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FULL PAPER

Bacterial network community in fecal and endoluminal Microbiota after colonoscopy

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SUMMARY

The gut microbiota is a complex and dynamic ecosystem with a strong influence on the host's health. Several factors can modify the gut's bacterial composition, often leading to the onset of intestinal dysbiosis. Therefore, it is essential not only to evaluate the quantitative bacterial changes occurring in the human microbiota but also to characterize relationships existing among all the microorganisms. This study aimed to evaluate the impact of bowel cleansing on the fecal microbiota network by highlighting differences between fecal microflora before and after colonoscopy, and luminal samples during colonoscopy. Fecal and luminal samples, previously analyzed by mean of Next-Generation Sequencing (NGS) for their bacterial abundance, were further processed by a method based on Artificial Neural Network (ANN) architecture. The bowel lavage had a strong effect on the intestinal microbiota network, leading to significant changes in the distribution of different bacterial hubs potentially involved in the microbiota homeostasis. Furthermore, the fecal and luminal microbiota showed a different bacterial network, characterized by distinct microbial hubs. In particular, the latter seemed to be rich in potentially pathogenic bacteria which, in physiological conditions, are counteracted by fecal microorganisms.

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INTRODUCTION

The gut microbiota is a complex and variable ecosystem composed of hundreds of different bacterial species. Commensal bacteria of the gastrointestinal tract (GI) perform many essential functions that are fundamental for the development and maintenance of the host's health (Jalanka *et al*., 2015; Lu *et al*., 2015). In particular, they protect the organism from the action of pathogens, regulate the host fat storage, stimulate the immune system and intestinal angiogenesis and digest numerous dietary components (Bäckhed *et al*., 2014; Andriessen *et al*., 2016; Sajib *et al*., 2018). However, the gut microbiota is often associated with several human pathologies, especially when dysbiosis occurs (Biedermann and Rogler, 2015).

Inflammatory bowel disease, obesity, colon cancer, and atopic diseases are only a small number of ailments in which the gut dysbiosis is involved, even if to date it is not clear if the dysregulation of bacterial homeostasis is the leading cause or only the consequence of the aforementioned diseases (Drago *et al*., 2012; Harley and Karp C, 2012; Chen *et al*., 2014, Tilg *et al*., 2018). To date, the main limitation of the majority of studies on the characterization of the gut microbiota has been the use of fecal samples as representative of the whole intestinal microbiota, without dwelling on potential differences due to specific anatomical sites (Jalanka *et al*., 2015; Lu *et al*., 2015). The GI, indeed, is a complex structure subjected to different influences and stimuli leading to significant metabolic, anatomical, physiological and biochemical differences along its entire length (Donaldson *et al*., 2016). Consequently, the intestinal bacterial population is also subjected to different factors and influences, depending on the specific GI site under consideration. Furthermore, the gut microbiota is influenced by several factors that modify its composition and diversity, such as diet, age, lifestyle, host genetics, antibiotic therapy and environment (Rodríguez *et al*., 2015). In addition, colonoscopy, and in particular the bowel lavage that precedes the endoscopic examination, has been observed to significantly alter the composition of the intestinal microflora up to one month after the bowel cleansing (Drago *et al*., 2016). The direct effect of colonoscopy is an induced intestinal dysbiosis that could hurt the host's health (Drago *et al*., 2016).

Furthermore, when the gut microbiota or the effect of specific events on the microflora composition has to be characterized, the relative abundance of each microorganism within the ecosystem but also the relation and interaction between all microorganisms should be considered as well. Bacteria, archaea, viruses, and fungi form intricate ecological connection webs that can have negative, positive or no impact on the different species involved in the network (Faust and Raes, 2012). Bacterial interactions are essential for microorganisms to survive in the host's organism, as they can cooperate to maximize the use of nutrients and energy sources, and for pathogens, to acquire antibi-

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otic resistance and escape from the host's immune system (Baldassano and Bassett, 2016). Microbial biofilm is an example of bacterial cooperation in which microorganisms interact not only with the environment but also with the other microorganisms present in the biofilm. These interactions lead to a selective advantage, allowing bacteria to persist and colonize the environment (Baldassano and Bassett, 2016).

This work aimed to characterize the fecal microbiota network before and after colonoscopy, to evaluate if bowel cleansing was able to modify bacterial relationships. Moreover, we characterized the luminal microbiota network and evaluated how bacteria interactions change in relation to the different intestinal environment samples under consideration. We used a methodology based on Artificial Neural Network (ANN) architecture, the Auto Contractive Map (AutoCM), to study bacterial relations in the microbiota network, performing data mining with advanced complex mathematic systems to assess the pattern of commensal clustering (Drago *et al.*, 2017).

MATERIALS AND METHODS

Study population

The population considered for this network evaluation was previously analyzed for their gut microbiota abundances after colonoscopy by Drago *et al.* Patients characteristics are described in the paper by Drago *et al.* (Drago *et al.*, 2016). The evaluation was conducted according to ICH guidelines for Good Clinical Practice and also positively approved by the Scientific Committee and Scientific Direction of Bolognini Hospital (Seriate, Italy). Network analysis was conducted on fecal samples collected one week before and one month after colonoscopy. Luminal samples (one for each patient) were collected during the endoscopic procedure.

Bacterial DNA extraction,16S gene sequencing, and data analysis

Total DNA extraction, DNA amplification, and gene sequencing were performed as previously commented (Drago *et al.*, 2016), and data were re-analyzed as described below according to the Auto Contractive Map.

The Auto Contractive Map (AutoCM)

The human-microbe mutualism is widely studied in system biology and can be explained by complex mathematical networks, based on the collection of large amounts of data per single subject. The Auto Contractive Map (AutoCM) approach was used in this study to try to depict this complex network.

The Auto-CM system is a fourth-generation unsupervised artificial neural network (ANN) able to illustrate the natural connections occurring among variables with a graph based on minimum spanning tree theory, in which distances among variables are related to the weights of the ANN after a successful training phase (Buscema *et al.*, 2008a; Buscema *et al.*, 2008b).

A learning algorithm finds a square matrix of "similarities" among the bacterial abundances. As described previously (Drago *et al.*, 2017), the learning algorithm of Auto-CM is based on four steps:

a) transfer of the signal from the Input into the Hidden layer;
b) adaptation of the connections value between the Input layer and the Hidden layer;
c) transfer of the signal from the Hidden layer into the Output layer; d) adaptation of the connections value between the Hidden layer and the Output layer.

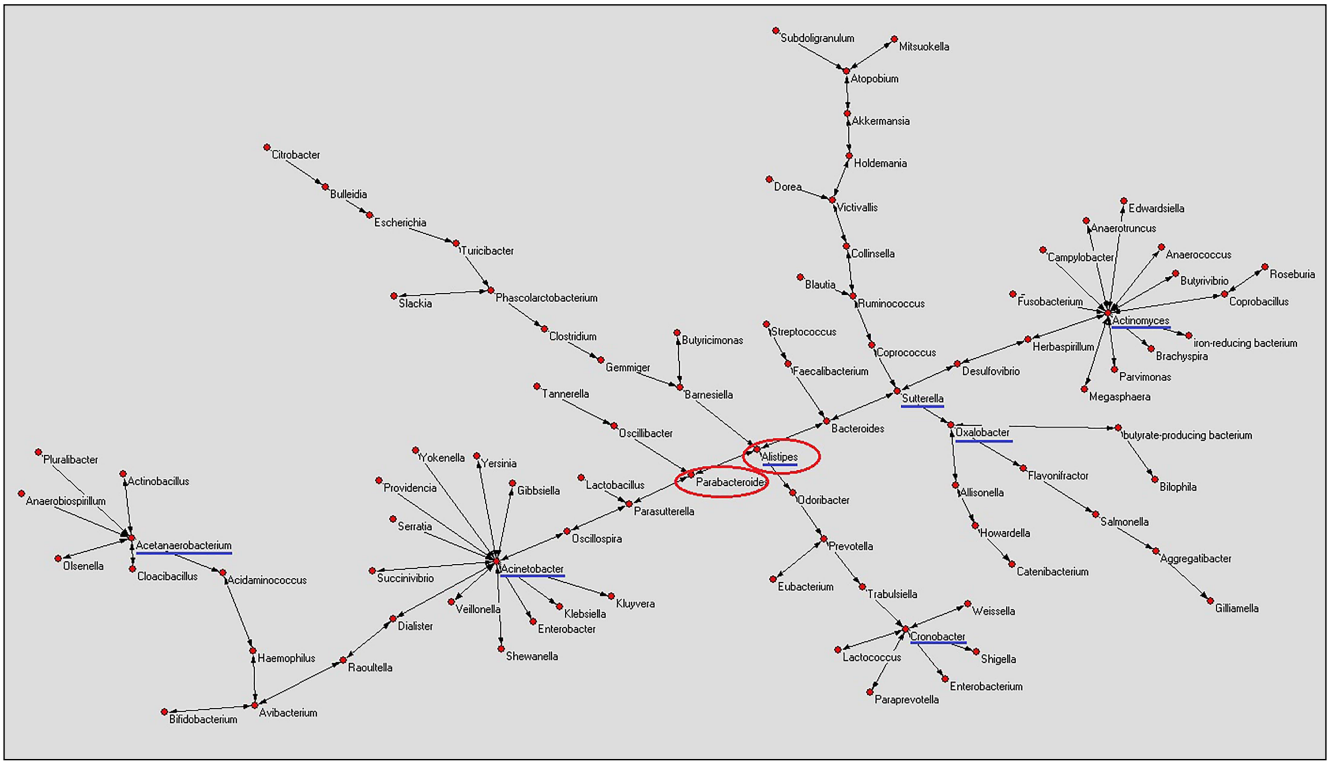

Figure 1 - Fecal microbiota network before the bowel cleansing. *The main* hubs *of the bacterial network are underlined with a blue line; red circles show the* central nodes *of the network.*

The AutoCM Neural Network does not arbitrarily assign initial weights but always starts with the same value. In other words, AutoCM can identify only the relevant connections and organizes them into a coherent picture, building a global representation of the whole pattern of variation.
After the determination of matrix weight, the Minimum Spanning Tree algorithm (MST) cleans the matrix (Kruskal *et al.*, 1956) and shows the shortest possible combination among all the possible ways to connect the bacterial abundances in a tree. All the connections that generate cycles are eliminated to reduce and make a simpler graphic representation. This proofreading check is based on the assumption that all biological systems naturally tend to minimal energetic states, making this graphic representation the fundamental biological source of information of the system. Therefore, the final graph is perfectly reproducible, along with many possible runs.
The last target of this mathematical model is to untangle hidden associations among variables. This approach generates the map of relevant inter- and intra-connections of variables and the principal hubs of the system. Hubs are the bacterial genera with the maximum amount of connections on the map.
Moreover, the "central node" is the inner node, the last remaining node after the isolated ends of the graph are cleaned. See the Supplementary Data for a graphic description of the MST concept and a detailed description of the theory and functioning of this analytic technique.

RESULTS

The bacterial hubs characterized in the present study were different in all groups analyzed.
In particular, in fecal samples taken one week before colonoscopy, the leading bacterial *hubs* were *Acetanaerobacterium* spp, *Acinetobacter* spp, *Actinomyces* spp, *Alistipes* spp, *Cronobacter* spp, *Oxalobacter* spp and *Sutterella* spp. The *central nodes* of this bacterial network were represented by *Alistipes* spp and *Parabacteroides* spp (*Figure 1*).
One month after bowel cleansing, the microbiota network showed a significantly different bacterial distribution, as the main *hubs* were *Acetanaerobacterium* spp, *Bacteroides* spp, *Brachyspira* spp, *Butyrivibrio*, *Catenibacterium* spp and *Faecalibacterium* spp, and *Phascolarctobacterium* spp.; *Paraprevotella* spp and *Butyricimonas* spp represented the *central nodes* of this bacterial network. Furthermore, *Acetanaerobacterium* represented the most significant *hub* of this network as it was connected with 46 bacterial branches (*Figure 2*). Finally, the luminal network showed different bacterial *hubs* when compared to fecal microbiota, such as *Acidaminococcus* spp, *Actinobacillus* spp, *Actinomyces* spp, *Brachyspira* spp, *Campylobacter* spp, *Dorea* spp and *Parabacteroides* spp, with *Parabacteroides* spp representing the *central node* of the whole network (*Figure 3*).

DISCUSSION

After bowel cleansing, a significant change in the fecal microbiota network was observed. Indeed, the intestinal lavage seemed able to change bacterial interactions in the fecal microbiota, as different bacterial *hubs* were highlighted up to one month after colonoscopy when compared with samples collected before the endoscopic examination. One of the main *hubs* observed before colonoscopy was represented by *Acinetobacter*, a multi-drug resistant bacterium often involved in urinary and respiratory infections in immunocompromised subjects (Cheng *et al.*, 2015). However, previous studies showed the abil-

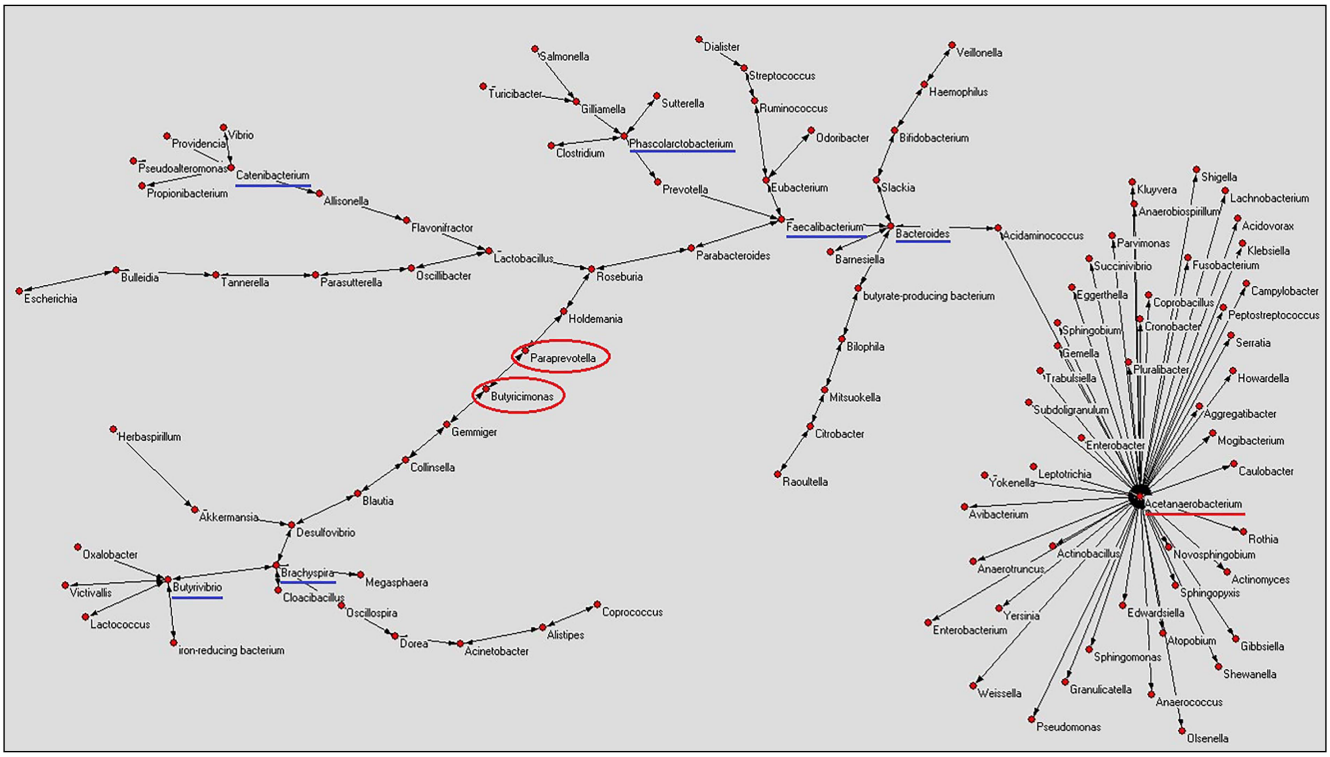

Figure 2 - Fecal microbiota network after the bowel cleansing. *The main* hubs *of the bacterial network are underlined with a blue line; red circles show the* central nodes *of the network.*

ity of some species belonging to the *Acinetobacter* genus to improve allergic responses in murine models, leading to a polarization of T-cell towards Th1 response by inducing the production of Interleukin 12 (IL-12) (Biedermann and Rogler, 2015; von Martels *et al*., 2017; Sommer *et al*., 2017). Interestingly, in our study, *Acinetobacter* was directly related to several bacteria belonging to the *Enterobacteriaceae* family, such as *Yersinia*, *Klebsiella*, *Shewanella*, *Providencia*, *Enterobacter*, *Kluyvera*, *Yokenella* and *Serratia*, which can act as opportunistic pathogens when intestinal dysbiosis occurs. Conversely, after bowel cleansing, *Acinetobacter* did not represent a network *hub,* and the majority of *Enterobacteriaceae* were connected to *Acetanaerobacterium*, a bacterial genus able to ferment glucose to acetate or butyrate (Hullar *et al*., 2015). Interestingly, this bacterial genus, which showed a higher number of bacterial connections after colonoscopy, represents the only bacterial *hub* shared by the microbiota network before and after bowel cleansing. Moreover, *Oxalobacter*, an anaerobic microorganism, represents another *hub* of the gut microbiota network before colonoscopy. *Oxalobacter formigenes*, in particular, is the main microorganism involved in oxalic acid homeostasis (Stewart *et al*., 2004). Oxalate is ingested with food and excreted in urine; however, the accumulation of a large amount of this compound may lead to paralysis of the nervous system, precipitation of blood calcium, and skin corrosion (Stewart *et al*., 2004). Furthermore, when urinary oxalate concentration is excessive, the development of calcium oxalate kidney stone disease has been observed (Stewart *et al*., 2004). Consequently, *O. formigenes* plays an essential role in the human organism, as its absence may predispose individuals to kidney stone disease (Mittal and Kumar, 2004). For this reason, the administration of *O. formigenes* or enzymes involved in oxalate degradation has been proposed as an effective and promising approach to reduce urinary oxalate levels (Mittal and Kumar, 2004). Also, *Alistipes* seemed to have a pivotal role in the physiological gut microbiota, as this bacterial genus has already been observed to be more abundant in the gut of healthy subjects than in patients with non-alcoholic fatty liver disease (NAFLD). The main hypothesis is that *Alistipes* may play a beneficial role in the human gut, protecting against the development of NAFDL (Jiang *et al*., 2015a). Other authors have reported the beneficial role of this genus (with *Butyricimonas* and *Oscillospira*) as a putative microbial biomarker of Clostridium Difficile Infections (CDI) (Milani *et al*., 2016). As *Alistipes* also represents a *central node* in the intestinal microbiota network before colonoscopy and not after bowel cleansing, we firmly believe that it may positively influence the host's health. However, this finding is not concordant with the literature, in which many authors have reported the negative role of *Alistipes* genus, as it is associated with abdominal pain in patients with irritable bowel syndrome (Saulnier *et al*., 2011; Jiang *et al*., 2015b), and with colorectal carcinoma via IL-6-STAT3 pathway activation (Moschen *et al*., 2016). The other *central hub* observed before colonoscopy is represented by *Parabacteroides*, an anaerobic bacterial genus able to produce a large spectrum of bacteriocins, proteins or peptides of different sizes characterized by their strong bactericidal activity (Nakano *et al*., 2006). The ability of *Parabacteroides* to release bacteriocins into the intestinal lumen is of great importance since these molecules are effective against several species of *Enterobacteriaceae*, gram-positive cocci and *Pseudomonas aeruginosa*, and contribute to the maintenance of intestinal homeostasis (Nakano *et al*., 2006). These results

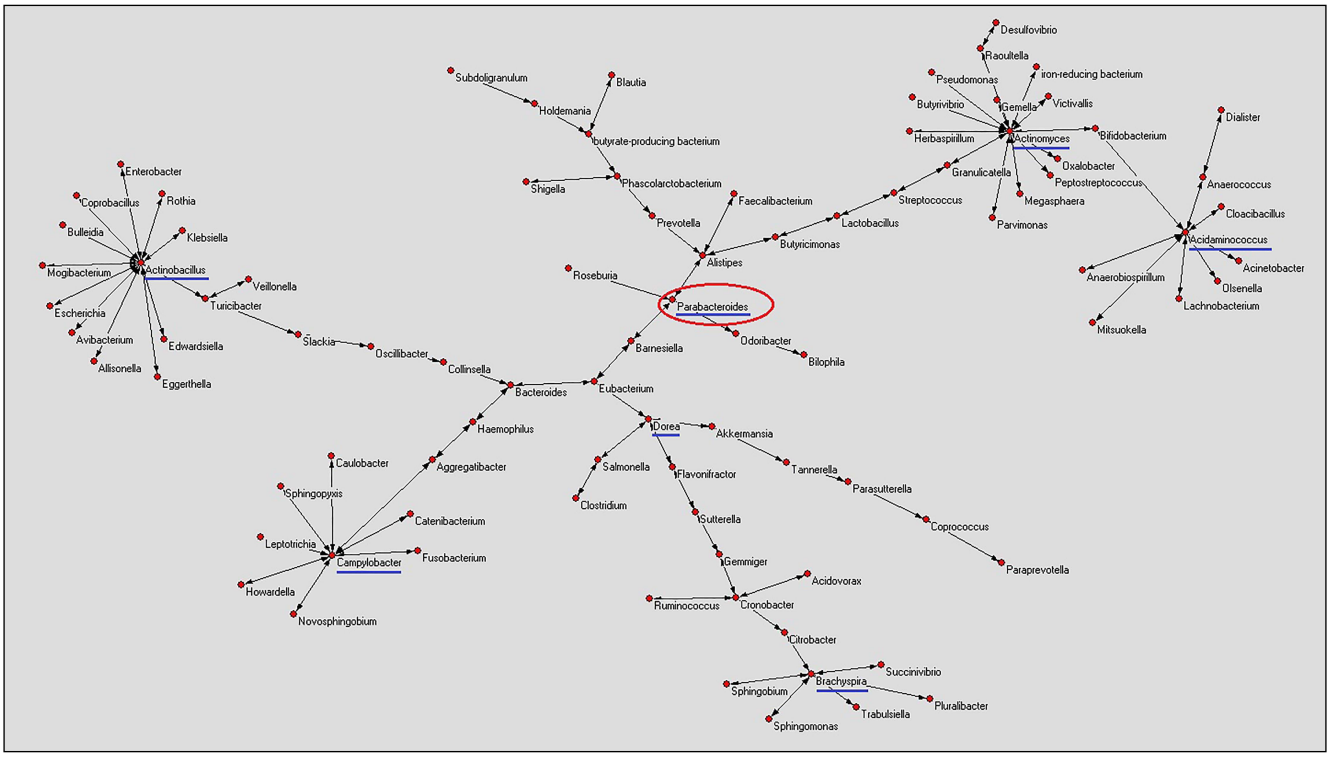

Figure 3 - Luminal microbiota network. *The main* hubs *of the bacterial network are underlined with a blue line; the red circle shows the* central node *of the network.*

suggest that in the physiological gut microbiota network, *Alistipes* and *Parabacteroides* may exert a pivotal role in establishing intestine health and also prevent the onset of intestinal dysbiosis. Instead, after bowel cleansing, the gut microbiota *central nodes* were represented by *Butyricimonas* and *Paraprevotella*. The former, characterized by anti-inflammatory properties, is reduced in patients affected by multiple sclerosis and is positively correlated with colon transit time and stool firmness (Vandeputte *et al*., 2016). The latter, conversely, seems to be increased in patients suffering from colonic Crohn's disease and is probably involved in the progression and worsening of the pathology (Walters *et al*., 2014). Although the role of *Butyricimonas* as a central node may have a positive outcome in intestinal microbiota physiology, the presence of the genus *Paraprevotella* as a second *central hub* could negatively impact intestinal homeostasis and the host's health. Indeed, this bacterial genus has been observed to be increased in individuals with colorectal cancer, having a potential negative impact on the host (Tilg *et al*., 2018). When fecal samples before and after colonoscopy were compared, some other interesting changes in the microbiota network were noted. Indeed, *Bacteroides* and *Butyrivibrio* represented two of the main bacterial *hubs* found one month after bowel cleansing; these were not detected before the endoscopic examination. These bacterial genera are of great importance in the host's intestine due to the beneficial roles they play. *Bacteroides*, indeed, produce several bacteriocins able to contrast colonization by pathogens (Braga *et al*., 2019), while *Butyrivibrio* is a butyrate-producing bacterium that has already been observed to have a beneficial effect on a colorectal cancer model (Ohkawara *et al*., 2005). Interestingly, the luminal microbiota network appeared significantly different when compared with the fecal one, both before and after colonoscopy. The main *hubs* of the luminal network were *Acidaminococcus*, *Dorea*, *Actinobacillus* and *Campylobacter*. *Acidaminococcus* and *Dorea* are often associated with growth deficits in infants and irritable bowel syndrome, respectively, while *Campylobacter* is the most common cause of enteritis in developed countries and is normally counteracted by the physiological intestinal microbiota (Dicksved *et al*., 2014). Consequently, the reduction of several fecal bacterial genera and species due to bowel cleansing may lead to a significant proliferation of *Campylobacter*, with negative impact on the host's health. In physiological conditions, colonization of potential pathogens is limited by the presence of commensal fecal microorganisms; however, when an intestinal dysbiosis occurs, harmful bacteria can proliferate, leading to negative effects on the human organism.

It is difficult to understand the biological role of microbial networks and their interaction meaning. Indeed, some bacterial clusters may have a biological meaning when they are alone, and another, or the opposite, when they interact with nearby bacteria. Much still needs to be done to give hubs a biological role in the microbiota.

Collectively, our results suggest that bowel cleansing may have a significant and sometimes negative impact on human health because, after this procedure, the gut environment is exposed to several pathobiont microorganisms that can interact negatively with the host. A better understanding of all bacterial interactions occurring in the gut microbiota may contribute to the development of new strategies to maintain and improve human health.

Competing interests

The authors declare that they have no competing interests.

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References

Andriessen E.M.M.A., Wilson A.M., Mawambo G., Dejda A., Miloudi K., et al. (2016). Gut microbiota influences pathological angiogenesis in obesity-driven choroidal neovascularization. *EMBO Molecular Medicine*. **8**, 1366-1379.

Bäckhed F., Ding H., Wang T., Hooper L.V., Koh G.Y., et al. (2014). The gut microbiota as an environmental factor that regulates fat storage. *PNAS*. **101**, 15718-15723.

Baldassano S.N., Bassett D.S. (2016). Topological distortion and reorganized modular structure of gut microbial co-occurrence networks in inflammatory bowel disease. *Scientific Reports*. **6**, 1-14.

Biedermann L., Rogler G. (2015). The intestinal microbiota: its role in health and disease. *Eur J Pediatr*. **174**, 151-167.

Braga M.N.P., Guimarães N.R., Oliveira J.S., dos Santos S.G., Bemquerer M.P., Magalhães P.P. (2019). Characterization of an antagonistic peptide produced by a Bacteroides Fragilis isolate obtained from a patient with intra-abdominal infection. *Braz. J. of Develop.* **12**, 32316-32345.

Buscema M., Grossi E., Snowdon D., Antonio P. (2008a). Auto-Contractive Maps: An Artificial Adaptive System for Data Mining. An Application to Alzheimer Disease. *Curr. Alzheimer. Res.* **5**, 481-498.

Buscema M., Grossi E. (2008b). The semantic connectivity map: an adapting self-organising knowledge discovery method in data bases. Experience in gastro-oesophageal reflux disease. *Int. J. Data Min. Bioinform.* **2**, 362-404.

Chen L., Wang W., Zhou R., Ng S.C., Li J, et al. (2014). Characteristics of fecal and mucosal-associated microbiota in Chinese patients with inflammatory bowel disease. *Medicine*. **93**, e51.

Cheng V.C.C., Chen J.H.K., So S.Y.C., Wong S.C.Y., Yan M.K., Chau P.H., et al. (2015). Use of fluoroquinolones is the single most important risk factor for the high bacterial load in patients with nasal and gastrointestinal colonization by multidrug-resistant Acinetobacter baumannii Eur J Clin *Microbiol Infect Dis.* **34**, 2359-2366.

Dicksved J., Ellström P., Engstrand L., Rautelin H. (2014). Susceptibility to Campylobacter Infection Is Associated with the Species Composition of the Human Fecal Microbiota. *MBio*. **5**, e01212-e01214.

Donaldson G.P., Lee S.M., Mazmanian S.K. (2016). Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.***14**, 20-32.

Drago L., Toscano M., De Grandi R., Grossi E., Padovani E.M., Peroni D.G. (2017) Microbiota network and mathematic microbe mutualism in colostrum and mature milk collected in two different geographic areas: Italy versus Burundi. *The ISME Journal*. **11**, 875-884.

Drago L., Toscano M., De Grandi R., Casini V., Pace F. (2016). Persisting changes of intestinal microbiota after bowel lavage and colonoscopy. *Eur. J. Gastroenterol. Hepathol.* **28**, 532-537.

Drago L., Toscano M., De Vecchi E., Piconi S., Iemoli E. (2012). Changing of Fecal Flora and Clinical Effect of *L. salivarius* LS01 in Adults With Atopic Dermatitis. *J. Clin. Gastroenterol.* **46**, S56-63.

Faust K., Raes J. (2012). Microbial interactions: from networks to models. *Nature.* **10**, 538-550.

Harley IT, Karp C. (2012). Obesity and the gut microbiome: Striving for causality. *Mol. Metab*. **1**, 21-31.

Hullar M.A., Lancaster S.M., Li F., Tseng E, Beer K. (2015). Enterolignan-producing phenotypes are associated with increased gut microbial diversity and altered composition in premenopausal women in the United States. *Cancer Epidemiol. Biomarkers Prev.* **24**, 546-554.

Jalanka J., Salonen A., Salojärvi J., Ritari J., Immonen O., Marciani L., et al. (2015). Effects of bowel cleansing on the intestinal microbiota. *Gut*. **64**, 1562-1568.

Jiang W., Wu N., Wang X., Chi Y., Zhang Y., Qiu X., et al. (2015a). Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci. Rep*. **5**, 8096.

Jiang H., Ling Z., Zhang Y., Mao H., Ma Z., Yin Y. (2015b). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, Behavior, and Immunity.* **48**, 186-194.

Weihong Wang E., Wenxin Tang C., Zhonglin Tan C., Jianfei Shi C., Lanjuan Li A., Bing Ruan A.,⇑

Kruskal J.B. Jr. (1956). On the Shortest Spanning Subtree of a Graph and the Traveling Salesman Problem. *Proc. Am. Math. Soc*. **7**, 48-50.

Lu K., Mahbub R., Fox J.G. (2015). Xenobiotics: Interaction with the Intestinal Microflora. *ILAR Journal*. **56**, 218-227.

Milani C., Ticinesi A., Gerritsen J., Nouvenne A., Lugli G.A., Mancabelli L., et al. (2016). Gut microbiota composition and *Clostridium difficile* infection in hospitalized elderly individuals: a metagenomic study. *Scientific Reports*. **6**, 1-12.

Mittal R.D., Kumar R. (2004). Gut-inhabiting bacterium *Oxalobacter formigenes*: role in calcium oxalate urolithiasis. *J. Endourol.* **18**, 418-424.

Moschen A.R., Gerner R.R., Wang J., Klepsch V., Adolph T.E., Reider S.J., et al. (2016). Lipocalin 2 Protects from Inflammation and Tumorigenesis Associated with Gut Microbiota Alterations. *Cell Host & Microbe.* **19**, 455-469.

Nakano V., Ignacio A., Fernandez M.R., Fukugaiti M.H., Campos M.J.A. (2006). Intestinal *Bacteroides* and *Parabacteroides* species producing antagonistic substances. *Microbiology.* **1**, 61-64.

Ohkawara S., Furuya H., Nagashima K., Asanuma N., Hino T. (2005). Oral administration of *Butyrivibrio fibrisolvens*, a butyrate-producing bacterium, decreases the formation of aberrant crypt foci in the colon and rectum of mice. *J. Nutr.* **135**, 2878-2883.

Rodríguez J.M., Murphy K., Stanton C., Ross R.P., Kober O.I., et al. (2015). The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb. Ecol. Health. Dis*. **26**, 26050.

Sajib S., Tuz Zahra F., Lionakis M.S., German N.A., Mikelis C.M. (2018). Mechanisms of angiogenesis in microbe-regulated inflammatory and neoplastic conditions. *Angiogenesis*. **21**, 1-14

Saulnier D.M., Riehle K., Mistretta T.A., Diaz M.A., Mandal D., Raza S., et al. (2011). Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology*. **141**, 1782-1791.

Sommer F., Anderson J.M., Bharti R., Raes J., Rosenstiel P. (2017). The resilience of the intestinal microbiota influences health and disease. *Nat Rev Microbiol*. **10**, 630-638.

Stewart C.S., Duncan S.H., Cave D.R. (2004). *Oxalobacter formigenes* and its role in oxalate metabolism in the human gut. *FEMS Microbiol. Lett.* **230**, 1-7.

Tilg H., Adolph T.E., Gerner R.R., Moschen A.R. (2018). The Intestinal Microbiota in Colorectal Cancer. *Cancer Cell.* **33**, 954-964.

Vandeputte D., Falony G., Vieira-Silva S., Tito R.Y., Joossens M., et al. (2016). Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes, and bacterial growth rates. *Gut*. **65**, 57-62.

von Martels J.Z.K., Sadabad M.S., Bourgonje A.R., Blokzijl T., Dijkstra G., Faber K.N., et al. (217). The role of gut microbiota in health and disease: In vitro modeling of host-microbe interactions at the aerobe-anaerobe interphase of the human gut. *Anaer*. **44**, 3-12.

Walters W.A., Xu Z., Knight R. (2014). Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Lett*. **588**, 4223-4233.