile that leads to decreased hepatotoxic effects of bile (Cabrera et al. 2019). Therefore, UDCA is used in liver diseases such as primary biliary cholangitis and nonalcoholic steatohepatitis (Floreani 2020; Sodum et al. 2021). UDCA directly arrests germination and vegetative overgrowth of *C. difficile in vitro* (Thanissery et al. 2017). However, the direct impact of UDCA on microbiota *in vivo* is not fully understood.

The study conducted by Winston et al. (2020) demonstrated that no significant difference in microbiome after UDCA application could be seen in a mouse model.

However, another study showed that the UDCA administration results in reducing the abundance of *Bifidobacterium*, *Lactobacillus*, and *Lactobacillaceae* in the human gut microbiota composition (Kim et al. 2018).

As mentioned above, CDI is associated with decreased secondary BA and increased primary BA levels in the stool. Importantly, UCDA treatment of mice with CDI is associated with the BA pool alteration leading to the enhanced concentration of β-MCA, tauro-β-MCA, TCA, tauroursodeoxycholic acid (TUDCA), and UDCA in feces (Palmieri et al. 2018; Winston et al. 2020). However, differences in BA profiles and metabolism among species may constitute major limitations in BA research, making it challenging to translate animal models into clinic (Winston and Theriot 2020).

Palmieri et al. (2018) revealed that UDCA administration failed in CDI prevention in a rodent model. Similar results were obtained by Winston et al. (2020). They demonstrated in a murine model that UDCA treatment did not prevent CDI; however, it was associated with a retardation of the onset of symptoms. Therefore, UDCA may impact on the early stages of CDI pathogenesis (Winston et al. 2020). The retrospective analysis showed that the UDCA treatment of primary sclerosing cholangitis in patients with inflammatory bowel disease did not protect from CDI development (Palmieri et al. 2018). In another study, UDCA was prescribed off-label to patients with recurrent CDI and risk factors of relapses. In those cases, UDCA treatment was associated with 87.5% effectiveness in CDI prevention at a median follow-up of 264 days (Webb et al. 2019). Weingarden et al. (2016a) presented a single case report on a patient with recurrent *C. difficile* ileal pouchitis. They showed that oral UDCA treatment influenced *C. difficile* elimination and long-term prevention from CDI recurrence (Weingarden et al. 2016a). Currently, ursodiol is in phase IV clinical trials to prevent *C. difficile* recurrence (Winston et al. 2020).

Noteworthy, UDCA can modulate the immune response through NF-κB signaling. In the study by Winston et al. (2020) in a mouse model, UDCA application was associated with decreased expression of genes encoding products mediating the inflammatory response to CDI, including IFN-γ, IL-6, TNF-α, and IL-1β. Therefore, it may be concluded that UDCA treatment could reduce the excessive inflammatory response during CDI, subsequently alleviating severe forms of CDI. Moreover, the immune response modulation was associated with the UDCA-induced activation of TGR5 and FXR. Pretreatment with UDCA resulted in a significantly enhanced expression of TGR5, FXR, and FGF15 during CDI compared to the no-UDCA treatment (Winston et al. 2020). However, this effect of UDCA on the FXR signaling was not confirmed in prior studies. It has been reported that UDCA administration might also lead to the suppression of FGF19 transcription and increment of CYP7A1 expression, thus suggesting that UDCA is an FXR antagonist (Gonzalez et al. 2015).

Moreover, studies performed on primary human hepatocyte cultures have demonstrated that UDCA has no effect on hepatic FXR (Liu et al. 2014; Zhang et al. 2017). However, the administration of UDCA at supratherapeutic concentrations results in the FGF19 enhancement and subsequent CYP7A1 suppression. It has been suggested that the UDCA effect in higher doses is most likely exerted through the PXR signaling pathway and not mediated by FXR mechanisms (Zhang et al. 2017). The PXR activation also downregulates the NF-κB signaling (Mohandas and Vairappan 2017).

Obeticholic acid. Obeticholic acid (OCA) is a synthetically modified BA, which was approved in 2016 for the treatment of primary biliary cholangitis (Fiorucci et al. 2019). Currently, OCA is under investigation to treat primary sclerosing cholangitis and non-alcoholic steatohepatitis (Novotny et al. 2021). Similarly to UDCA, OCA administration leads to its absorption in the distal ileum and occurrence in bile. OCA as a potent FXR ligand contributes to the diminished synthesis of primary BAs in the liver. Consequently, OCA administration leads to a decrease in hepatotoxic properties of bile and lower primary BA content in the cecum (Novotny et al. 2021). Due to the affinity to FXR, OCA promotes NF-κB signaling and afterward takes part in the modulation of the intestinal immune response (Matsubara et al. 2013).

Given that obesity is a risk factor for a severe CDI, Jose et al. (2021) conducted a study administering OCA to obese mice after exposition to *C. difficile*. The result of the treatment was associated with a reduction in severity and duration of CDI symptoms. OCA contributed to the alleviation of CDI later phases by decreasing the number of *C. difficile* bacteria in stool and the cecal contents, and diminishing the concentrations of the toxins. Consequently, treatment with OCA resulted in a significant reduction of intestinal damage (Jose et al. 2021).

Conclusions and perspectives

It is well known that gut microbiota plays a pivotal role in CDI prevention. The CDI risk factors contribute to dysbiosis leading to dysregulation of the BA metabolome. Since BAs regulate *C. difficile* life cycle, they become promising therapeutic targets. FMT is also associated with a shift in fecal BA profile, which proves their important role in CDI. The role of UDCA, OCA, and other FXR and TGR5 agonists in CDI treatment, requires further investigation because the exact mechanism of BA-mediated FXR and TGR5 activation remains to be elucidated. The modulation

of the inflammatory response through NF-κB signaling and inhibition of BA synthesis seem particularly important. Therefore, BAs constitute an encouraging treatment option in CDI. Nonetheless, further studies concerning the definite relationship between BAs and the pathogenesis of CDI and the BA impact on the course of CDI are required.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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