Impact of gut microbiota on colorectal anastomotic healing (Review)

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Abstract. Intestinal anastomosis is a critical procedure in both emergency and elective surgeries to maintain intestinal continuity. However, the incidence of anastomotic leakage (AL) has recently increased, reaching up to 20%, imposing major clinical and economic burdens. Substantial perioperative alterations in the intestinal microbiota composition may contribute to AL, particularly due to disruptions in key microbial populations essential for intestinal health and healing. The intricate interplay between the intestinal microbiota and the host immune system, along with microbial changes before and during surgery, significantly influences anastomotic integrity. Notably, specific pathogens such as *Enterococcus* and *Pseudomonas aeruginosa* have been implicated in AL pathogenesis. Preventive strategies including dietary regulation, personalized intestinal preparation, microbiota restoration and enhanced recovery after surgery protocols, may mitigate AL risks. Future research should focus on elucidating the precise mechanisms linking intestinal microbiota alterations to anastomotic healing and developing targeted interventions to improve surgical outcomes.

Contents

1. Introduction

Intestinal anastomosis is a fundamental surgical procedure performed in both emergency and elective settings to restore intestinal continuity following bowel resection. It is indicated in various conditions, including colorectal, bowel obstruction, blunt/penetrating abdominal trauma and intestinal perforation leading to peritonitis. Despite advancements in surgical techniques, including minimizing tissue damage, ensuring adequate vascularization, reducing anastomotic tension and employing optimal suturing methods, the rate of anastomotic leak (AL) has remained high, reaching up to 20% (1). AL is associated with increased morbidity, prolonged hospitalization and substantial healthcare costs, underscoring the urgent need for innovative therapeutic strategies to enhance anastomotic healing and reduce complications.

Previous studies have reignited interest in the role of the gut microbiota in intestinal wound healing and AL pathogenesis (2,3). The perioperative period is characterized by profound shifts in gut microbiota composition and function, driven by factors such as chemotherapy, radiotherapy, fasting, bowel preparation, antibiotic prophylaxis (4,5), surgical trauma, environmental exposure and ischemia-reperfusion injury (6). These disruptions can cause microbial dysbiosis, impaired gut barrier function and bacterial translocation, all of which have been implicated in AL pathogenesis (7). Despite increasing evidence linking the microbiome to tissue repair and immune regulation, the precise mechanisms through which microbial alterations influence anastomotic healing remain poorly understood. This knowledge gap highlights the urgent need for research into microbiome-based therapeutic approaches to significantly mitigate AL risks.

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The present review hypothesized that the gut microbiome is a pivotal player in anastomotic healing and that targeted modulation of microbial communities during the perioperative period could reduce AL incidence. Understanding the interactions between gut microbiota, host epithelial cells and immune responses may enable the identification of predictive biomarkers and novel therapeutic targets. Potential translational applications include microbiome-based diagnostics for AL risk assessment, probiotics and prebiotics to restore microbial balance and targeted antimicrobial interventions to counteract pathogenic shifts. Such interventions directly improve patient outcomes by augmenting anastomotic healing, reducing postoperative complications and shortening recovery times. The present review explored the dynamic perioperative changes in gut microbiota, their impact on AL and emerging microbiome-targeted strategies to improve surgical outcomes.

2. Co-evolution of microbiota and host

The human gut harbors >100 trillion microbial cells, ~10-fold more than the combined number of human somatic and germ cells (8). Advances in metagenomic sequencing have facilitated the characterization of the gut microbiome, comprising complex microbial communities and their collective genomes. These approaches integrate high-throughput sequencing of specific 16S ribosomal RNA hypervariable regions and whole-genome shotgun sequencing to analyze microbial diversity and function (9,10). Most gut microorganisms reside in the lumen of the gastrointestinal tract, where they serve essential roles in metabolism, immune modulation and epithelial integrity. During infancy, the gut microbiome undergoes dynamic changes before stabilizing into four dominant bacterial phyla: Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria. The composition and prevalence of these microbes vary significantly based on environmental conditions, genetics, immune status, dietary habits and early encounters with infections or antibiotics (11). The gut microbial community consists of bacteria, viruses, fungi, archaea and protozoa, forming an intricate ecological network with profound physiological implications. The symbiotic relationship between gut microbiota and the host has co-evolved to support metabolic homeostasis, immune defense and intestinal barrier integrity. The microbiome serves a critical role in maintaining the host's nutritional and energy balance while also being essential for developing and sustaining a robust immune system (12). It facilitates nutrient assimilation by breaking down carbohydrates, fatty acids and proteins into bioavailable forms, thereby influencing nutrient metabolism and delivery to the host. This process includes regulating the activity of host genes related to nutrient transport and processing. Conversely, malnutrition can negatively affect both the innate and adaptive immune systems, as well as the microbiome itself (13). The gut microbiome also serves as a protective barrier against intestinal pathogens by producing antimicrobial compounds such as short-chain fatty acids (SCFAs), secondary bile acids and bacteriocins, which help maintain intestinal integrity (14,15). At the cellular level, SCFAs can directly or indirectly affect processes such as cell proliferation, differentiation and gene expression. Additionally, they act as ligands for G protein-coupled receptors (GPCRs), including GPR109A, GPR43 and GPR41, triggering anti-inflammatory signaling cascades (16). Among these, butyrate enhances intestinal barrier function in IPEC-J2 cells by selectively upregulating tight junction proteins and activating the Akt signaling pathway (17). Despite these insights, the precise mechanisms by which the gut microbiome confers resistance to pathogen colonization remain incompletely understood. These mechanisms likely include the production of antimicrobial substances, competition for nutrients, maintenance of intestinal barrier integrity and bacteriophage-mediated bacterial regulation. The interplay between the host's genetic makeup and microbiota composition significantly influences susceptibility to diseases (18). Disruptions in microbial populations, including the depletion of certain beneficial bacteria or significantly reduced microbial diversity, can elevate the risk of infections and complications, such as AL.

3. Perioperative gut microbiota changes associated with AL

Baseline alterations in gut microbiota related to AL. As aforementioned, the human gut harbors numerous microorganisms. The gut microbiota-host interaction creates a balanced microecological environment crucial for maintaining normal biological functions. The gut microbiota composition can be altered through surgical interventions but also by changes in host physiology and immune responses (19). Several factors can influence gut microbiota composition and function, such as dietary habits, physical activity levels, medications and chronic health conditions (20,21). Diet also serves a fundamental role in determining the structure and function of gut microbial communities (22). Additionally, specific dietary components can directly affect the gut microbiota or indirectly modulate it by influencing the host's metabolism and immune system. Regulatory T cells, also known as Treg cells, are essential to sustain intestinal balance. SCFAs, essential for Treg cell homeostasis, are produced through bacterial fermentation of dietary fibers (23,24). A deficiency in Treg cells leads to inflammation, illness (including type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease) and an imbalance in the gut microbiota composition. Dietary components can interfere with the protective role of the intestinal barrier. Western-style, high-fat, and low-fiber diets impair intestinal barrier function in animal models. However, fiber supplementation may help restore barrier integrity (25-27). In addition to diet, physical activity significantly influences the gut microbiota composition (28), but its effects depend on intensity. While moderate exercise can be beneficial, intense physical activity may compromise epithelial integrity, leading to increased intestinal permeability, bacterial translocation and inflammation (29,30). Moreover, certain medications including opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) can adversely affect anastomotic healing by altering the gut microbiota. A rat study demonstrated that morphine administration impaired surgical site healing and increased AL risk, which was associated with the presence of collagenase-producing *Enterococcus faecalis* in anastomotic tissue (31). Furthermore, alterations in the gut microbiota composition have been linked to different chronic diseases and lifespan variations in both human and animal studies. Conditions such as type 2 diabetes (32), depression

Table I. Comparative effects of MBP vs. MBP + oral antibiotics on the microbiome.

Aspect	MBP alone	MBP + oral antibiotics
Microbial diversity	-Reduces microbial diversity (4). -Significant reduction in obligate anaerobes (for example, *Bacteroides* and *Clostridia*) (48).	-Further reduction in microbial diversity, with a longer time to restore baseline levels (4,49). -Obligate anaerobes nearly disappear, while facultative anaerobes (for example, *Enterococcus faecalis*) increase (48).
Intestinal barrier function	-Intestinal barrier function may be temporarily impaired but recovers quickly (48).	-Intestinal barrier function is severely impaired, recovery is slow and the risk of bacterial translocation increases (4).
Postoperative infection risk	-Reduces postoperative infection (48,50,51).	-Significantly reduces surgical site infections, anastomotic leakage and intestinal obstruction after colorectal surgery (46,50-52).

MBP, mechanical bowel preparation.

and chronic stress (33) and liver injury (34) have all been associated with microbiota imbalances.

Preoperative gut microbiota changes associated with AL. Preoperative preparation can significantly alter the gut microbiome, especially around the surgical anastomosis site (35). While achieving a complete pathological response following neoadjuvant chemoradiotherapy is widely recognized as beneficial in cancer treatment, its impact on anastomotic healing or leakage rates remains unclear. Radiation therapy has been shown to alter the microbiome composition, but there is no conclusive evidence linking it to an increased AL risk (36,37). A previous study developed a rat AL model to investigate the effects of preoperative radiation on AL. The rats underwent distal colon resection after radiation exposure, mimicking treatment protocols for advanced rectal cancer. Subsequently, *Pseudomonas aeruginosa*, a common colonizer of the radiated intestine, was introduced into their intestines. The irradiated intestinal tissues exhibited a considerably higher AL risk when infected with *P. aeruginosa* (37). However, a previous systematic review and meta-analysis concluded that neoadjuvant chemotherapy is not significantly associated with an increased incidence of AL or other adverse postoperative outcomes (38).

Surgeons recognize that the physical trauma of surgery, such as incisions, tissue dissection, organ removal and vascular reconnection, heightens a patient's susceptibility to infections. Current standard clinical protocols aim to minimize microbial presence and prevent postoperative complications (39,40). Common preoperative strategies include mechanical bowel preparation (MBP) with laxatives, administration of oral and/or intravenous antibiotics and topical application of disinfectant solutions to sterilize the intestines and skin (41). Despite these measures, the gut microbiota remains a potential reservoir of pathogens that may contribute to post-surgical infections, particularly in intestinal surgery. The effects of preoperative bowel preparation on gut microbiota preservation and bacterial phenotype transformation are still not fully understood and warrant further investigation. Research indicates that MBP alone induces transient microbiome alterations, the magnitude of this effect is minimal, and the gut microbiome generally returns to its baseline state within 10 days following a colonoscopy. However, patients undergoing systemic bowel preparation (SBP), which includes a combination of MBP and oral antibiotics, experience more pronounced disruptions in microbiome composition, requiring at least 30 days for the gut microbiome composition to reach the initial state (4). MBP functions by removing solid fecal matter and reducing bacterial load in the colon, leading to temporary shifts in microbial diversity. Specifically, it decreases the abundance of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, while increasing the abundance of *Escherichia coli* and *Staphylococcus*. When combined with SBP, microbial diversity is further reduced, nearly eliminating obligate anaerobes while favoring facultative anaerobes such as *E. faecalis*. Additionally, SBP has been associated with increased antibiotic resistance (40). Moreover, the indiscriminate use of preoperative intravenous antibiotics may not provide any substantial advantages in reducing postoperative infections (42). Emerging evidence suggests that almost 50% of severe post-surgical infections involve antibiotic-resistant microbes. *E. faecalis* and *P. aeruginosa* frequently colonize leaking anastomoses, despite the use of strong broad-spectrum antibiotics (43). In rats, localized antibiotic application, rather than systemic intravenous administration, effectively eliminates *E. faecalis* and lowers AL risk in colorectal surgery (44). A combination of preoperative oral antibiotics and MBP has been shown to reduce Al incidence of colorectal surgery by nearly 50%. However, neither MBP alone nor oral antibiotics alone provide significant protection against AL (Table I) (45,46). Furthermore, the impact of MBP on microbiome composition and postoperative infection risk, including AL, may be influenced by individual patient characteristics such as body mass index and metabolic profile. A randomized controlled trial investigating microbiome alterations following MBP in patients undergoing elective colorectal surgery found substantial reductions in overall bacterial counts, particularly in *Clostridium*,

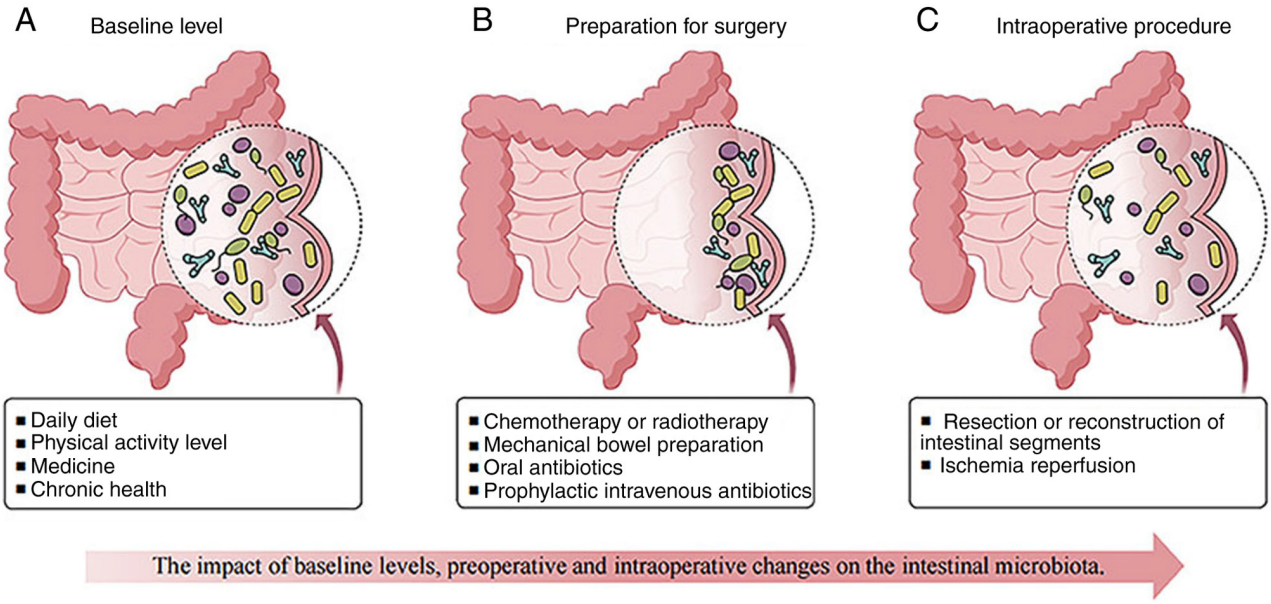

Figure 1. Effect of baseline, preoperative, and intraoperative events on the gut microbiota. (A) Baseline level. (B) Preparation for surgery. (C) Intraoperative procedure.

Bifidobacterium, *Lactobacillus* and Enterobacteriaceae, with no significant reductions noted in *Enterococcus* and *Staphylococcus* (47). Thus, the benefits of bowel preparation remain controversial.

Intraoperative gut microbiota changes associated with AL. Surgical resection of diseased, obstructed or ischemic bowel often requires complex procedures to restore intestinal continuity. These interventions significantly affect the gut microbiome, leading to substantial compositional shifts (53-55). Intestinal manipulation induces tissue damage and inflammation, which contribute to AL. Specifically, matrix metalloproteinase-9 is upregulated after surgery, facilitating leukocyte migration and inflammation, thereby impairing anastomotic healing. Additionally, intestinal ischemia-reperfusion (IIR) injury triggers dynamic changes in colonic microbiota. Although the precise mechanisms remain unclear, IIR induces inflammation and oxidative stress. The gut microbiota typically provides colonization resistance, preventing the overgrowth of indigenous pathogens, which suggests the suppression of potentially pathogenic commensals within the microbial community (56). However, IIR disrupts the intestinal mucosal barrier, reducing commensal bacteria and fostering pathogenic proliferation, ultimately exacerbating intestinal inflammation (57). In a mouse model, mesenteric ischemia-reperfusion resulted in increased *E. coli* levels in the ileum and colon while reducing the *Lactobacillus* levels, leading to gut barrier dysfunction and bacterial translocation (58). Fang *et al* (59) conducted a clinical study analyzing 332 fecal samples from 129 individuals (50 with ulcerative colitis and 79 with Crohn's disease). Their findings demonstrated that intestinal surgery reduces microbial diversity and metabolite concentrations in patients with inflammatory bowel disease, with long-lasting effects (including reduced diversity of microbes and metabolites, and further increase of the instability of the gut microbiome in patients with IBD). Previous research corroborates that preoperative interventions and surgical stress disrupt microbiota balance, causing metabolic disruptions and increasing susceptibility to AL (60). Therefore, surgical reconfiguration fundamentally alters the gut microbial ecosystem, impacting digestion, nutrient absorption, and immune function (55). The extent to which these alterations contribute to postoperative AL warrants further investigation (Fig. 1).

4. Interaction between gut microbiota changes and factors affecting intestinal wound healing

AL, a serious postoperative complication, is associated with both increased local recurrence rates and reduced disease-free survival in colorectal cancer patients compared with non-leakage cases (5). It has been a longstanding worry for surgeons, despite their meticulous surgical technique. AL remains unpredictable, occurring even in patients without identifiable risk factors. Efforts to identify high-risk patients for anastomotic failure after colorectal surgery have led to extensive research on potential contributors to anastomotic failure. A prospective, multicenter snapshot study by Gao *et al* (61) analyzed 1,854 patients undergoing right hemicolectomy for colorectal cancer. Multivariate analysis identified side-to-side anastomosis, intraoperative blood loss >50 ml, and neoadjuvant chemotherapy as independent risk factors for AL (61). Importantly, the gut microbiota significantly influences various factors affecting intestinal healing (including the microbial barrier, immune regulation, tissue repair and alterations in the metabolic microenvironment), making it a potential target for novel prevention and treatment strategies. Following colorectal anastomosis, tissue repair progresses through four stages: Hemostasis, inflammation, proliferation and wound remodeling (62). AL is influenced by a range of biological processes, including gut microbiota composition, inflammation, host genetics and immune responses. Among

these, the gut microbiota serves a particularly crucial role in intestinal wound healing (63,64).

Inflammation. During the inflammatory phase, the gut microbiota can promote or hinder wound healing by modulating cellular activation and fibrosis (65,66). While the precise mechanisms remain incompletely understood, studies suggest that lipoxin A4 and annexin-1 contribute to IL-10-dependent attenuation of inflammation in germ-free mice (67). Notably, administering lipoxin A4 or annexin-1 peptides to conventional mice mitigates tissue damage, TNF release and mortality following IIR injury (67). Various anti-inflammatory treatments (for instance, prolonged use of synbiotics, prebiotics and probiotics may modulate inflammatory status and potentially impact perioperative outcomes) have been explored for their role in preventing AL in animal and human studies. However, the results have been highly variable. Suppressing inflammation alone does not appear to prevent AL, as anti-inflammatory medications have failed to reduce its incidence (68). Interestingly, NSAIDs may disrupt microbial function, leading to dysbiosis and impaired anastomotic healing. Postoperative NSAID use has been correlated with increased AL rates, possibly due to its effects on the gut microbiota (69). Although NSAIDs are primarily prescribed for pain management rather than inflammation reduction, NSAIDs have been consistently associated with higher AL incidence (70,71).

Host genetics. While some single nucleotide polymorphisms (SNPs) have been identified in association studies, evidence for the influence of host genetics on AL is limited, and this research field remains in its early stages. COX-2, also known as Ptgs2, serves a crucial role in intestinal wound healing following colorectal surgery, primarily through its effects on angiogenesis, which is essential for anastomotic healing (72,73). A specific SNP in human PTGS2 (-765G>C; rs20417) has been associated with reduced COX-2 expression levels and an increased risk of AL (74,75). Studies on mice deficient in COX-2 have demonstrated a higher incidence of AL and mortality, which can be partially mitigated by the administration of prostaglandin E2 (PGE2). As the primary product of COX-2, PGE2 is essential for preserving intestinal homeostasis (74).

Intestinal immune regulation. The intestinal barrier and mucosal immune system work together to maintain a delicate balance between the host and external microorganisms, regulating immune responses to beneficial organisms while protecting against harmful pathogens. Increasing evidence highlights the crucial role of the gut microbiome in immune system maturation. The gut microbiota influences intestinal wound healing and epithelial repair through various molecular pathways (76). For example, gut microbes affect cellular immunity by modulating lymphocyte polarization, transportation and cross-reactivity. The microbiota can influence the adaptive immune system in various ways, including the bystander effect on T cell populations and molecular mimicry impacting antigen responses (77,78). Intestinal symbionts contribute to the development of gut-associated lymphoid tissue, secretory IgA and Th17 cells. It has been previously reported that cytokine levels fluctuate in the blood or peritoneum of patients undergoing colorectal resection, suggesting that the interleukin (IL)-Th17 pathway is a contributing factor to AL. Transforming growth factor-beta and IL-6 stimulate the differentiation of naïve $CD4^+$ T cells into Th17 cells by increasing the expression of IL-23 and IL-1 receptors, which are essential for Th17 cell expansion and survival (79). These factors help maintain intestinal homeostasis and the host-microbiome balance (76). The presence of resident microbiota, influenced by the gut microbiome, affects Th17 cell development (80) and may contribute to AL (81). Additionally, microbial signaling pathways serve a crucial role in wound healing. Pattern recognition receptors, such as toll-like receptors (TLRs), are key components of the innate immune system (82) and recognize pathogen-associated molecular patterns from microorganisms (83,84). TLRs, expressed in both immune and non-immune cells, detect gut microbial elements such as lipopolysaccharides (LPS) and flagellin (85), which help maintain intestinal homeostasis and promote epithelial healing. Rakoff-Nahoum *et al* (86) demonstrated that under normal homeostatic conditions, symbiotic bacteria are recognized by TLRs. Mice deficient in TLR2, TLR4 or MyD88 exhibit exacerbated acute gastrointestinal epithelial injury and inflammation. Cox-2 serves a key role in intestinal inflammation and healing. Its activity is regulated by TLR4 signaling and is essential for intestinal mucosal regeneration. Furthermore, TLR4-deficient mice fail to upregulate Cox-2 expression levels in response to epithelial damage (73). Cox-2 is expressed by both intestinal epithelial cells and lamina propria macrophages in a TLR4- and MyD88-dependent manner (73). Overall, these findings suggest that direct interactions between gut bacteria and the immune regulatory mechanisms of the intestinal lining may enhance wound healing and tissue repair. Intestinal wound healing should be considered in conjunction with the microbiome, host genetic composition, immune regulation and their relationship with the perioperative inflammatory state, as well as the influence of interdependent factors.

5. Depletion of factors promoting anastomotic healing

Reduced microbial diversity. The gut microbiota serves a crucial role in wound healing. In 1955, Cohn and Rives (87) first proposed that the intestinal microbial composition influences AL development. Disruptions in gut microbiota diversity are frequently observed due to preoperative or perioperative interventions, as well as surgical stress. Such disruptions can lead to metabolic disarray and a decreased ability to resist pathogenic invasion, potentially increasing the risk of AL (60). The significant depletion of beneficial bacteria in anastomotic tissue may facilitate the colonization of pathogenic microbes, ultimately hindering the recovery process (88,89).

Changes in gut microbiota metabolites. Imbalances in the gut microbiome also alter the production of metabolites such as SCFAs and bile acids, as well as bacterial components such as LPS and peptidoglycan. These modifications can cause disturbances in the intestinal barrier, impair hormonal regulation and trigger immune dysregulation, ultimately impacting gut function and overall health (90). Butyrate, a key SCFA, is the primary energy source for colonic epithelial cells and serves

a crucial role in promoting cell proliferation and maintaining barrier integrity. It also has anti-inflammatory properties helping to reduce pro-inflammatory cytokines (91,92). Molecular studies suggest that butyrate lowers the AL risk by suppressing the proliferation of *P. aeruginosa* (93,94). Formyl peptide receptors (FPRs), along with reactive oxygen species (ROS) and reactive nitrogen species, are considered to contribute to the beneficial effects of gut bacteria on intestinal regeneration and healing. Butyrate-producing bacteria exhibit strong protective effects and may regulate extracellular signal-regulated kinase pathways via FPR-mediated signaling, which triggers ROS production in intestinal epithelial cells. These processes improve gut repair and regeneration, helping to maintain mucosal integrity and function (62,95,96). Additionally, research indicates that specific gut microbiota can confer infection resistance by converting host bile salts into metabolites that inhibit *Clostridioides difficile* (97). However, bacteria can trigger systemic inflammation and sepsis (98). In sepsis, LPS-induced inflammation is a crucial pathological occurrence that disrupts metabolic and immune functions (99-101). Furthermore, bacterial peptidoglycan has been shown to stimulate the production of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-10, contributing to tissue damage and immune dysregulation (102). In a rat model, injection of peptidoglycan-polysaccharide from *Streptococcus pyogenes* into the distal colon induced chronic granulomatous colitis (103). These studies provide new insights into how gut microbial derivatives and metabolism influence postoperative healing and AL risk.

Decline in angiogenesis regulation. Probiotics serve a crucial role in aiding wounds at various stages of intestinal recovery (104). In the gut, symbiotic bacteria regulate the formation of new blood vessels (104), which helps reduce intestinal inflammation and promotes mucosal tissue healing during the inflammatory phase of wound repair. This process occurs through the signaling of the vascular endothelial growth factor receptor (VEGFR). For example, the yeast *Saccharomyces boulardii* modulates angiogenesis by adjusting VEGFR signaling. This regulation decreases intestinal inflammation and supports mucosal tissue repair. Inflammation can have both beneficial and detrimental effects on intestinal AL and angiogenesis is a crucial component of the inflammatory response necessary for mucosal remodeling during recovery (105). In 2002, Stappenbeck *et al* (106) conducted a study on rodents highlighting the essential role of the microbiome in rebuilding the mesenchymal microvascular system by involving Paneth cells, key components of the innate immune response. Their research revealed that *Bacteroides thetaiotaomicron* can increase the capillary network in the small intestine two-fold by acting on Paneth cells, thereby promoting angiogenesis (106).

6. Intestinal barrier disruption

Perioperative procedures for colorectal surgery can lead to a reduction in bacterial populations and dysbiosis, which can further cause intestinal barrier dysfunction. The roles of the four intestinal wall layers (the mucosa, submucosa, muscularis propria and serosa) in anastomotic healing remains an area of ongoing research. During colorectal resection, all four layers are transected before the formation of an anastomosis. Nevertheless, the mucosa and submucosa are in close proximity to the intestinal microbiota, making them particularly relevant to healing.

Submucosa. The submucosa, composed of connective tissue, has the highest tensile strength among the four intestinal layers. The submucosa is recognized as the main structural component responsible for anastomotic healing (107). As the most resilient fibrous layer, it consists mainly of elastin fibers and collagen, making it the most resistant of these layers to mechanical stress (108). This layer serves as a major source of fibroblasts, which are activated after gastrointestinal surgery to produce and release collagen. Bacterial adhesion to collagen is a crucial factor in anastomotic healing (109,110). Mechanistic studies have discovered that imbalanced gut microbiota can inhibit epithelial cell migration and repair by interfering with integrin α2β1 or laminin-332 in the extracellular matrix, thereby suppressing Rac1 expression (111).

Mucus layer. Considering that bacteria reside within the colonic mucus; the mucosa serves a more significant role in healing than previously considered (15). The gastrointestinal tract relies on mucus as its first line of defense against external factors. Preoperative bowel preparation can alter the composition and production of the protective mucus layer, potentially triggering bacterial translocation. Everard *et al* (112) demonstrated that mucus-degrading bacteria, such as *Akkermansia muciniphila*, which inhabit the mucus layer, can restore gut barrier function. These bacteria influence the mucus layer by increasing mucus production and stimulating the secretion of specific antimicrobial proteins, which help reduce weight gain, fat accumulation and low-grade inflammation. Reduced mucus levels expose collagen at the anastomotic site, enabling pathogens to colonize and produce collagenases (113).

Mucosal layer. Li *et al* (114) demonstrated that the gut microbiota enhances the mucosal layer's ability to defend against infections. Indigenous microorganisms inhabit microbial niches, competing with intestinal pathogens and opportunistic bacteria to avert infections. The intestinal epithelium serves as a physical barrier, forming a monolayer of interconnected biofilms that prevent bacterial invasion and the translocation of pathogens and microbial products (115,116). Additionally, intestinal epithelial cells possess inducible innate defense mechanisms that enable rapid responses to pathogenic challenges. These include the secretion of salt, water, antimicrobial peptides and complex glycoproteins, such as mucins (117). Mucins, produced by mucosal epithelial cells, form a protective barrier that limits the penetration of environmental substances into the epithelial layer (15). Certain probiotic lactobacilli species adhering to intestinal epithelial cells can rapidly induce the expression of the eukaryotic MUC3 mucin gene. This secreted mucin inhibits the adhesion of intestinal pathogens to epithelial cells, enhancing barrier function (89). A foundational study reported that *Muc2* mucin gene knockout mice exhibited increased leukocyte infiltration, reduced collagen deposition and impaired angiogenesis. Consequently, colonizing bacteria came into direct contact

with intestinal epithelial cells, exacerbating inflammation and tissue damage (118). Bacteria adhere to mammalian cells via the AIDA-1 adhesin protein. A clinical study reported that patients with AL had a higher abundance of mucin-degrading bacteria from the Lachnospiraceae and Bacteroidaceae families at the anastomotic site. This bacterial composition may serve to predict AL (119). Tight junctions, which function as intracellular boundary structures, serve a crucial role in preserving the integrity of the intestinal epithelial barrier. They regulate the passage of solutes and molecules while preventing the translocation of harmful substances, such as lipids and microbial peptides, from the gut into the bloodstream (120). Previous findings indicate that the microbiome serves a crucial role in effective wound healing, influencing systemic physiological processes. Activation of β-catenin signaling through interactions between the microbiome and epithelial cells is essential for regulating epithelial cell proliferation (121). This highlights the complex molecular and cellular interactions required for repairing intestinal epithelial repair, involving host cells, luminal growth factors and the gut microbiota (122,123). During surgical anastomosis, the proximity of two suture lines may lead to an imprecise circumferential connection between the proximal and distal segments. Complete epithelial coverage across the anastomotic site is a critical prerequisite for early-stage healing. *A. muciniphila* serves a key role in this process by activating pathways that enhance epithelial cell movement and proliferation (124). Bacterial fermentation of dietary fibers produces SCFAs such as acetate, propionate, butyrate, valerate and isovalerate, which serve as the primary energy source for colonic epithelial cells. SCFAs also exert a direct nutritional impact on the colonic mucosa, promoting its integrity and function (16). *In vitro* research has demonstrated that *A. muciniphila* and *Bacteroides fragilis* enhance intestinal epithelial integrity despite being anaerobic organisms (125). Of note, these bacteria can survive in aerobic environments, a critical feature given that surgical procedures can temporarily elevate oxygen levels in the lower gastrointestinal tract, reducing the number of obligate anaerobes. Mouse studies have replicated these bacterial effects, demonstrating faster mucosal re-epithelialization and improved healing outcomes (124,126).

7. Common pathogens associated with AL

Under the stress of surgery or perioperative treatment, gut-colonizing symbiotic and pathogenic microbes can potentially develop aggressive and toxic tissue-degrading phenotypes (producing collagenases that directly degrade collagen fibers and activate host-derived proteases, collectively contributing to tissue breakdown). Previous research has explored the cause-and-effect relationship between AL and the intestinal microbiome, revealing that anastomotic damage results in notable alterations in the structure and operation of the microbiome associated with anastomotic tissues. Komen *et al* (127) identified *E. faecalis* and *P. aeruginosa* as the predominant pathogens at leakage sites, showing significant collagenolytic activity (127). Tissue-degrading bacteria, such as *E. faecalis*, *P. aeruginosa*, *Pseudomonas putida* and *Fusobacterium nucleatum*, produce collagenases that break down collagen and activate host proteases such as MMP9 (42,128).

E. faecalis. Collagens I and IV serve critical roles in maintaining and repairing the extracellular matrix. *E. faecalis* is of particular interest due to its collagen-degrading activity, which may serve as a potential distinguishing factor between AL and non-AL. Significant upregulation of *Enterococcus* and *Enterobacter* species has been observed in the intestinal mucosa near colonic anastomoses in rats (46). As a major cause of enterococcal bacteremia, *E. faecalis* is a common pathogen causing nosocomial bloodstream infections. It is known for its strong adhesion to extracellular matrix proteins such as fibronectin, laminin and type IV collagen (129,130). Additionally, *E. faecalis* has been shown to induce the activation and cleavage of intestinal MMP9, which is associated with AL, in a GelE/SprE-dependent manner (42). These findings suggest that *E. faecalis* may impair anastomotic healing through a dual mechanism.

P. aeruginosa. A study conducted by Schardey *et al* (131) was the first to suggest the potential involvement of *P. aeruginosa* in AL. One of its most critical virulence factors is the type III secretion system (T3SS). Jin *et al* (132) reported that MexT regulates T3SS through MexS and PtrC, and mutations in the *mexT* gene contribute to *P. aeruginosa* virulence transformation, serving a crucial role in cytotoxicity. Sequence analysis of *P. aeruginosa* at anastomotic sites has revealed an SNP mutation in the *mexT* gene, conferring swarming ability, enhanced collagenase activity and an epithelial-destructive phenotype (37). Consequently, bacteria residing at the anastomotic site can detect subtle changes in cytokines, chemokines and ischemic byproducts and respond by increasing their virulence, particularly through the expression of collagenolytic phenotypes (Fig. 2).

8. Preventing AL from a microbiome perspective

Previous studies highlight the essential role of the gut microbiota in maintaining intestinal health, protecting the intestinal barrier and supporting normal digestive functions (133,134). As the microbiome serves a crucial and pathogenic role in AL etiology and pathogenesis, it represents a promising target for intervention. Preventing AL may involve identifying high-risk patients with unfavorable microbiome compositions, including an overabundance of collagenase-producing pathogens. Preventative strategies include preoperative prehabilitation through dietary modifications, personalized antibiotic treatments (oral, intravenous or enema), microbiome restoration and/or adherence to Enhanced Recovery After Surgery (ERAS) protocols (41).

Healthy eating habits. Unlike the host genome, the microbiome is highly adaptable to environmental and dietary influences. Among these factors, dietary habits profoundly shape microbial composition. Preoperative dietary preparation with a low-fat, high-fiber diet has been shown to enhance anastomotic healing by modulating the microbiome. Studies have explored how short-term dietary interventions can counteract the negative effects of high-fat Western diets on anastomotic healing in mice. A Western diet significantly disrupted both the gut microbiota composition and anastomotic healing, whereas a preoperative low-fat, high-fiber diet restored microbial

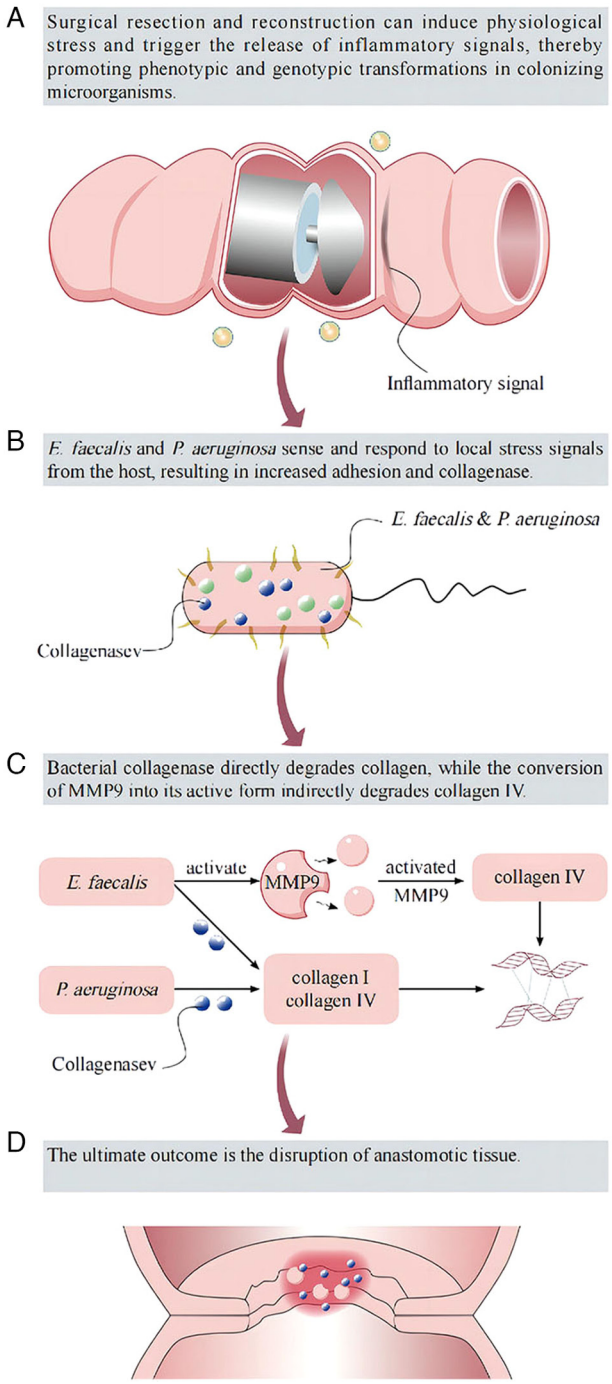

Figure 2. Microbial pathogenesis of AL (common pathogens associated with AL). (A) Surgical resection in the patient's intestine induces physiological stress. (B) *Enterococcus faecalis* and *Pseudomonas aeruginosa* sense and respond to local host stress signals, leading to increased adhesion and collagenase production. (C) Bacterial collagenases directly degrade collagen, while MMP-9 activation indirectly cleaves type IV collagen. (D) Progressive tissue destruction culminates in anastomotic leakage. AL, anastomotic leakage. MMP9, matrix metalloprotease 9.

diversity and improved outcomes (135). Guo *et al* (136) further confirmed that a preoperative low-fat, high-fiber diet enhances microbial diversity postoperatively and accelerates anastomotic healing. This dietary adjustment can mitigate the harmful effects of a Western diet, leading to improved survival rates after surgery (137). Furthermore, modifying the gut microbiome using dietary inulin and 5-aminosalicylic acid can strengthen the intestinal barrier and prevent anastomotic tumors and metastatic spread in mice (138). Mice receiving diets supplemented with inulin, galacto-oligosaccharides (GOS) or cellulose for 2 weeks before colonic surgery exhibited improved results as inulin and GOS increased butyrate production and improved anastomotic healing. Therefore, dietary supplementation with inulin and GOS can strengthen intestinal barriers and promote recovery in mice (139).

Personalized bowel preparation. Perioperative procedures disrupt the gut microbiome, leading to reduced microbial diversity and an increased risk of opportunistic pathogen overgrowth (4,49). Localized decontamination strategies have been successfully employed to prevent colorectal AL. Applying a non-absorbable antibiotic mixture of polymyxin B, gentamicin and vancomycin locally every 6 h for 5 days postoperatively prevented AL (140). Schardey *et al* (141) also confirmed that local antibiotic decontamination is highly effective in preventing AL in rectal surgery. Scarborough *et al* (46) analyzed data from the American College of Surgeons National Surgical Quality Improvement Program, studying 4,999 patients. They found that a combination of preoperative oral antibiotics and MBP reduced the incidence of AL in colorectal surgery from 5.7 to 2.8%, compared with patients who received no preparation. Neither oral antibiotics nor MBP alone were effective in reducing AL rates (46). Further research indicates that a double-dose bowel cleansing regimen has a lesser impact on the intestinal microbiome than a single dose, leading to a faster microbial recovery period, making it a more suitable clinical application (43). The bowel preparation protocol offers two options: a split-dose regimen (1 L twice) or a single-dose regimen (2 L once). While the total volume remains identical, the administration frequency differs. Laboratory studies in rats have shown that preoperative treatment and surgery result in an increase in facultative anaerobes and a depletion of SCFAs (21,58). This highlights the importance of preoperative administration of SCFAs, such as butyrate, to counteract microbial imbalances. Additionally, surgical procedures, cancer treatment, antibiotics and painkillers can reduce *Lactobacillus* levels at anastomotic sites. This decrease in *Lactobacillus* leads to reduced phosphate levels in the surrounding intestine and an increase in collagenase-producing bacteria. These bacteria release collagenases that activate inflammatory responses, further increasing the AL risk. Therefore, incorporating non-absorbable oral phosphate supplementation into bowel cleansing protocols may help mitigate these effects (142).

Microbiome restoration. Animal studies have shown that a diverse microbiome significantly promotes intestinal anastomotic healing, whereas germ-free mice or those with a single bacterial colony exhibit poor healing (62,143). Probiotics are preparations of live microorganisms that provide health benefits when consumed in sufficient quantities. They are mainly composed of species from the *Bifidobacterium* and *Lactobacillus* genera (144). These beneficial microbes enhance the intestinal epithelial barrier in both mice and humans, reducing infections in patients with colorectal cancer after colon surgery and strengthening gut mucosal

Table II. Therapeutic interventions for preventing anastomotic leakage from a microbiome perspective and their mechanisms.

Intervention	Specific methods	Mechanisms
Low-fat, high-fiber diet	-Increase dietary fiber (for example, whole grains, vegetables and fruits) preoperatively. -Reduce intake of saturated fats and processed foods.	-Promotes the growth of beneficial bacteria (for example, *Bacteroides* and *Lactobacillus*) and maintains gut microbiota balance (137). -Enhances intestinal barrier function and reduces inflammation (152).
Preoperative bowel preparation with personalized antibiotics	-Mechanical bowel cleansing (for example, polyethylene glycol). -Select antibiotics (for example, neomycin, metronidazole or vancomycin) based on microbiota testing. -Administer orally, intravenously or via enema.	-Reduces the load of potential pathogens (for example, *Enterococcus faecalis* and *Escherichia coli*). -Prevents postoperative infections and bacterial translocation (50,52). -Restores gut microbiota balance and inhibits pathogen proliferation (144).
Microbiota restoration	-Supplement with probiotics (for example, *Lactobacillus* and *Bifidobacterium*). -Use prebiotics (for example, fructo-oligosaccharides and inulin). -Fecal microbiota transplantation.	-Enhances intestinal barrier function, reduces inflammation and prevents bacterial translocation (145).
Enhanced recovery after surgery	-Early enteral nutrition. -Limit intraoperative fluid infusion. -Multimodal analgesia. -Early mobilization.	-Promotes recovery of intestinal function and reduces postoperative ileus. -Reduces inflammatory response and improves anastomotic healing (151). -Reduces postoperative complications (149,153).

barrier integrity (145). Studies on perioperative probiotic use, particularly with *Bifidobacterium*, have reported a reduction in harmful bacteria, including Enterobacteriaceae, *Clostridium difficile* and *Pseudomonas,* postoperatively (144). However, while animal studies suggest that probiotics and synbiotics promote anastomotic healing, clinical evidence supporting their effectiveness as standalone treatments remains inconclusive. A network meta-analysis attempted to identify the most effective intervention between prebiotics, probiotics, synbiotics or oral antibiotics for reducing infection rates in patients undergoing elective colorectal surgery. It was demonstrated that oral antibiotics were the most effective in reducing both infection rates and AL rates. While synbiotics and probiotics appeared to reduce postoperative infections, they did not significantly reduce AL rates (146).

Fecal microbiota transplantation (FMT), also known as fecal bacteriotherapy, involves transplanting stool from a healthy donor into the recipient's gastrointestinal tract to restore gut microbiota diversity and balance (147,148). Mice that received FMT from patients with AL exhibited impaired anastomotic healing. In an experimental setup, antibiotic-conditioned mice received FMT from patients with AL prior to surgery. These results showed that these mice had compromised colonic healing, characterized by lower concentrations of extracellular matrix components and higher concentrations of E-cadherin, indicating impaired cell migration at the wound edges (138). The aforementioned study collected preoperative fecal samples from 77 patients with colorectal cancer. Among them, 9 patients subsequently developed anastomotic leakage (AL), who were then matched with 9 non-AL patients by age, sex, and tumor location (non-AL vs. AL groups). Through FMT, the preoperative fecal samples were transferred to antibiotic-pretreated mice, which subsequently underwent surgical procedures.

ERAS. A key strategy for minimizing perioperative microbiome disruption is the implementation of ERAS protocols. ERAS aims to reduce surgical stress responses and organ dysfunction, leading to fewer postoperative complications and faster recovery. Emerging consensus guidelines emphasize early mobilization, immediate resumption of food intake and reduced opioid analgesia use, all of which help maintain a healthier microbiome and reduce postoperative bacterial growth (149,150). ERAS protocols recommend preoperative oral carbohydrate drinks to improve nutritional status and early reintroduction of oral intake after surgery. SCFAs such as butyrate, propionate and acetate, primarily produced through the fermentation of complex carbohydrates like fiber, may exhibit significant metabolic and protective effects. SCFAs serve as primary energy sources for intestinal epithelial cells, activate GPCRs and inhibit histone deacetylases, thereby promoting gut barrier integrity (Table II) (151).

9. Conclusion

Despite advancements in surgical techniques, AL rates have not significantly decreased in recent years. AL arises from

a complex and dynamic interplay of multiple factors and biological processes, including gut microbiome dysbiosis, inflammation, host genetics, immune responses and pathogenic microbes capable of compromising the intestinal barrier. Future therapeutic strategies should focus on fostering and maintaining a diverse and balanced microbiome rather than merely eradicating specific microbes. Interventions that mitigate collagen degradation, reduce free radical production and regulate control matrix metalloproteinase activation may prove more effective compared with antimicrobial treatments alone. ERAS protocols, which reduce hospital stays and healthcare costs, offer a promising framework for improving surgical outcomes by addressing microbiome-related factors. Microbiome-based biomarkers must be developed and validated to predict AL risk preoperatively, enabling targeted interventions for high-risk patients. Microbiome-modulating drugs, such as probiotics, prebiotics, synbiotics or targeted antibiotics, must be investigated and designed according to the unique needs of surgical patients to promote a healing-friendly microbial environment. Artificial intelligence and machine learning must be implemented to analyze and predict individual microbiome profiles, enabling personalized perioperative care strategies that optimize gut microbiota composition for improved wound healing. Mechanisms through which specific microbial metabolites and immune-modulating factors influence anastomotic healing must be investigated, with a focus on collagen metabolism, epithelial barrier integrity and inflammatory responses. ERAS protocols must be combined with microbiome-targeted therapies to enhance recovery, reduce complications and improve long-term surgical outcomes. By prioritizing these research areas, understanding of the gut microbiome's role in anastomotic healing can be advanced, and innovative, personalized approaches can be developed to reduce AL rates and improve patient outcomes.

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YC, NW, TS, LK and JF conceived and designed the review. YC and NW drafted the manuscript. ZZ, GO, CX and XY conducted the literature search and data analysis. TS, LK, JF and GO critically revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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