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Impact of microbial derived secondary bile acids on colonization resistance against *Clostridium difficile* in the gastrointestinal tract

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Abstract

Clostridium difficile is an anaerobic, Gram positive, spore-forming bacillus that is the leading cause of nosocomial gastroenteritis. *Clostridium difficile* infection (CDI) is associated with increasing morbidity and mortality, consequently posing an urgent threat to public health. Recurrence of CDI after successful treatment with antibiotics is high, thus necessitating discovery of novel therapeutics against this pathogen. Susceptibility to CDI is associated with alterations in the gut microbiota composition and bile acid metabolome, specifically a loss of microbial derived secondary bile acids. This review aims to summarize *in vitro, ex vivo*, and *in vivo* studies done by our group and others that demonstrate how secondary bile acids affect the different stages of the *C. difficile* life cycle. Understanding the dynamic interplay of *C. difficile* and microbial derived secondary bile acids within the gastrointestinal tract will shed light on how bile acids play a role in colonization resistance against *C. difficile*. Rational manipulation of secondary bile acids may prove beneficial as a treatment for patients with CDI.

Graphical Abstract

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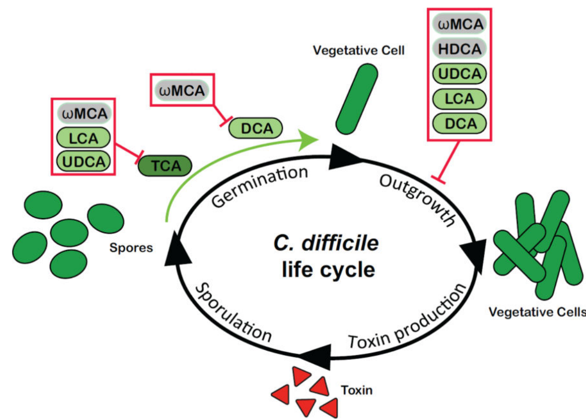

Keywords

Clostridium difficile; secondary bile acids; colonization resistance; gut microbiota; antibiotics

Introduction

Clostridium difficile is an anaerobic, Gram positive, spore forming bacillus that was first isolated from newborn infants by Hall and O'Toole in 1935[1]. Currently, *C. difficile* is a leading nosocomial enteric pathogen that causes significant human morbidity, mortality, and results in over $4.8 billion per year in healthcare costs[2–5]. In 2013, the Centers for Disease Control and Prevention (CDC) categorized *C. difficile* as an urgent antibiotic resistance threat negatively impacting public health[5]. A major risk factor for infection with *C. difficile* is the use of antibiotics[6, 7]. Antibiotics lead to significant and long lasting shifts in the gastrointestinal (GI) microbiota and metabolome[8–10] resulting in a loss of colonization resistance against *C. difficile*[11–14]. Colonization resistance is the ability of the indigenous gut microbiota to protect against invasion by enteric pathogens[15]. Although the exact mechanisms of colonization resistance against *C. difficile* are unknown, there is increasing evidence that gut microbiota derived secondary bile acids play an important role[11, 12, 16, 17].

Antibiotic treatment with vancomycin and metronidazole is considered standard of care for *C. difficile* infection (CDI)[18]. Unfortunately this treatment further disrupts the gut microbiota composition and recurrence of CDI after cessation of antibiotics is high, occurring in 20–30% of patients[2, 18–21]. Consequently, antibiotic treatment is insufficient for some patients with CDI thus necessitating the discovery of novel therapeutics against *C. difficile*. In this review we aim to highlight the dynamic interplay between *C. difficile* and the secondary bile acids within the GI tract. In particular, we will review *in vitro*, *ex vivo* and *in vivo* studies done by our group and others that focus on how bile acids affect the different stages of the *C. difficile* life cycle. Rational manipulation of secondary bile acids in the GI tract may prove beneficial as a therapeutic strategy against *C. difficile* [12, 22].

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Formation of Microbial Derived Secondary Bile Acids

Bile acids are water-soluble, cholesterol derived amphipathic molecules synthesized by hepatocytes[23]. Cholate (CA) and chenodeoxycholate (CDCA) are the primary bile acids synthesized in humans and rodents (Figure 1A)[23–25]. In rodents, a significant amount of CDCA is converted by 6-β-hydroxylation to muricholate (MCA) [26]. The host further metabolizes primary bile acids via N-*acyl* amination to glycine or taurine forming conjugated bile acids, such as glycocholate (GCA) or taurocholate (TCA). Primary bile acids enter the small intestine where they aid in fat emulsification and absorption[23, 27]. Bile acids are also biological detergents and induce expression of antimicrobial peptides, thus contributing to the host defense system against both commensal microbes and some enteric pathogens[28, 29]. Once host derived primary bile acids enter into the GI tract, members of the gut microbiota transform them into over 50 chemically diverse secondary bile acids[23, 30]. Secondary bile acids are formed by two main bacterial reactions: deconjugation predominantly within the small intestine and epimerization/dehydroxylation within the large intestine[23].

Deconjugation of conjugated primary bile acids occurs rapidly by extracellular bile salt hydrolases (BSH), which are widespread in the gut microbiota[23, 28, 31]. Based on metagenomic screening, 3 major phyla in the gut microbiota possess BSHs: Firmicutes (30%), Bacteroidetes (14.4%), and Actinobacteria (8.9%)[31]. Within these phyla, BSHs from the following genera are heavily studied: *Clostridium*, *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* [28]. BSHs appear to enhance bacterial colonization within the lower GI tract potentially by detoxification of bile acids[23]. Thus the presence of BSHs are included in probiotic selection criteria to improve strain competitiveness within the gut[32]. However, the host and microbial physiologic function of BSHs is still being investigated[23, 28].

The second bile acid modifying reaction results in oxidization and epimerization of specific hydroxyl groups by three distinct bacterial hydroxysteroid dehydrogenases (HSHD), 3-α, 7-α, and 12-α[23]. HSHDs can produce 27 unique metabolites from the primary bile acid CA alone[23, 33]. In the colon nearly 100% of bile acids are bacterial derived and a broad spectrum of bacteria can dehydrogenate unconjugated bile acids[23, 34]. In contrast, 7α-dehydroxylation is performed by only a few anaerobic species, representing less than 0.025% of the total gut microbiota and 0.0001% of total colonic microbiota[23, 35, 36]. These are largely represented by *Clostridium spp. (C. hiranonis, C. hylemonae, C. sordelli, C. absonum*, and *C. scindens*) and *Eubacterium spp.*, which are all members of the Firmicutes phylum[12, 23, 33, 37–43]. Removal of the 7-α hydroxyl group of primary bile acids requires multiple intracellular enzymatic steps, which are encoded in the *bai* (bile acid inducible) operon[12, 23, 33, 44–46]. Ultimately these reactions lead to the formation of secondary bile acids, deoxycholate (DCA) from CA and lithocholate (LCA) from CDCA (Figure 1A)[34, 47]. DCA and LCA can be modified further by gut microbes into additional secondary bile acids, such as ursodeoxycholate (UDCA) from LCA (Figure 1A)[47].

The diverse chemical structures of bile acids are a collaborative effort by the host (production of primary bile acids) and the gut microbiota (production of secondary bile

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acids). The lack of secondary bile acids in a germfree mouse GI tract illustrates the bacterial contribution to bile acid modification[48]. Alterations in bile acid profiles (primary vs. secondary) are also observed in the antibiotic treated mouse gut, which have an altered gut microbial composition[11, 49, 50]. The host and microbial physiologic functions of secondary bile acids remain complex. In the host, secondary bile acids DCA and LCA can be cytotoxic leading to oxidative stress, membrane damage, and colonic carcinogenesis[51]. However, the secondary bile acid UDCA can protect colonic cells against apoptosis and oxidative damage[51]. For gut microbes, secondary bile acids are postulated to have three potential roles: serve as terminal electron acceptors for production of energy, form less hydrophobic membrane damaging bile acids pools, and alter the virulence of enteric pathogens[23, 28]. Secondary bile acids modulate the virulence of the enteric pathogen *C. difficile*, by inhibiting different stages of its life cycle[11, 23, 28, 52–55]. These examples highlight the diverse and potentially divergent roles of bile acids in relation to host and microbial physiology.

Impact of Secondary Bile Acids on the *C. difficile* Life Cycle

Bile acids play a dynamic yet critical role in the life cycle of *C. difficile*. The impact of bile acids on *C. difficile* dates back to 1982 when Wilson *et al.* demonstrated that bile acids TCA, desoxycholate or deoxycholate (DCA), and CA stimulated germination of *C. difficile* spores *in vitro*[56]. It is well accepted that the primary bile acid TCA triggers *C. difficile* spore germination and permits outgrowth of vegetative cells, which can culminate in toxin production at high cell densities[53] (Figure 1B). Variations in the efficiency of primary bile acids to stimulate spore germination *in vitro* are also documented in clinical isolates of *C. difficile*[57–59].

In Vitro Studies

Despite the ability of CA derived primary bile acids to initiate spore germination, many other bacterial derived secondary bile acids are able to inhibit spore germination (ωMCA, LCA, UDCA) and growth (ωMCA, HDCA, UDCA, LCA, and DCA) of *C. difficile in vitro* (Figure 1B and Table 1)[11, 12, 50, 53–55, 60–62]. In particular, the secondary bile acids ωMCA, LCA, and UDCA inhibit TCA-mediated spore germination and [50, 54, 55] ωMCA interferes with DCA-mediated spore germination [50]. Growth of *C. difficile* is altered by most secondary bile acids including ωMCA, HDCA, UDCA, LCA, and DCA [12, 22, 50, 53, 54, 63].

Bile acid concentrations in the murine gut determined by targeted bile acid liquid chromatography-mass spectrometry (LC-MS) revealed that at physiologic concentrations ωMCA and LCA inhibited TCA-mediated *C. difficile* spore germination, while HDCA, UDCA, LCA, and DCA decreased *C. difficile* growth in a dose dependent manner[50]. Additionally, bile acid concentrations in CDI patients' feces before and after fecal microbiota transplantation (FMT) were tested against ten clinical isolates of *C. difficile in vitro*[17, 63]. Primary bile acids TCA (0.55 +/− 0.25 mM), CA (1.45 +/−0.29 mM), and CDCA (0.37 +/− 0.09 mM) were detected in the feces prior to FMT[17, 63]. At physiological concentrations, TCA, CA, and CDCA induced germination of *C. difficile*

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spores from all clinical isolates *in vitro*[63]. Following FMT, only secondary bile acids DCA (1.24 +/− 0.24 mM) and LCA (0.95 +/−0.15 mM) were detected in feces[17, 63]. At physiological concentrations, DCA and LCA abated spore germination and growth of *C. difficile* in 9 out of 10 clinical isolates *in vitro*[63].

Collectively, these studies emphasize the major impact that secondary bile acids have on the life cycle of *C. difficile in vitro*. Direct comparison of these studies is challenging since different strains of *C. difficile* were used (Table 1). Evaluation of additional strains of *C. difficile* exposed to physiologically relevant concentrations of secondary bile acids is warranted.

Ex vivo **Studies**

In order to evaluate the impact of secondary bile acids on the life cycle of *C. difficile* in the presence of the gut microbiota outside of the host, *ex vivo* models are utilized. *Ex vivo* studies remove intestinal content from mice at necropsy for use in *C. difficile* spore germination and outgrowth assays *in vitro* (Table 2). Multiple studies have shown prior to antibiotic treatment murine ileal content supports *C. difficile* spore germination *ex vivo* whereas cecal content inhibits spore germination and outgrowth [11, 50, 64, 65]. After disruption of the gut microbiota with specific antibiotics, cecal content allows for spore germination and outgrowth of *C. difficile* [11, 50, 64, 65]. Since microbial derived secondary bile acids are predominantly produced in the large intestine, we will focus on this section of the GI tract.

Giel *et al.* determined that cecal content from clindamycin treated mice could stimulate some germination and outgrowth of *C. difficile* CD196 spores *ex vivo* [64]. Based on an enzymatic assay, they found that the cecal content was dominated by primary bile acids (100 μM)[64]. Cecal content from cefoperazone treated C57BL/6 mice also allowed for spore germination and growth of *C. difficile* VPI 10463 vegetative cells [11]. Targeted bile acid metabolomics revealed the cecal content had decreased secondary bile acid DCA and increased primary bile acids TCA and CA[11]. Koenigsknecht *et al.* examined cecal content of C57BL/6 mice using a targeted bile acid LC-MS assay with an extended bile acid library that included 30 unique bile acids[65]. Prior to antibiotic treatment the cecal content of mice was made up of many secondary bile acids including DCA, UDCA, LCA, and ωMCA[65]. Multiple studies suggest that cecal content from mice prior to antibiotic treatment does not support spore germination or outgrowth of *C. difficile* [11, 65].

Taking it a step further, Theriot *et al.* 2016 treated groups of mice with a variety of different antibiotics (cefoperazone plus 1–6 weeks recovery off of antibiotic, clindamycin, vancomycin, metronidazole, and kanamycin) to create distinct microbial and metabolic (bile acids) environments. Only specific antibiotic treatments (cefoperazone, clindamycin and vancomycin) allowed for spore germination and outgrowth of *C. difficile* VPI 10463 in mouse cecal content *ex vivo*. Cecal contents were associated with significantly more primary bile acid TCA and a loss of all secondary bile acids[50]. Cecal content that did not support *C. difficile* spore germination and outgrowth was associated with secondary bile acids, such

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as ωMCA (average concentration 0.004%), HDCA (0.002%), UDCA (0.004%), LCA (0.001%), and DCA (0.023%).

In summary, the *ex vivo* studies reveal that non-antibiotic treated cecal content with secondary bile acids, specifically ωMCA, HDCA, UDCA, LCA, and DCA conferred resistance to spore germination and outgrowth of *C. difficile*[11, 50, 64, 65]. Whereas, after specific antibiotic treatment cecal content with low secondary bile acids and high primary bile acids TCA and CA were able to support some stages of the *C. difficile* life cycle[11, 50]. The alteration of microbial derived secondary bile acids in the GI tract impacts *C. difficile* spore germination and outgrowth.

In vivo Studies

The impact of secondary bile acids on the life cycle of *C. difficile* is also evident *in vivo* (Table 2). Susceptibility of mice to *C. difficile* colonization after antibiotics is associated with alterations in gut bile acids, specifically a decrease in secondary bile acids and an increase in primary bile acids[11]. The same trend is being seen in patients with recurrent CDI, where high levels of primary bile acids and reduced secondary bile acids were observed in feces when compared to healthy individuals[17, 66]. After successful treatment of CDI with FMT, patients restored the level of fecal secondary bile acids, specifically, DCA and LCA [17]. Weingarden *et al.* 2014 suggested that FMT restores the gut microbiota, specifically bacteria that are important for conversion of primary bile acids into secondary bile acids[17].

More recently, comparison of the human and murine intestinal microbiota in CDI susceptible and resistant states revealed that the loss of several bacterial taxa was associated with infection[12]. Buffie *et al.* 2015 used mathematical modeling to demonstrate that *C. scindens*, a 7α-dehydroxylating gut microbe capable of transforming primary bile acids into secondary bile acids, was associated with resistance to CDI[12]. C57BL/6 female mice treated with an antibiotic cocktail were deemed susceptible to CDI (Table 2). Administration of *C. scindens* alone or with a consortium of three other bacteria (*Barnesiella intestihominis*, *Pseudoflavonifractor capillosus*, *Blautia hansenii*) in antibiotic treated mice resulted in partial protection against CDI[12]. The observed colonization resistance against *C. difficile* was associated with restoration of the relative abundance of secondary bile acids DCA and LCA in the cecum[12].

The current literature collectively suggests that bile acids play an important role in the *C. difficile* life cycle *in vitro*, *ex vivo*, and *in vivo*. Bile acids directly impact *C. difficile* physiology and thus the pathogenesis. Further studies exploring the dynamics between the gut microbiota and the bile acid metabolome are essential for identifying novel therapeutics against this enteric pathogen.

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Antibiotic Mediated Alterations in the Gut Microbiota Alters the Bile Acid Metabolome Contributing to a Loss of Colonization Resistance Against *C. difficile*

Antibiotics cause collateral damage to the indigenous gut microbiota and loss of colonization resistance against pathogens, such as *C. difficile*[8, 9, 12, 67]. Susceptibility to CDI after antibiotic treatment in mouse models is associated with a decrease in gut bacterial diversity, an increase in the relative abundance of members from the Proteobacteria phylum and a decrease in the Bacteroidetes phylum [11, 13, 14, 68]. However, it is important to acknowledge that no single gut microbial community permits susceptibility to CDI[67]. Based on the current literature, it is postulated that depletion of specific gut microbes responsible for converting primary bile acids into secondary bile acids reduces colonization resistance against *C. difficile*.

In 2010, Sorg and Sonenshein first demonstrated inhibition of *C. difficile* using secondary bile acid producing bacterium *C. scindens in vitro* [55]. As mentioned previously, Buffie *et al.* 2015 demonstrated that the presence of *C. scindens* significantly correlated with resistance to CDI *in vivo*[12]. *C. scindens* encodes the *bai* operon responsible for formation of microbial derived secondary bile acids[69]. Using metagenomic analysis in the antibiotic treated mouse gut, the abundance of the *bai* operon genes correlated strongly with resistance to CDI, however BSH encoding genes did not. Furthermore, using PCR for *baiCD*, the gene that specifically encodes the 7-α dehydroxylating enzyme, they established that mice with restored colonization resistance against *C. difficile* following antibiotic treatment were *baiCD+* compared to susceptible mice which lacked this gene[12].

More recently, C57BL/6 mice treated with various antibiotics (detailed in the *Ex vivo* section), resulted in distinct gut microbial compositions and thus impacted bile acid profiles[50]. Following antibiotic treatment, gut microbial composition analysis revealed that a significant loss of secondary bile acids correlated with a loss of members from the Lachnospiraceae and Ruminococcaceae families. Interestingly, several of these family members are known to be involved in the formation of secondary bile acids[34]. Overall, this study demonstrated that antibiotics induced changes in the gut microbial composition and subsequently modified bile acid profiles. Such alterations had a direct impact on the *C. difficile* life cycle *ex vivo* and are regionally specific within the mouse GI tract[50].

The current literature supports the hypothesis that following antibiotics, alterations in gut microbial composition and a subsequent alteration in the bile acid metabolome result in a loss of colonization resistance against *C. difficile*[11, 12, 50]. Restoration of gut microbes that possess the ability to modulate intestinal bile acid profiles, specifically via production of secondary bile acids, may prove beneficial in the treatment of CDI.

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Manipulation of Microbial Derived Secondary Bile Acids to Restore Colonization Resistance Against *C. difficile*

Studies showing the contribution of secondary bile acids to colonization resistance against *C. difficile* are increasing. However, evidence of administering bile acids or bile acid modifying bacteria to manipulate bile acid profiles against this enteric pathogen is limited[12, 22, 70]. In a single case report, daily UDCA administration successfully eliminated and prevented recurrence of *C. difficile* ileal pouchitis [22]. The *C. difficile* strain from the patient was isolated and UDCA was able to inhibit spore germination and vegetative growth *in vitro* (Table 1)[22]. Others have administered bacteria to restore colonization against *C. difficile* in humans and in antibiotic treated and germfree mice, however the impact of these microbes on bile acids was not investigated[71–76].

Conclusions

The necessity of novel therapeutics against *C. difficile* is evident. The dynamic and pivotal role bile acids play in the *C. difficile* life cycle creates a potential target for such therapeutics. Although the exact mechanisms of colonization resistance are unknown, current literature suggests that microbial derived secondary bile acids could play an important role. Studies evaluating the rational manipulation of bile acid pools by either administration of bile acids directly or by administering bile acid modifying bacteria are needed. Such orchestration of collaborative bile acid metabolism may provide an innovative therapeutic strategy against *C. difficile* infection. Additional studies investigating the interplay between *C. difficile*, bile acids, the gut microbiota, and the host are essential for understanding the complexity of colonization resistance. Such information may also prove beneficial in other disease processes displaying bile acid dysmetabolism such as metabolic disease, obesity, and inflammatory bowel disease[77].

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Abbreviations

GI	Gastrointestinal
CDI	*Clostridium difficile* infection
LC-MS	Liquid chromatography mass spectrometry
CA	cholate
CDCA	chenodeoxycholate
DCA	deoxycholate
HCA	hyocholate

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HDCA	hyodeoxycholate
LCA	lithocholate
MDCA	murideoxycholate
UDCA	ursodeoxycholate
αMCA	α-muricholate
βMCA	β-muricholate
ωMCA	ω-muricholate

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Highlights

- The chemical diversification of bile acids is a collaborative effort by the host (production of primary bile acids) and the gut microbiota (production of secondary bile acids).
- Bile acids play an important and dynamic role in the *C. difficile* life cycle and this can be seen with *in vitro*, *ex vivo*, and *in vivo* approaches.
- Alterations in the gut microbiota that result in a loss of secondary bile acids are associated with a loss of colonization resistance against *C. difficile*.

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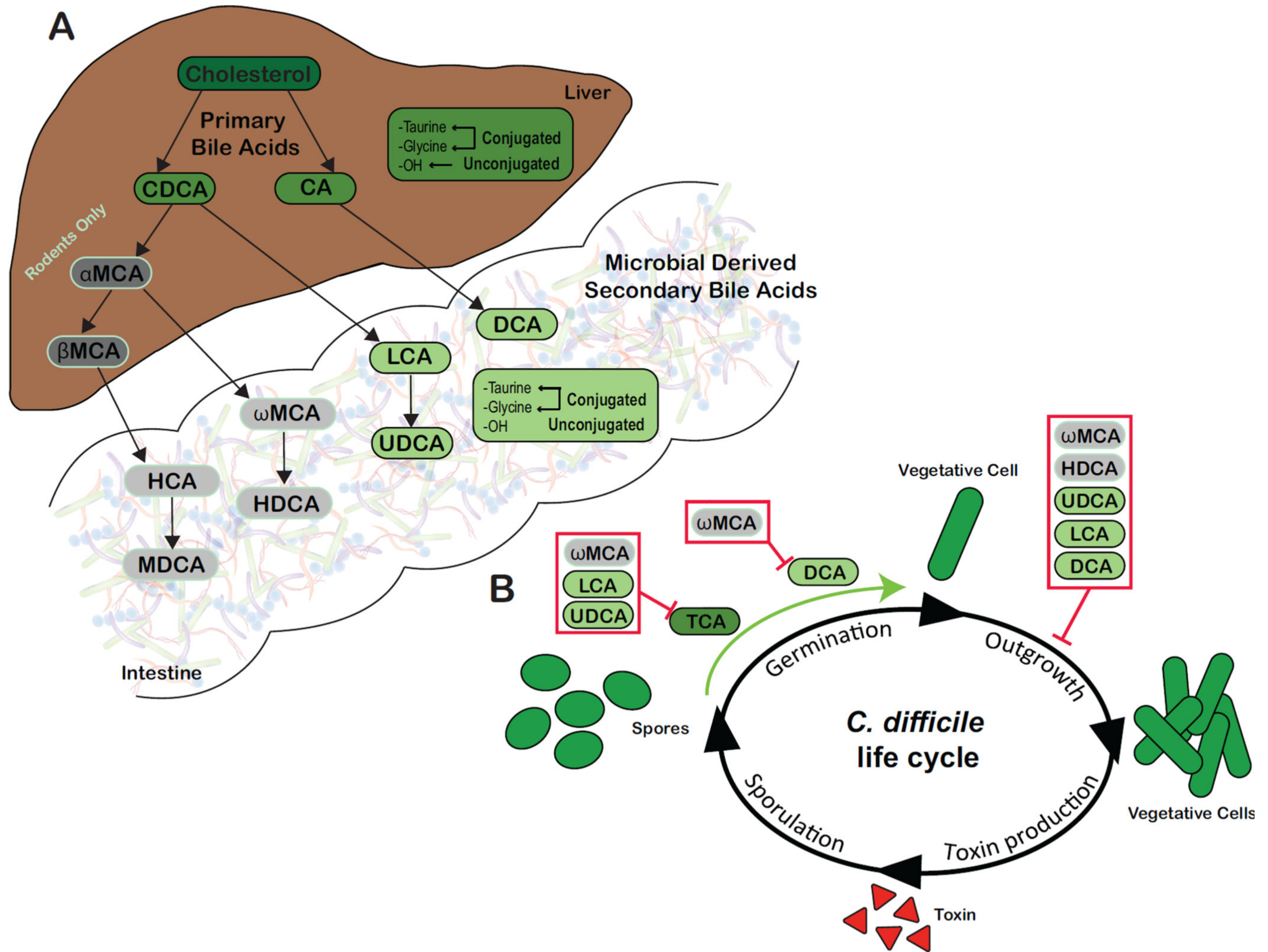

Fig 1.

A: Production of microbial derived secondary bile acids. Primary bile acids, chenodeoxycholate (CDCA) and cholate (CA) are synthesized from cholesterol by hepatocytes in humans and rodents. In rodents, a portion of CDCA is further converted into α-muricholate (αMCA) and β-muricholate (βMCA), which are not recognized in humans (represented in gray). Primary bile acids can be unconjugated or further modified via conjugation to taurine or glycine within the liver. Once synthesized, host derived primary bile acids (represented in darker shades) enter into bile. Bile is stored in the gallbladder until release in the duodenum following ingestion of a meal. Once within the GI tract, the gut microbiota can convert host derived primary bile acids into secondary bile acids (represented in lighter shades). Microbial derived secondary bile acids can also be unconjugated or conjugated to taurine or glycine. B: Effects of various secondary bile acids on the life cycle of *C. difficile in vitro*. TCA is essential for germination of *C. difficile* spores (green arrow). TCA-mediated spore germination can be blocked by specific secondary bile acids (red box). DCA can also stimulate germination of *C. difficile* spores, a process that is inhibited by ωMCA in mice. Outgrowth of *C. difficile* vegetative cells is inhibited by multiple secondary bile acids (red box).

Abbreviations: CA, cholate; CDCA, chenodeoxycholate; DCA, deoxycholate; HCA, hyocholate; HDCA, hyodeoxycholate; LCA, lithocholate; MDCA, murideoxycholate; UDCA, ursodeoxycholate; αMCA, α-muricholate; βMCA, β-muricholate; ωMCA, ω-muricholate

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Table 1

The impact of secondary bile acids on the life cycle of *C. difficile* using *in vitro* approaches.

Strain (*ribotype*)	Methods to measure spore germination and growth	Secondary bile acid concentrations	Main findings of the study	Ref.
CD196 UK14 *(027)*	- Relative spore germination determined by drop in OD_{600} assay in BHIS media - Growth was determined by OD_{600} in BHIS media for 7 h	0.1% DCA 1% DCA	- DCA induced colony formation by spores - DCA prevented growth	53
UK1 *(027)*	Relative spore germination determined by drop in OD_{600} assay in BHIS media	2 mM UDCA 0.2 mM LCA	UDCA and LCA can inhibit TCA-mediated spore germination	55
VPI 10463 *(003)* 630 *(012)*	Relative spore germination determined by drop in OD_{580} assay in BHIS media	6 mM 7-keto-LCA	7-keto-LCA did not induce nor inhibit spore germination	62
UK1*(027)* M68 *(017)*	- Relative spore germination determined by drop in OD_{600} assay in BHIS media - Growth was determined by OD_{600} in BHIS media for 24 h	0.29, 0.2 mM ωMCA 2 mM ωMCA	- ωMCA inhibited TCA-mediated spore germination of UK1 and M68 - ωMCA inhibited growth	54
VPI 10463 *(003)*	- Growth was determined by OD_{600} in BHIS media for 10 h	0.001, 0.01, 0.1% DCA 0.001, 0.01% LCA	DCA and LCA inhibited growth in a dose dependent manner	12
VPI 10463 *(003)*	- Percentage of spore germination was calculated by [(CFU on BHI)/(CFU on BHI + TCA)] × 100 after a 30 min incubation - Growth was determined by OD_{620} in BHI media for 24 h	*0.001, 0.004% ωMCA *0.001, 0.01% LCA *0.0001, 0.001% HDCA *0.001, 0.01% HDCA *0.01% UDCA *0.001% LCA *0.1% DCA	- Interference of TCA-mediated spore germination in concentration dependent manner seen with ωMCA and LCA - HDCA enhanced spore germination - Interference of DCA-mediated spore germination in concentration dependent manner seen with ωMCA - Secondary bile acids, HDCA, UDCA, LCA, and DCA resulted in decreased growth rates in a dose dependent manner	50
10 strains *(NAP1/027)*	- Relative spore germination determined by drop in OD_{600} assay in BHIS media	*0.5, 1, 2 mM DCA *0.5, 1, 2 mM LCA 0.5, 1, 2 mM UDCA	- Spores did not germinate in the presence of DCA or LCA - UDCA inhibited spore germination and vegetative growth	22, 63

*Concentrations based on *in vivo* targeted bile acid LC-MS assay (see Table 2)

Abbreviations: OD, Optical density; BHIS, Brain heart infusion-supplemented

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Table 2

The impact of secondary bile acids on the life cycle of *C. difficile* using *ex vivo* and *in vivo* approaches.

Host	Antibiotic treatment	Bile acid analysis	Main findings of the study	Strain (*ribotype*)	Ref.
***Ex vivo* studies**					
CD-1 female mice	Clindamycin	Measure NADH during oxidation of hydroxyl groups of bile salts by HSDHs	Able to stimulate a high level of colony formation from spores in antibiotic treated mouse cecal contents, made up of primary bile acids and a reduction in secondary bile acids.	CD196 *(027)*	64
5–14 wk C57BL/6 WT male and female mice (colony established from Jax)	Cefoperazone	Untargeted and targeted bile acid LC-MS assay (limited bile acids library)	Cecal content of mice after antibiotics had a decrease in secondary bile acid DCA and increased primary bile acids, TCA and CA, and allowed for spore germination and outgrowth and growth of vegetative cells. No spore germination and outgrowth was seen in mouse cecal content prior to antibiotics.	VPI 10463 *(003)* BI-9 *(027)*	11
See above	Cefoperazone	Targeted bile acid LC-MS assay (expanded bile acid library)	Cecal content of mice prior to antibiotic treatment contained higher concentrations of secondary bile acids, including DCA, UDCA, LCA and ωMCA. Inhibition of spore germination and outgrowth was seen in cecal content.	VPI 10463 *(003)*	65
See above	- Cefoperazone plus 1–6 wk period off - Clindamycin - Vancomycin - Metronidazole - Kanamycin	Targeted bile acid LC-MS assay (expanded bile acid library)	Cecal content that provided resistance against spore germination and outgrowth had an average concentration of secondary bile acids: ωMCA 0.004%, HDCA 0.002%, UDCA 0.004%, LCA 0.001%, and DCA 0.023%. Cecal content that allowed for susceptibility to spore germination and outgrowth showed a significant loss in the secondary bile acids listed above and increased TCA.	VPI 10463 *(003)*	50
***In vivo* studies**					
5–14 wk C57BL/6 WT male and female mice (colony established from Jax)	Cefoperazone	Targeted bile acid LC-MS assay (expanded bile acid library)	Susceptibility to *C. difficile* colonization in mice was associated with significant changes to the gut metabolome, specifically a decrease in secondary bile acid DCA and an increase in primary bile acid TCA.	VPI 10463 *(003)*	11
Fecal transplant patients and donors		LC-MS assay	Increased fecal DCA and LCA were associated with recovery from *C. difficile* infection in post-FMT patients.	NAP1 *(027)*	17
6–8 wk C57BL/6J female mice from Jax	Combination of kanamycin, gentamycin, colistin, metronidazole, vancomycin in followed by single dose of clindamycin	LC-MS assay	*C. scindens* alone and in concert with three other bacteria restored partial colonization resistance against *C. difficile* in mice. This was associated with restored relative abundance of secondary bile acids DCA and LCA in the cecum and no changes in primary bile acid relative abundance.	VPI 10463 *(003)*	12
CDI Relapse patient		LC-MS assay	Oral therapy of UDCA prevents relapse of *C. difficile* infection in a patient with ileal pouchitis (n=1).	NAP1 *(027)*	22

Abbreviations: HSDH: Hydroxysteroid dehydrogenases; LC-MS: Liquid chromatography–mass spectrometry