Review

Gut Microbiota Composition Associated with Clostridioides difficile Colonization and Infection

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Abstract: Clostridioides difficile is an anaerobic Gram-positive and spore-forming bacterium. The majority of C. difficile strains produce two toxins, A and B, associated with the development of acute diarrhea and/or colitis. In this review, two situations are distinguished: C. difficile infection (CDI) and asymptomatic colonization (AC). The main objective of this review is to explore the available data related to the link between the gut microbiota and the development of CDI. The secondary aim is to provide more information on why some people colonized with toxigenic C. difficile develop an infection while others show no signs of disease. Several factors, such as the use of antibiotics and proton pump inhibitors, hospitalization, and age, predispose individuals to C. difficile colonization and/or C. difficile infection. The gut microbiota of people with AC showed decreased abundances of Prevotella, Alistipes, Bacteroides, Bifidobacterium, Dorea, Coprococcus, and Roseburia. The gut microbiota of people suffering from CDI showed reductions in the abundances of Lachnospiraceae, Ruminococcaceae, Blautia spp., Prevotella spp., Dialister spp., Bifidobacterium spp., Roseburia spp., Anaerostipes spp., Faecalibacterium spp. and Coprococcus spp., in comparison with healthy people. Furthermore, increases in the abundances of Enterococcaceae and Enterococcus were associated with C. difficile infection.

Keywords: gut microbiota; Clostridioides difficile; Clostridioides difficile infection; asymptomatic colonization; human

1. Introduction

Clostridioides difficile is an anaerobic, Gram-positive, and spore-forming bacterium recognized as the leading cause of health care-associated diarrhea. In the United States of America (USA), in 2017, the Centers for Disease Control and Prevention (CDC) considered C. difficile infection (CDI) to be a major health threat (with 223,900 national cases) among hospitalized patients, eventually leading to 12,800 deaths [1]. In France, in 2016, CDI incidence in acute care was estimated to be 3.6 cases per 10,000 patient days [2]. In 2016, a total of 7711 CDI cases were reported to the ECDC (European Centre for Disease Prevention and Control) in Europe (20 EU countries), of which 74.7% were associated with health care settings [3].

In this review, two situations are distinguished in order to understand the trigger symptoms: CDI and AC of C. difficile. CDI is defined by the presence of diarrhea and at least one other following criterion: the carriage of a toxigenic strain of C. difficile, the presence of toxins in the stool and/or a colonoscopy result showing pseudomembranous colitis. AC by C. difficile can be defined as the absence of diarrhea with at least one other criterion: carrying a strain of C. difficile and/or the presence of toxins in the stool [4]. Crobach et al., defined AC as the presence of C. difficile but without symptoms of CDI.

The main risks factors are PPI use, antibiotics use, corticoid use, hospital stay and age.
PPI use is a risk factor for rCDI (recurrent CDI) [5]. Their use increased stomach pH from 1.2 to 5, raising the possibility of developing CDI or even carrying the bacterium asymptomatically compared with subjects without this treatment [6]. The increase in stomach pH to a value of 5 during digestion does not influence the resistance of the spores of C. difficile, as they are able to survive at a normal stomach pH [6–8]. The vegetative cells of C. difficile can survive in the gastric content only if the pH is equal to or greater than 5 [7].

The use of antibiotics is a well-known risk factor for C. difficile asymptomatic colonization or infection due to modification of the gut microbiota. Most of these cases are associated with the use of four antibiotics: clindamycin, cephalosporins, carbapenem and fluoroquinolone [9–11]. Other antibiotics, including macrolides, sulfonamide, trimethoprim and penicillin, are less associated with CDI [9–11]. Antibiotic exposure increases the possibility of C. difficile colonization by 3.7-fold [12] and of developing CDI by 3.55-fold [9]. A previous study showed that among antibiotic-associated diarrhea (AAD) cases, the incidence of CDI was 1.14–1.89% [13], and the frequency of toxigenic C. difficile carriage was 18.1–19.0% [13].

The use of corticosteroids increases the risk of C. difficile colonization in adults admitted to the hospital [14] and immunosuppressive therapy is a risk factor for complicated CDI [15]. Immunocompromised patients have a higher risk of developing rCDI during hospitalization [16].

A recent hospitalization or a recent intensive care unit stay increases the risk of developing CDI by 2.2 and 6.5, respectively [11]. Hospitalization in the previous 6 months increases the risk of colonization by 2.18 [14]. Previous studies showed that in an ambulatory group (n = 43), in patients with short hospital stays (n = 48) and in patients with long hospital stays (n = 102), the percentages of C. difficile carriage were 9.5%, 8% and 13%, respectively [17]. CDC hospitalization rates are significantly higher among those 65 years of age and older (by 4-fold) and those over 85 years of age (by 10-fold) compared to those under 65 years [18,19].

The percentage of AC evolves as a function of age. It is high in the first months of life and decreases until adult age, and then it increases with advancing age. The percentage of individuals with AC over time is shown in Figure 1 [4,20–27]. Patients aged >65 years old have a 10-fold higher risk of developing CDI than patients in the other age groups [28,29].

Figure 1. Box plot illustrating the mean relative proportion of C. difficile asymptomatic carriers in function of category of age. These data were found in articles that studied the prevalence of AC [4,20–27].

There are three lines of defense against pathogens: the epithelial barrier, innate immunity, and adaptive immunity. The first step is intestinal colonization by C. difficile. Once the bacterium can produce toxins, these toxins transgress the epithelial barrier through the activation of Rho glycosylation, which causes disruption of tight junctions. Secondly,
pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs). This interaction induces a rapid innate immunity response. Finally, adaptive immunity provides a highly specific immune response against *C. difficile* [30].

The intestinal mucus is composed of two types of mucins: MUC1 (cell-surface) and MUC2 (secreted forms). Secreted MUC2 is found mostly in the feces of healthy people, while people who suffer from CDI have an imbalanced mucus composition; their stool mucus is composed mainly of MUC1, with significantly decreased MUC2 levels [31]. They also present an increase in terminal galactose residues (a known receptor for *C. difficile* toxin A in mice, hamster, rabbits and pigs) and N-acetyl glucosamine (GlcNAc) [31] and a decrease in N-acetyl galactosamine (GalNAc).

Essential to *C. difficile* spore germination is the presence of primary bile acids (PBAs). PBAs is produced by the liver, is discharged into the small intestine and helps with fatty digestion [20]. PBAs stimulates germination of *C. difficile* spores [32], and secondary bile acids (SBAs) inhibit germination [32].

Several studies have tried to understand the *C. difficile* pathogenesis in order of reduce the risks of development of the disease and find new therapeutic strategy. The animal experimentations using hamster have allowed to test the transplantation fecal efficiency, the use of non-toxigenic strain of *C. difficile* and use of monoclonal antibodies against toxin A and toxin B [33–35]. The piglet model of CDI is representative of the key characteristics of human CDI and helped to understand the virulence and new treatment [36]. Then, *C. difficile* studies use different methods in vitro in order to limit using animal experimentation: feces cultures [37], the continuous culture model [38,39], the triple-stage chemostat human gut model [40,41], and the Tim-2 model [42].

The main objective of this review is to explore the available data about the link between the gut microbiota and the development of CDI. The secondary aim is to provide more information on why some people colonized with toxigenic *C. difficile* develop CDI and others show no signs of disease.

2. Microbiota Associated with Asymptomatic Colonization and CDI

2.1. Composition of the Normal Human Gut Microbiota

The composition of the gut microbiota is influenced by diet, age, the use of antibiotics, etc. [43]. In a normal gut, the dominant phyla are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Firmicutes and Bacteroidetes represent 90% of the gut microbiota [44,45]. Gut Bacteroidetes is mainly composed of two genera, *Bacteroides* and *Prevotella* [44,45]. The main genera in the Firmicutes phylum are *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, *Faecalibacterium*, *Roseburia*, and *Ruminococcus*. The Actinobacteria phylum is represented mainly by the *Bifidobacterium* genus [44,45]. The Metahit consortium classified human fecal metagenomic samples from three continents into three groups called “enterotypes”: *Bacteroides* (enterotype I), *Prevotella* (enterotype II) and *Ruminococcus* (enterotype III) [45,46]. The enterotype concept is very controversial in the scientific community [46]. Gorvitovskaia et al., chose to validate the first two enterotypes (*Prevotella* and *Bacteroides*) [47], while Cheng et al., have shown that enterotypes are not constant over time and are influenced by age and diet [43].

The gut microbiota of infants is rich in *Bifidobacterium* spp. and the gut microbiota of elderly individuals has decreased proportions of *Ruminococcaceae, Bifidobacterium, Lactobacillus* and *Faecalibacterium* and increased proportions of Proteobacteria, Bacteroidetes and *Clostridium* spp. [44,48,49].

2.2. Factors Influencing the Healthy Gut Microbiota

The use of proton pump inhibitors (PPIs) influences the pH of the stomach and therefore the gut microbiota. The use of PPIs decreased gut microbial diversity and the abundances of *Clostridiales* and *Ruminococcaceae* [50,51] and increased the abundances of the *Enterococcaceae* and *Staphylococcaceae* families [50,52] and *Veillonella parvula* and *Streptococcus*
mutans [53]. These modifications of the gut microbiota are strongly correlated with CDI development.

In human feces, clindamycin, cephalosporins and fluoroquinolone treatments impact the microbiota, resulting in an increase in Enterococcus abundance [54]. These three categories of antibiotics induce a reduction in the abundances of Streptococcus spp., Anaerococcus spp., Peptoniphilus spp., Porphyromonas spp. and Prevotella spp., and increase the abundance of Sphingomonas spp. [54]. Antibiotics also seem to decrease the proportions of Ruminococcaceae, Lachnospiraceae and Bifidobacterium and to increase the proportions of Lactobacillaceae and Streptococcaceae [49]. Werkhoven and collaborators (2021) showed that the carriage of toxigenic C. difficile increased the incidence of developing CDI 10-fold after antibiotic treatment. Specifically, the use of carbapenem increased the incidence of CDI 5-fold and increased the abundance of Enterococcus 5-fold [13].

The age-modified gut microbiota. The gut microbiota of elderly individuals has decreased proportions of Ruminococcaceae, Bifidobacterium, Lactobacillus and Faecalibacterium and increased proportions of Proteobacteria, Bacteroidetes and Clostridium spp. [44,48,49].

Two interesting recent studies have addressed the gut microbiota composition in hospitalized elderly patients with CDI, showing lower microbial diversity, lower proportions of gut commensals with putative functions and a reduction in butyrate-producing bacteria in CDI samples [55,56].

The state of dysbiosis can be defined as a decrease in the obligate anaerobic bacteria and an increase in the relative abundance of facultative anaerobic bacteria, such as Enterobacteriaceae [57,58], a decrease in microbial diversity and a decrease in anti-inflammatory species such Faecalibacterium prausnitzii [59]. In Inflammatory Bowel Disease (IBD), a decrease in microbial diversity, a decrease in F. prausnitzii and an increase in Streptococcus and Escherichia/Shigella are observed [59,60].

2.3. Composition of Microbiota among Patients with AC

As previously described, AC by C. difficile can be defined as the absence of diarrhea with at least one other criterion: carrying a strain of C. difficile and/or the presence of toxins in the stool [4]. In the literature, few studies differentiate between AC by C. difficile and CDI. The composition of the microbiota of patients with AC was similar to that of the control group and included the phyla Bacteroidetes (40.95%), Firmicutes (36.23%), and Proteobacteria (15.73%) [61].

Within the phylum Bacteroidetes, decreases in two families (Bacteroidaceae and Prevotellaceae) were observed [17]. Zhang et al., showed decreases in the AC of Prevotella spp., Alistipes spp., Bacteroides spp. and an increase in the AC of Parabacteroides spp. [61].

In the phylum Firmicutes, increases in the abundances of Ruminococcaceae, Erysipelotrichaceae and Clostridiae and decreases in the abundances of Leuconostocaceae and Erysipelotrichaceae were observed [17]. Zhang et al., showed decreases in AC by Dorea spp., Coprococcus spp., and Roseburia spp. [61] and increases in AC by Lactobacillus spp. [61], Enterococcus spp. [51] and Oscillospira spp. [62]. Another study showed increases in AC by Blautia spp., Flavonifractor spp., and Lachnospiraceae_unclassified [63].

Within the phylum Proteobacteria, several studies showed an increase in AC by Enterococcus spp. and Klebsiella spp. [61]. In the phylum Actinobacteria, Zhang et al., showed a decrease in AC by Bifidobacterium spp. [61]. Within the phylum Verrucomicrobia, a decrease in AC by Akkermansia spp. was observed [63].

Some studies showed no differences in microbial diversity between an AC group and a healthy group (HG) who presented a negative stool test for C. difficile [63]; however, some studies showed that the microbial richness (Chao index) decreased in patients with AC compared with patients in the HG [61].

2.4. Microbiota Composition of Adults Suffering from CDI

Many studies have described the gut microbiota composition of patients with CDI. In Table 1, the modifications of gut microbiota with increases (red) and decreases (green)
in the abundances of various phyla and genera when a patient is suffering from CDI are represented. These data are from the original research publications.

The prevalence of the phylum Proteobacteria is increased in adults with CDI [61,62,64–66]. The main family responsible for this increase is Enterobacteriaceae [62,66–69], and the main genera exhibiting increases are Klebsiella spp. [56,61,62,66,70], Escherichia/Shigella [56,61,64,66,69–71], Proteus spp. [56,66] and Providencia spp. [66].

The prevalence of the phylum Firmicutes is decreased in adults with CDI [61,62,65]. The main families responsible for this decrease are Lachnospiraceae and Ruminococcaceae [54,56,62,66,67,72], and the main genera are Blautia spp. [54,61,64,66,70,74], Roseburia spp. [54,61,64,66,74], Anaerostipes spp. [62,64,74], Faecalibacterium spp. [54,56,61,62,64,66,69,70,74] Collinsella spp. [56,64,66,70,74] and Coprococcus spp. [61,62,69,70,74]. Some of the genera that have been shown to exhibit increases are Enterococcus spp. [54,56,61,62,69–71], Veillonella spp. [56,61,62,74] and Lactobacillus spp. [61,70,71,74]. Metabolization of PBAs to SBAs is provided by populations from the Firmicutes phylum: Ruminococcaceae, Blautia and Lachnospiraceae [20,75]. The decreases in Lachnospiraceae and Ruminococcaceae also lead to decreases in the concentration of SCFA and the transformation of SBAs from PBAs, providing advantages to these bacteria [20,56,62,64,67]. If the abundances of these bacterial groups decrease, there is a decrease in SBAs and therefore a decrease in the inhibition of germination in the ileum. This decrease in SBAs facilitates the development of CDI. Clostridium scindens is able to restore SBAs metabolism and inhibit the germination of C. difficile [20,32,76].

Regarding the phylum Bacteroidetes, some studies showed a general decrease in abundance [61,62,67] while others showed an increase [64,68]. Depending on the studies, the abundance of the genus Bacteroides spp. has been shown to decrease [54,56,61,71] or increase [62,64,74]. Only one study showed that the abundance of Parabacteroides spp. increased [61]. Some genera, such as Prevotella spp. [54,61,62,64,70,73], Paraprevotella spp. [66], Alistipes spp. [54,56,61,69,70], and Porphyromonas spp., showed decreased abundances [54].

The abundance of the phylum Actinobacteria seems to decrease with adult CDI [61,62,64]. The main genus responsible for the decrease is Bifidobacterium spp.

Within the phylum Verrucomicrobia, some studies showed that an increase in the abundance of A. muciniphila is associated with CDI [62,68,71,77], while others reported that an increase in the abundance of Akkermansia protected against CDI [56,63,69].

Hernandez et al., (2019) classified CDI into two groups according to prognosis. Cluster A showed high abundances of Enterococccae and Enterococcus and decreases in the abundances of Bacteroidaceae and Lachnospiraceae. This cluster was associated with more severe diarrhea, more aggressive therapy and a poor prognosis. Cluster B had high abundances of Bacteroidaceae and Lachnospiraceae. This cluster was associated with less severe diarrhea, less aggressive therapies and a good prognosis [67].

Several recent studies have focused on comparing the gut microbiota of people with CDI with people with negative C. difficile detection and symptomatology. A significantly decrease in the bacterial population diversity (Shannon index) [54,64–66,70,74] and a significantly lower richness (Chao1 index) [64,66] has often been observed in patients with CDI.

People who developed a single CDI had higher levels of IgM anti-toxin A, toxin B and non-toxigenic antigens on Day 3 and significantly higher IgG anti-toxin A on Day 12 than people who developed recurrent CDI forms [30].

In Table 2, more details about these studies are provided.
Table 1. Variation in the abundance of the main phyla and genera of the gut microbiota of CDI people versus healthy people.

| Study       | Genus                                      |
|-------------|--------------------------------------------|
| **Phylum**  |                                            |
| **Proteobacteria** | **Escherichia coli/\text{Shigella}** | **Proteobacteria** | **Klebsiella spp.** | **Proteus spp.** | **Providencia spp.** |
| **Proteobacteria** | **Enterococcus spp.** | **Finegoldia spp.** | **Lactobacillus spp.** | **Faecalibacterium spp.** | **F. prausnitzii** |
| **Proteobacteria** | **Ruminococcaceae** | **Ruminococcus spp.** | **Lachnospiraceae** | **Roseburia spp.** | **Blautia spp.** |
| **Proteobacteria** | **Dorea spp.** | **Coprococcus spp.** | **Anaerostipes spp.** | **Eubacterium spp.** | **Collinsella spp.** |
| **Proteobacteria** | **Streptococcus spp.** | **Fusicatenibacter spp.** | **Subdoligranulum spp.** | | |
| **Firmicutes** | **Enterococcus spp.** | **Finegoldia spp.** | **Lactobacillus spp.** | **Faecalibacterium spp.** | **F. prausnitzii** |
| **Firmicutes** | **Ruminococcaceae** | **Ruminococcus spp.** | **Lachnospiraceae** | **Roseburia spp.** | **Blautia spp.** |
| **Firmicutes** | **Dorea spp.** | **Coprococcus spp.** | **Anaerostipes spp.** | **Eubacterium spp.** | **Collinsella spp.** |
| **Firmicutes** | **Streptococcus spp.** | | | | |
### Table 1. Cont.

| Study | [65] | [64] | [62] | [66] | [61] | Study | [54] | [71] | [70] | [64] | [62] | [69] | [68] | [66] | [56] | [61] | [74] |
|-------|------|------|------|------|------|-------|------|------|------|------|------|------|------|------|------|------|------|------|
| **Phylum** |     |      |      |      |      | Genus |      |      |      |      |      |      |      |      |      |      |      |      |
| **Firmicutes** |     |      |      |      |      | Veillonella spp. | * |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | Oscillospira spp. |  | * |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | Lactobacillus spp. |  |  | * |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | Clostridium spp. |  |  |  | * |  |  |  |  |  |  |  |
| **Bacteroidetes** | **+** |  |  |  |  | Bacteroides spp. |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | Prevotella spp. |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | Paraprevotella spp. |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | Alistipes spp. |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | Porphyromonas spp. |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | Parabacteroides spp. |  |  |  |  |  |  |  |  |  |  |  |
| **Actinobacteria** | **+** |  |  |  |  | Bifidobacterium spp. |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  | A.muciniphila |  |  |  |  |  |  |  |  |  |  |  |
| **Fusobacteria** |     |     |     |     |     | Fusobacterium spp. |  |  |  |  |  |  |  |  |  |  |  |

*p*-values are the original value from research article, where * * * * * represents an increase of +10 to 33%, red ( ) an increase of +34 to 66%, dark red ( ) an increase of +67 to 100%. Bright green ( ) represents a decrease of −10 to −33%, green ( ) a decrease of −34 to −66%, dark green ( ) a decrease of −67 to −100%. Striped red ( ) represents an increase by unspecified value and striped green ( ) a decrease by unspecified value.
Table 2. Analysis of the most important taxa exhibiting increased and decreased abundances in the CDI group compared with the control group.

| Analysis Method | Study | Study Group | Diversity | Increase in CDI Group Compared with Control Group | Decrease in CDI Group Compared with Control Group |
|-----------------|-------|-------------|-----------|-----------------------------------------------|-----------------------------------------------|
| 16S rRNA gene amplicon analysis a Illumina | [54] | CDI group (n = 15) Control group (n = 669) | The alpha diversity (Chao1 and Shannon index) was lower in the CDI group. * | Enterococcus spp. * | Bifidobacterium spp., Blautia spp., Faecalibacterium spp., Bacteroides spp. and Prevotella spp. |
| qPCR | [71] | CDI group (n = 28; 79 y) Control group (n = 56; 75 y) | NR | A. muciniphila *, Lactobacillus spp. *, and Escherichia coli * | Bacteroides spp. *, Bifidobacterium spp.* and F. prausnitzii * |
| 16S rRNA gene amplicon analysis a Illumina HiSeq | [70] | CDI group (n = 26; 66.5 y) Control group (n = 61) | The alpha diversity (Shannon index) was lower in the CDI group. * | Enterococcus *, Lactobacillus *, Escherichia * and Klebsiella *. | Bifidobacterium *, Ruminococcus *, Eubacterium *, Faecalibacterium spp.*, Prevotella *, Blautia *, Collinsella *, Dorea *, Alistipes *, Lachnospiraceae * and Coprococcus * (p < 0.05) |
| 16S rRNA gene amplicon analysis a MiSeq Illumina | [65] | CDI group (n = 11; 70.81 ± 20.1 y) Control group (n = 8; 18 to 45 y) Country: France | The alpha diversity (Shannon index) was lower in the CDI group. * | Proteobacteria **ns Actinobacteria **ns Bacteroidetes * | |
| 454 pyrosequencing analysis of bacteria e 454 GS FLX Titanium Sequencing System (Roche) | [64] | CDI group (n = 24, 64.8 ± 15.7 y) Control group (n = 13, 49.2 ± 11.5 y) Country: Korea | The richness (Chao1 index; 283.3 vs. 642.9) and the alpha diversity (Shannon index; 3.6 vs. 4.5) were lower in the CDI group. * | Proteobacteria **ns Bacteroidetes **ns Verrucomicrobia Bacteroides, Streptococcus spp., and Escherichia | Actinobacteria * Bifidobacterium, Faecalibacterium, Collinsella, Dorea, Eubacterium, Fusitetenbacter, Prevotella, Roseburia, Subdoligranum, Ruminococcus, Clostridium, Catenibacterium, Dialister and Anaerostipes |
| 16S rRNA gene amplicon analysis Metatranscriptomic Illumina Hiseq4000 platform | [69] | CDI group (n = 18, 65.3 ± 17 y) Control group (n = 31, 60 ± 18 y) Country: United States | A lower average species evenness was observed in the CDI group with Heip’s evenness (93.4 ± 23.1 in the CDI group vs. 121.8 ± 58.2 in the control group). **ns | Metagenetic Increases in the proportions of Clostridiaceae, Peptostreptococcaceae, and Enterococcus | Metagenetic Increases in the proportions of Faecalibacterium and Collinsella |
| | | | | Metatranscriptomic Increases in the proportions of Clostridiales difficile, E. coli, unclassified Peptostreptococcaceae, and Enterobacteriaceae | Metatranscriptomic Decreases in the proportions of A. muciniphila, Faecalibacterium prausnitzii, Coprococcus, Alistipes shahii, Collinsella and Verrucomicrobiaceae * |
Table 2. Cont.

| Analysis Method                        | Study                                                                 | Study Group                          | Diversity                                                                 | Increase in CDI Group Compared with Control Group                                                                 | Decrease in CDI Group Compared with Control Group |
|----------------------------------------|----------------------------------------------------------------------|---------------------------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|
| 16S rRNA gene amplicon analysis b      | CDI group ($n = 13; 55.5 \pm 20.5$ y)                                | No difference was observed in richness or evenness between groups. *ns | Enterobacteriaceae *, Peptostreptococcaceae *, A. muciniphila *           | Bacteroidales, Clostridales                                                                      |
| MiSeq technology Illumina              | Control group ($n = 13; 51.2 \pm 16.6$ y)                            |                                       |                                                                          |                                                                                                                      |
|                                        | tcdB-positive group ($n = 79; 62.5 \pm 19.9$ y) divided into two groups: CDI group ($n = 58$) and colonized group ($n = 21$). Control group ($n = 20; 62.2 \pm 14.4$ y). Country: Korea | The richness (Chao1 index; 60 vs. 95) was lower in the tcdB group. * | Proteobacteria *, Enterobacteriaceae *, Porphyromonadaceae *, Enterococcaceae * Parabacteroides *, Enterococcus *, Veillonella *, Klebsiella * and Akkermansia * | Lachnospiraceae *, Ruminococcaceae *, Prevotellaceae *, Prevotella, Phascolarctobacterium, Haemophilus, Lachnospira, Coprococcus, Dialister, Butyricimonas, Catenibacterium, Faecalibacterium, Paraprevotella, Odoribacter and Anaerostipes |
| 16S rRNA gene amplicon analysis c      | CA group ($n = 94$)                                                   | No difference in diversity between the groups. | Blautia Flavonifractor Lachnospiraceae unclassified | Akkermansia                                                                                                           |
| Ion Chez System and Ion S5 L system    | Control group ($n = 24$) Mean age: 78.66 y                           |                                       |                                                                          |                                                                                                                      |
|                                        | CA group ($n = 7$)                                                   | No difference in the alpha diversity or beta diversity (Shannon index: 3.47 in the CA group vs. 3.12 in the control group). | Firmicutes, Actinobacteria Akkermansia spp., Dermabacter spp., Romboutsia spp., Meiothermus spp., Peptoclostridium spp., Ruminococcaceae UGC 009 * | Bacteroidetes, Proteobacteria                                                                                      |
|                                        | Control group ($n = 25$) Mean age: 89.3 y Country: United States.    |                                       |                                                                          |                                                                                                                      |
| Pyrosequencing e Roche GS Junior       | CA group ($n = 8, 58.9 \pm 22.2$ y)                                  | Reductions in richness (Chao) *ns and diversity (Simpson and Shannon indexes) * in the CDI and CD+ groups. | Proteobacteria Fusobacteria Clostridium cluster XI Parabacteroides Escherichia/Shigella Klebsiella Enterococcus Veillonella Lactobacillus | Bacteroidetes and Firmicutes. Prevotella, Bacteroides, Faecalibacterium, Coprococcus, Roseburia |
|                                        | CA group ($n = 8, 60.5 \pm 20.8$ y)                                  |                                       |                                                                          |                                                                                                                      |
|                                        | Control group ($n = 9, 60.8 \pm 16.02$ Country: China                |                                       |                                                                          |                                                                                                                      |
Table 2. Cont.

| Analysis Method                        | Study                                                                 | Study Group                                                                 | Diversity                                                                 | Increase in CDI Group Compared with Control Group | Decrease in CDI Group Compared with Control Group |
|----------------------------------------|-----------------------------------------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Pyrosequencing d                       | [17]                                                                  | Control group (n = 252)                                                      | NR                                                                       | Bacteroidaceae                                    | Enterococcaceae                                  |
|                                        |                                                                       | CA group (n = 22)                                                           |                                                                           | Ruminococcaceae                                  | Prevotellaceae                                    |
|                                        |                                                                       | Age: 65 y                                                                  |                                                                           | Clostridiaceae                                    | Leuconostoccaceae                                |
|                                        |                                                                       | Country: Ireland                                                           |                                                                           | Erysipelothrichaceae                              | Spirochetaceae                                   |
|                                        |                                                                       |                                                                               |                                                                           | Aerococcaceae (one patient)                       |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | Flavobacteriaceae (one patient)                   |                                                  |
| 16S rRNA gene amplicon analysis b      | [56]                                                                  | CDI group (n = 25, 82.9 ± 8.5)                                              | Decreased diversity index (Chao1 and Shannon index) in the CDI group      | Klebsiella *, Escherichia/shigella *,              | Faecalibacterium, Bifidobacterium,                |
| Illumina MiSeq sequencer               |                                                                       | AB group (n = 29, 84.2 ± 8.1)                                               | compared to the AB group.                                                | Sutterella, Enterococcus *, Citrobacter,          | Akkermansia, Bacteroides *, Lachnospira *,        |
|                                        |                                                                       | Control group (n = 30, 82.3 ± 6.8)                                         |                                                                           | Veillonella, Proteus, Morganella, Hafnia,          | Alstipes *                                       |
|                                        |                                                                       | Country: Italy                                                              |                                                                           | Corynebacterium, Staphylococcus                   |                                                  |
| 16S rRNA gene amplicon analysis b      | [66]                                                                  | CDI group (n = 15; 61 y)                                                    | Decreased alpha diversity (Shannon index) and richness (Chao1) in the    | Proteobacteria *, Enterococcaceae, Streptococcaceae| Firmicutes and Bacteroidetes *, Lachnospiraceae,  |
| QMiSeq Illumina                        |                                                                       | Diarrhea group (n = 18; 56.5 y).                                            | CDI group and diarrhea group. *                                          | Lactobacillaceae and Peptostreptococcaceae*       | Blautia–Lachnospiraceae_incertae_sedis, Roseburia |
|                                        |                                                                       | Control group (n = 25, 58 y)                                                |                                                                           | Clostridium spp *                                 | and Dorea                                        |
|                                        |                                                                       | Country: China                                                              |                                                                           | Actinomycetes and Rickettsia                       | Ruminococcaceae *                                 |
|                                        |                                                                       |                                                                               |                                                                           | Escherichia/Shigella, Klebsiella, Proteus and      |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | Providencia                                      |                                                  |
| 454-pyrosequencing f                   | [73]                                                                  | All samples with diarrhea                                                  | NR                                                                       | Bacteroides                                      | CDI group with and without toxins:               |
| FLX Titanium platform                  |                                                                       | CDI–toxins group (71 ± 15 y)                                                |                                                                           | Parabacteroides                                  | Blautia, Holdemania, Enterobacteriaceae, and      |
|                                        |                                                                       | CDI–without toxins group (66.3 ± 27 y)                                      |                                                                           | Faecalibacterium                                  | Lachnospiraceae                                  |
|                                        |                                                                       | Control group (52.0 ± 13 y)                                                 |                                                                           | Clostridium XIVa                                  |                                                  |
|                                        |                                                                       | Country: Spain                                                              |                                                                           | Clostridium cluster XI                           |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | CDI group with and without toxins:               |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | Phascolarctobacterium, Enterococcus,              |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | Clostridium XI cluster, flavonifractor, and       |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | Erysipelothrichaceae incertae_sedis *             |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | CDI group with toxins: Enterococcus, Clostridium  |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | XI cluster, and Erysipelothrichaceae incertae     |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | sedis                                            |                                                  |
|                                        |                                                                       |                                                                               |                                                                           | CDI group with toxins: Fusobacterium, Prevotella,|                                                  |
|                                        |                                                                       |                                                                               |                                                                           | Vovibrio, and Dialister                          |                                                  |
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### Table 2. Cont.

| Analysis Method | Study | Study Group | Diversity | Increase in CDI Group Compared with Control Group | Decrease in CDI Group Compared with Control Group |
|-----------------|-------|-------------|-----------|--------------------------------------------------|--------------------------------------------------|
| **454-Pyrosequencing**<sup>§</sup> | [78]  | CDI group (n = 94; 55.9 ± 18.3) CDN (=C. difficile-negative nosocomial diarrhea) group (n = 89; 58.7 ± 14.9) Control group (n = 155; 52.2 ± 21.5) | Reduction in the diversity (inverse Simpson index) * in the CDI and CDN groups compared with the control group. | Enterococcus * Lachnospiraceae * Erysipelotrichaceae * | Bacteroides species * |
| **Pyrosequencing**<sup>e</sup> | [74]  | CDI group (n = 39; 54.7 ± 20.1) CDN (=C. difficile-negative nosocomial diarrhea) group (n = 36; 54.6 ± 20.0) Control group (n = 40; 60.9 ± 9.1) | Reduction in the diversity (Shannon index) * in the CDI and CDN groups compared with the control group. | Bacteroides Veillonella Enterococcus Lactobacillus Fusobacterium | Bacteroides Lachnospiraceae and Ruminococcaceae Blautia * Pseudobutyrivibrio, Roseburia, Faecalibacterium, Anaerostipes, Subdoligranulum, Ruminococcus, Streptococcus, Dorea * and Coprococcus. |
| **16S rRNA gene amplicon analysis**<sup>a</sup> MiSeq technology Illumina | [79]  | CDI group (n = 57; 69.5 y): GDH+ and TcdB No control group Country: Spain | The richness (Chao1) was 134.32 and the alpha-diversity (Shannon index) was 4.01. | Bacteroides (46.51%), Firmicutes (34.70%), Proteobacteria (13.49%), Bacteroidaceae (31.01%), Enterobacteriaceae (9.82%), Lachnospiraceae (9.33%), Tannerllaceae (6.16%) and Ruminococcaceae (5.64%) | |
| **16S rRNA gene amplicon analysis**<sup>d</sup> MiSeq technology Illumina | [80]  | CDI group (n = 31; 64.0 y). Three periods: pretreatment ATB, two days after treatment, seven days after treatment or discharge. No control group | The alpha diversity (Shannon index) was lower with CDI pretreatment in the recurrent group. * | Veillonella dispar * predictor of recurrence. | / |
Table 2. Cont.

| Analysis Method                        | Study                          | Study Group                                      | Diversity                                                                 | Increase in CDI Group Compared with Control Group | Decrease in CDI Group Compared with Control Group |
|----------------------------------------|--------------------------------|--------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| 16S rRNA gene amplicon analysis \(^d\) | [81]                           | CDI group (n = 88; 52.7 y)                       | Decreased alpha diversity (Chao1 index) in the CDI group than in the control group. | G1: Ruminococcaceae; Rikenellaceae; Clostridiaceae; Bacteroides; Faecalibacterium; Rothia | /                                                |
|                                       |                                | G1 (ATB responder)                               |                                                                           | G2: Clostridiaceae, Lachnospiraceae, Blautia, Coprococcus, Streptococcus, Bifidobacterium, Ruminococcaceae and Actinomyces. |                                                   |
|                                       |                                | G2 (non-ATB responder)                           |                                                                           |                                                   |                                                   |
|                                       |                                | G3 (recurrent CDI)                               |                                                                           |                                                   |                                                   |
|                                       |                                | G4 (non-recurrent CDI)                           |                                                                           |                                                   |                                                   |
|                                       |                                | No control group (other studies)                 |                                                                           |                                                   |                                                   |

\(^a\) V3–V4 regions of the 16S rRNA gene. \(^b\) V3 region of the 16S rRNA gene. \(^c\) V2–V3 regions of the 16S rRNA gene. \(^d\) V4 regions of the 16S rRNA gene. \(^e\) V1–V3 regions of the 16S rRNA gene. \(^f\) V1–V2 regions of the 16S rRNA gene. \(^g\) V3–V5 regions of the 16S rRNA gene. * Significant. \(^{ns}\), non-significant.
3. Fecal Microbiota Transplantation (FMT)

FMT is the transfer of the fecal microbiota containing bacteria from a healthy volunteer into a diseased patient [82,83]. In 1958, Dr Ben Eiseman described the “fecal enema” as a treatment for pseudomembranous enterocolitis [83]. FMT is administered in several ways: with capsules for ingestion, with an endoscope and with a nasoenteric tube [84]. The criteria for selecting a healthy donor have been described in several papers [84,85]. Briefly, the blood and the stool must be tested to ensure that no infectious diseases or pathogenic bacteria are present, and a series of criteria are checked, including recent antibiotic use, history of diarrhea, and history of immune disorders [84,85].

In the case of primary CDI, this treatment could be used before using antibiotics or in addition of antibiotics to avoid rCDI [86]. In the case of rCDI, FMT is the second line of treatment. FMT has been reported to be successful in 80–92% of patients with rCDI [84,87–89] and with primary CDI [89]. This treatment is safe and effective in adults [87,88], in elderly [90–92] and in children [93].

Regarding the composition of the gut microbiota of patients with FMT, the alpha diversity (Shannon index) seems to be lowest pretreatment among patients with CDI, and the alpha diversity is restored after FMT [87,88,90,91]. The composition of the microbiota has an impact on the recurrence of CDI and the success of FMT. After FMT, the phylum Firmicutes increased significantly in rCDI (<65 y) and the phylum Proteobacteria decreased significantly in rCDI (>65 y) [90]. Lachnospiraceae, Ruminococcaceae and Bifidobacteriaceae increased significantly [90] and Enterobacteriaceae decreased significantly after FMT in rCDI [89,90]. Blautia, Ruminococcus, Coprococcus, Bifidobacterium [90] and Faecalibacterium [89] spp. increased significantly after FMT in rCDI [89,90]. These modifications of the gut microbiota after FMT strongly suggested that these bacterial populations are associated with healthy people (See Table 1) and will favor a good prognosis. Staley et al. (2018) showed that a follow-up analysis of 16S rDNA extracted from feces can be used to predict an eventual recurrence of CDI. After FMT, high proportions of Lactobacillales, Enterobacteriaceae, Enterococcus, Klebsiella, Streptococcus and Veillonella and reductions in Roseburia, Blautia, Lachnospiraceae, Ruminococcaceae, Anaerostipes, Coprococcus, Dorea and Clostidiales [88,91] will disadvantage the patient and promote rCDI. These bacterial population are associated with the gut microbiota of CDI cases (see in Table 1).

Before using the FMT, a preventive probiotic administration before the antibiotics use is effective [94]. The use of probiotics before and at the same time as antibiotics reduces the risk of CDI by >50% in hospitalized adults [94].

4. Conclusions

Many studies have characterized the gut microbiota composition of patients with CDI, but confusion is still present in the literature between CDI and AC. Few studies have differentiated AC by C. difficile and CDI. This review explores the available data related to the link between the gut microbiota and the development of C. difficile infection. The causes of the development of CDI are clearly multifactorial. An external cause (such as a medication) can disrupt the homoeostasis of the gut microbiota. PPI and antibiotic use decrease the richness of the gut microbiota [95]. This imbalance promotes the growth of some bacteria (for example, A. muciniphila), and these bacteria can degrade the mucus layer and allow the pathogenic bacteria and toxins access to the epithelium. Moreover, the abundances of some bacteria (Lachnospiraceae, Ruminococcaceae and Blautia) decrease, and these bacteria are involved in bile metabolism and can increase the primary bile acid concentration. Higher PBAs concentrations are favorable to C. difficile germination and multiplication. Some bacteria also have a positive correlation (Enterococcus, Enterobacteriaceae) or negative correlation (Blautia, Prevotella, Roseburia, Dorea, Collinsella, Coprococcus, Ruminococcus, Ruminococcaceae, Lachnospiraceae) with C. difficile colonization and/or CDI. The gut microbiota will promote the development of the CDI. Through all the studies, the CDI has a gut microbiota footprint with the decrease and the increase in some bacteria. In this review, a lot of bacteria are singled out for giving an advantage or a disadvantage when developing CDI. Some of
these bacteria have an impact on gut health. *Faecalibacterium prausnitzii* is considered as a species of the healthy gut microbiota. This bacterium is reduced in gut dysbiosis, in IBD, in obesity, in diabetes, etc. [96]. *Lachnospiraceae* is protective against CDI [20]. *C. scindens*, member of *Lachnospiraceae*, have a protective effect against CDI [20,32]. Amrane et al. (2018) showed that *C. scindens* is present in the feces when patient developing CDI [97]. *Alistipes* spp. indicated a controversial pathogenicity. On the one hand, the bacteria have protective effects against liver fibrosis, cancer immunotherapy and cardiovascular disease [98]. On the other hand, this genus is associated with colorectal cancer and mental disease [98].

In this review, CDI can be associated with an increase or a decrease in *A. municiphila* and AC is associated with a decrease *A. municiphila*. The presence of this bacteria is positive against obesity, diabetes, cardiometabolic disease and low-grade inflammation [99]. It is actually used to manage obesity [100]. In human intestinal organoids, *C. difficile* is capable of decreasing MUC2 production, but it is not responsible for altering host mucus oligosaccharide composition [31]. Furthermore, it has been reported that *C. difficile* is not capable of degrading mucin glycans, although coculture with mucin-degrading *Akknansia muciniphila*, *Bacteroides thetaiotaomicron* and *Ruminococcus torques* allowed the pathogen to grow in media that lacked glucose but contained purified MUC2 [101]. When mucus is degraded by bacteria, oligosaccharides (GlcNAc, GalNAc, galactose, mannose and fucose) are salted out [101], and *C. difficile* is capable of using these oligosaccharides [31].

The *Enterobacteriaceae* family is associated with the dysbiosis state [58,59]. *Enterococcus* spp. is a controversial bacterium [102]. It is a commensal bacterium of intestinal flora, vagina, and mouth microbiota [102]. *E. faecium* and *E. faecalis* are used as probiotics [102,103] and *Enterococcus* spp. is used in meat and cheese [102,104] fermentation. Recently, it was shown that *E. faecalis* and *E. faecium* are potentially pathogenic bacteria due to their ability to adapt in new environment [102,105]. Additionally, a resistance to vancomycin has emerged in this genus [102,105]. Romyasamit et al. (2020) exhibited that six *E. faecalis* strains have a probiotic effect and anti-*C. difficile* activity [106]. *Klebsiella pneumoniae* is present in the mucus layer with *C. difficile* [107].

The second objective of this review was to provide more information on why some people colonized with toxigenic *C. difficile* develop *C. difficile* infection and others show no signs of disease. The answer to this question is still unknown, but some facts will improve the understanding. The response of the adaptative immune system impacts the development of the disease. Patients exhibiting AC were shown to have higher antibody levels against *C. difficile* than people who developed CDI. It has been reported that sixty percent of the general population has had AC or has been infected with *C. difficile*, as determined by the observation of detectable seric IgG and IgA antibodies to toxins A and B [108]. IgG and IgA titers against toxins A and B are significantly higher in children positive for toxigenic strains than in people carrying non-toxigenic strains [109]. IgG antibody levels against toxin A are significantly higher within 3 days of colonization in AC patients than in those who develop CDI [30,108,110]. IgG levels against toxin B and non-toxin antigens seem to be higher among individuals who develop AC [30,108,110].

Some risks factors will predispose patients to developing CDI (antibiotics use, PPI use, age, etc.). The decrease in the diversity described in elderly gut microbiota [18], the decrease in some bacterial population (*Ruminococcaceae*, *Bifidobacterium*, *Faecalibacterium*) and the increase in some bacterial population (*Proteobacteria*, *Bacteroidetes* and *Clostridium* spp.) suggest why this population have a 10-fold higher risk of developing CDI.

Some treatments involving bacteria are commonly used and effective against CDI. FMT allows the recovery of patients with recurrent CDI with an increase in the abundances of some bacteria (*Blausia, Collinsella*, *Anaeroestipes*, *Coprococcus*, *Dorea* and *Roseburia*) and a decrease in the abundances of others (*Lactobacillales*, *Enterobacteriaceae*; *Enterococcus*, *Klebsiella*, *Streptococcus* and *Veillonella*). More research with strict inclusion criteria is needed to measure AC and CDI gut microbiota analysis. The purpose of this work was to study the impact of in vivo control measures for the gut microbiota to decrease colonization and CDI or to improve recovery.
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