Identification of Specific Oral and Gut Pathogens in Full Thickness Colon of Colitis Patients: Implications for Colon Motility

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Impaired colon motility is one of the leading problems associated with inflammatory bowel disease (IBD). An expanding body of evidence supports the role of microbiome in normal gut function and in progression of IBD. The objective of this work is to determine whether diseased full thickness colon specimens, including the neuromuscular region (critical for colon motility function), contain specific oral and gut pathogens. In addition, we compared the differences in colon microbiome between Caucasians (CA) and African Americans (AA). Thirty-nine human full thickness colon (diseased colon and adjacent healthy colon) specimens were collected from Crohn’s Colitis (CC) or Ulcerative Colitis (UC) patients while they underwent elective colon surgeries. We isolated and analyzed bacterial ribosomal RNA (rRNA) from colon specimens by amplicon sequencing of the 16S rRNA gene region. The microbiome proportions were quantified into Operational Taxonomic Units (OTUs) by analysis with Quantitative Insights Into Microbial ecology (QIIME) platform. Two hundred twenty-eight different bacterial species were identified by QIIME analysis. However, we could only decipher the species name of fifty-three bacteria. Our results show that proportion of non-detrimental bacteria in CC or UC colon samples were altered compared to adjacent healthy colon specimens. We further show, for the first time in full thickness colon specimens, that microbiome of CC and UC diseased specimens is dominated by putative oral pathogens belonging to the Phyla Firmicutes (Streptococcus, Staphylococcus, Peptostreptococcus), and Fusobacteria (Fusobacterium). In addition, we have identified patterns of differences in microbiome levels between CA and AA specimens with potential implications for health disparities research. Overall, our results suggest a significant association between oral and gut microbes in the modulation of colon motility in colitis patients.

Keywords: colitis, colon motility, nitric oxide (NO), antioxidants, oral microbiome, operational taxonomic units (OTUs), gut microbiome
INTRODUCTION

Inflammatory bowel disease (IBD) is comprised of Crohn’s disease / Crohn’s colitis (CC) and Ulcerative colitis (UC). The term Colitis, refers to general inflammation of the inner lining of the colon arising from numerous underlying causes including idiopathic infection, IBD (either CC or UC), ischemic colitis, allergic reactions, and/or microscopic colitis. Distally, gingivitis, and periodontal disease are chronic inflammatory gum diseases associated with orange, red, yellow, purple, and green complex bacterial infections in sub-gingival areas of oral cavity (Popova et al., 2013).

Previous studies have shown that periodontal disease (PD) is a significant risk factor and contributor to many systemic diseases, including IBD (Vavricka et al., 2013). Several factors including genetic, dietary, and environmental factors could influence the pathogenesis of microbiome (oral and gut) which in turn may increase the incidence of periodontitis and IBD (Lira-Junior and Figueredo, 2016; Agossa et al., 2017). In addition, Porphyromonas gingivalis known to cause PD altered the gut microbiota leading to increased gut epithelial permeability and endotoxaemia, which causes systemic inflammation (Hajishengallis, 2015). In addition, many earlier studies have shown intestinal colonization of oral bacteria in the pathogenesis of IBD (Strauss et al., 2011; Atarashi et al., 2017).

Innumerable number of studies have shown that the gut microbiome including Phyla Proteobacteria, Firmicutes, and Bacteroidetes contribute to normal gut function (Mariat et al., 2009; Koliada et al., 2017; Walker et al., 2018; Zhao et al., 2018). Colon motility is mainly regulated by neuromuscular portion of the colon and this was shown to be impaired in colitis patients; putatively due to a reduction in neuronal nitric oxide (NO) synthase (nNOS) protein expression and/or neuronal degeneration (Bassotti et al., 2014; Gangula et al., 2017). Previous studies have analyzed the microbiome in feces and/or colon mucosal biopsy specimens of colitis patients (Gibson et al., 1991; Bibiloni et al., 2006). However, the relationship/interaction between oral and gut bacteria in the development and/or exacerbation of inflammatory disease in the colon (containing neuromuscular tissue) was under studied. In addition, data is limited on how oral bacteria interact with and influence the large intestinal flora, thereby contributing to colitis. Since motility of the colon is impaired in colitis patients and neuromuscular tissue play a role in the motility function (Geboes and Collins, 1998; Poli et al., 2001), we hypothesize that the interaction between oral and gut microbiome may play a significant role in the inflammatory processes associated with the development and progression of colitis seen in certain patient populations. Furthermore, we hypothesize that difference in microbiome may exist between CA and AA colitis patients, potentially contributing to health disparities in IBD.

METHODS

Ethics Statement

The participants provided both written and verbal informed consent to Collaborative Human Tissue Networking (CHTN) Consortium to collect specimens while they underwent elective colon surgeries.

Collection of Specimens

Frozen full thickness colon specimens were obtained from Cooperative Human Tissue Networking (CHTN). Thirty-nine human full thickness colon (moderate to severe diseased colon and adjacent healthy colon) specimens were collected from CC and UC male and female patients (ages between 18 and 75 years old) while they underwent elective colon surgeries. The specimens include Ulcerative (n = 13), Crohn’s (n = 13) and adjacent healthy (n = 13) specimens. Characteristics of participants included Caucasians (CA) (n = 30) and African Americans (AA) (n = 9). CC male and female patients presented with symptoms like fever, fatigue, diarrhea, blood in stool, mouth sores, abdominal cramping, and pain around the anus, reduced appetite, and weight loss. While UC male and female patients presented with additional signs like rectal pain, rectal bleeding, and inability to defecate despite urgency.

Extraction of DNA, Amplification of 16S rRNA Gene and Amplicon Sequencing

DNA extraction and microbial analysis were performed in the University of North Carolina at Chapel Hill School of Medicine Microbiome Core Facility (UNC: MC). We identified a conserved region of the 16s rRNA gene of 550 bp to amplify. This encompassed variable regions V3–V4 from the colon genomic DNA using primers 16s rRNA-F 5′-AGAGTTTGATCCTGGCTCAG-3′ and 16s rRNA-R 5′-GCTG CCTCCCGT AGGAGT-3′ and overhang adapter sequences appended to the primer pair for compatibility with Illumina index and sequencing adapters. Briefly, each 16s rRNA amplicon was purified using AMPure XP reagent (Beckman Coulter, Indianapolis, IN, USA). Specifically, each sample was amplified using a limited cycle PCR program, adding Illumina sequencing adapters and optional dual-index barcodes [index 1(i7) and index 2(i5)] (Illumina, San Diego, CA, USA) to the amplicon target. The final libraries were purified using AMPure XP reagent, quantified and normalized prior to pooling. The DNA library pool was denatured with NaOH, diluted with hybridization buffer and heat denatured before loading on to the MiSeq reagent cartridge and to the MiSeq instrument (Illumina). The standard Illumina paired-end 250 base pair (PE250) protocol was used for sequencing the 16s rRNA amplicons (Illumina, CA, USA).

Processing of Sequence Reads

Data was analyzed and microbial proportions using Operational Taxonomic Units (OTUs) were determined using Quantitative Insights Into Microbial ecology (QIIME) pipeline (Caporaso et al., 2010a) in the Meharry Medical College Bioinformatics Core. Briefly, generated raw reads were preprocessed for adapter removal. Processed sequence reads were obtained as fastq files and were converted into fasta, quality and flow files using Mothur package (Schloss et al., 2009). The initial number of fasta sequences obtained were 31,09,793. First, the fasta files were cleaned by host reads of mapping to on 9 mm mouse
The primer sequences and barcode sequences were removed, demultiplexed and quality filtered. The number of high quality sequences remaining after quality filtering was 16,64,769. The OTUs were picked by de novo strategy. The high quality sequences were clustered at 97% identity using UCLUST inbuilt in QIIME pipeline to generate 3994 OTUs and taxonomy was assigned to OTU representative sequences using UCLUST (Edgar, 2010). The picked sequences were aligned using PyNAST aligner (Caporaso et al., 2010b). The chimeric sequences and singleton OTUs were removed using ChimeraSlayer (Haas et al., 2011). We constructed a phylogenetic tree for the sequences using FastTree version 2.1.3 (data not shown) (Price et al., 2010). Next, an OTU table was constructed and taxa were summarized using the 894 OTUs obtained from QIIME pipeline. α-diversity metrics was computed using Chao1 (abundance-based richness estimator) and Shannon analysis (diversity index) and Rarefaction plots were constructed (data not shown). β-diversity metrics was computed using weighted and unweighted Principal Coordinates Analysis (PCoA) (data not shown) (Gower, 2005). A Taxonomic Summary Bar plot showing OTUs assigned to Phyla-level taxonomy per sample was subsequently constructed (Figure 1). Bar Plots showing the relative abundance of bacteria at the Phyla-level between races, diseased tissue and healthy tissue groups is shown in Figure 3. Sample-specific sequences were deposited in the MGRAST database (accession number: b3b851ba2c6d676d343739393937332e33) and was assigned an MG-RAST project ID (mgs675214) (Keegan et al., 2016). In addition, sample-specific sequences were deposited in the NCBI (BioProject: PRJNA496071).

The pathogenic and beneficial oral and gut bacteria were identified using the NCBI Genome database (https://www.ncbi.nlm.nih.gov/genome/). This analysis was performed to assess the pathogenic and healthy bacterial proportions in human full thickness colon specimens (Tables 1–3).

**Statistical Analysis and Evaluation**

Statistical analysis was performed between the healthy and diseased groups and based on race classification ($n = 13$ CC, $n = 13$ UC, $n = 13$ non-disease healthy patients, $n = 30$ CA and $n = 9$ AA). A non-parametric Mann-Whitney $U$ Test $p$-value < 0.05 of bacterial 16S rRNA OTUs between the groups was considered statistically significant. IBM SPSS software package version 23 (IBM Analytics, USA) was used to conduct statistical analysis.

**RESULTS**

**Relative Abundance Analysis**

QIIME analysis showed about two hundred twenty-eight bacterial species in entire 39 specimens (Tables 1–5). However, non-ambiguous annotation at the species name resulted in fifty-three bacterial identifications. The dominant phyla across all samples (both diseased and healthy specimens) were Bacteroidetes (46.92%), followed by Firmicutes.
| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD                                                                 | NCBI genome database link |
|--------|----------------|-----------------|---------------|----------------|--------------------------------------------------------------------------------|----------------------------|
| **ADJACENT HEALTHY COLON** |                 |                 |               |                |                                                                                  |                            |
| 1      | Prevotella     | stercorea       | 2.3           | Bacteroidetes  | Alters mucosal microbiota in the colon of patients with IBD                      | H                          |
| 2      |Prevotella     | Other           | 0.3           | Bacteroidetes  | A microbial signature of Crohn's disease                                        | GS                         |
| 3      |Gemella s__    |                 | 0.1           | Firmicutes     | Microbiome in New-Onset Crohn's Disease                                          | CP                         |
| 4      |Staphylococcus | sciuri          | 0.1           | Firmicutes     | Develops intestinal inflammation in acute and chronic colitis                   | I                          |
| 5      |Staphylococcus | aureus          | 0.6           | Firmicutes     | Causes Crohn's disease                                                          | AU                         |
| 6      |Abiotrophia    | s__             | 0.1           | Firmicutes     | Causes fecal microbial dysbiosis in IBD                                          | CS                         |
| 7      |Lactobacillus  | zeae            | 1.9           | Firmicutes     | Maintains remission of ulcerative colitis                                       | A                          |
| 8      |Lactobacillus  | s__             | 0.4           | Firmicutes     | Maintains remission of ulcerative colitis                                       | CW                         |
| 9      |Lactococcus    | s__             | 0.7           | Firmicutes     | Used in the treatment of Crohn's disease                                        | CX                         |
| 10     |Peptostreptococcus | anaerobius   | 11.6          | Firmicutes     | Causes dysbiosis in IBD                                                         | AW                         |
| 11     |Peptostreptococcus | s__         | 0.5           | Firmicutes     | Causes gut microbiota dysbiosis in IBD                                          | DR                         |
| 12     |Selenomonas     | s__             | 0.2           | Firmicutes     | Causes dysbiosis in colorectal cancer                                            | EB                         |
| 13     |Eubacterium     | dolichum        | 1.0           | Firmicutes     | Causes dysbiosis of the intestinal microbiota                                    | AL                         |
| 14     |Fusobacterium  | s__             | 2.2           | Fusobacteria   | Identified from colonic biopsies of IBD patients                                | EN                         |
| 15     |Pseudomonas     | alcaligenes     | 1.8           | Proteobacteria  | Identified in the gut microbiota of IBD                                        | AX                         |
| 16     |Pseudomonas     | s__             | 0.1           | Proteobacteria  | Causes infection in Children with Early-onset Crohn's Disease                   | GG                         |
| 17     |Pseudomonas     | Other           | 0.2           | Proteobacteria  | Gut microbe in children with early onset Crohn's disease                        | HR                         |
| 18     |Corynebacterium | durum           | 0.1           | Actinobacteria  | Gut microbe in IBD patients                                                     | AK                         |
| 19     |Corynebacterium | s__             | 0.8           | Actinobacteria  | Causes experimental colitis                                                     | BI                         |
| 20     |Pseudoramibacter_Eubacterium | s__ | 1.6 | Firmicutes | Metabolizes Linoleic acid in the Gut                                              | DF                         |

| **DISEASED COLON (ULCERATIVE COLITIS)** |                 |                 |               |                |                                                                                  |                            |
| 1      | Prevotella     | stercorea       | 1.0           | Bacteroidetes  | Alters mucosal microbiota in the colon of patients with IBD                      | H                          |
| 2      |Prevotella     | s__             | 0.3           | Bacteroidetes  | A microbial signature of Crohn's disease                                        | BZ                         |
| 3      |Prevotella     | Other           | 0.3           | Bacteroidetes  | A microbial signature of Crohn's disease                                        | GS                         |
| 4      |Staphylococcus | aureus          | 0.3           | Firmicutes     | Causes Crohn's disease                                                          | AU                         |
| 5      |Lactobacillus  | zeae            | 7.6           | Firmicutes     | Maintains remission of ulcerative colitis                                       | A                          |
| 6      |Lactobacillus  | s__             | 0.3           | Firmicutes     | Maintains remission of ulcerative colitis                                       | CW                         |
| 7      |Lactococcus    | s__             | 0.6           | Firmicutes     | Used in the treatment of Crohn's disease                                        | CX                         |
| 8      |Peptostreptococcus | anaerobius   | 12.7          | Firmicutes     | Causes dysbiosis in IBD                                                         | AW                         |
| 9      |Peptostreptococcus | s__         | 0.3           | Firmicutes     | Causes gut microbiota dysbiosis in IBD                                          | DR                         |
| 10     |Selenomonas     | s__             | 0.1           | Firmicutes     | Causes dysbiosis in colorectal cancer                                            | EB                         |
| 11     |Eubacterium     | dolichum        | 0.5           | Firmicutes     | Causes dysbiosis of the intestinal microbiota                                    | AL                         |

(Continued)
| Sl. No. | Bacteria genus          | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD                                                                 | NCBI genome database link |
|--------|-------------------------|------------------|---------------|----------------|--------------------------------------------------------------------------------|--------------------------|
| 12     | Pseudoramibacter        | Eubacterium s___ | 1.9           | Firmicutes      | Metabolizes Linoleic acid in the Gut                                             | DF                       |
| 13     | Fusobacterium           | s___             | 3.0           | Fusobacteria    | Identified from colonic biopsies of IBD patients                                 | EN                       |
| 14     | Pseudomonas             | s__              | 0.4           | Proteobacteria  | Identified in the gut microbiota of IBD                                         | AX                       |
| 15     | Pseudomonas             | s__              | 0.8           | Proteobacteria  | Infection in Children with Early-onset Crohn’s Disease                          | GG                       |
| 16     | Aggregatibacter         | s___             | 1.4           | Proteobacteria  | Causes fungal microbiota dysbiosis in IBD                                       | BI                       |
| 17     | Corynebacterium         | s___             | 1.0           | Actinobacteria  | Causes experimental colitis                                                     | BI                       |

**DISEASED COLON (CROHN’S COLITIS)**

| Sl. No. | Bacteria genus       | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD                                                                 | NCBI genome database link |
|---------|----------------------|------------------|---------------|----------------|--------------------------------------------------------------------------------|--------------------------|
| 1       | Prevotella           | tannerae         | 0.2           | Bacteroidetes  | Prevalent in colitis                                                           | F                        |
| 2       | Prevotella           | stercorea        | 3.3           | Bacteroidetes  | Alters mucosal microbiota in the colon of patients with IBD                    | H                        |
| 3       | Prevotella           | melaninogenica   | 0.4           | Bacteroidetes  | Gut microbiome biomarker in ankylosing spondylitis                            | U                        |
| 4       | Prevotella           | Other            | 3.3           | Bacteroidetes  | A microbial signature of Crohn's disease                                      | GS                       |
| 5       | Gemella              | s___             | 0.1           | Firmicutes     | Microbiome in New-Onset Crohn's Disease                                       | CP                       |
| 6       | Staphylococcus       | sciuri           | 0.1           | Firmicutes     | Develops intestinal inflammation in acute and chronic colitis                | I                        |
| 7       | Staphylococcus       | aureus           | 0.5           | Firmicutes     | Causes Crohn's disease                                                        | AU                       |
| 8       | Abiotrophia          | s___             | 0.2           | Firmicutes     | Causes fecal microbial dysbiosis in IBD                                       | CS                       |
| 9       | Lactobacillus        | zeae             | 6.8           | Firmicutes     | Maintains remission of ulcerative colitis                                     | A                        |
| 10      | Lactobacillus        | reuteri          | 0.1           | Firmicutes     | Prevents colitis as a probiotic                                               | M                        |
| 11      | Lactobacillus        | s___             | 0.6           | Firmicutes     | Maintains remission of ulcerative colitis                                     | CW                       |
| 12      | Lactococcus          | s___             | 0.7           | Firmicutes     | Used in the treatment of Crohn's disease                                      | CX                       |
| 13      | Peptostreptococcus   | anaerobius       | 4.0           | Firmicutes     | Causes dysbiosis in IBD                                                        | AW                       |
| 14      | Peptostreptococcus   | s___             | 0.1           | Firmicutes     | Causes gut microbiota dysbiosis in IBD                                        | DR                       |
| 15      | Selenomonas          | s___             | 0.4           | Firmicutes     | Causes dysbiosis in colorectal cancer                                          | EB                       |
| 16      | Eubacterium          | dolichum         | 0.8           | Firmicutes     | Causes dysbiosis of the intestinal microbiota                                  | AL                       |
| 17      | Pseudoramibacter     | Eubacterium s___ | 1.3           | Firmicutes     | Metabolizes Linoleic acid in the Gut                                           | DF                       |
| 18      | Fusobacterium        | s___             | 2.4           | Fusobacteria    | Identified from colonic biopsies of IBD patients                               | EN                       |
| 19      | Pseudomonas          | alcaligenes      | 0.8           | Proteobacteria  | Identified in the gut microbiota of IBD                                        | AX                       |
| 20      | Pseudomonas          | s___             | 1.0           | Proteobacteria  | Infection in Children with Early-onset Crohn’s Disease                         | GG                       |
| 21      | Corynebacterium      | durum            | 0.2           | Actinobacteria  | Gut microbe in IBD patients                                                    | AK                       |
| 22      | Corynebacterium      | s___             | 0.1           | Actinobacteria  | Causes experimental colitis                                                    | BI                       |
| 23      | Pyramidobacter       | piscolens        | 0.1           | Synergistetes   | Oral bacteria in IBD                                                          | P                        |

Specific Information of functions was adapted from NCBI Genome Database (https://www.ncbi.nlm.nih.gov/genome/).

The bacterial species that could not be identified at the genus level are mentioned as g___ and the bacterial species that could not be identified at the species level are mentioned as s___.

(27.8%), and Proteobacteria (24.5%). Most importantly, our results indicate that putative oral pathogens (belonging to mostly Phylum Firmicutes) dominated the microbiome of diseased specimens (Figure 1). Adjacent healthy specimens show an increased abundance of Phylum Bacteroidetes (~ 57%, containing mostly symbiotic and/or beneficial bacteria) population, which is altered in disease categories (Figure 2).
| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD | NCBI genome database link |
|--------|----------------|-----------------|---------------|----------------|----------------|------------------------|
| **ADJACENT HEALTHY COLON** | | | | | | |
| 1 | Shuttleworthia | sataelles | 0.2 | Firmicutes | Identified in the human ileum | J |
| 2 | Bifidobacterium | longum | 0.1 | Actinobacteria | Attenuates acute murine experimental model of IBD | Y |
| 3 | Rhizobium | leguminosarum | 0.1 | Proteobacteria | Identified commensal gut microbe | AB |
| 4 | Lysinibacillus | boronitolerans | 12.1 | Firmicutes | Identified commensal gut microbe | AT |
| 5 | Aloiococcus | s__ | 1.7 | Firmicutes | Identified commensal gut microbe | CT |
| 6 | Christensenella | s__ | 2.2 | Firmicutes | Identified gut microbe | DC |
| 7 | Blautia | s__ | 0.3 | Firmicutes | Butyrate-producing bacterial species in Gut | DI |
| 8 | Coprococcus | s__ | 0.1 | Firmicutes | Butyrate-producing bacterial species in Gut | DI |
| 9 | g__ | s__ | 0.7 | Gemmatimonadetes | Identified commensal gut microbe | EP |
| 10 | g__ | s__ | 0.7 | Lentisphaerae | Normal gut microbe | EQ |
| 11 | g__ | s__ | 0.4 | Proteobacteria | Identified commensal gut microbe | FC |
| 12 | Comamonas | s__ | 1.0 | Proteobacteria | Identified commensal gut microbe | FL |
| 13 | Desulfovibrio | s__ | 0.3 | Proteobacteria | Sulfate reducing bacteria in IBD | FV |
| 14 | Paracoccus | Other | 0.1 | Proteobacteria | Identified commensal gut microbe | HH |
| 15 | Other | Other | 0.2 | Proteobacteria | Mucosal and fecal microbe | HP |
| **DISEASED COLON (ULCERATIVE COLITIS)** | | | | | | |
| 1 | Lysinibacillus | boronitolerans | 8.2 | Firmicutes | Identified commensal gut microbe | AT |
| 2 | Vanibaculum | s__ | 0.2 | Actinobacteria | Identified in the gut of a premature infant | BH |
| 3 | Aloiococcus | s__ | 3.1 | Firmicutes | Identified commensal gut microbe | CT |
| 4 | Christensenella | s__ | 0.6 | Firmicutes | Identified gut microbe | DC |
| 5 | Blautia | s__ | 0.3 | Firmicutes | Butyrate-producing bacterial species in Gut | DH |
| 6 | Coprococcus | s__ | 0.2 | Firmicutes | Butyrate-producing bacterial species in Gut | DI |
| 7 | g__ | s__ | 0.3 | Gemmatimonadetes | Identified commensal gut microbe | EP |
| 8 | g__ | s__ | 0.3 | Proteobacteria | Identified commensal gut microbe | FC |
| 9 | Comamonas | s__ | 2.7 | Proteobacteria | Identified commensal gut microbe | FL |
| 10 | Desulfovibrio | s__ | 0.1 | Proteobacteria | Sulfate reducing bacteria in IBD | FV |
| 11 | Morganella | s__ | 0.1 | Proteobacteria | Sulfate reducing bacteria in IBD | FU |
| 12 | g__ | s__ | 0.6 | TM7 | Identified commensal gut microbe | FZ |
| 13 | Other | Other | 0.1 | Actinobacteria | Commensal gut bacteria in IBD | GN |
| 14 | Other | Other | 0.5 | Proteobacteria | Adult fecal microbe | HO |
| **DISEASED COLON (CROHN’S COLITIS)** | | | | | | |
| 1 | Akkermansia | muciniphila | 0.1 | Verrucomicrobia | Adheres to enterocytes and strengthens the integrity of the epithelial cell layer | S |
| 2 | Bifidobacterium | longum | 0.4 | Actinobacteria | Attenuates acute murine experimental model of IBD | Y |

(Continued)
Differential Expression of Microbiomes in the Colon of CA and AA Patients

Figure 3 shows racial differences of various bacterial phyla in adjacent healthy, UC and CC full thickness colon specimens. The tissue specimens from Caucasians represented a significantly higher proportion \( (p < 0.05) \) of the oral pathogen, *Fusobacterium*, and gut bacteria, *Parabacteroides* (Bacteroidetes). CA specimens also showed significantly higher levels \( (p < 0.05) \) of Phyla Proteobacteria including *Citrobacter*, *Hemophilus*, *Acinetobacter*, *Pseudomonas*, and *Stenotrophomonas* as compared to AA. Whereas, the AA specimens were observed to have a significantly higher proportion \( (p < 0.05) \) of *Prevotella* (Bacteroidetes) and *Clostridia* (Firmicutes) (Figure 3; Table 4).

As depicted in Figure 3, the adjacent healthy colon specimens, UC and CC contained ~1%, ~7% and ~7% of sequence reads, respectively that were un-assignable to any taxon with a larger proportion of them identified in AA Colitis patients. Other major phyla observed among these specimens also include Proteobacteria (Adjacent healthy: 23.8%; UC: 26.5% and CC: 23.1%), Actinobacteria (Adjacent healthy: 6.7%; UC: 8.1% and CC: 14.1%), *Fusobacteria* (Adjacent healthy: 4.2%; UC: 3.6% and CC: 4.0%), and Synergistetes (Adjacent healthy: 0.2%; UC: 0.04% and CC: 1.5%). The Phylum Proteobacteria did not show...
TABLE 3 | Functions and proportions of specific pathogenic Gut bacteria colonized in full thickness colon specimens.

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD                                                                 | NCBI Genome database link |
|---------|----------------|-----------------|----------------|-----------------|--------------------------------------------------------------------------------|----------------------------|
| 1       | Ochrobactrum    | s__             | 0.1            | Proteobacteria  | Causes early bacterial dependent induction of inducible nitric oxide synthase (iNOS) in epithelial cells in experimental colitis | EU                         |
| 2       | Sphingomonas    | s__             | 0.2            | Proteobacteria  | Tissue associated intestinal microflora                                           | FF                         |
| 3       | Burkholderia    | s__             | 1.2            | Proteobacteria  | causes dysfunction of GALT and gut flora in IBD                                 | Fi                         |
| 4       | Acinetobacter   | rhizosphaerae   | 0.3            | Proteobacteria  | Identified gut bacteria in IBD                                                   | K                          |
| 5       | Acinetobacter   | lwoffii         | 0.3            | Proteobacteria  | gut bacteria in multiple sclerosis patients                                      | W                          |
| 6       | Stenotrophomonas| geniculata      | 1.2            | Proteobacteria  | Identified gut bacteria in IBD                                                   | AG                         |
| 7       | Staphylococcus  | sciuri          | 0.1            | Firmicutes      | Develops intestinal inflammation in acute and chronic colitis                   | I                          |
| 8       | Staphylococcus  | aureus          | 0.6            | Firmicutes      | Causes Crohn's disease                                                           | AU                         |
| 9       | Lactobacillus   | zeae            | 1.9            | Firmicutes      | Maintains remission of ulcerative colitis                                        | A                          |
| 10      | Lactobacillus   | s__             | 0.3            | Firmicutes      | Maintains remission of ulcerative colitis                                        | W                          |
| 11      | Lactococcus     | s__             | 0.7            | Firmicutes      | Used in the treatment of Crohn's disease                                        | X                          |
| 12      | Pseudomonas     | alcaligenes     | 1.8            | Proteobacteria  | Identified in the gut microbiota of IBD                                         | AX                         |
| 13      | Pseudomonas     | s__             | 0.1            | Proteobacteria  | Infection in Children with Early-onset Crohn's Disease                          | GG                         |
| 14      | Pseudomonas     | Other           | 0.2            | Proteobacteria  | Gut microbe in children with early onset Crohn's disease                        | HR                         |
| 15      | Bacillus        | s__             | 0.2            | Firmicutes      | Increases cytokine levels in IBD                                                 | CL                         |
| 16      | Bacteroides     | Other           | 0.1            | Bacteroidetes    | Commensal bacteria that induces colitis                                         | GR                         |
| 17      | Microbiotum     | maritipum       | 0.1            | Actinobacteria   | Fecal microbiome in Obesity                                                      | V                          |
| 18      | Eggerthela      | lenta           | 0.8            | Actinobacteria   | Causes bacteremia in Crohn's disease patient                                    | AA                         |
| 19      | Brevundimonas   | diminuta        | 0.1            | Proteobacteria  | Identified in the adult fecal microbiota of allergy patients                    | AO                         |
| 20      | Propionibacterium| acnes           | 5.3            | Actinobacteria   | Intestinal microbe in Liver disease                                              | BA                         |
| 21      | Methanobrevibacter| s__            | 0.7            | Euryarchaeota   | Identified in the gut of IBD                                                     | BB                         |
| 22      | g__             | s__             | 2.4            | Acidobacteria    | Identified in the gut microbiome of Type 2 Diabetes patients                    | BD                         |
| 23      | g__             | s__             | 1.1            | Actinobacteria   | Identified in gut microbiota in IBD                                              | BF                         |
| 24      | Actinomyces     | s__             | 0.1            | Actinobacteria   | Identified in Abdominopelvic actinomycosis involving the GIT                    | BG                         |
| 25      | Varibaculum     | s__             | 0.2            | Actinobacteria   | Identified in the gut of a premature infant                                     | BH                         |
| 26      | Microbacterium  | s__             | 0.1            | Actinobacteria   | Identified in the duodenum of children with ulcerative colitis                 | BK                         |
| 27      | g__             | s__             | 0.2            | Actinobacteria   | Identified in fecal microbiota of pediatric IBD patients                        | BP                         |
| 28      | Atopobium       | s__             | 0.1            | Actinobacteria   | Altered intestinal microbiota in Crohn's disease                                 | BR                         |
| 29      | Slackia         | s__             | 0.2            | Actinobacteria   | Human gut bacteria in Multiple Sclerosis                                         | BS                         |
| 30      | g__             | s__             | 0.1            | Bacteroidetes    | Characterized in intestinal biopsies in IBD patients                            | CB                         |
| 31      | g__             | s__             | 0.1            | Bacteroidetes    | Human gut microbe in Obesity and IBD                                             | CC                         |
| 32      | Clostridium     | s__             | 1.1            | Bacteroidetes    | Identified in the rectum of human colorectal adenoma patients                  | CG                         |
| 33      | g__             | s__             | 0.2            | Cyanobacteria    | Identified in the gut microbiome of IBD patients                                | CI                         |
| 34      | g__             | s__             | 0.6            | Firmicutes       | Causes microbiota dystbiosis in IBD                                              | CO                         |
| 35      | g__             | s__             | 0.7            | Firmicutes       | A microbial signature of Crohn's disease                                        | DB                         |
| 36      | Clostridium     | s__             | 0.9            | Firmicutes       | Causes infection of the gut in IBD                                              | DE                         |
| 37      | Dorea            | s__             | 0.1            | Firmicutes       | Causes dysfunction of the intestinal microbiome in IBD                           | DJ                         |
| 38      | Lachnospira     | s__             | 0.1            | Firmicutes       | Gut bacteria in Crohn's disease patients                                        | DK                         |
| 39      | Ruminococcus    | s__             | 0.1            | Firmicutes       | Dominant in gut microbiome of IBD patients                                      | DO                         |
| 40      | g__             | s__             | 0.2            | Firmicutes       | Gut microbe in IBD                                                              | DP                         |

(Continued)
### TABLE 3 | Continued

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum      | Function in IBD                                                                 | NCBI genome database link |
|---------|----------------|------------------|----------------|----------------------|-------------------------------------------------------------------------------|--------------------------|
| 41      | g__            | s__              | 0.6            | Firmicutes           | A microbial signature of Crohn’s disease                                      | DQ                      |
| 42      | Anaerotruncus  | s__              | 0.2            | Firmicutes           | Tissue associated intestinal microflora                                        | DT                      |
| 43      | Oscillospira   | s__              | 0.4            | Firmicutes           | Gut microbe in IBD patients                                                   | DU                      |
| 44      | Ruminococcus  | s__              | 0.7            | Firmicutes           | Dominant in gut microbiome of IBD patients                                    | DV                      |
| 45      | g__            | s__              | 0.4            | Firmicutes           | Gut microbe underlying the onset of IBD                                        | DW                      |
| 46      | Acidaminoccocus | s__            | 1.3            | Firmicutes           | Gut microbe in IBD                                                            | DX                      |
| 47      | Phascolarctobacterium | s__      | 1.9            | Firmicutes           | Causes dysfunction of the intestinal microbiome in IBD                        | DZ                      |
| 48      | Schwartzia     | s__              | 0.5            | Firmicutes           | Causes fecal microbial dysbiosis in IBD                                       | EA                      |
| 49      | g__            | s__              | 0.2            | Firmicutes           | A microbial signature of Crohn’s disease                                      | EC                      |
| 50      | Anaerococcus   | s__              | 1.3            | Firmicutes           | Microbe in Inflammatory Pouch Complications                                   | EE                      |
| 51      | Finegoldia     | s__              | 0.3            | Firmicutes           | Intestinal microbe in colorectal cancer                                        | EF                      |
| 52      | g__            | s__              | 0.3            | Firmicutes           | Gut microbe in GI diseases                                                    | EI                      |
| 53      | Buleidia       | s__              | 0.1            | Firmicutes           | Fecal-associated and mucosal-associated microbiota in irritable bowel syndrome patients | EJ                      |
| 54      | Coprobacillus  | s__              | 0.3            | Firmicutes           | Alters Gut Microbiota in Psoriatic Arthritis                                  | EL                      |
| 55      | Leptotrichia   | s__              | 0.7            | Fusobacteria         | Causes gut mucosal inflammation in Rheumatoid arthritis patients             | EO                      |
| 56      | g__            | s__              | 4.2            | Proteobacteria       | Intestinal microbe in children with severe and complicated acute viral gastroenteritis | EV                      |
| 57      | Methylobacterium | s__         | 0.1            | Proteobacteria       | Causes microbial dysbiosis in pediatric Crohn’s disease                    | EW                      |
| 58      | g__            | s__              | 0.1            | Proteobacteria       | Intestinal microbe in children with severe and complicated acute viral gastroenteritis | EX                      |
| 59      | g__            | s__              | 1.2            | Proteobacteria       | Involved in host-microbial cross talk in IBD                                 | FG                      |
| 60      | Lautropia      | s__              | 0.3            | Proteobacteria       | causes fecal microbial dysbiosis in IBD                                       | FJ                      |
| 61      | g__            | s__              | 0.1            | Proteobacteria       | Fecal and mucosa associated microbe in IBD                                   | FK                      |
| 62      | Citrobacter    | s__              | 0.1            | Proteobacteria       | Gut microbe in newly diagnosed with treatment-naive Crohn’s disease patients | FY                      |
| 63      | Halomonas      | s__              | 1.4            | Proteobacteria       | Intestinal microflora in chronic kidney disease                             | GB                      |
| 64      | g__            | s__              | 0.1            | Proteobacteria       | Microbe in colon tissue from IBD subjects                                    | GE                      |
| 65      | g__            | s__              | 0.1            | Proteobacteria       | bacteria in human Ulcerative Colitis patients                                | GH                      |
| 66      | Other          | Other            | 0.1            | Actinobacteria       | Alters fecal microbiota in pediatric IBD patients                             | GO                      |
| 67      | Other          | Other            | 3.6            | Firmicutes           | gut microbe in experimental colitis                                           | GT                      |
| 68      | Other          | Other            | 12.5           | Firmicutes           | Fecal and mucosa associated microbe in IBD                                   | GW                      |
| 69      | Weissella      | Other            | 0.2            | Firmicutes           | Gut microbe in IBD patients                                                  | GX                      |
| 70      | Other          | Other            | 0.1            | Proteobacteria       | Fecal and mucosa associated microbe in IBD                                   | HL                      |
| 71      | Other          | Other            | 0.1            | Proteobacteria       | Involved in host-microbial cross talk in IBD                                 | HM                      |

**DISEASED COLON (ULCERATIVE COLITIS)**

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum      | Function in IBD                                                                 | NCBI genome database link |
|---------|----------------|------------------|----------------|----------------------|-------------------------------------------------------------------------------|--------------------------|
| 1       | Ochrobactrum   | s__              | 0.1            | Proteobacteria       | Causes early bacterial dependent induction of inducible nitric oxide synthase (NOS) in epithelial cells in experimental colitis | EU                      |
| 2       | Delftia        | s__              | 0.1            | Proteobacteria       | Fecal and mucosa associated microbe in IBD                                    | FM                      |
| 3       | Sphingomonas   | s__              | 0.5            | Proteobacteria       | Tissue associated intestinal microflora                                        | FF                      |
| 4       | Burkholderia  | s__              | 0.2            | Proteobacteria       | Causes dysfunction of GALT and gut flora in IBD                               | FI                      |
| 5       | Acinetobacter  | rhizosphaerae    | 0.7            | Proteobacteria       | Identified gut microbe in IBD                                                 | K                       |
| 6       | Acinetobacter  | lwolfii          | 0.1            | Proteobacteria       | Gut bacteria in multiple sclerosis patients                                   | W                       |
| 7       | Acinetobacter  | s__              | 0.5            | Proteobacteria       | Tissue associated intestinal microflora                                        | GF                      |
| 8       | Stenotrophomonas | geniculata     | 0.1            | Proteobacteria       | Identified gut microbe in IBD                                                 | AG                      |
| 9       | Enterococcus  | sciuri           | 0.8            | Firmicutes           | Induces experimental IBD                                                      | CV                      |
| 10      | Staphylococcus | sciuri           | 0.1            | Firmicutes           | Develops intestinal inflammation in acute and chronic colitis                | I                       |
| Sl. No. | Bacteria genus   | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD                                                | NCBI genome database link |
|--------|------------------|------------------|----------------|----------------|---------------------------------------------------------------|---------------------------|
| 11     | Staphylococcus   | aureus           | 0.6            | Firmicutes     | Causes Crohn's disease                                        | AU                        |
| 12     | Lactobacillus    | zeae             | 1.9            | Firmicutes     | Maintains remission of ulcerative colitis                    | A                         |
| 13     | Lactobacillus    | s__              | 0.4            | Firmicutes     | Maintains remission of ulcerative colitis                    | CW                       |
| 14     | Lactococcus      | s__              | 0.7            | Firmicutes     | used in the treatment of Crohn's disease                     | CX                       |
| 15     | Pseudomonas      | alcaligenes      | 1.8            | Proteobacteria  | Identified in the gut microbiota of IBD                      | AX                       |
| 16     | Pseudomonas      | s__              | 0.1            | Proteobacteria  | Infection in Children with Early-onset Crohn's Disease       | GG                       |
| 17     | Pseudomonas      | Other            | 0.2            | Proteobacteria  | Gut microbe in children with early onset Crohn's disease     | HR                       |
| 18     | Bacillus         | s__              | 0.1            | Firmicutes     | Increases cytokine levels in IBD                             | CL                       |
| 19     | Bacteroides      | cacaoe           | 0.1            | Actinobacteria  | Identified in the gut of ulcerative colitis patients         | AS                       |
| 20     | Bacteroides      | Other            | 0.2            | Bacteroidetes   | Commensal bacteria that induces colitis                       | GR                       |
| 21     | Blautia          | producta         | 0.1            | Firmicutes     | Gut microbe in Obesity and IBD                               | N                        |
| 22     | Faecalibacterium | prausnitzii      | 0.1            | Firmicutes     | Gut microbe in Crohn's disease patients                      | O                        |
| 23     | Microbacterium   | maritipicum      | 0.1            | Actinobacteria  | Fecal microbiome in Obesity                                  | V                        |
| 24     | Eggerthelia      | lenta            | 5.1            | Actinobacteria  | Causes bacteremia in Crohn's disease patient                 | AA                       |
| 25     | Propionibacterium| acnes            | 2.8            | Actinobacteria  | Intestinal microbe in Liver disease                          | BA                       |
| 26     | Methanoreovibacter| s__              | 0.3            | Euryarchaeota   | Identified in the gut of IBD                                  | BB                       |
| 27     | g__ s__          | 1.8  | Acidobacteria  | Identified in the gut microbiome of Type 2 Diabetes patients | BD                       |
| 28     | g__ s__          | 0.3  | Actinobacteria  | Identified in gut microbiota in IBD                           | BF                       |
| 29     | Adlercreutzia     | s__              | 0.2  | Actinobacteria  | Causes dysbiosis in IBD patients                              | BQ                       |
| 30     | Slackia          | s__              | 0.2  | Actinobacteria  | Alters human gut microbiome in Multiple Sclerosis            | BS                       |
| 31     | g__ s__          | 0.8  | Bacteroidetes   | Identified in gut microbiome of IBD patients                 | CA                       |
| 32     | g__ s__          | 0.4  | Bacteroidetes   | Characterized in intestinal biopsies in IBD patients          | CB                       |
| 33     | g__ s__          | 0.1  | Bacteroidetes   | Human gut microbe in Obesity and IBD                         | CC                       |
| 34     | Cloacibacterium  | s__              | 0.4  | Bacteroidetes   | Identified in the rectum of human colorectal adenoma patients | CG                       |
| 35     | g__ s__          | 0.1  | Firmicutes      | Causes microbiota dysbiosis in IBD                            | CO                       |
| 36     | g__ s__          | 0.1  | Firmicutes      | Gut microbe in IBD                                           | DA                       |
| 37     | g__ s__          | 1.7  | Firmicutes      | A microbial signature of Crohn's disease                     | DB                       |
| 38     | Clostridium      | s__              | 0.8  | Firmicutes      | Causes infection of the gut in IBD                            | DE                       |
| 39     | Dorea            | s__              | 0.1  | Firmicutes      | Causes dysfunction of the intestinal microbiome in IBD       | DJ                       |
| 40     | Lachnospira      | s__              | 0.3  | Firmicutes      | gut bacteria in Crohn's disease patients                     | DK                       |
| 41     | Ruminococcus     | s__              | 0.1  | Firmicutes      | Dominant in gut microbiome of IBD patients                   | DO                       |
| 42     | g__ s__          | 0.3  | Firmicutes      | A microbial signature of Crohn's disease                     | DQ                       |
| 43     | Oscillospira     | s__              | 0.1  | Firmicutes      | Gut microbe in IBD patients                                  | DU                       |
| 44     | Ruminococcus     | s__              | 0.9  | Firmicutes      | Dominant in gut microbiome of IBD patients                   | DV                       |
| 45     | g__ s__          | 0.3  | Firmicutes      | Gut microbe underlying the onset of IBD                       | DW                       |
| 46     | Acidaminococcus  | s__              | 1.1  | Firmicutes      | Gut microbe in IBD                                           | DX                       |
| 47     | Phascolarctobacterium| s__          | 2.2  | Firmicutes      | Causes dysfunction of the intestinal microbiome in IBD       | DZ                       |
| 48     | Schwartzia       | s__              | 0.5  | Firmicutes      | Causes fecal microbial dysbiosis in IBD                       | EA                       |
| 49     | Anaerococcus     | s__              | 0.6  | Firmicutes      | Microbe in Inflammatory Pouch Complications                   | EE                       |
| 50     | Finegoldia       | s__              | 0.3  | Firmicutes      | Intestinal microbe in colorectal cancer                      | EF                       |
| 51     | g__ s__          | 0.6  | Firmicutes      | Gut microbe in GI diseases                                   | EI                       |
| 52     | Bulleidia        | s__              | 0.1  | Firmicutes      | Fecal-associated and mucosal-associated microbiota in irritable bowel syndrome patients | EJ                       |
| 53     | Coproaceticus    | s__              | 0.1  | Firmicutes      | Alters Gut Microbiota in Psoriatic Arthritis                  | EL                       |
| 54     | Leptotrichia     | s__              | 0.5  | Fusobacteria    | Causes gut mucosal inflammation in Rheumatoid arthritis patients | EO                       |
### TABLE 3 | Continued

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD | NCBI genome database link |
|---------|----------------|------------------|----------------|-----------------|-----------------|--------------------------|
| 55      | g__            | s__              | 3.6            | Proteobacteria  | Intestinal microbe in children with severe and complicated acute viral gastroenteritis | EV                       |
| 56      | g__            | s__              | 0.5            | Proteobacteria  | Causes chronic inflammation in IBD | FB                       |
| 57      | g__            | s__              | 0.1            | Proteobacteria  | Microbial factor associated with postoperative Crohn’s disease | FD                       |
| 58      | g__            | s__              | 1.5            | Proteobacteria  | Involved in host-microbial cross talk in IBD | FG                       |
| 59      | Sutterella     | s__              | 0.1            | Proteobacteria  | Gut microbe in experimental colitis | FH                       |
| 60      | Lautropia      | s__              | 0.2            | Proteobacteria  | Causes fecal microbial dysbiosis in IBD | FJ                       |
| 61      | g__            | s__              | 0.5            | Proteobacteria  | Fecal and mucosa associated microbe in IBD | FK                       |
| 62      | Citrobacter    | s__              | 0.3            | Proteobacteria  | Gut microbe in newly diagnosed with treatment-naive Crohn’s disease patients | FY                       |
| 63      | Halomonas      | s__              | 0.8            | Proteobacteria  | Intestinal microflora in chronic kidney disease | GB                       |
| 64      | g__            | s__              | 0.3            | Proteobacteria  | Bacteria in human Ulcerative Colitis patients | GH                       |
| 65      | Other          | Other            | 0.2            | Actinobacteria  | Alters fecal microbiota in pediatric IBD patients | GO                       |
| 66      | Eggerthella    | Other            | 0.1            | Actinobacteria  | Causes bacteremia in Crohn’s disease patient | GQ                       |
| 67      | Other          | Other            | 4.0            | Firmicutes      | Gut microbe in experimental colitis | GT                       |
| 68      | Other          | Other            | 4.2            | Firmicutes      | Fecal and mucosa associated microbe in IBD | GW                       |
| 69      | Weissella      | Other            | 1.2            | Firmicutes      | Gut microbe in IBD patients | GX                       |
| 70      | Other          | Other            | 2.5            | Proteobacteria  | Causes microbial dysbiosis in pediatric Crohn’s disease | HD                       |
| 71      | Other          | Other            | 1.4            | Proteobacteria  | Fecal and mucosa associated microbe in IBD | HL                       |

### DISEASED COLON (CROHN’S COLITIS)

| Sl. No. | Bacteria genus     | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD                                                                 | NCBI genome database link |
|---------|-------------------|------------------|----------------|-----------------|--------------------------------------------------------------------------------|--------------------------|
| 1       | Ochrobactrum      | s__              | 0.1            | Proteobacteria  | Causes early bacterial dependent induction of inducible nitric oxide synthase (iNOS) in epithelial cells in experimental colitis | EU                       |
| 2       | Sphingomonas      | s__              | 0.1            | Proteobacteria  | Tissue associated intestinal microflora | FF                       |
| 3       | Burkholderia      | s__              | 1.6            | Proteobacteria  | Causes dysfunction of GALT and gut flora in IBD | FI                       |
| 4       | Acinetobacter rhizosphaerae | s__ | 1.8 | Proteobacteria  | Identified gut microbe in IBD | K                       |
| 5       | Acinetobacter lwoffii | s__ | 0.5 | Proteobacteria  | Gut bacteria in multiple sclerosis patients | W                       |
| 6       | Acinetobacter     | Other            | 1.6            | Proteobacteria  | Tissue associated intestinal microflora in colitis patients | HQ                       |
| 7       | Stenotrophomonas  | geniculata       | 0.3            | Proteobacteria  | Identified gut microbe in IBD | AG                       |
| 8       | Enterococcus      | s__              | 0.1            | Firmicutes      | Induces experimental IBD | CV                       |
| 9       | Staphylococcus    | sciuri           | 0.1            | Firmicutes      | Develops intestinal inflammation in acute and chronic colitis | I                       |
| 10      | Staphylococcus    | aureus           | 0.6            | Firmicutes      | Causes Crohn’s disease | AU                       |
| 11      | Lactobacillus     | zeae             | 1.9            | Firmicutes      | Maintains remission of ulcerative colitis | A                       |
| 12      | Lactobacillus     | s__              | 0.4            | Firmicutes      | Maintains remission of ulcerative colitis | CW                       |
| 13      | Lactococcus      | s__              | 0.7            | Firmicutes      | Used in the treatment of Crohn’s disease | CX                       |
| 14      | Pseudomonas       | alicigenes       | 1.8            | Proteobacteria  | Identified in the gut microbiota of IBD | AX                       |
| 15      | Pseudomonas       | s__              | 0.1            | Proteobacteria  | Infection in Children with Early-onset Crohn’s Disease | GG                       |
| 16      | Pseudomonas       | Other            | 0.2            | Proteobacteria  | Gut microbe in children with early onset Crohn’s disease | HR                       |
| 17      | Bacillus          | thermoamylvorans | 0.2            | Firmicutes      | A probiotic- normal flora of the gut | E                        |
| 18      | Bacillus          | s__              | 2.6            | Firmicutes      | Increases cytokine levels in IBD | CL                       |
| 19      | Bacteroides       | eggerthii        | 0.1            | Bacteroidetes   | Enhances colitis in mice | AJ                       |
| 20      | Bacteroides       | Other            | 0.4            | Bacteroidetes   | Commensal bacteria that induces colitis | GR                       |
| 21      | Microbacterium    | mantypticum      | 0.1            | Actinobacteria  | Fecal microbiome in Obesity | V                        |
| 22      | Eggerthelia       | lenta            | 4.5            | Actinobacteria  | Causes bacteremia in Crohn’s disease patient | AA                       |
| 23      | Brevundimonas     | diminuta         | 0.2            | Proteobacteria  | Identified in the adult fecal microbiota of allergy patients | AO                       |
TABLE 3 | Continued

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD | NCBI genome database link |
|--------|----------------|------------------|----------------|----------------|----------------|---------------------------|
| 24     | Propionibacterium | acnes            | 1.2            | Actinobacteria | Intestinal microbe in Liver disease | BA            |
| 25     | Methanobrevibacter | s__              | 0.8            | Euryarchaeota  | Identified in the gut of IBD patients | BB            |
| 26     | g__             | s__              | 0.7            | Acidobacteria  | Identified in the gut microbiome of Type 2 Diabetes patients | BD            |
| 27     | g__             | s__              | 0.2            | Actinobacteria | Identified in gut microbiota in IBD | BE            |
| 28     | g__             | s__              | 0.5            | Actinobacteria | Identified in gut microbiota in IBD | BF            |
| 29     | Microbacterium | s__              | 0.1            | Actinobacteria | Identified in the duodenum of children with ulcerative colitis | BK            |
| 30     | Bifidobacterium | s__              | 0.2            | Actinobacteria | Identified in gut microbiota of IBD patients | BO            |
| 31     | g__             | s__              | 0.3            | Actinobacteria | Identified in fecal microbiota of pediatric IBD patients | BP            |
| 32     | Atopobium       | s__              | 0.2            | Actinobacteria | Altered intestinal microbiota in Crohn’s disease | BR            |
| 33     | Slackia         | s__              | 1.3            | Actinobacteria | Alters human gut microbiome in Multiple Sclerosis | BS            |
| 34     | g__             | s__              | 0.1            | Bacteroidetes  | Identified in gut microbiome of IBD patients | CA            |
| 35     | g__             | s__              | 0.3            | Bacteroidetes  | Human gut microbe in Obesity and IBD | CC            |
| 36     | g__             | s__              | 0.7            | Firmicutes     | Causes microbiota dysbiosis in IBD | CO            |
| 37     | g__             | s__              | 0.2            | Firmicutes     | Gut microbe in IBD | DA            |
| 38     | g__             | s__              | 0.9            | Firmicutes     | A microbial signature of Crohn’s disease | DB            |
| 39     | Clostridium     | s__              | 0.6            | Firmicutes     | Causes infection of the gut in IBD | DE            |
| 40     | Lachnospira     | s__              | 0.6            | Firmicutes     | Gut bacteria in Crohn’s disease patients | DK            |
| 41     | Morvella        | s__              | 0.1            | Firmicutes     | Microbe in Inflammatory Pouch Complications | DL            |
| 42     | g__             | s__              | 0.1            | Firmicutes     | Gut microbe in IBD | DP            |
| 43     | g__             | s__              | 0.6            | Firmicutes     | A microbial signature of Crohn’s disease | DQ            |
| 44     | Oscillospira    | s__              | 0.2            | Firmicutes     | Gut microbe in IBD patients | DU            |
| 45     | Ruminococcus   | s__              | 0.9            | Firmicutes     | Dominant in gut microbiome of IBD patients | DV            |
| 46     | g__             | s__              | 0.2            | Firmicutes     | Gut microbe underlying the onset of IBD | DW            |
| 47     | Acidaminococcus | s__              | 1.0            | Firmicutes     | Gut microbe in IBD | DX            |
| 48     | Phascolarctobacterium | s__ | 0.6          | Firmicutes     | Causes dysfunction of the intestinal microbiome in IBD | DZ            |
| 49     | Schwartzia      | s__              | 0.2            | Firmicutes     | Causes fecal microbial dysbiosis in IBD | EA            |
| 50     | g__             | s__              | 0.1            | Firmicutes     | A microbial signature of Crohn’s disease | EC            |
| 51     | Anaerococcus    | s__              | 0.3            | Firmicutes     | Microbe in Inflammatory Pouch Complications | EE            |
| 52     | Finegoldia      | s__              | 0.4            | Firmicutes     | Intestinal microbe in colorectal cancer | EF            |
| 53     | g__             | s__              | 0.5            | Firmicutes     | Gut microbe in GI diseases | EI            |
| 54     | Bulleidia       | s__              | 0.2            | Firmicutes     | Fecal-associated and mucosal-associated microbiota in irritable bowel syndrome patients | EJ            |
| 55     | Coprobacillus   | s__              | 0.3            | Firmicutes     | Alters Gut Microbiota in Psoriatic Arthritis | EL            |
| 56     | Leptotrichia    | s__              | 0.5            | Fusobacteria   | Causes gut mucosal inflammation in Rheumatoid arthritis patients | EO            |
| 57     | g__             | s__              | 4.1            | Proteobacteria | Intestinal microbe in children with severe and complicated acute viral gastroenteritis | EV            |
| 58     | g__             | s__              | 0.2            | Proteobacteria | Microbial factor associated with postoperative Crohn’s disease | FD            |
| 59     | g__             | s__              | 1.2            | Proteobacteria | Involved in host-microbial cross talk in IBD | FG            |
| 60     | Sutterella      | s__              | 0.1            | Proteobacteria | Gut microbe in experimental colitis | FH            |
| 61     | Lautropia       | s__              | 0.1            | Proteobacteria | Causes fecal microbial dysbiosis in IBD | FJ            |
| 62     | g__             | s__              | 0.4            | Proteobacteria | Fecal and mucosa associated microbe in IBD | FK            |
| 63     | g__             | s__              | 0.1            | Proteobacteria | Bacteria in Mucosal and Submucosal Intestinal Tissues in Advanced Crohn’s Disease | FN            |
| 64     | Ralstonia       | s__              | 0.1            | Proteobacteria | Microbiota in the Mucosa of Patients With Ulcerative Colitis | FP            |
| 65     | Halomonas       | s__              | 0.5            | Proteobacteria | Intestinal microflora in chronic kidney disease | GB            |
TABLE 3 | Continued

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD | NCBI genome database link |
|--------|----------------|------------------|----------------|-----------------|-----------------|---------------------------|
| 66     | Haemophilus     | s__              | 0.7            | Proteobacteria  | Treatment naïve microbiome in new onset Crohn’s disease | GD |
| 67     | Other           | s__              | 0.1            | Proteobacteria  | Microbe in colon tissue from IBD subjects | GE |
| 68     | Other           | Other            | 1.1            | Actinobacteria  | Alters fecal microbiota in pediatric IBD patients | GO |
| 69     | Eggerthella     | Other            | 0.1            | Actinobacteria  | Causes bacteremia in Crohn’s disease patient | GQ |
| 70     | Other           | Other            | 1.7            | Firmicutes      | Gut microbe in experimental colitis | GT |
| 71     | Other           | Other            | 2.2            | Firmicutes      | Fecal and mucosa associated microbe in IBD | GW |
| 72     | Weissella       | Other            | 2.2            | Firmicutes      | Gut microbe in IBD patients | GX |
| 73     | Other           | Other            | 0.1            | Proteobacteria  | Causes microbial dysbiosis in pediatric Crohn’s disease | HD |
| 74     | Methylobacterium| Other            | 0.1            | Proteobacteria  | Causes gut microbial dysbiosis in pediatric Crohn’s disease patients | HG |
| 75     | Other           | Other            | 0.2            | Proteobacteria  | Fecal and mucosa associated microbe in IBD | HK |
| 76     | Other           | Other            | 0.6            | Proteobacteria  | Fecal and mucosa associated microbe in IBD | HL |

Specific information of functions was adapted from NCBI Genome Database (https://www.ncbi.nlm.nih.gov/genome/).

The bacterial species that could not be identified at the genus level are mentioned as g___ and the bacterial species that could not be identified at the species level are mentioned as s___.

any significant difference between healthy colon specimens and diseased colon specimens (Table 4).

Bacterial Species Identified in a Significantly Higher Proportion in Diseased Colon Tissues

As shown in Figure 3, diseased colon specimens represented a significantly higher proportion (p < 0.05) of gut bacteria belonging to Phylum Firmicutes including Blautia producta, Faecalibacterium prausnitzii, Anoxybacillus kestanbolensis, Ruminococcus gnavus, Eubacterium dolichum, Lysinibacillusboronitolerans, and oral bacteria including Staphylococcus sciuri, Staphylococcus aureus, Streptococcus anginosus.

In contrast, healthy colon specimens were significantly dominated (p < 0.05) by oral bacteria belonging to Phylum Actinobacteria that includes; Corynebacterium kroppenstedtii, Corynebacterium duros. Additionally, healthy colon specimens were dominated by gut bacteria belonging to Phylum Actinobacteria that includes; Collinsella stercoris, Collinsella aerofaciens, Kocuria rhizophila, Eggerthella lenta, Propionibacterium granulosum, Propionibacterium acnes, Actinomyces europaeus, Rothia dentocariosa, and Phylum Bacteroidetes that includes; Bacteroides fragilis, Bacteroides eggerthii, Bacteroides caccae, Parabacteroides distasonis (Figure 3).

Alpha Diversity and Beta Diversity Analyses

Alpha diversity and beta diversity metrics were computed to analyse the diversity of bacterial species within each sample and between samples. To assess our sampling efficiency, we plotted rarefaction curves (Chao1 and Shannon) for all 39 specimens. Increased diversity (Shannon) in the diseased samples compared to control samples was observed. From the rarefaction curves, it is evident that most AA samples require additional sampling whereas Caucasian samples do not (data not shown).

Since, outliers exhibiting different microbiome profiles were observed both in the healthy and disease groups, we performed principle coordinate analysis (PCoA analysis) and hierarchical clustering to obtain a holistic view of the microbiome profile in each sample. Two dimensional PCoA plots revealed that control samples which had similar microbiome profiles as suggested by histograms and OTU heat map clustered together (data not shown).

Pathogenic Oral and Gut Flora Abundantly Colonized in Diseased Colon Specimens

The pathogenic oral bacteria identified abundantly in diseased colon specimens as compared to healthy colon specimens were Porphyromonas, Prevotella, Gemella, Staphylococcus, Streptococcus, Abiotrophia, Granulicatella, Lactobacillus, Lactococcus, Peptostreptococcus, Selenomonas, Veillonella, Parvimonas, Eubacterium, Fusobacterium, Pseudomonas, Aggregatibacter, and Corynebacterium (Table 1). Pathogenic gut bacteria identified abundantly in diseased colon specimens as compared to healthy colon specimens include Ochrobactrum, Delftia, Sphingomonas, Burkholderia, Acinetobacter, Stenotrophomonas, Enterococcus, Granulicatella, Staphylococcus, Streptococcus, Lactobacillus, Lactococcus, Pseudomonas, Bacillus, Campylobacter, and Bacteroides (Table 3).

DISCUSSION

Our study demonstrates significant perturbations among bacteria belonging to Phyla Bacteroidetes and Firmicutes in full-thickness diseased colon specimens containing neuromuscular compartment (Figure 2). Our studies further show that the proportion of pathogenic bacteria are higher in diseased
### TABLE 4 | Functions and Proportions of bacterial species identified in the full thickness human colon specimens of Caucasians and African Americans.

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD | NCBI genome database link |
|---------|----------------|------------------|----------------|-----------------|-----------------|---------------------------|
|         |                |                  |                |                 |                 |                           |
| **CAUCASIAN AMERICANS** | | | | | | |
| 1       | Lactobacillus  | zeae             | 6.8            | Firmicutes      | Maintains remission of ulcerative colitis | A |
| 2       | Bacillus       | thermoamylovorans| 0.1            | Firmicutes      | A probiotic- normal flora of the gut       | E |
| 3       | Prevotella     | tannerae         | 0.1            | Bacteroidetes   | Prevalent in colitis                        | F |
| 4       | Collinsella    | stercoris        | 0.0            | Actinobacteria  | Used for treatment of IBD                   | G |
| 5       | Prevotella     | stercorea        | 1.4            | Bacteroidetes   | Alters mucosal microbiota in the colon of patients with IBD | H |
| 6       | Staphylococcus | sciuri           | 0.1            | Firmicutes      | Develops intestinal inflammation in acute and chronic colitis | I |
| 7       | Shuttleworthia | satelles         | 0.0            | Firmicutes      | Identified in the human leum                | J |
| 8       | Acinetobacter  | rhizosphaerae    | 1.1            | Proteobacteria  | Identified gut microbe in IBD               | K |
| 9       | Blautia        | producta         | 0.1            | Firmicutes      | Gut microbe in Obesity and IBD              | N |
| 10      | Akkermansia    | muciniphila      | 0.1            | Firmicutes      | Adheres to enterocytes and strengthens the integrity of the epithelial cell layer | S |
| 11      | Prevotella     | melaninogenica   | 0.2            | Bacteroidetes   | Gut microbiome biomarker in ankylosing spondylitis | U |
| 12      | Acinetobacter  | lwoffii          | 0.2            | Proteobacteria  | Gut bacteria in multiple sclerosis patients  | W |
| 13      | Bifidobacterium| longum           | 0.2            | Actinobacteria  | Attenuates acute murine experimental model of IBD | Y |
| 14      | Eggerthella    | lenita           | 4.3            | Actinobacteria  | Causes bacteremia in Crohn's disease patient | AA |
| 15      | Rhizobium      | leguminosarum    | 0.1            | Proteobacteria  | Identified gut microbe in IBD patients       | AB |
| 16      | Anoxybacillus  | kestanbolensis   | 0.1            | Firmicutes      | Identified gut microbe in IBD patients       | AD |
| 17      | Stenotrophomonas| geniculata       | 0.1            | Proteobacteria  | Identified gut microbe in IBD patients       | AG |
| 18      | Corynebacterium| durum            | 0.1            | Actinobacteria  | Identified gut microbe in IBD patients       | AK |
| 19      | Eubacterium    | dolichum         | 0.8            | Firmicutes      | Causes dysbiosis of the intestinal microbiota | AL |
| 20      | Brevundimonas  | diminuta         | 0.1            | Proteobacteria  | Identified in the adult fecal microbiota of allergy patients | AO |
| 21      | Lysinibacillus | boronitolerans   | 12.3           | Firmicutes      | Identified gut microbe in IBD patients       | AT |
| 22      | Staphylococcus | aureus           | 0.6            | Firmicutes      | Causes Crohn's disease                       | AU |
| 23      | Peptostreptococcus| anaerobius     | 4.8            | Firmicutes      | Causes dysbiosis in IBD                      | AW |
| 24      | Pseudomonas    | aalcaligens      | 1.0            | Proteobacteria  | Identified in the gut microbiota of IBD      | AX |
| 25      | Propionibacterium| acnes           | 3.9            | Actinobacteria  | Intestinal microbe in Liver disease          | BA |
| 26      | Methanobrevibacter| s__            | 0.6            | Euryarchaeota   | Identified in the gut of IBD                 | BB |
| 27      | g__            | s__              | 0.8            | Acidobacteria   | Identified in the gut microbiome of Type 2 Diabetes patients | BD |
| 28      | g__            | s__              | 0.9            | Actinobacteria  | Identified in gut microbiota in IBD          | BF |
| 29      | Vanbaculum     | s__              | 0.1            | Actinobacteria  | Identified in the gut of a premature infant  | BH |
| 30      | Corynebacterium| s__              | 0.7            | Actinobacteria  | Causes experimental colitis                  | BI |
| 31      | Microbacterium | s__              | 0.1            | Actinobacteria  | Identified in the duodenum of children with ulcerative colitis | BK |
| 32      | Bifidobacterium| s__              | 0.1            | Actinobacteria  | Identified in gut microbiota of IBD patients | BO |
| 33      | g__            | s__              | 0.2            | Actinobacteria  | Identified in fecal microbiota of pediatric IBD patients | BP |
| 34      | Adlercreutzia  | s__              | 0.1            | Actinobacteria  | Causes dysbiosis in IBD patients             | BQ |
| 35      | Atopobium      | s__              | 0.1            | Actinobacteria  | Altered intestinal microbiota in Crohn's disease | BR |
| 36      | Slackia        | s__              | 0.7            | Actinobacteria  | Alters human gut microbiome in Multiple Sclerosis | BS |
| 37      | Prevotella     | s__              | 0.1            | Bacteroidetes   | A microbial signature of Crohn's disease     | BZ |
| 38      | g__            | s__              | 0.4            | Bacteroidetes   | Identified in gut microbiome of IBD patients | CA |
| 39      | g__            | s__              | 0.2            | Bacteroidetes   | Characterized in intestinal biopsies in IBD patients | CB |
| 40      | g__            | s__              | 0.2            | Bacteroidetes   | Human gut microbe in Obesity and IBD         | CC |
| 41      | Cloacibacterium| s__              | 0.7            | Bacteroidetes   | Identified in the rectum of human colorectal adenoma patients | CG |
| 42      | SHD-231        | s__              | 0.1            | Chloroflexi     | Identified in the fecal microbiome of Gout patients | CH |
| 43      | g__            | s__              | 0.1            | Cyanobacteria   | Identified in the gut microbiome of IBD patients | Cl |

(Continued)
### TABLE 4 (Continued)

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD                                                                 | NCBI genome database link |
|---------|----------------|------------------|---------------|----------------|---------------------------------------------------------------------------------|----------------------------|
| 44      | Calothrix      | s__              | 0.1           | Cyanobacteria   | Identified gut microbe in IBD patients                                           | CK                         |
| 45      | Bacillus       | s__              | 0.5           | Firmicutes      | Increases cytokine levels in IBD                                                | CL                         |
| 46      | g__            | s__              | 0.6           | Firmicutes      | Causes microbiota dysbiosis in IBD                                              | CO                         |
| 47      | Gemella        | s__              | 0.1           | Firmicutes      | Microbiome in New-Onset Crohn’s Disease                                         | CP                         |
| 48      | Abiotrophia    | s__              | 0.1           | Firmicutes      | Causes fecal microbial dysbiosis in IBD                                          | CS                         |
| 49      | Aloiccoccus    | s__              | 3.4           | Firmicutes      | Identified gut microbe in IBD patients                                           | CT                         |
| 50      | Enterococcus   | s__              | 0.4           | Firmicutes      | Induces experimental IBD                                                        | CV                         |
| 51      | Lactobacillus  | s__              | 0.5           | Firmicutes      | Maintains remission of ulcerative colitis                                       | CW                         |
| 52      | Lactococcus    | s__              | 0.7           | Firmicutes      | Used in the treatment of Crohn’s disease                                        | CX                         |
| 53      | g__            | s__              | 0.1           | Firmicutes      | Gut microbe in IBD                                                              | DA                         |
| 54      | g__            | s__              | 1.1           | Firmicutes      | A microbial signature of Crohn’s disease                                         | DB                         |
| 55      | Christensenella| s__              | 1.2           | Firmicutes      | Identified gut microbe                                                          | DC                         |
| 56      | Clostridium    | s__              | 0.9           | Firmicutes      | Causes infection of the gut in IBD                                              | DE                         |
| 57      | Pseudorambacter| Eubacterium      | s__           | Firmicutes      | Metabolizes Linoleic acid in the Gut                                            | DF                         |
| 58      | g__            | s__              | 0.1           | Firmicutes      | Commensal gut bacteria in IBD                                                   | DG                         |
| 59      | Blautia        | s__              | 0.3           | Firmicutes      | Butyrate-producing bacterial species in Gut                                    | DH                         |
| 60      | Coprococcus    | s__              | 0.2           | Firmicutes      | Butyrate-producing bacterial species in Gut                                    | DI                         |
| 61      | Dorea          | s__              | 0.1           | Firmicutes      | Causes dysfunction of the intestinal microbiome in IBD                         | DJ                         |
| 62      | Lachnospira    | s__              | 0.3           | Firmicutes      | Gut bacteria in Crohn’s disease patients                                        | DK                         |
| 63      | g__            | s__              | 0.1           | Firmicutes      | Gut microbe in IBD                                                              | DP                         |
| 64      | g__            | s__              | 0.6           | Firmicutes      | A microbial signature of Crohn’s disease                                         | DQ                         |
| 65      | Peptostreptococcus| s__         | 0.3           | Firmicutes      | Causes gut microbiota dysbiosis in IBD                                          | DR                         |
| 66      | Anaerotruncus  | s__              | 0.1           | Firmicutes      | Tissue associated intestinal microflora                                         | DT                         |
| 67      | Oscillospira   | s__              | 0.2           | Firmicutes      | Gut microbe in IBD patients                                                     | DU                         |
| 68      | Ruminococcus   | s__              | 0.4           | Firmicutes      | Dominant in gut microbiome of IBD patients                                      | DV                         |
| 69      | g__            | s__              | 0.3           | Firmicutes      | Gut microbe underlying the onset of IBD                                         | DW                         |
| 70      | Acidaminococcus| s__              | 1.2           | Firmicutes      | Gut microbe in IBD                                                              | DX                         |
| 71      | Phascolarctobacterium| s__     | 1.9           | Firmicutes      | Causes dysfunction of the intestinal microbiome in IBD                         | DZ                         |
| 72      | Schwartzia     | s__              | 0.5           | Firmicutes      | Causes fecal microbial dysbiosis in IBD                                          | EA                         |
| 73      | Selenomonas    | s__              | 0.2           | Firmicutes      | Causes dysbiosis in colorectal cancer                                           | EB                         |
| 74      | g__            | s__              | 0.1           | Firmicutes      | A microbial signature of Crohn’s disease                                         | EC                         |
| 75      | Anaerococcus   | s__              | 0.7           | Firmicutes      | Microbe in Inflammatory Pouch Complications                                      | EE                         |
| 76      | Finegoldia     | s__              | 0.3           | Firmicutes      | Intestinal microbe in colorectal cancer                                          | EF                         |
| 77      | g__            | s__              | 0.4           | Firmicutes      | Gut microbe in GI diseases                                                      | EI                         |
| 78      | Bulleidia      | s__              | 0.1           | Firmicutes      | Fecal-associated and mucosal-associated microbiota in irritable bowel syndrome patients | EJ                         |
| 79      | Coprobacillus  | s__              | 0.3           | Firmicutes      | Alters Gut Microbiota in Psoriatic Arthritis                                     | EL                         |
| 80      | Fusobacterium  | s__              | 0.8           | Fusobacteria    | Identified from colonic biopsies of IBD patients                                 | EN                         |
| 81      | Leptotrichia   | s__              | 0.2           | Fusobacteria    | Causes gut mucosal inflammation in Rheumatoid arthritis patients                 | EO                         |
| 82      | g__            | s__              | 0.3           | Gemmatimonadetes| Identified gut microbe in IBD patients                                           | EP                         |
| 83      | g__            | s__              | 0.4           | Lentisphaerae   | Normal gut microbe                                                              | EQ                         |
| 84      | Ochrobactrum   | s__              | 0.1           | Proteobacteria   | Causes early bacterial dependent induction of inducible nitric oxide synthase (NOS) in epithelial cells in experimental colitis | EU                         |
| 85      | g__            | s__              | 4.7           | Proteobacteria   | Intestinal microbe in children with severe and complicated acute viral gastroenteritis | EV                         |
| 86      | g__            | s__              | 0.2           | Proteobacteria   | Causes chronic inflammation in IBD                                              | FB                         |
| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD | NCBI genome database link |
|--------|----------------|-----------------|----------------|-----------------|----------------|---------------------------|
| 87     | g__ s__        | 0.3             | Proteobacteria | Identified gut microbe in IBD patients | FC             |
| 88     | g__ s__        | 0.1             | Proteobacteria | Microbial factor associated with postoperative Crohn's disease | FD             |
| 89     | Sphingomonas   | s__             | 0.3           | Proteobacteria | Tissue associated intestinal microflora | FF             |
| 90     | g__ s__        | 1.7             | Proteobacteria | Involved in host-microbial cross talk in IBD | FG             |
| 91     | Sutterella     | s__             | 0.1           | Proteobacteria | Gut microbe in experimental colitis | FH             |
| 92     | Burkholderia   | s__             | 1.2           | Proteobacteria | Causes dysfunction of GALT and gut flora in IBD | FI             |
| 93     | Lautopia       | s__             | 0.2           | Proteobacteria | Causes fecal microbial dysbiosis in IBD | FJ             |
| 94     | g__ s__        | 0.4             | Proteobacteria | Fecal and mucosa associated microbe in IBD | FK             |
| 95     | Comamonas      | s__             | 2.5           | Proteobacteria | Identified gut microbe in IBD patients | FL             |
| 96     | Delfia         | s__             | 0.1           | Proteobacteria | Fecal and mucosa associated microbe in IBD | FM             |
| 97     | Desulfovibrio  | s__             | 0.2           | Proteobacteria | Sulfate reducing bacteria in IBD | FV             |
| 98     | Citrobacter    | s__             | 0.2           | Proteobacteria | Gut microbe in newly diagnosed with treatment-naïve Crohn's disease patients | FY             |
| 99     | Halomonas      | s__             | 0.9           | Proteobacteria | Intestinal microflora in chronic kidney disease | GB             |
| 100    | Aggregatibacter| s__             | 0.6           | Proteobacteria | Causes fungal microbiota dysbiosis in IBD | GC             |
| 101    | Haemophilus    | s__             | 0.3           | Proteobacteria | Treatment naïve microbiome in new onset Crohn's disease | GD             |
| 102    | Pseudomonas    | s__             | 0.8           | Proteobacteria | Infection in Children with Early-onset Crohn's Disease | GG             |
| 103    | g__ s__        | 0.2             | Proteobacteria | Bacteria in human Ulcerative Colitis patients | GH             |
| 104    | g__ s__        | 0.1             | TM7           | No role in IBD |                |
| 105    | Other          | Other           | 0.5           | Actinobacteria | Alters fecal microbiota in pediatric IBD patients | GO             |
| 106    | Eggerthella    | Other           | 0.1           | Actinobacteria | Causes bacteremia in Crohn’s disease patient | GQ             |
| 107    | Bacteroides    | Other           | 0.1           | Bacteroidetes | Commensal bacteria that induces colitis | GR             |
| 108    | Prevotella     | Other           | 0.7           | Bacteroidetes | A microbial signature of Crohn's disease | GS             |
| 109    | Other          | Other           | 3.2           | Firmicutes     | Gut microbe in experimental colitis | GT             |
| 110    | Other          | Other           | 7.5           | Firmicutes     | Fecal and mucosa associated microbe in IBD | GW             |
| 111    | Weissella      | Other           | 1.5           | Firmicutes     | Fecal microbe in IBD patients | GX             |
| 112    | Other          | Other           | 0.1           | Firmicutes     | Commensal gut bacteria in IBD | HA             |
| 113    | Other          | Other           | 1.1           | Proteobacteria | Causes microbial dysbiosis in pediatric Crohn's disease | HD             |
| 114    | Paracoccus     | Other           | 0.3           | Proteobacteria | Identified gut microbe in IBD patients | HH             |
| 115    | Other          | Other           | 0.1           | Proteobacteria | Fecal and mucosa associated microbe in IBD | HK             |
| 116    | Other          | Other           | 0.7           | Proteobacteria | Fecal and mucosa associated microbe in IBD | HL             |
| 117    | Other          | Other           | 0.1           | Proteobacteria | Identified gut microbe in IBD patients | HN             |
| 118    | Other          | Other           | 0.5           | Proteobacteria | Adult fecal microbe | HO             |

**African Americans**

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD | NCBI genome database link |
|---------|----------------|-----------------|----------------|-----------------|----------------|---------------------------|
| 1       | Lactobacillus | zeae             | 1.1            | Firmicutes      | Maintains remission of ulcerative colitis | A             |
| 2       | Prevotella    | stercordia      | 4.6            | Bacteroidetes   | Alters mucosal microbiota in the colon of patients with IBD | H             |
| 3       | Shuttleworthia| satellies       | 0.4            | Firmicutes      | Identified in the human ileum | J             |
| 4       | Acinetobacter | rhizosphaerae   | 0.2            | Proteobacteria  | Identified gut microbe in IBD patients | K             |
| 5       | Microbacterium| manntypicum     | 0.2            | Actinobacteria  | Fecal microbiome in Obesity | V             |
| 6       | Acinetobacter | lwolffii        | 0.5            | Proteobacteria  | Gut bacteria in multiple sclerosis patients | W             |
| 7       | Eggerthella   | lenta           | 0.7            | Actinobacteria  | Causes bacteremia in Crohn's disease patient. | AA            |
| 8       | Stenotrophomonas | geniculata   | 2.0            | Proteobacteria  | Identified gut microbe in IBD patients | AG            |
| 9       | Corynebacterium| durum           | 0.1            | Actinobacteria  | Identified gut microbe in IBD patients | AK            |
| 10      | Eubacterium   | dolichum        | 0.5            | Firmicutes      | Causes dysbiosis of the intestinal microbiota | AL            |
| 11      | Brevundimonas | diminuta        | 0.1            | Proteobacteria  | Identified in the adult fecal microbiota of allergy patients | AO            |

(Continued)
| Sl. No. | Bacteria genus     | Bacteria species         | Proportion (%) | Bacteria phylum | Function in IBD                                                                 | NCBI genome database link |
|---------|-------------------|--------------------------|----------------|-----------------|---------------------------------------------------------------------------------|---------------------------|
| 12      | Lysinibacillus    | boronitolerans           | 5.0            | Firmicutes      | Identified gut microbiome in IBD patients                                        | AT                        |
| 13      | Staphylococcus    | aureus                   | 0.2            | Firmicutes      | Causes Crohn's disease                                                           | AU                        |
| 14      | Peptostreptococcus| anaerobius               | 24.9           | Firmicutes      | Causes dysbiosis in IBD                                                           | AW                        |
| 15      | Pseudomonas       | alcaligenes              | 1.1            | Proteobacteria  | Identified in the gut microbiota of IBD                                          | AX                        |
| 16      | Propionibacterium | acnes                    | 0.6            | Actinobacteria  | Intestinal microbe in Liver disease                                               | BA                        |
| 17      | Methanobrevibacter| s__                      | 0.4            | Euryarchaeota   | Identified in the gut of IBD                                                      | BB                        |
| 18      | g__               | s__                      | 0.2            | Acidobacteria   | Identified in human gut microbiota                                               | BC                        |
| 19      | g__               | s__                      | 4.3            | Acidobacteria   | Identified in the gut microbiome of Type 2 Diabetes patients                    | BD                        |
| 20      | g__               | s__                      | 0.3            | Actinobacteria  | Identified in gut microbiota of IBD                                              | BE                        |
| 21      | Vanbaculum        | s__                      | 2.7            | Firmicutes      | Increases cytokine levels in IBD                                                  | CL                        |
| 22      | Cornyebacterium   | s__                      | 0.6            | Actinobacteria  | Identified in the gut of a premature infant                                      | BH                        |
| 23      | Arthrobacter      | s__                      | 0.5            | Actinobacteria  | Causes experimental colitis                                                       | BI                        |
| 24      | Slackia           | s__                      | 0.1            | Actinobacteria  | Fecal microflora in chronic IBD patients                                         | BL                        |
| 25      | Chrysobacterium   | s__                      | 0.3            | Bacteroidetes   | Alters human gut microbiome in Multiple Sclerosis                                | BS                        |
| 26      | Cloacibacterium   | s__                      | 0.2            | Bacteroidetes   | Fecal and mucosa associated microbe in IBD                                       | CF                        |
| 27      | g__               | s__                      | 0.2            | Cyanobacteria   | Gut microbe in IBD                                                               | DA                        |
| 28      | Bacillus          | s__                      | 0.3            | Firmicutes      | Gut microbe underlying the onset of IBD                                          | DB                        |
| 29      | Alloccoccus       | s__                      | 0.2            | Firmicutes      | Identical gut microbe                                                            | DC                        |
| 30      | Lactobacillus     | s__                      | 2.2            | Firmicutes      | Causes dysbiosis in IBD                                                           | DD                        |
| 31      | Lactococcus       | s__                      | 0.3            | Firmicutes      | Causes dysbiosis in IBD                                                           | DE                        |
| 32      | g__               | s__                      | 0.4            | Firmicutes      | Metabolizes Lincolic acid in the Gut                                              | DF                        |
| 33      | g__               | s__                      | 0.1            | Firmicutes      | Gut bacteria in Crohn's disease patients                                         | DG                        |
| 34      | Christensenella   | s__                      | 0.3            | Firmicutes      | A microbial signature of Crohn's disease                                         | DH                        |
| 35      | Clostridium       | s__                      | 0.3            | Firmicutes      | Increases cytokine levels in IBD                                                  | DJ                        |
| 36      | Pseudoramibacter  | Eubacterium              | 0.9            | Firmicutes      | Gut bacteria in Crohn's disease patients                                         | DK                        |
| 37      | Lachnospira       | s__                      | 0.3            | Firmicutes      | Gut bacteria in Crohn's disease patients                                         | DL                        |
| 38      | g__               | s__                      | 0.3            | Firmicutes      | A microbial signature of Crohn's disease                                         | DM                        |
| 39      | Peptostreptococcus| s__                      | 0.4            | Firmicutes      | Causes dysbiosis in IBD                                                           | DN                        |
| 40      | Oscillospira      | s__                      | 0.2            | Firmicutes      | Causes dysbiosis in IBD                                                           | DO                        |
| 41      | Ruminococcus      | s__                      | 0.2            | Firmicutes      | Causes dysbiosis in IBD                                                           | DP                        |
| 42      | g__               | s__                      | 0.4            | Firmicutes      | Causes dysbiosis in IBD                                                           | DQ                        |
| 43      | Acidaminococcus   | s__                      | 1.0            | Firmicutes      | Causes dysbiosis in IBD                                                           | DR                        |
| 44      | Phascolactobacterium | s__              | 0.5            | Firmicutes      | Causes dysbiosis in IBD                                                           | DS                        |
| 45      | Schwartzia        | s__                      | 0.1            | Firmicutes      | Causes dysbiosis in IBD                                                           | DT                        |
| 46      | Selenomonas       | s__                      | 0.3            | Firmicutes      | Causes dysbiosis in IBD                                                           | DU                        |
| 47      | Anaerococcus      | s__                      | 0.5            | Firmicutes      | Causes dysbiosis in IBD                                                           | DV                        |
| 48      | Finegoldia        | s__                      | 0.4            | Firmicutes      | Causes dysbiosis in IBD                                                           | DW                        |
| 49      | g__               | s__                      | 0.8            | Firmicutes      | Causes dysbiosis in IBD                                                           | DX                        |
| 50      | Bulleidia         | s__                      | 0.1            | Firmicutes      | Causes dysbiosis in IBD                                                           | EY                        |
| 51      | Fusobacterium     | s__                      | 0.4            | Fusobacteria    | Causes dysbiosis in IBD                                                           | EY                        |
| 52      | Leptotrichia      | s__                      | 1.7            | Fusobacteria    | Causes dysbiosis in IBD                                                           | EY                        |
| 53      | g__               | s__                      | 0.7            | Gemmatimonadetes | Identified gut microbiota of IBD patients                                         | EP                        |
| 54      | Ochrobactrum      | s__                      | 0.1            | Proteobacteria  | Causes early bacterial dependent induction of inducible nitric oxide synthase (iNOS) in epithelial cells in experimental colitis | EU                        |
| 55      | g__               | s__                      | 1.5            | Proteobacteria  | Intestinal microbe in children with severe and complicated acute viral gastroenteritis | EV                        |
TABLE 4 | Continued

| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD | NCBI genome database link |
|--------|----------------|------------------|----------------|-----------------|-----------------|--------------------------|
| 56     | g__ s__   | 0.1             | Proteobacteria | Involved in host-microbial cross talk in IBD | FG              |
| 57     | Sutterella | 0.2             | Proteobacteria | Gut microbe in experimental colitis | FH              |
| 58     | Burkholderia| 0.4             | Proteobacteria | Causes dysfunction of GALT and gut flora in IBD | FI              |
| 59     | Lautropia | 0.2             | Proteobacteria | Causes fecal microbial dysbiosis in IBD | FJ              |
| 60     | Comamonas | 0.4             | Proteobacteria | Identified gut microbe in IBD patients | FL              |
| 61     |Ralstonia | 0.2             | Proteobacteria | Microbiota in the Mucosa of Patients With Ulcerative Colitis | FP              |
| 62     | Bilophila | 0.1             | Proteobacteria | Causes irritable bowel syndrome | FU              |
| 63     | Desulfovibrio| 0.1           | Proteobacteria | Sulfate reducing bacteria in IBD | FV              |
| 64     | Halomonas | 0.7             | Proteobacteria | Intestinal microflora in chronic kidney disease | GB              |
| 65     | g__ s__   | 0.1             | Proteobacteria | Microbe in colon tissue from IBD subjects | GE              |
| 66     | Acinetobacter | 0.7         | Proteobacteria | Tissue associated intestinal microflora | GF              |
| 67     | Pseudomonas | 0.1             | Proteobacteria | Infection in Children with Early-onset Crohn's Disease | GG              |
| 68     | g__ s__   | 0.8             | TM7            | No role in IBD |                 |
| 69     | Other     | 0.3             | Actinobacteria | Alters fecal microbiota in pediatric IBD patients | GO              |
| 70     | Eggerthella| 0.1             | Actinobacteria | Causes bacteremia in Crohn's disease patient | GQ              |
| 71     | Bacteroides| 0.7             | Bacteroidetes | Commensal bacteria that induces colitis | GR              |
| 72     | Prevotella | 3.2             | Bacteroidetes | A microbial signature of Crohn's disease | GS              |
| 73     | Other     | 2.8             | Firmicutes     | Gut microbe in experimental colitis | GT              |
| 74     | Paenibacillus| 0.1            | Firmicutes     | Gut microbe in a healthy infant | GU              |
| 75     | Other     | 2.2             | Firmicutes     | Fecal and mucosa associated microbe in IBD | GW              |
| 76     | Paracoccus | 0.1             | Proteobacteria | Identified gut microbe in IBD patients | HH              |
| 77     | Other     | 0.6             | Proteobacteria | Fecal and mucosa associated microbe in IBD | HL              |
| 78     | Other     | 0.1             | Proteobacteria | Adult fecal microbe | HO              |
| 79     | Other     | 0.4             | Proteobacteria | Mucosal and fecal microbe | HP              |
| 80     | Acinetobacter | 2.2            | Proteobacteria | Tissue associated intestinal microflora in colitis patients | HQ              |
| 81     | Pseudomonas | 0.2             | Proteobacteria | Gut microbe in children with early onset Crohn's disease | HR              |

Specific Information of functions was adapted from NCBI Genome Database (https://www.ncbi.nlm.nih.gov/genome/).
The bacterial species that could not be identified at the genus level are mentioned as g___ and the bacterial species that could not be identified at the species level are mentioned as s___.

Compared to adjacent healthy colon specimens. We suggest that pathogenic bacteria belonging to these two phyla have a greater impact on colon motility function in colitis patients (Tables 1, 3). Although the incidence of IBD is increasing among African Americans (AA), the underlying causes are completely unknown (Sofia et al., 2014). Our study further highlight a significant disparity in bacterial dysbiosis among AA compared to CA colitis patients (Figure 3).

CA specimens had significantly higher levels of *Fusobacterium, Parabacteroides, Citrobacter, Haemophilus, Acinetobacter, Pseudomonas, and Stenotrophomonas*. *Fusobacterium nucleatum* is known to have a well-characterized role in the oral cavity. We have determined that *Fusobacterium* can be recovered from human full thickness colon specimens and this could indicate their ability to survive and proliferate inside host cells. *Parabacteroides* was found to be dominant in the acute phase of IBD in CA patients. *Citrobacter* is an epithelial cell adherent pathogen and can subvert inflammation in colitis. *Pseudomonas* interacts with the mucosal layer of colon and disrupts the mucosal barrier integrity leading to colitis in CA patients.

The AA specimens had significantly higher levels of *Prevotella* and *Clostridia*. *Prevotella* augments T-helper cells mediated colon mucosal inflammation by activating Toll-like receptor 2 leading to production of T-helper cells polarizing cytokines by antigen-presenting cells, including interleukins. In addition, *Prevotella* induce epithelial cells to produce interleukins and cytokines that can promote recruitment of neutrophils and mucosal T-helper cell immune responses. *Prevotella* could mediate inflammation of the mucosa leading to the circulation of bacteria, bacterial products and other inflammatory mediators. *Prevotella* could augment release of inflammatory mediators from immune cells and various stromal cells in colitis in AA patients. *Clostridium* can disrupt gut immune dormancy and cause infectious colitis in AA patients. Collectively, our data suggest that the presence of pathogenic bacteria in AA...
| Sl. No. | Bacteria genus | Bacteria species | Proportion (%) | Bacteria phylum | Function in IBD | NCBI genome database link |
|--------|----------------|-----------------|----------------|-----------------|-----------------|---------------------------|
| **ADJACENT HEALTHY COLON** | | | | | | |
| 1 | Micrococcus | luteus | 0.01 | Actinobacteria | No role in IBD | X |
| 2 | Arthrobacter | s__ | 0.01 | Actinobacteria | No role in IBD | BL |
| 3 | Propionicimonas | s__ | 0.01 | Actinobacteria | No role in IBD | BL |
| 4 | Paludibacter | s__ | 0.01 | Bacteroidetes | No role in IBD | BW |
| 5 | Chryseobacterium | s__ | 0.02 | Bacteroidetes | No role in IBD | CF |
| 6 | Calothrix | s__ | 0.03 | Cyanobacteria | No role in IBD | CK |
| 7 | Novosphingobium | s__ | 0.02 | Proteobacteria | No role in IBD | FE |
| **DISEASED COLON (ULCERATIVE COLITIS)** | | | | | | |
| 1 | Micrococcus | luteus | 0.01 | Actinobacteria | No role in IBD | X |
| 2 | Arthrobacter | s__ | 0.02 | Actinobacteria | No role in IBD | BL |
| 3 | Propionicimonas | s__ | 0.1 | Actinobacteria | No role in IBD | BN |
| 4 | Paludibacter | s__ | 0.03 | Bacteroidetes | No role in IBD | BW |
| 5 | Chryseobacterium | s__ | 0.1 | Bacteroidetes | No role in IBD | CF |
| 6 | Calothrix | s__ | 0.1 | Cyanobacteria | No role in IBD | CK |
| 7 | Novosphingobium | s__ | 0.04 | Proteobacteria | No role in IBD | FE |
| **DISEASED COLON (CROHN’S COLITIS)** | | | | | | |
| 1 | Micrococcus | luteus | 0.02 | Actinobacteria | No role in IBD | X |
| 2 | Arthrobacter | s__ | 0.2 | Actinobacteria | No role in IBD | BL |
| 3 | Propionicimonas | s__ | 0.02 | Actinobacteria | No role in IBD | BN |
| 4 | Paludibacter | s__ | 0.02 | Bacteroidetes | No role in IBD | BW |
| 5 | Chryseobacterium | s__ | 0.3 | Bacteroidetes | No role in IBD | CF |
| 6 | Calothrix | s__ | 0.1 | Cyanobacteria | No role in IBD | CK |
| 7 | Novosphingobium | s__ | 0.01 | Proteobacteria | No role in IBD | FE |

Specific Information of functions was adapted from NCBI Genome Database [https://www.ncbi.nlm.nih.gov/genome/].

The bacterial species that could not be identified at the genus level are mentioned as g__ and the bacterial species that could not be identified at the species level are mentioned as s__.

full thickness diseased specimens could adversely affect colon motility.

Additionally, our data in UC and CC specimens show the presence of several orange (Prevotella, Peptostreptococcus, Eubacterium, Fusobacterium, and Campylobacter), red (Porphyromonas), purple (Veillonella), and yellow (Streptococcus) complex putative oral pathogens known to cause gingivitis and periodontitis among IBD patients (Tables 1, 3). Previous studies using mucosal biopsies and feces have shown that gut microbiota in bowel diseases is characterized by an increase in certain phyla such as Proteobacteria, Firmicutes, genus Bifidobacterium, as well as a reduction in the amounts of genera Ruminococcus, Clostridia and (in some cases) Faecalibacterium (Lane et al., 2017; Nishida et al., 2018).

However, none of the earlier studies using feces have shown a shift in the balance between Phyla Bacteroidetes and Firmicutes among UC or CC patients; even though this was observed in healthy individuals (Mariat et al., 2009; Koliada et al., 2017). In contrary to our results, one study using mucosal biopsies has shown a significantly decreased Firmicutes to Bacteroidetes ratio in both UC and CC compared with controls (Kabeerdoss et al., 2015). Collectively, our data suggest that the putative oral pathogens found in diseased colon specimens may modulate the proportion of non-detrimental gut bacteria, thus potentially worsening the condition of the colon in colitis patients.

Oral bacterial species like Porphyromonas, Peptostreptococcus, Eubacterium, Fusobacterium, Streptococcus salivarius, S. mitis, S. bovis, Veillonella spp., Staphylococcus aureus, S. epidermidis, and Campylobacter spp. can convert nitrate to nitrite. A large amount of bioactive NO is found in the gastrointestinal tract, generated by dietary sources and by conversion of anaerobic bacteria in the oral cavity, or by anaerobic reaction with nitrate in the colon by Escherichia coli spp. The entero-salivary nitrate conversion pathway provides a rich source of bioactive NO and nitrate-reducing bacteria, such as Veillonella. In this pathway, nitrate is obtained by the salivary gland and is then concentrated in the saliva. Various facultative anaerobic bacteria on the top of the tongue effectively reduces nitrate to nitrite. The bacteria then use the nitrate and the nitrite as electron acceptors in their respiration process. This also helps the host in the first steps of converting nitrate to NO. The salivary nitrate then reaches the systemic circulation, various enzymatic reactions occur leading to reduction to NO, and other reactive nitrogen intermediates. The oral cavity plays an important role the production of nitric oxide, and specifically, employs the nitrate-nitrite-NO pathway in the oral cavity. It is well known that oral cavity bacteria can migrate to the colon. Taken together, our data suggest that the
putative oral pathogens found in diseased colon specimens may survive by exploiting the nitrate-nitrite-NO pathway to modulate the proportion of non-detrimental gut bacteria, thus potentially worsening the condition of colon in colitis patients (Figure 4).

Previous studies suggest that enteric neurons and smooth muscle mediated gut motility is impaired in colitis patients (Snape et al., 1991; Vermillion et al., 1993). IBD associated gut inflammation affects the morphological and functional changes in the myenteric/enteric nervous system (ENS) and nitric oxide (NO) synthesis (Takahashi, 2003; Kono et al., 2004). Experimental studies have also shown that gut bacteria have a role in oxidative stress induced gut inflammation by controlling metabolic endotoxemia in obese mice (Cani et al., 2008). We have shown that polybacterial oral infection decrease the expression of nNOS and NRF2-phase II enzymes in the gut and this could lead to impaired colon motility (Gangula et al., 2015; Walker et al., 2018).

Some of the gut bacteria we have identified in the full thickness colon specimens in the present study, including Bacteroides, Prevotella, Pseudomonas, etc., have been identified in colon mucosal biopsies in earlier studies (Bibiloni et al., 2006). These bacteria evoke inflammatory responses affecting the innermost lining of colon. Many specific beneficial bacteria, including members of Bacteroides and Prevotella groups, C. coccoides, and Lactic acid bacteria were known to be decreased in colitis patients (Gibson et al., 1991). Specimens used in prior studies were colon mucosal biopsies or stool samples; but not full thickness colon specimens (Gibson et al., 1991; Bibiloni et al., 2006). Full-thickness colon consists of four layers of tissue including mucosa, submucosa, muscularis, and serosa.

Novel to this research design, full thickness colon specimens were obtained because colitis patients often experience colon motility abnormalities (Snape et al., 1991; Annese et al., 1997; Vrees et al., 2002). Several lines of evidence suggest that nitrergic neurons that releases NO via nNOS are known to play a pivotal role in colon motility (Kono et al., 2004; Winston et al., 2013). Previous studies have demonstrated that nitrergic neurons are degenerated in colitis (Onori et al., 2005; Sung et al., 2006). Recent studies from our laboratory indicate that nNOS, as well as antioxidants (NRF2 regulated-Phase II enzymes) protein expression are down-regulated in diseased colon specimens (Myers et al., 2014; Gangula et al., 2017). Furthermore, our previous studies demonstrated that polybacterial infection led to a decrease in nNOS, NRF2 and antioxidants protein expression in the colon tissues (Gangula et al., 2015). In addition, studies have shown that NO may play homeostatic role in gut inflammation (Kolios et al., 2004). Taken together, our data suggest that elevated levels of oral and gut pathogens in diseased colon full thickness specimens could contribute to impaired nNOS-NO-NRF2-Phase II system and colon motility abnormalities in IBD patients (Figure 4).

To our knowledge, our study is the first to report the presence of several microbiota of unknown function in IBD including Micrococcus luteus, Chloracidobacteria, Arthrobacter, Propionibacterium, Paludibacter, Chryseobacterium, Calothrix, and Novosphingobium (Table 5). These new microbiota members have not been identified in mucosal/fecal specimens in previous studies, suggesting that these bacteria are primarily colonized in the neuromuscular compartment. Additional studies are warranted to characterize the novel bacteria and investigate their specific role in colon motility and constipation in IBD patients.

In summary, this study have identified specific bacterial pathogens potentially associated with colon motility in IBD patients. The observations showed that some putative oral pathogens belonging to the Phyla Firmicutes (Streptococcus, Staphylococcus, Peptostreptococcus), and Fusobacteria (Fusobacterium) dominated in the microbiomes of CC and UC diseased specimens and might involve the modulation of colon motility in IBD.

**STUDY LIMITATIONS**

The limitations of the study include the smaller sample size across disease and race groups making this as a preliminary study. In spite of the limitations in sample size and the fact that some of the identified bacteria were not significantly altered in colitis specimens, we were still able to observe differences in the microbiome between CA and AA colitis patients. This could be due to amplicon sequencing of a shorter conserved region of 16S rRNA gene instead of in depth shotgun sequencing.

**FIGURE 2 | Perturbation of the full thickness colon microbiome in Colitis specimens as compared to adjacent healthy specimens.** Specimens are categorized into adjacent healthy colon (n = 13), Ulcerative colitis (UC, n = 13) and Crohn’s colitis (CC, n = 13). Data represented are the mean of relative abundances of each phylum identified in specimens belonging to each group while error bars indicate standard error. Diseased specimens demonstrate a balance between the Phyla Firmicutes and Bacteroidetes. Conversely, healthy colon specimens demonstrate a significantly higher proportion of Phyla Bacteroidetes. *p < 0.05 by Mann-Whitney U Test (n = 13 under each group).
FIGURE 3 | Summary of major bacterial taxa showing the relative abundance of oral and gut bacteria at the Phylum level in the colitis and adjacent healthy specimen groups under each race. Data represented are the mean of relative abundances of each Phyla detected in samples belonging to each group. The dominant phyla across all samples (both diseased and healthy specimens) were Bacteroidetes, followed by Firmicutes and Proteobacteria. Other major phyla observed among these specimens also include Actinobacteria, Fusobacteria, and Synergistetes. The Phylum Proteobacteria did not show any significant difference between healthy colon specimens and diseased colon specimens. A larger proportion of unassigned bacteria (0.3%) was identified in AA Crohn’s Colitis patients compared to other groups.

FIGURE 4 | Schematic Representation of the suggested mechanism involved in the development of colitis by oral and gut microbiome. We propose that the increase in the concentrations of putative oral pathogens elevates the cytokine and chemokine levels in oral cavity. When putative oral pathogens travel to the gut, they can colonize locally and lead to the elevated levels of proinflammatory cytokines. This can effect on nNOS-NO-NRF2-Phase II system in the large intestine and could lead to colon dysmotility and colitis.
Moreover, we did not profile the oral microbiome from oral specimens (dental plaque, etc.) in the same IBD patients from whom full thickness colon specimens were collected. Finally, host-microbiome interaction studies are needed to better discern specific roles of the oral and gut bacteria in the development of colitis. Future studies are aimed to collect oral and fecal specimens therefore a comparative experiments in regards to changes in microbiome, along with specific key proteins will be conducted from the same patient.

**AUTHOR CONTRIBUTIONS**

VD, SM, KS, SP, SS, PG, and MT have contributed both for data analysis and manuscript preparation. DS, CF-D, LK, SA, and JS have contributed in manuscript preparation.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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