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Baigiamasis darbas

Žarnų mikrobiomos reikšmė pooperacinių komplikacijų vystymesi po didžiųjų pilvo organų operacijų dėl onkologinių susirgimų

Gut Microbiome Impact on Postoperative Morbidity After Major Abdominal Oncological Surgery

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SANTRAUKA

Maždaug trečdalis pacientų operuojamų dėl virškinimo trakto vėžio patiria pooperacines komplikacijas. Šiandieną mokslinėje literatūroje daugėja duomenų, kad pooperacinių komplikacijų patogenezėje gali dalyvauti žarnų mikrobiota. Vis dėlto tikslus jos vaidmuo nėra visiškai aiškus. Todėl, siekiant apibendrinti šiandieną egzistuojančius įrodymus apie žarnų mikrobiotos galima vaidmenį pooperacinių komplikacijų patogenezėje, atlikta ši išsami literatūros apžvalga. Literatūros paieška atlikta naudojantis PubMed duomenų baze, atrenkant tyrimus su žmonėmis, kuriuose buvo tiriamas ryšys tarp žarnyno mikrobiotos ir trumpalaikių komplikacijų po virškinamojo trakto vėžio operacijų. Atlikus literatūros paiešką atrinkti penki tyrimai, paskelbti laikotarpiu nuo 2018 iki 2023 metų. Visuose tyrimuose buvo nagrinėjama anastomozės nesandarumo komplikacija: keturiuose tyrimuose daugiausia dėmesio skirta anastomozės nesandarumui po storosios žarnos vėžio operacijos, o viename tyrime aptartas anastomozės nesandarumas po stemplės pašalinimo operacijos. Dviejuose tyrimuose *Bacteroides fragilis* ir *Ruminococcus torques* buvo nustatytos kaip vyraujančios bakterijos pacientams, kuriems nustatytas anastomozės nesandarumas, o tai rodo galimą jų vaidmenį didinant šios komplikacijos riziką. Tolesnės diskusijos atskleidė, kad nemažai bakterijų rūšių, siejamų su anastomozės nesandarumo pasireiškimu, skatina interleukino-17 gamybą . Ryšio tarp anastomozės nesandarumo ir interleukino-17 galimybę parodo interleukino-17 veikoje padidintas matrikso metaloproteinazių, kurios ardo kolageną, aktyvumas. Manoma, kad bakterijų rūšys skatinančios žarnyno gijimą, gali būti naudingos dėl trumposios grandinės riebalų rūgščių gamybos ir žarnyno uždegimo mažinimo skatinant interleukino-10 gamybą.

Raktažodžiai: žarnyno mikrobiota, operaciniai rezultatai, virškinamojo trakto vėžys, Bacteroides fragilis, interleukinas-17, trumposios grandies riebalų rūgštys, žarnyno uždegimas.

ABSTRACT

Approximately one third of gastrointestinal cancer patients experience post-operative complications. In today's literature, the microbiome is acknowledged as a potential contributor to the pathogenesis of post-operative complications. However, the exact role is not clear. Consequently, this comprehensive review was conducted to summarize existing data on the possible role of intestinal microbiome in the pathogenesis of postoperative complications. A literature search was conducted using the PubMed database, selecting human studies that investigated the link between intestinal microbiome and short-term outcomes of gastrointestinal cancer surgery. After the search, five studies published between 2018 and 2023 were included. All studies addressed the complication of anastomotic leakage, with four studies focusing on anastomotic leakage following colorectal cancer surgery and one study

examining anastomotic leakage after esophagectomy. *Bacteroides fragilis* and *Ruminococcus torques* were identified as prevalent bacteria in patients with anastomotic leakage in two studies, suggesting a potential role in increasing the risk of this complication. Further discussion revealed that some of the bacterial species, related to increased rates of anastomotic leakage, induced interleukin-17 production. The possibility of a link between anastomotic leakage and interleukin-17 is supported by the interleukin-17 induced upregulated activity of matrix metalloproteinases, which degrade collagen. Bacterial species thought to promote intestinal healing were found to be potentially beneficial through the production of short-chain fatty acids and the reduction of intestinal inflammation, primarily via induced production of interleukin-10.

Keywords: intestinal microbiome, surgical outcomes, gastrointestinal cancer, Bacteroides fragilis, interleukin-17, short-chain fatty acid production, intestinal inflammation.

INTRODUCTION

Epidemiology

The prevalence of solid gastrointestinal tumors constitutes nearly one-fourth of all cancer cases, as reported by the Global Cancer Observatory. In 2022, colorectal cancer accounted for 12% of all cancer incidences, positioning it as the second most prevalent cancer type. Pancreatic cancer was diagnosed in 3.3% of cancer patients, while stomach cancer affected 3%, liver and intrahepatic bile duct cancer comprised 2%, and esophageal cancer represented 1.2% of all cancer cases in the same year (1).

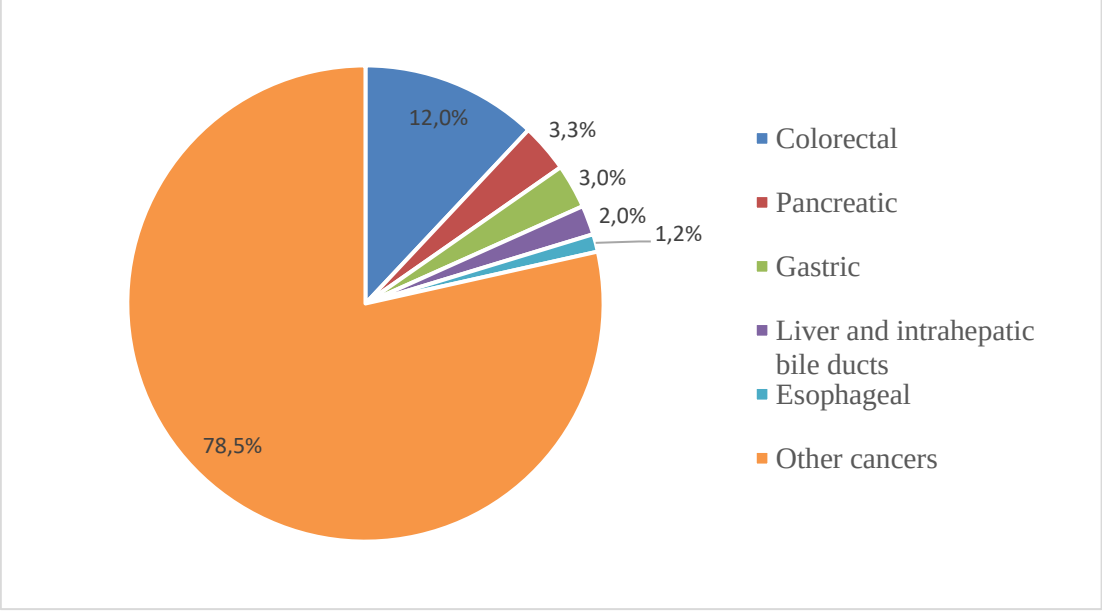

Figure 1 Incidence of gastrointestinal cancers in Europe in 2022, Global Cancer Observatory

The role of surgery in treatment of gastrointestinal cancers

Radical surgery remains the cornerstone treatment for all types of solid gastrointestinal tumors. Surgical tumor removal is typically a major intervention resulting in a higher risk of various complications. Multiple studies show that about one third of gastrointestinal malignancy patients experience postsurgical complications (2,3). In colorectal cancer surgery ileus and anastomotic leakage are the most prevalent complications followed by general surgical complications such as pneumonia, significant blood loss and electrolyte imbalances (4). Following pancreaticoduodenectomy, common complications include postoperative pancreatic fistulae, delayed gastric emptying, surgical site infections, and pancreatitis (5). In gastric cancer surgery, general surgical complications such as respiratory complications, bleeding, wound infections, and cardiovascular complications are most prevalent, followed by anastomotic leaks (6). After hepatectomy, post-hepatectomy liver failure and biliary leakage are common liver related complications, other complications include acute renal failure and ascites (7,8). Esophagectomy remains the main esophageal cancer treatment with complications varying from pneumonia, atrial dysrhythmia, anastomotic leakage and chyle leaks to recurrent nerve injury (9).

Postoperative complication pathogenesis

Surgical procedures inherently create a traumatic and stressful environment for the body, leading to impaired wound healing, inflammation, blood loss, and ischemia. These interconnected mechanisms significantly elevate the risk of infections (10). Naturally preoperative organ function, individual patients' parameters and other comorbidities also influence the risk of complications, but more emphasis is put on adjustable mechanisms of the complications.

Recent studies indicate that the gut microbiome may play a pivotal role in either exacerbating or mitigating postoperative complications by impacting wound healing and inflammation. For instance, experiments conducted on rats have demonstrated that *Enterococcus faecalis* increases the risk of anastomotic leakage by degrading collagen and activating tissue matrix metalloprotease-9 (MMP-9) (11). Conversely, targeting gut bacteria that produce short-chain fatty acids (SCFAs) and enhancing butyrate production could potentially improve colonic healing (12). Despite promising findings, there is a lack of robust data on humans (13,14). Further research is needed to elucidate the mechanisms underlying these interactions and to develop novel microbiome-based interventions for lowering postoperative complication rates. Therefore, the objective of this study was to conduct a comprehensive review of the

available literature, to summarize the current evidence, and to identify the gaps in knowledge. This endeavor aims to facilitate the formulation of future research directions more effectively.

METHODS

The search was conducted on the PubMed database using provided MeSH Terms: "gastrointestinal microbiome" OR "brain gut axis" OR "dysbiosis" OR "probiotics" AND "postoperative complications" OR (surgical procedures, operative AND treatment outcome) OR (surgical procedures, operative AND Recovery of Function). The search concluded on November 29th, 2023. Only English language manuscripts were considered for inclusion. The selection process began by screening articles based on their titles. Subsequently, the author meticulously reviewed the titles and identified relevant abstracts for further evaluation. Exclusion criteria included articles published in languages other than English, opinion manuscripts, letters, conference reports, abstracts, reviews, and systematic reviews. Additionally, studies conducted exclusively on animals or in vitro, those focused on the pediatric population, and investigations into long-term consequences were excluded. Inclusion criteria were defined to incorporate studies centered on gastrointestinal cancer surgery, incorporating microbiome analysis, and facilitating a comparative assessment between groups with and without complications. Full-text manuscripts were retrieved for confirmation of inclusion (Figure 1).

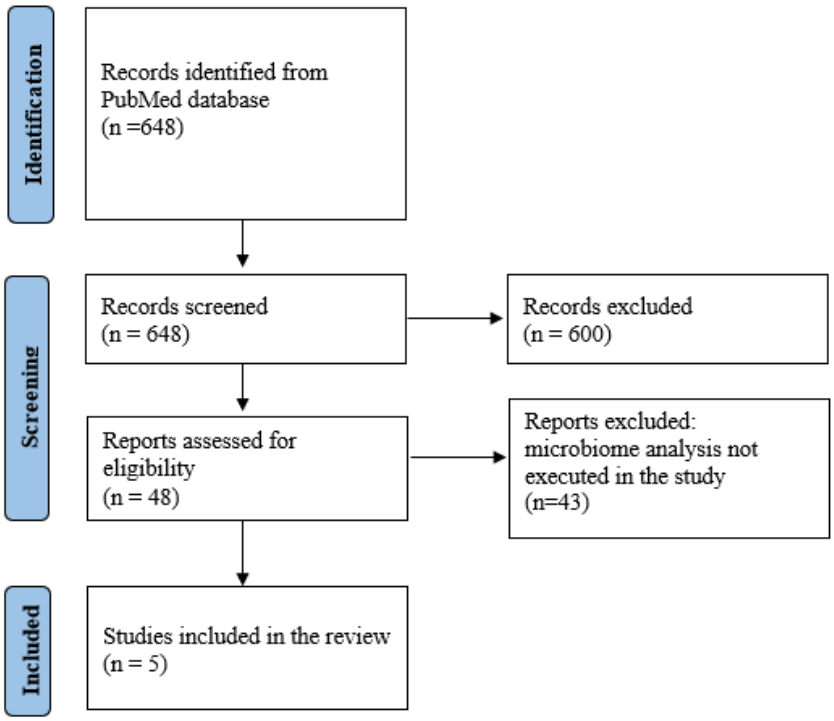

Figure 2. Flow diagram on the identification, screening and inclusion of studies.

RESULTS

Study characteristics

During the literature search, a total of 648 manuscripts published between 1997 and 2023 were identified. Subsequently, following the literature review process described above, five publications were included in the final comprehensive review. These studies are summarized in Table 1.

All included studies were observational studies. Detailed characteristics of the studies are shown in Table 1. Among them, four (80%) studies focused on colorectal cancer patients, while one (20%) examined patients with esophageal cancer. The main aim of all included studies was to establish the association between the gut microbiome composition and anastomotic leakage. Altogether, studies included 305 patients, of whom 66 developed anastomotic leakage. One study additionally investigated the impact of gut microbiomes' composition on postoperative pneumonia rates (15). All studies conducted a comparative analysis of microbiomes between patients with and without complications. Two (40%) of the studies employed fecal microbiota transplantation in animal models to assess the impact of microbiomes from patients with and without anastomotic leakage and explore potential biomarkers associated with complications or the facilitation of healing (16,17).

Microbiome identification across all studies utilized 16s rRNA, with three (60%) of the studies relying on stool samples. The snapshot approach of collecting samples a single time was adopted by three (60%) studies, however, the timing of sample collection varied among the studies (17–19). Palmisano et al. opted for the collection of stool samples exclusively before surgical intervention or following neoadjuvant therapy when indicated (18). Jin X et al. selected stool samples from the first postoperative defecation (17). In a unique approach, Van Praah JB et al. utilized samples from the resected intestinal "doughnut" obtained during anastomotic surgery (19). The other two studies chose a longitudinal approach (15,16). Hajjar et al. collected stool samples before surgery and prior to the administration of prophylactic antibiotics and mechanical bowel preparation followed by the resection of mucosal samples adjacent to the anastomosis during surgery and the acquisition of rectal swabs on the third postoperative day (16). Reddy RM et al. expanded the scope of sample types by including saliva samples collected before and after surgery, along with esophageal and gastric mucosa samples acquired during the operation. Additionally, in instances of postoperative complications, this study further detailed the collection of neck wound swabs in cases of anastomotic leakage and sputum samples when pneumonia was present (15).

Gut microbiome impact on anastomotic leakage

Microbiome findings related to anastomotic leakage exhibited considerable heterogeneity among the studies, with discernible patterns primarily emerging at the genus rather than species level. Specifically, the genera *Blautia, Bacteroides*, and *Ruminococcus* were recurrently identified in two (40%) studies (17,19). At the species level, *Bacteroides fragilis* and *Ruminococcus torques* exhibited higher prevalence rates among patients experiencing anastomotic leakage in two (40%) studies (16,19). Notably, in one of these studies, these findings were not directly investigated but were included as supplementary materials (16). Other bacterial species prevalent in patients with anastomotic leakage included *Acinetobacter lwoffii, Acinetobacter johnsonii, Hafnia alvei, Blautia obeum, Blautia glucerasei, Alistipes onderdonki, and Ruminococcus gnavus* (16–19). At the genus level, *Romboutsia, Streptococcus, Blautia, Bacteroides, Clostridium sensu stricto 1, Eggerthella, and Coprococcus* were prevalent in patients with leakage (17,19). The relative proportion of *Escherichia/Shigella* was elevated in patients who experienced leakage in one (20%) study (17). In cases of esophageal leakages, *Bacteroidetes* emerged as a common organism in neck swabs post-leakage, alongside a noted cluster of *Firmicutes/Clostridia* (15).

Certain bacterial species were found to be more common in patients without the complication, namely *Barnesiella intestinihominis, Faecalibacterium prausnitzii, Streptococcus salivarius, Prevotella copri, Eubacterium biforme, and Parabacteroides goldsteinii* (16,18,19). Higher microbial proportions of strains such as *Ruminococcaceae, Streptococcaceae, Lactobacillus*, and *Comamonas* were linked with lower anastomotic leakage rates in two (40%) studies (17,19).

Microbial diversity

Microbial diversity was explored in four (80%) of the studies reviewed (16–19). α-Diversity, a measure of within-sample diversity, was investigated in four (80%) studies, with variations in findings observed among the studies (16–19). Lower α-diversity was reported to be more prevalent in patients with anastomotic leakage in two (40%) studies, with one study indicating that a microbial composition consisting of 60% or more from families *Lachnospiraceae* and *Bacteroidaceae* was associated with an increased risk of anastomotic leakage (18,19). However, contrary to such findings, one study (20%) noted that patients who experienced anastomotic leakage exhibited higher α-diversity (17). In the fourth study the difference in α-diversities showed no statistical significance (16).

β-diversity, which measures differences in microbial composition between samples, was investigated in two (40%) studies, both of which utilized mouse models to extend the research (16,17).

Jin et al. found that β-diversity was higher in patients who experienced anastomotic leakage, while Hajjar et al. noted differences in microbial compositions between cohorts, although it was not specified whether β-diversity was higher or lower in patients with the complication (16,17). Regarding leakages in the esophageal region, the authors observed a higher variance of bacteria, with neck swab results indicating a mixture of oral and gastric microbial sources (15).

Other findings

Since the included studies in the review exhibited heterogeneity in study designs and measured outcomes, several other important findings were also recorded.

Studies employing animal models and fecal microbiota transplantation (FMT) investigated the mechanisms of anastomotic healing and inflammation and their relation to anastomotic leakage and the microbiome (16,17). Hajjar R et al. detected elevated levels of pro-inflammatory cytokines, including macrophage inflammatory protein 1 alpha and 2 (MIP-1α and MIP-2), monocyte chemoattractant protein 1 (MCP-1), and interleukin-17A/F (IL-17A/F), in mucosal biopsies from surgery patients. These levels correlated with subsequent complications and were increased by *A. onderdonkii* but decreased by *P. goldsteinii* (16). In the same study, patients with anastomotic leakage exhibited poorer colonic healing, lower concentrations of extracellular matrix components (ECM), higher concentrations of E-cadherin, and lower fibronectin and hydroxyproline concentrations, which were associated with increased collagen degradation, primarily involving matrix metalloproteinase-2 (MMP-2) (16). Conversely, Jin X et al. found that collagen synthesis was upregulated through epithelial-mesenchymal transition (EMT) pathways and increased gene expression in patients without leakage, characterized by elevated expression of transforming growth factor-β1 (TGF-β1), p-Smad3, Snail, N-cadherin, vimentin, α-smooth muscle actin (α-SMA), and S100A4, while E-cadherin expression was decreased (17). Both studies revealed a positive correlation, whereby microbiomes associated with increased anastomotic leakage rates also exhibited elevated expression levels of E-cadherin.

Regarding other complications mentioned in the studies, Reddy RM et al. noted pneumonia but found no significant differences in microbiomes between patients with and without this complication (15).

Table 1. Studies investigating the role of gut microbiomes in postoperative complications following major abdominal surgery.

Authors, year	**Study design and number of patients**	**Patient inclusion and exclusion criteria**	**Microbiome identification method and material used for analysis**	**Main findings**	**Other findings**
Palmisano S, et al., 2020 (18)	• Group A consisted of 27 healthy subjects and group B consisted of 21 patient with CRC eligible for surgery, of whom 5 developed AL. • Group A (healthy subjects) did not receive surgery, only fecal samples were taken. • After surgery, group B was divided into subgroups: BL – patients who developed AL and BNL – patients who had uneventful stay. • BNL patients were not enrolled consecutively but matched with the clinical characteristics of BL patients. • Mechanical bowel preparation was performed only in patients who were eligible for anterior rectal resection.	Inclusion criteria for healthy subjects: • Age >18 years, • Negative family history of CRC. Inclusion criteria for patients eligible for surgery: • Age >18 years, • Histologically documented CRC. Exclusion criteria for healthy subjects: • Antibiotic therapy <1 month before study enrollment, • Reported rectal blood loss, • Clinical history of colorectal polyps, • Inflammatory bowel disease. Exclusion criteria for patients with CRC: • Antibiotic therapy <1 month before study enrollment; • History of previous gastrointestinal surgery; • Presence of diverting stoma;	16S rRNA. Stool samples at the day of enrollment in healthy subjects. Group B stool samples were taken before surgery (or after neoadjuvant therapy if it was indicated).	• *Acinetobacter lwoffii, Acinetobacter johnsonii* concentrations were increased in patients, with AL while absent in patients without AL. • *Hafnia alvei* was more represented in patients with CRC compared to healthy subjects, increased even more in patients who experienced AL. • *Barnesiella intestinihominis* represented 4% of flora in patients without AL and was absent in patients with AL. • *Faecalibacterium prausnitzii* was more represented in healthy patients, decreased in AL patients compared to patients without AL. • One patient who developed AL had a significant concentration of *Odoribacter ianeaus* (35%), while it was absent in healthy subjects and subjects without AL	• α-diversity analysis results showed a higher richness of species in patients without AL compared with patients who developed AL.

		• Inflammatory bowel disease.			
Van Praagh JB, et al., 2019 (19)	• The study analyses 118 CRC patients undergoing surgery. • 25 patients who developed AL were matched on sex, age, and preoperative chemotherapy and radiotherapy with 93 patients without AL. • All patients participated in the C-seal trial, a trial to evaluate the effect of the C-seal in the prevention of clinical AL in the stapled colorectal anastomosis.	Inclusion criteria: • Elective colorectal surgery with a circular stapled colorectal anastomosis; • Age >18 years; • ASA score <4; • Mechanical pre-operative bowel preparation; • No clinical signs of peritonitis. Exclusion criteria: • Major surgical or interventional procedures <30 days prior to this surgery; • Other interventional procedures planed within 30 days of entry in this study; • Psychological, familial, sociological, or geographical conditions which could potentially hamper compliance with the study protocol or the follow-up schedule.	16S rRNA; resected intestinal tissue "doughnut" from the area of anastomosis	• *Blautia genus (Blautia obeum, Blautia glucerasea), Tenericutes phylum, Ruminococcus torques, Bacteroides fragilis, Coprococcus* were found in patient with AL. • More *Ruminococcaceae, Prevotella copri,* and/or more *Streptococcaceae* (especially *s. salivarius*) were found in patients without AL. • *Eubacterium biforme, Bacteroides uniformis, Bacteroides ovatus* were related to lower AL rates.	• Low microbial α-diversity was found in patients with AL. • The Microbial constitution of 60% or more from families *Lachnospiraceae* and *Bacteroidaceae* was found to be related to increased AL risk.
Hajjar R, et al., 2023 (16)	• 77 patients participated in the study and underwent surgery. • 9 patients later experienced AL and were matched for age, sex and tumor location with 9 patients who did not experience AL.	Exclusion criteria: • No anastomosis; • Perforated tumor or intestinal perforation before surgery; • Emergency surgery for bowel obstruction;	16S rRNA. Stool before surgery. Mucosal samples day 0. Rectal swabs day +3.	• Patients with AL presented a higher abundance of *Alistipes onderdonkii,* which was associated with higher AL score. • Patients without AL presented increased levels of *Parabacteroides*	• Difference in β-diversity of mice after FMT from non-AL and AL donors. • AL patients had higher basal levels of pro-inflammatory cytokines: MIP-1α, MIP-2,

	• Preoperative stool samples were transferred into antibiotic-conditioned mice by FMT. The mice were then subjected to surgery and further microbiome and biomarker analyses were conducted. • Patients had blood samples taken before surgery for analysis of inflammatory markers.	• Active confirmed infection; • Hematological malignancy; • Hyperthermic intraperitoneal chemotherapy; • Palliative surgery; • No available preoperative complete blood count within 3 months before surgery.		*goldsteinii*. *P.goldsteinii* abundance correlated with lower AL score, lower MMP levels.	MCP-1, IL-17A/F. Respectively *A. onderdonki* increased and *P. goldsteinii* inhibited the production of the cytokines. • Rectal swabs of patients with AL showed increased TNF-α and IL-1β levels. • Higher expression of MMP-2 was found in poorly healing anastomotic wounds. • FMT from patients with AL resulted in poorer colonic healing, lower concentration of ECM components and a higher concentration of E-cadherin in mice. • FMT from donors with AL led to increased bacterial load in the spleen.
Jin X, et al., 2022 (17)	• 34 CRC patients who underwent colon surgery were included in the study. • 17 developed AL and 17 did not. The study model provided 1:1 individual match between the two groups.	Not specified.	16S rRNA. Stool samples of first postoperative defecation.	• *Romboutsia, Blautia, Bacteroides, Clostridium sensu stricto 1, Ruminococcus gnavus, Eggerthella, Escherichia/Shigella* were increased in patients with AL • Lower relative proportion of *Lactobacillus* also	• α-diversity and β-diversity of AL patients was significantly higher. • TGF-β1, p-Smad3, Snail, N-cadherin, vimentin, α-SMA, and S100A4 expression levels were

	• 5 samples from AL and without AL patients were randomly selected and mixed at equal weight and transplanted to rat models. • Further experiments (on the impact of specific bacteria, on the mechanism of AL healing) were conducted on FMT rat models.			correlated with higher AL rates. • Higher relative proportion of *Lactobacillus* and *Comamonas* correlated with lower AL rates.	significantly upregulated in mice with FMT from patients without AL. • The expression of E-cadherin was decreased in mice with FMT from patients without AL.
Reddy RM, et al., 2018 (15)	• 55 patients underwent successful transhiatal esophagectomy and were chosen for the analysis. • Primary surgical complications, including postoperative pneumonia and postoperative AL, were documented, and analyzed. • AL occurred in 10 patients. Pneumonia occurred in 2.	Not specified.	16S rRNA. • Saliva before the operation and 1-3 days after. • Esophageal and gastric mucosal samples collected intraoperatively. • Neck wound swabs of patients with cervical anastomotic leaks. • Induced sputum samples were collected when pneumonia developed.	• Neck swab flora showing a mixed composition between the oral and gastric sources – *Bacteroidetes,* with one cluster of *Firmicutes/Clostridia* in patients with AL.	• No differences were noted in patients who developed postoperative pneumonia.

Basic characteristics of studies; AL score grades macroscopic healing of the anastomosis as follows: 0 - normal healing; 1 - flimsy adhesion; 2 - dense adhesion; 3 - phlegmon/abscess; 4 - overt leak with peritonitis; AL – anastomotic leakage; ASA score - American Society of Anaesthesiologists score; CRC – colorectal cancer; ECM – extracellular matrix; FMT – fecal microbiota transplantation; IL-1β – interleukin 1 beta ; IL-17A/F - interleukin-17A/F; MCP-1 - monocyte chemoattractant protein 1; MIP-1α - macrophage inflammatory protein 1 alpha; MIP-2 - macrophage inflammatory protein 2; MMP-2 - matrix metalloproteinase 2; α-SMA - alpha-smooth muscle actin; TGF-β1 - transforming growth factor-beta 1; TNF-α - tumor necrosis factor alpha.

DISCUSSION

The aim of this comprehensive review was to summarize the existing evidence concerning the link between microbial species and the incidence of postoperative complications in gastrointestinal cancer surgery, particularly focusing on the role of gut microbiome. Our thorough literature search yielded five clinical studies dedicated to this subject. Among these, four studies centered on anastomotic leakage subsequent to colorectal cancer resection (16–19), while one study delved into complications post-esophagectomy for cancer (15). Although the findings across these studies varied, they collectively underscored the association between intestinal microbiome and postoperative complications. Despite the heterogeneity in the results, the overarching conclusion remains that gut microbiome plays a significant role in the pathogenesis of such complications.

Our present study is not the first in the field. Previous narrative review summarized complications following surgeries for various visceral diseases, highlighting the insufficient data and ambiguous relationships between the microbiome composition and complication development (20). This indicates the need of more elaborate approach in investigating short term complications after gastrointestinal cancer surgery conducted in this review.

Bacteroides fragilis was identified as a recurrent species in patients experiencing anastomotic leakage across two studies (16,19). The most virulent strains of this species are enterotoxin producing *B. fragilis*, with studies showing toxin contribution to increased inflammation in the gut and diarrhea (21). Comparative analyses demonstrated a higher prevalence of *Enterotoxinogenic B. fragilis* in patients with colorectal cancer compared to healthy controls, although the overall isolation rate of *B. fragilis* remained consistent (22). Notably, the molecular activity of *B. fragilis* toxin includes the cleavage of E-cadherin, a protein crucial for cell adhesion, signaling, proliferation, and differentiation within the intestinal epithelium. (23–25). Disruption of E-cadherin is associated with enhanced cell proliferation, suggesting a potential role for *B. fragilis* toxin in intestinal oncogenesis and increased intestinal permeability, thereby exacerbating harm to colon cells (26,27). Additionally, the influence of *B. fragilis* on postoperative complication rates may be mediated through the initiation of pro-inflammatory cytokines IL-17 and interleukin-8 (IL-8) (28–30).

A notable observation in the prevalence of bacterial species among patients with anastomotic leakage is the identification of bacteria from the *Ruminococcus genus*, specifically *Ruminococcus torque*s and *Ruminococcus gnavus*. These bacteria are recognized for producing SCFAs such as acetate and formate, though not butyrate, and are integral to the commensal human intestinal microbiome(31). SCFAs are an energy supply for colonocytes, with inhibited SCFA synthesis resulting in diarrhea (32).

SCFAs also participate in mitigating inflammation and potentially inhibiting pro-oncogenic activities in the gastrointestinal tract (33,34). *Ruminococci* metabolize mucin glycans derived from the host's intestinal mucosal layer to produce SCFAs, but there is no evidence to suggest that they degrade the mucus layer sufficiently to compromise the gut barrier (31). Interestingly, short glycan chain mucins are more prevalent in patients with inflammatory bowel disease, correlating with an increased presence of *Ruminococci* in these conditions (35,36). The available studies propose a hypothesis that *Ruminococci* may function as opportunistic bacteria in inflamed intestines rather than exhibiting outright pathogenic behavior.

The role of the *Blautia* genus in increasing anastomotic leakage rates remains ambiguous. Opinions vary on whether *Blautia* species are pathogenic or beneficial; some research indicates a higher prevalence of *Blautia* in patients with inflammatory bowel disease, while other studies suggest it may reduce inflammation in cases of ileal pouch-anal anastomosis (37,38). Consequently, definitive conclusions about the *Blautia* genus should be approached with caution. *Blautia obeum* is recognized as part of the healthy gut microbiota, although information on *Blautia glucerasea* remains limited (39). The potential mechanism by which *B. obeum* could induce anastomotic leakage may involve the stimulation of pro-inflammatory responses. A study in mice demonstrated that *B.obeum* aggravated colitis and elevated levels of IL-17, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) (40).

Acinetobacter lwoffi and *Acinobacter johnsonii* are considered nosocomial opportunistic pathogens, although *Acinobacter baumannii* is more commonly associated with hospital-acquired infections and *A. lwoffi* and *A. johnsonii* are considered a part of the normal skin and mucous membranes flora (41). *Acinetobacter* are proved to be quite resistant to diluted disinfectants and show resistance to antibiotics (42,43). *A. lwoffii* has been implicated in inducing gastritis, indicative of pro-inflammatory activity (44). While the relationship between the inflammatory activity of *A. lwoffii* or *A. johnsonii* and gastrointestinal diseases remains poorly defined, studies using asthma murine models have shown *A. lwoffii* to enhance the activity of interleukin-6 (IL-6) and interleukin-10 (IL-10), supporting the hypothesis of its pro-inflammatory effects (45). Given the scarcity of studies on *Acinetobacter lwoffii* and *Acinetobacter baumanni*i, it can only be hypothesized that their potential contribution to the development of anastomotic leakage lies in the pro-inflammatory activity.

Hafnia alvei is another opportunistic nosocomial pathogen, which rarely presents as ethiological pathogen of the infection (46). Strains of *H. alvei* affect cell permeability by reducing the expression of zonula occludens (ZO) proteins, which are crucial for the structural integrity of tight junctions, or by

inhibiting the phosphorylation of occludin, which is essential for the assembly and maintenance of tight junctions, eventually suggesting a possible impact in aggravating anastomotic leakage (47–49).

Any conclusions regarding the impact of *Alistipes onderdonkii* on anastomotic leakage rates can be drawn from its previous isolation from abdominal abscesses, suggesting its pathogenic potential (50). Experiments conducted in mice have further supported this notion. Hajjar et al. facilitated a study in which mice, six days post-surgery and having received *A. onderdonkii* isolated from human stool samples, exhibited a higher incidence of leaks and abscesses. Additionally, these mice demonstrated lower hydroxyproline and fibronectin concentrations at the anastomotic site compared to control mice that received saline (16). As fibronectin plays a role in cell adhesion, migration, and wound healing – lower levels indicate reduced tissue remodeling, while lower hydroxyproline, an amino acid found in collagen, levels show reduced collagen turnover (51). Hajjar et al. also documented an elevation in cytokine and chemokine concentrations, notably MIP-1α, MIP-2, and IL-17A/F, in mice exposed to *A. onderdonkii* (16). This finding further confirms the association between heightened susceptibility to compromised wound healing at anastomotic sites and the presence of *A. onderdonkii* species.

Several bacterial species discussed in this review, including *Bacteroides fragilis, Blautia obeum*, and *Alistipes onderdonkii*, have been observed to increase the expression of IL-17, necessitating a discussion on this cytokine. IL-17 acts in concert with other cytokines such as TNF and interleukin-22 (IL-22) to activate matrix metalloproteinases (MMPs), and it also stimulates the production of interleukin-1β (IL-1β), IL-6, TNF, and the chemokines MCP-1 and MIP-2 (52). MMPs contribute to the pathogenesis of impaired wound healing by cleaving collagen (53). While a balanced microbiome can produce cytokines and chemokines that support inflammation conducive to wound healing, dysregulated expression of MCP-1, MIP-2, and other cytokines can lead to persistent intestinal inflammation (54). This concludes that degradation of collagen and persistent inflammation are consistent with the pathogenesis of impaired anastomotic healing.

The potential of certain bacterial species to mitigate the risk of anastomotic leakage and enhance wound healing may be associated with their production of SCFAs or anti-inflammatory activities. Among the species presented in the results section, *Faecalibacterium prausnitzii*, *Eubacterium biforme* and *Bacteroides ovatus* were highlighted as butyrate producing bacteria (55,56). Butyrate serves as a crucial energy source for colonic epithelial cells, modulates immune responses, reduces colonic hypersensitivity, decreases intestinal-cell permeability and intestinal motility, thereby facilitating intestinal wound healing (57). Although *Prevotella copri* does not directly produce butyrate, it generates succinate, a precursor to butyrate, enhancing butyrate production in the intestine (58).

Another mechanism for reducing the risk of anastomotic leakage involves the anti-inflammatory activity of interleukin-10 (IL-10) produced by *Faecalibacterium prausnitzii* and *Bacteroides uniformis* (59,60). The anti-inflammatory effects of IL-10 include suppression of various pro-inflammatory cytokines and chemokines, such as IFN-γ, TNF, IL-1α/β, IL-6, and IL-8 (61). Other bacterial species exhibit unique mechanisms that could potentially exacerbate or reduce intestinal inflammation and wound healing. *Streptococcus salivarius* appears to exhibit anti-inflammatory activity through the inhibition of peroxisome proliferator-activated receptor gamma (PPARγ) (62). *Bacteroides ovatus* may facilitate wound repair by stimulating the production of IL-22 (62). The influence of *Parabacteroides goldsteinii* on anastomotic healing has been demonstrated in murine models (16). Research indicates that treatment with *P. goldsteinii* increases hydroxyproline and fibronectin concentrations in wounds, exerts an anti-inflammatory effect by suppressing the Myeloid differentiation primary response 88 and Nuclear Factor kappa-light-chain-enhancer of activated B cells (MyD88/NF-κB) signaling pathway, and inhibits the production of MIP-1α, MIP-2, IL-17A/F, and MCP-1 in mice.

Changes in microbial diversity may also impact occurrence of anastomotic leakage. Studies have associated lower α-diversity with an increased risk of anastomotic leakage, although one study demonstrated a correlation between increased α-diversity and anastomotic leakage (17–19). Other studies also linked lower diversity with higher rates of postoperative complications (63,64). It is estimated that reduced bacterial diversity affects nearly one-quarter of colorectal cancer patients prior to surgery, with mechanical bowel preparation and antibiotic use potentially exacerbating this condition (64). Patients with low bacterial diversity are predicted to experience a loss of butyrate-producing bacteria, with a compositional shift toward facultative anaerobes typical of low diversity dysbiosis (65). No studies have elucidated the mechanism by which high diversity might impair intestinal health, but it is noteworthy that studies indicating lower diversity typically collected samples before or during surgery, whereas the study suggesting higher diversity utilized samples from the first postoperative defecation.

Among the studies included in the review, the most notable methodological difference is the timing at which samples for microbiome identification were collected. This timing varied from pre-surgery to three days post-surgery, with no studies matching in terms of timing and sample material. Consequently, the differing timing and materials used for microbial identification likely contributed to the varying results observed between studies. There was limited consistency in bacterial species identification, making it challenging to establish reliable correlations between specific bacterial species and the risk of postoperative complications. While certain studies demonstrated congruent findings at the genus level, the variability of species within genera precludes the establishment of reliable connections

at this taxonomic level. In essence, only one study substantiated a correlation between specific bacteria and leakage rates, while others merely correlated the prevalence of bacteria with anastomotic leakage rates. To achieve conclusive results, further studies are warranted, particularly those that focus on bacteria directly associated with anastomotic leakage rates. Moreover, future research efforts would benefit from standardized timing for sample collection to enable more robust comparisons across studies.

This study utilizes several limitations. Firstly, only anastomotic leakage was examined among a range of postoperative complications, with pneumonia also noted but with a sample size too small to yield statistically significant findings. Furthermore, most studies focused on colorectal cancer surgeries, with only one instance of esophageal cancer surgery, thus not encompassing all gastrointestinal cancer surgeries and their associated complications. The limited number of studies complementary to the aim of this review prohibited a wider approach on different gastrointestinal cancer and complications. The cumulative sample size of patients remained relatively small and given the variety of microbial species discussed in the results, definitive conclusions were difficult to draw.

CONCLUSION

Overall, the findings from studies demonstrate correlations between certain bacterial species and either increased or decreased rates of anastomotic leakage. Among the bacteria exacerbating anastomotic leakage, *Bacteroides fragilis* was identified in two studies, with potential mechanisms of action including toxin-induced E-cadherin cleavage and facilitation of interleukin-17. Conversely, among bacteria promoting anastomotic healing, *Faecalibacterium prausnitzii* emerged as noteworthy, as it produces both butyrate and interleukin-10, which contribute to intestinal cell health. Further research is warranted to establish more definitive associations between specific bacterial species and complication rates, as the current literature only allows for hypotheses. Nevertheless, the recurrence of certain bacteria across studies highlighted in this review holds promise for future research endeavors.

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