Annals of Gastroenterology (2017) **30,** 45-53

Gut microbiome, surgical complications and probiotics

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Abstract

The trigger for infectious complications in patients following major abdominal operations is classically attributed to endogenous enteral bacterial translocation, due to the critical condition of the gut. Today, extensive gut microbiome analysis has enabled us to understand that almost all "evidence-based" surgical or medical intervention (antibiotics, bowel preparation, opioids, deprivation of nutrition), in addition to stress-released hormones, could affect the relative abundance and diversity of the enteral microbiome, allowing harmful bacteria to proliferate in the place of depressed beneficial species. Furthermore, these bacteria, after tight sensing of host stress and its consequent humoral alterations, can and do switch their virulence accordingly, towards invasion of the host. Probiotics are the exogenously given, beneficial clusters of live bacteria that, upon digestion, seem to succeed in partially restoring the distorted microbial diversity, thus reducing the infectious complications occurring in surgical and critically ill patients. This review presents the latest data on the interrelationship between the gut microbiome and the occurrence of complications after colon surgery, and the efficacy of probiotics as therapeutic instruments for changing the bacterial imbalance.

Keywords Gut microbiome, surgical complications, colon surgery, colon anastomosis, probiotics

Ann Gastroenterol 2017; 30 (1): 45-53

Introduction

Complications after colorectal surgery – especially those performed for malignancy – are often a result of bacterial infections, leading to a significant increase in morbidity and mortality, as well as the duration of hospitalization and the subsequent costs. In this process, the gut seems to play a crucial part. Failure of the gut barrier function has long been considered to lead to a process called "bacterial translocation", where whole bacteria or their virulent products enter the systemic circulation and provoke systemic inflammatory response syndrome (SIRS), which may lead to multiple organ failure or even death. Human studies have shown that at least 11% of individuals who undergo an open-abdomen surgical operation have experienced translocation of live bacteria to the mesenteric lymph nodes or to the serosa of the bowel wall. Evidence of bacterial DNA in the blood of approximately 50% of patients in the intensive care unit (ICU) also suggests bacterial translocation, but there is still a great deal of controversy as to whether this is only an epiphenomenon, or whether it really contributes to morbidity [1,2].

In recent years, there has been ongoing interest in the human gut microbial ecosystem, which ultimately appears to be involved in both disease onset and progression, as well as in the development of complications. Moreover, there is increasing recognition of the important fact that microbes can obtain information from their host environment, which they then utilize to determine whether to colonize or express a virulent phenotype to invade the host, a scenario especially prevalent during prolonged critical illness [3-5].

In this review, efforts were made to present the newest data on the interrelationship between gut microbiome and the emergence of complications after colon surgery, and the efficacy of probiotics as therapeutic instruments for changing the bacterial imbalance.

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Conflict of Interest: None

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Received 05 May 2016; accepted 11 July 2016;
published online 6 September 2016

DOI: http://dx.doi.org/10.20524/aog.2016.0086

Intestinal microbiota: symbiosis and dysbiosis

The gastrointestinal tract hosts a particularly complex microbial ecosystem, consisting of more than 10^{14} microbes representing 500-1500 species. This ecosystem remains relatively stable throughout life, leading to the speculation that individuals might possess a unique microbial "fingerprint", despite daily variations attributable to diet, lifestyle, age,

and the host's physiological and immunological health. All microorganisms residing within or on the human body are called microbiota, and their genomes are known as the human microbiome [6,7].

The four dominant phyla inhabitants of the human gut are *Firmicutes* and *Bacteroidetes*, accounting for more than 90% of the bacteria cells, with a smaller representation of *Actinobacteria* and *Proteobacteria*. Species from the genus *Bacteroides* alone constitute about 30% of all bacteria in the human microbiome, while the well-known family *Enterobacteriaceae*, which contains medically relevant genera such as *Escherichia, Klebsiella, Pseudomonas,* and *Salmonella*, actually represents less than 1% [4,8-11] (Fig. 1).

This complex ecosystem coexists in a fragile balance (symbiosis), that can easily be disturbed (dysbiosis). This occurs when a disturbance in the composition and function of beneficial bacteria makes them incapable of controlling the harmful bacteria successfully. Today, dysbiosis has been linked with important human diseases, not only infections, but also autoimmune and autoinflammatory disorders, [8,12]. In this context, there is now clear evidence that every direct or indirect manipulation of gut microbiota – by means, for example, of antibiotics or surgery – contributes to disease development or the opposite: a broad range of medical and surgical problems are linked to perturbations of the microbiome (Table 1).

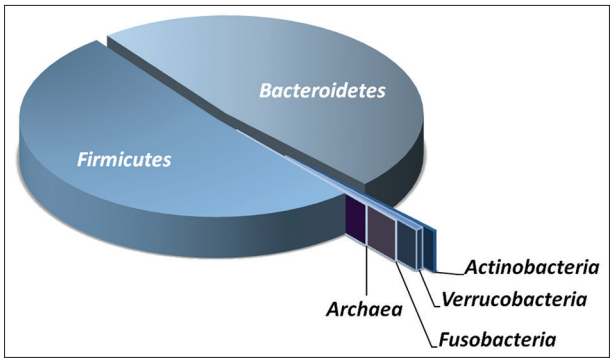

Figure 1 Distribution of the intestinal microbiota phyla

Table 1 Iatrogenic factors affecting the gut microbiome

Perioperative manipulations affecting gut microbiome
Mechanical bowel cleansing
Antibiotics
Stress-released hormones (catecholamines)
Vasoactive drugs (norepinephrine)
Endogenous and exogenous opioids (morphine)
Enteral feeding restriction
Micronutrients insufficiency
Operation/gut manipulation/resection/anastomosis

Intestinal microbiome and colon surgery

Intestinal microbiota and the human gut epithelium, serving as the host, maintain a long-term, well-tolerated symbiotic relationship. When the host "alters" the conditions of "hospitality", as occurs with the physiologic changes in the human body caused by surgical stress, and more specifically of the intestinal microenvironment, a disturbance in ecological balance occurs [13].

However, the fact that most surgical patients do not experience infectious complications simply underlines the adaptability of both the host and microbe in response to surgical stress [1,4,14]. It is also recognized that, besides the extent and severity of surgical stress, the variability of the inflammatory response is also mediated by genetic predisposition, the presence of comorbidities and the side-effects of pharmacologic treatments.

In a recent study in piglets, DNA sequencing of the colonic content was studied comparatively in the "transection surgery" group and in the "no-surgery" group, two weeks after operation. Changes in the relative abundance of bacterial species were confined to *Proteobacteria* and *Bacteroidetes* phyla, while, at family level, there was evidence of a reduction in *Enterobacteriaceae, Bacteroidaceae*, and *Rhodospirillaceae* versus controls [15].

In colon surgery patients, there is not only the operative stress itself, but also a variety of perioperative interventions imposed by modern intensive care therapy, including preoperative bowel cleansing, multiple antibiotic exposure, prolonged starvation, exclusively intravenous nutrition, the administration of vasoactive agents, inhibitors of gastric acidity, and opioids; and finally, the intense manipulation of the gut, which could disrupt the host-microbe relationship and thus could yield heightened virulence expression by bacteria and a fulminant inflammatory response in the host [1,15-18].

Intestinal microbiome and mechanical bowel cleansing

Mechanical bowel preparation for colorectal surgery has been normal routine for surgeons for more than a century; however, the Cochrane Database of Systematic Reviews, in an analysis of 18 trials with 5805 participants aimed at determining the safety and effectiveness of this preparation on morbidity and mortality in colorectal surgery patients, concluded that bowel cleansing can be safely omitted, as it is considered not to reduce rates of surgical site infections, unless it is combined with both oral and systemic antibiotics [17,19].

By approaching the issue from the perspective of gut bacteria, a randomized controlled trial evaluated the effect of preoperative mechanical bowel cleansing on the fecal flora of patients undergoing colorectal surgery. They found a significant reduction in the total number of bacteria: *Clostridium coccoides* group, *Clostridium leptum* subgroup, *Bifidobacteria*, total *Lactobacillus* and *Enterobacteriaceae* were found to be significantly reduced, but there was no effect on the number of *Enterococci* and *Staphylococci* [16]. Similarly, from the 16S rRNA gene sequences analysis of mucosal biopsies obtained during sigmoidoscopies from unprepared and prepared gut of the same individuals, it became clear that standard colonic lavage alters

the composition and diversity of not only the intestinal lumen microbiota, but also the mucosa-associated, the differences being more prominent at the genus level [20]. It is now well accepted that the intestinal luminal and the mucosa-associated microbiota differ significantly from each other in diversity and composition, and appear as two distinct ecosystems with different metabolic and immunological functions [21].

Furthermore, when the intestinal microbiota composition was analyzed at baseline, immediately after bowel cleansing, and after 14 and 28 days, the number of bacteria in samples collected immediately after bowel cleansing was on average 34.7-fold lower than at baseline ($P<0.001$), and the number of methanogenic archaea was also decreased 20-fold ($P<0.001$); these had returned to baseline by the 14- and 28-day samples [22]. So far, it seems that bowel cleansing could be salutary for patients who are to undergo colon surgery. However, further analysis revealed that immediately after the lavage, the intestinal microbiota was significantly different from baseline, even at class or family level: there was a significant decrease in *Bacilli* and *Clostridium* cluster IV genera and a parallel significant increase in members of the *Proteobacteria* phylum and *Clostridium* cluster XIVa; additionally, the ratio of Gram-positive to Gram-negative species changed significantly after the lavage (from 5.3±4.8 to 9.2±7.5 at the 14-day time-point, $P<0.05$, after which a trend towards baseline was evidenced), while *Proteus* genera were still significantly increased after 28 days (Fig. 2).

In the same manner, a very recently published paper further underlines that, immediately after bowel lavage, a significant reduction in *Lactobacillaceae* and an increase in *Enterobacteriaceae* abundance were prominent; 30 days later these families were still significantly lower, while *Streptococcaceae* had increased 4-fold compared with samples collected before lavage [23].

These recent findings seem to provide clear evidence that the widely used polyethylene glycol bowel-cleansing preparation could be considered bacterial genocide, as it has a long-lasting effect on the composition and homeostasis of gut microbiota. It is well-known that laxatives in general introduce an osmotic flow of fluids into the gut, washing out the fecal luminal content with a substantial reduction in intestinal bacteria [24], while the concomitant rapid increase in gut motility further contributes to flushing out all bacteria incapable of adhering to the gut mucosa, thus distorting the fecal bacterial composition [22,25].

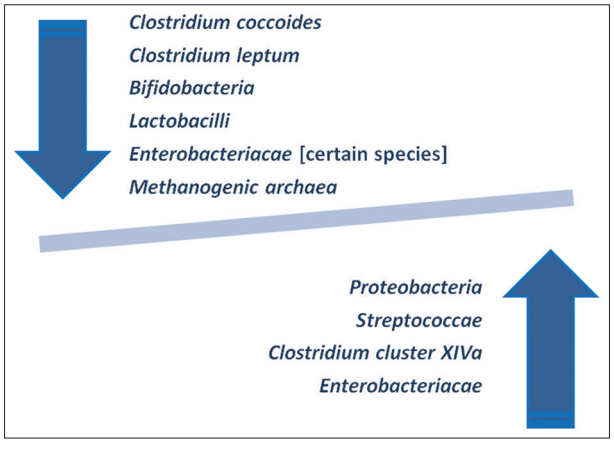

Figure 2 Alterations of the gut microbiome after mechanical bowel cleansing

Moreover, bowel purgation affects the quality and production of the protective mucus layer, while the fact that *Proteobacteria* flourish after lavage and in the long-term thereafter could be completely explained by the knowledge that purging leads to the introduction of oxygen into the normally anaerobic ecosystem and to an increase in pH, via the loss of short-chain fatty acids [25,26].

Finally, it has also been suggested that the sheer mechanical effect of colonic lavage may alter the intracellular signaling pathways involved in cell proliferation and influence the interaction between intestinal mucosal cells and the extracellular matrix, all of which are key elements of the mucosal gut barrier [27] (Fig. 3).

Intestinal microbiome and antibiotics

Antibiotic administration has long been known to have detrimental effects on the ecology of commensal bacteria, ranging from self-treated "functional" diarrhea to life-threatening pseudomembranous colitis [28,29]. Recent studies have demonstrated that beyond the prolonged disruption of the intestinal microbial content at the taxa level, antibiotics also affect gene expression, protein activity and more than 87% of all metabolites, thus deranging the majority of metabolic pathways of critical importance to host physiology. They have also underlined that antibiotics lead to a significant alteration of the gut microbiome and a parallel decrease in microbial diversity of between one fourth and one third [12,30-32].

Today, it is more or less clear that even short-term antibiotic treatment can cause detrimental damage to the intestinal microbiome that can last more than 24 months. Panta *et al* [32] investigated the number and composition of the fecal microbiota just before and after a 7-day treatment in 21 patients who received fluoroquinolones, β-lactams and other commonly used antibiotics. Quantitative polymerase chain reaction analysis and pyrosequencing of the 16S rRNA gene reveal that both fluoroquinolones and β-lactams significantly decrease microbial diversity by 25%, reducing the core phylogenetic taxa from 29 to only 12. At the phylum level, both antibiotics resulted in a 2.5-fold ($P=0.0003$) decrease in *Firmicutes* and an increase in *Bacteroidetes*, although this phenomenon was not prominent after treatment with piperacillin/tazobactam and levofloxacin/metronidazole.

Earlier studies showed that, during a 10-day amoxicillin-clavulanic acid administration, *Bifidobacterium spp*. (one of the major groups seen on day 0) disappeared as early as day 4, and had not returned by day 24. In contrast, *Enterobacteriaceae* (which represented only 2% of the day 0 sequences) increased to 34% on day 4, but were partially restored, as were the other major bacterial clusters, on day 24 [33]. Similarly, during a 5-day amoxicillin treatment, the dominant species presented on day 0 showed a major shift starting from day 1, reaching an average similarity of only 74% after 4 days, after which they were partially restored to 88% on day 30 and to 89% only on day 60 [34].

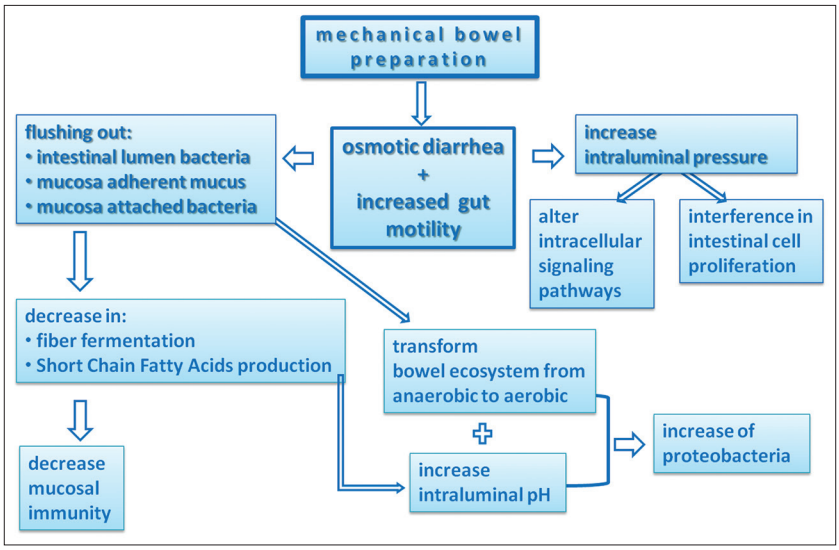

Figure 3 Effects of mechanical bowel cleansing on the intestinal microbiota

Finally, a 5-day ciprofloxacin administration was found to reduce the intestinal microbiota diversity, with significant effects on about one third of the bacterial taxa [31], the effects being less pronounced than those of clindamycin or amoxicillin-clavulanic acid [35]. This taxonomic disturbance had recovered to almost the pre-treatment state at 4 weeks post-treatment, but several taxa failed to recover within 6 months [31].

Other interventions

Today, it is generally known that many of the infectious bacteria species acquire the capacity not only to recognize stress-related hormones, but also to synthesize the very same neurochemicals, which can influence the host. In other words, pathogenic bacteria in the stressed host may use stress-released hormones as environmental cues by which to sense their surroundings [36,37]. It is also well known that microbes constantly assess their microenvironment and alter their phenotypic expression to optimize their survival, which means they tightly modify the expression of virulence in response to specific environmental cues [4,10].

It has been shown that catecholamines directly affect the growth and expression of virulence-related factors in some bacteria, such as *Yersinia enterocolitica*, *Escherichia coli (E. coli)*, *Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhii* or *Campylobacter jejuni* [38,39]. Furthermore, there is evidence that the *in-vitro* growth of the respiratory pathogen *Bordetella bronchiseptica* (*B. bronchiseptica*) is greatly enhanced in the presence of norepinephrine and that this ability is, in part, mediated by the ability of norepinephrine to increase the acquisition of transferrin-bound iron by *B. bronchiseptica* [40]. In the same manner, norepinephrine was found to increase the proliferation of *Streptococcus pneumoniae* by assisting the delivery of iron from host iron-binding proteins, while at the same time enhancing the formation of biofilms and thus increasing antibiotic resistance [39].

Morphine is produced endogenously during the inflammatory processes by different cell types, including neutrophils, which rapidly transfer it to sites of inflammation and infection [41]. Additionally, morphine, one of the most commonly used analgesics, is considered a powerful immunosuppressant [42]; therefore, the sustained exposure of tissues to morphine, either endogenously produced or exogenously supplied, is a virtual certainty for all surgical patients, those with trauma, or the critically ill.

Morphine treatment in mice whose gut had been contaminated with *P. aeruginosa* caused a shift towards a more virulent phenotype of *P. aeruginosa*, able to cause lethal gut-derived sepsis, and a tendency for biofilm formation, thus increasing its antibiotic resistance. Moreover, *P. aeruginosa* possesses the ability to switch phenotype from being mucus-enhancing to mucus-suppressing - having the ability to destroy gut epithelial integrity - depending on the presence or absence of morphine [43]. Additionally, Banerjee *et al* [44] have very recently shown for the first time that chronic morphine treatment significantly alters the gut microbiome composition and induces a preferential expansion of Gram-positive as well as a reduction in bile-deconjugating bacterial strains.

Last but not least, food restriction, even in the setting of complete intravenous nutrition, leads to a scarcity of macronutrients for the bacteria within the gut, and thus to a relative loss of *Firmicutes* and to an expansion of *Proteobacteria* and *Bacteroidetes*. The hostile environment may favor *Proteobacteria*, because they have been shown to survive in states of relative starvation, versus *Firmicutes*, which dominate in a nutrient-rich environment [45,46]. Furthermore, a micronutrient insufficiency in the host, such as a lack of iron and phosphate, results in an analogously deprived environment, and it is well-known that local tissue phosphate concentration functions as an important cue through which endogenous bacteria "taste" the resources of the host to determine whether they should colonize or invade the host [4].

Decreased microbial diversity, virulence and postoperative complications

It is increasingly recognized that the gut microbiome plays a fundamental role in the health maintenance of the host, and that any alteration in the diversity, the number or the virulent phenotype can have a critical effect on host morbidity and even mortality. The concept that bacteria are able to sense the host environment, and adjust their behavior and virulence accordingly, is a new dimension in the area of intense research in severe-infection patients that breaks new ground in our understanding of how the gut acts as the driving force of critical illness [47]. Based on these observations, it is obvious that when, for whatever reason, the symbiotic relationship with the host is turned to dysbiosis, the newly pathogenic bacteria can further trigger and promote harm to the already compromised host, in a positive spiral feedback.

Since medical interventions and surgical manipulation of the host are part of everyday practice, it would be of great interest and importance to examine the precise mechanisms and correlate the reported alterations of the microbiome with the infectious complications in the surgical and/or critically ill patient. Shimizu *et al* [3,13] found a significant reduction in the total anaerobic bacteria, as well as 2-log higher counts of the hazardous *Staphylococcus* and *Pseudomonas* groups, in the fecal flora of patients with SIRS, compared to healthy volunteers. Furthermore, they correlated key bacteria in the gut and derived their cutoff values in relation to infectious complications and mortality. The equilibrium between obligate anaerobes and total facultative anaerobes seems to play a critical role in causing septic complications: during the unfavorable evolution of SIRS, alterations in gut bacteria usually progress from a diverse pattern to a single pattern and then on to a depleted pattern, the three types representing a continuum of abnormality, depending on the severity of the patient's condition. Bacteremia was evident in 35% of those with a diverse pattern versus 71% with the single pattern, resulting in a mortality rate of 6% in the former, 52% in the letter, and 64% in those with a depleted pattern ($P<0.05$) [48].

Liu *et al* [49] analyzed the feces of patients undergoing colorectal surgery and found a reduction in microbial diversity, including *Bifidobacteria* and *Lactobacilli*. In contrast, the numbers of *Enterobacteriaceae, Pseudomonas* and *Candida*, showed a significant increase, which in turn was well correlated with the higher rate of infectious complications, 46% versus 14%, in probiotics-treated patients ($P<0.05$). Likewise, Komatsu [50] reported a significant reduction in the total number of bacteria and the number of dominant obligate anaerobes and a significant increase in the number of *Enterobacteriaceae*, *Staphylococcus* (MSCNS), *Pseudomonas*, and *Clostridium difficile* after colorectal surgery, compared with data from the same group before surgery.

Finally, in a recent study, neonatal piglets that underwent intestinal resection and received parenteral nutrition and antibiotics or placebo were examined at day 7 against age-matched sow-fed piglets. Ileal and colonic contents revealed dramatic differences in diversity and an almost complete loss of *Lactobacillus,* along with a remarkable increase in the *Fusobacteriaceae* and *Enterobacteriaceae* families in both the ileum and the colon. In addition, there was an increase in the *Bacteroidaceae* family in the colon [51]. These results strongly support similar findings in humans undergoing small bowel resection, who lacked exposure to enteral nutrition for 2 weeks. The reported loss in fecal bacterial diversity in this study was clearly associated with a higher incidence of postoperative infectious and anastomotic complications [52].

Anastomotic leaks

In colorectal surgery, an anastomotic leak represents the most dreaded of all complications, since it is often perceived as a failure of the operation or the surgeon, although the real cause of dehiscence is not fully elucidated. However, it has long been known that the intestinal bacterial population plays rather an important role: inoculation of rats with 10^9 *P. aeruginosa* led to an increase in the incidence of anastomotic insufficiency up to 95% after gastrectomy and to a significant increase in mortality [53].

This concept has re-emerged as a result of advances in microbial isolation and identification using 16S rRNA analysis. Olivas *et al* [54], working in an rat model of preoperative irradiation plus colonic resection and anastomosis, demonstrated that intestinal colonization with *P. aeruginosa* resulted in a significantly higher incidence of leaks, compared to the non-colonized group. What is even more striking is that the *Pseudomonas* colonizing anastomotic sites had become, *in vivo*, transformed to express a tissue-destroying phenotype; that is, one that had undergone a single nucleotide polymorphism mutation in the *mexT* gene that resulted in a much more virulent phenotype with increased collagenase activity, high swarming motility, and an increased ability for tissue destruction.

It is well known that important human mucosal pathogens have evolved virulence mechanisms to circumvent the mucosal epithelium barrier [55,56]. *P. aeruginosa* seems to favor damaged epithelial tissues to initiate colonization [57]; then, upon binding to epithelial cells, it activates a phosphatidylinositol 3-kinase, which is absolutely necessary for *P. aeruginosa* to enter from the apical surface of polarized epithelial cells, by subverting the epithelial cell polarity [56].

Further studies have demonstrated that the anastomosis construction itself causes significant alterations to the bacterial composition at the anastomotic site, but not to the luminal microbial content [58]. The most interesting observations are that the *Enterococcus* and the *Escherichia*/*Shigella* populations increased by 500-fold and 200-fold, respectively; at the same time, populations of beneficial bacteria were reduced [59,60]. For an in-depth analysis of the marked *Enterococcus* increase [58] and the associated high collagen-degrading activity, they inoculated *Enterococcus faecalis* (*E. faecalis*) strains obtained just after completion of the colorectal anastomosis in a rat model and on the sixth postoperative day; by collecting the liquids drained from the gut, they demonstrated that the collagen-degrading activity of the bacteria recovered from the anastomotic area enabled

discrimination between leaking and non-leaking anastomotic sites. They also found that *E. faecalis* exhibited an increased ability to activate tissue matrix metalloproteinase-9, operated through the *gelE* and *sprE* genes, both of which contributed to anastomotic leakage [61].

The microbiome of eight patients who experienced colorectal anastomotic rupture and of another eight matched for age, gender and adjuvant therapy, was investigated by studying the rings of colon and rectum tissues cut by the circular stapler to make the anastomosis [62]. The investigators surprisingly reported a significantly higher proportion of the *Lachnospiraceae* family versus controls-although these bacteria tend to be rather friendly to the bowel, as most of them belong to butyrate producing genera. However, further analysis revealed that a large fraction of the *Lachnospiraceae* were identified to be of the mucin-degrading *Ruminococcus*, and that *Lachnospiraceae* levels were strongly negatively correlated with microbial diversity levels, which in turn are associated with anastomotic leakage.

Probiotics - prebiotics - synbiotics

Probiotics are live microbial food supplements that may beneficially affect the host by improving its intestinal microbial balance, while prebiotics are indigestible fibers that promote the growth and function of probiotics; their combination is called synbiotics [7]. Probiotics are able to maintain gut barrier function by restoring intestinal permeability and ameliorating the intestinal inflammatory response and the release of cytokines, and can also maintain the homeostasis of the normal gut microbiota. As a result, they have been extensively studied as an adjuvant perioperative treatment modality for surgical patients [7,11]. In the field of gastrointestinal surgery, it has been shown that probiotics may be effective in restoring gut microbiota diversity, enhancing immunological response, reducing the systemic inflammatory response released postoperatively, and improving patients' quality of life. Moreover, as a consequence of all the above, they appear to work positively in reducing the total length of hospital stay, the number of days of ventilator support required and of days in intensive care, and the overall infectious complications [63-66]. However, other investigators have reported no benefits after the perioperative use of probiotics in patients undergoing elective abdominal surgery. A possible explanation of these differences may be related to the rather short administration period (median of 4 days) in the majority of the studies, the low concentration of bacteria present in the formulation prescribed, the one probiotic strain only of the regimen used, and the small number of participants in most studies. Last but not least, consideration must be given to the open-gut manipulation strategies applied, which fortify the bacterial contamination of the peritoneal cavity and the interruption of blood supply to the viscera, due both to the ligation of major vessels and the use of heat-coagulation for the smaller ones [67,68]. Finally, many clinicians start with the negative assumption that, given the degree of diversity and metabolic functions of the normal core microbiome, it appears naïve to believe that some *Lactobacilli* strains could fully supplant the degree of functionality required of the intestinal microbiome to bolster systemic immune function during disease states.

Probiotics and infectious complications

Multiple studies have been performed regarding the potential benefit of enteral administration of probiotics in reducing infectious complications in surgical as well as in critically ill patients, based on the idea that they may modify the gastrointestinal bacteria in a manner that preferentially favors the growth of minimally virulent species [69]. He *et al* [68] analyzed six randomized controlled trials dealing with pro/synbiotic administration in 361 colon cancer patients undergoing colorectal resection and found a significant decrease in the total number of infections (P=0.001), mainly due to the decreased cases of pneumonia (P=0.04), while other infectious complications, such as surgical site or intra-abdominal infections or bacteremia, remained unaffected.

This is in line with a previous meta-analysis that demonstrated a significant reduction in the rate of nosocomial pneumonia (P=0.03) in critically ill patients treated with probiotics [70], as was also reported in relation to ventilator-acquired pneumonia (VAP) [71]. Various other studies have demonstrated similar effects, while a recent meta-analysis suggests that probiotic treatment results in a 39% reduction in VAP, along with a subsequent reduction in the length of ICU stay [72]. A possible mode of action is considered to be the ability of probiotics to inhibit or ameliorate gastrointestinal and systemic bacterial colonization [73], since it has been shown that probiotic-treated patients exhibit smaller rates of *P. aeruginosa* colonization versus controls [72]. In a meta-analysis of 5 trials (844 patients), probiotics showed a trend towards a lower incidence of VAP; when one trial was excluded, a statistically significant conclusion could be drawn. Thus, the administration of probiotics seems to significantly reduce the risk of VAP caused by *P. aeruginosa* [74].

Another study suggested that modification of the upper aerodigestive flora by means of applying probiotics could reduce nosocomial infections [69]; in any case, *Lactobacillus* administration resulted in a significant delay in the time to onset of VAP (P<0.001), as has also been documented in a study of our group, where a statistically significant delay in the time of blood infection onset was prominent [75].

In a randomized controlled study of colorectal cancer patients, Zhang *et al* [64] demonstrated a significant reduction in septic complications (33.3% in controls versus 10% in probiotic-treated), along with a significant decrease in *E. coli* and a significant increase in the *Bifidobacterium* counts in the same group. Likewise, fecal cultures in ICU multiple trauma patients, symbiotic-treated, revealed a decrease in *Enterobacteriaceae*, coagulase-negative *Staphylococci*, and Gram-negative anaerobes, and an increase in *Enterococcus* spp. and Gram-positive anaerobes [76]. These patients demonstrated a 13.9% incidence of bacteremia versus 36.1% in those receiving placebo (P=0.028), a finding

related to the reduced incidence of *Acinetobacter baumanii*-related, ventilator-associated pneumonia [77]. Various other studies have produced similar results, with Liu [78], in a study of colorectal cancer patients, reporting rates of bacteremia of 55% in the probiotics group versus 72% in controls, P=0.017.

Furthermore, a recent meta-analysis demonstrated a reduction of postoperative sepsis after elective general surgery, both in pro- and synbiotic-treated patients compared to placebo (P=0.003 and 0.002, respectively). However, no significant difference in the incidence of pneumonia, urinary tract or surgical site infections was found [79], while a very recently published study revealed a significant decrease of 38% in the incidence of postoperative sepsis. Separate analysis according to the type of operation revealed a statistically significant difference among the types, with a 35% risk reduction in colorectal surgery, 73% in hepato-pancreatico-biliary, and a 56% risk reduction in liver transplant operations; these findings add to the evidence that colorectal surgery patients might be the most difficult group for the manipulation of gut microbial balance [80].

Finally, in a recently conducted systematic review, the mean incidence of surgical site infection was 6.8% in treated patients and 11.1% in controls, representing a 37% reduction. This study also underlined the potential benefit in relation to urinary tract infections and composite infections, as well as the non-occurrence of serious adverse events related to study products [81].

Moreover, it is now common knowledge that, because of the complexity of the individual gut microbiome, probiotics are not a one-species-fits-all approach [82]; thus, when a single probiotic regimen (*Lactobacillus plantarum* [*L. plantarum*] *299v*) was used in patients undergoing colectomy, no benefit was found regarding postoperative complications [67].

Probiotics and colon anastomosis failure

Taking into account the further progress in the research of Shogan *et al* [58], demonstrating that anastomosis construction itself causes significant alterations to the bacterial composition at the anastomotic site, and the recent knowledge that all medical and surgical interventions related to anastomosis construction contribute to a reduction in bacteria diversity, including the eradication of beneficial and the increasing virulence of noxious species [54,61], it is reasonable to seriously reconsider the crucial role of bacteria in undermining the healing process and to look for a mode of enrichment of the scarce or destroyed bacterial species, the simplest and easiest mode being probiotics.

The first single-center randomized clinical study to evaluate the effect of probiotic treatment on the incidence of colon anastomotic failure was that of Zhang *et al* [64]. They demonstrated only a slight reduction in the rate of anastomotic leaks (0/30 in probiotics-treated individuals versus 2/30 controls (P=0.49). However, the patients had received 3 oral viable capsules/day for 3 days only (10^8 cfu/g of *Bifidobacterium longum*, *Lactobacillus acidophilus* [*L. acidophilus*], and *E. faecalis*), from day -5 to day -3, followed by conventional bowel preparation plus oral gentamicin for 3 days.

In a recent randomized study by our group, involving patients undergoing colorectal surgery for cancer, a four-probiotic formulation (*L. acidophilus, L. plantarum, Bifidobacterium lactis* and *Saccharomyces boulardii*) or placebo was administered, starting one day before surgery – after mechanical bowel cleansing – and continuing for 15 days postoperatively, the patients being followed-up for complications for 30 days. The probiotic-treated group exhibited a significantly lower rate of all postoperative major complications (28.6% versus 48.8% in the placebo arm, P=0.010), postoperative pneumonia (2.4% versus 11.3%, P=0.029), surgical site infections (7.1% versus 20.0%, P=0.020), and anastomotic leakage (1.2% vs. 8.8 %, P=0.031) [63].

Moreover, after total RNA extraction, it was also clearly found that in probiotics versus controls, there was modulation of *suppressor of cytokine stimulation-3* (*SOCS3*) expression which encodes for the protein SOCS3 that finally suppresses overwhelming cytokine responses. In other words, the prophylactic action of probiotics in these colon-cancer patients is exerted through modulation of the immune response and is linked with the prevention of immunosuppression development after a bacterial challenge [64].

From all the above analyses, we would summarize that exogenously given probiotics contribute at least partially to the restoration of the decreased gut microbial diversity, but mainly preserve the host's immune function; i.e., they prevent immunosuppression, which might otherwise be "detected" by the pathogens and trigger changes in their virulence and lethality as they then attack the host.

Concluding remarks

Modulation of the intestinal microbiota with probiotics seems to be an effective method of reducing infectious complications in surgical patients, although their effect on mortality has still not been elucidated. Further studies need to be conducted to establish the best possible combination of probiotics, as well as to determine the subgroup of patients who could benefit most from such an intervention.

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