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THE INFLUENCE OF THE MICROBIOTA ON ANASTOMOSIS COMPLICATIONS AFTER PRIMARY COLORECTAL SURGERY: A LITERATURE REVIEW

FINAL MASTER'S THESIS

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1. SUMMARY

Final master's thesis by Sandra Ruseckaitė

Title – The influence of the microbiota on anastomosis complications after primary colorectal surgery: a literature review.

Relevance of the problem and aim of the work – Recent research in the field of molecular biology has proposed that the intestinal microbiome plays a pivotal role in determining colorectal surgery outcomes. Therefore, a call has been made for more reviews to focus on the available clinical data. Herein, the purpose of this systematic literature review was to analyze this theory and report the current evidence on the association between the composition of the gut microbiota and the development of postoperative complications such as colorectal anastomotic leaks (CAL), surgical-site infections (SSIs) or post-operative ileus (POI).

Methodology – The systematic literature review was conducted in accordance with the 2009 PRISMA guidelines. A thorough search of the literature was carried out in a systematic manner via online databases such as, *PubMed* and *ScienceDirect* to identify randomized and observational human clinical studies published between 2014 and 2024 addressing postoperative complications after colorectal surgery in association to the intestinal microbiome evaluation. The risk of bias for the reviewed case-control and cohort (observational) studies was assessed using the ROBINS-I tool, while Rob2 was used for randomized and prospective studies.

Results – Overall, after checking for eligibility, 19 studies (16 observational and 3 randomized) with a total of 615 patients who were diagnosed with anastomosis complications post colorectal surgery (155 had CAL, 421 had SSIs and 39 had POI) and had bacteriological data from either mucosa-associated microbiome or luminal microbiome specimens evaluated were presented. All studies focused on short-term surgical outcomes, and their results indicated that CAL, SSIs and POI were influenced/provoked by a combination of low microbial diversity and a shift towards a pathogenic strain dominant flora, however, the underlying mechanisms were yet to be entirely clarified. Clinical evidence also showed that the manipulation of the GM with surgical bowel preparation (SBP) or probiotics had uncertain benefits and could not guarantee lower rates of anastomosis complications.

Conclusions – Based on the limited evidence available, a statistically relevant difference exists between the bacterial flora in patients with colorectal anastomosis complications and those with an uneventful post-operative course of recovery. An imbalance between aggressive and protective strains pre-, peri- and post-operatively has reinforced the hypothesis that dysbiosis of intestinal flora is a risk factor for the development of anastomotic complications. These findings may lay the groundwork for the utilization of the microbiome as a prognostic biomarker for predicting anastomotic complications.

2. ACKNOWLEDGMENTS

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3. CONFLICTS OF INTEREST

The author reports no conflicts of interest.

4. PERMISSION ISSUED BY THE ETHICS COMMITTEE

Approval by the Ethics Committee permitting realization of the research was issued on the 9th of February 2024 (No. 2024-BEC2-172). See **Annex No. 1.**

5. ABBREVIATIONS

GM – gut microbiome

CAL – colorectal anastomotic leakage

SSI – surgical site infection

POI – post-operative ileus

CRC – colorectal cancer

MBP – mechanical bowel preparation

SBP – surgical bowel preparation

MAMC – mucosal-associated microbial community

ILMC – intra-luminal microbial community

SCFA – short-chain fatty acids

6. TERMS

Anastomotic leakage – a disruption in the surgical connection between two hollow internal organs, resulting in a leakage of the organ contents that may manifest at the surgical incision, drainage site, or in the vicinity of the anastomosis.

Collagen – the predominant structural protein in the intestinal submucosa crucial for maintaining the strength and integrity of the intestinal wall.

Intestinal ischemia – temporary or permanent disruption of the blood supply to the neighbouring gastrointestinal tract caused by surgical intervention involving the bowel and its vasculature.

Microbial taxa – different kinds of microbes classified into groups (like species, genus, family, etc.) based on how they're related or how similar they are.

Postoperative ileus – cessation of normal intestinal activity beyond the fifth postoperative day, accompanied by nausea and/or vomiting, abdominal distension, delayed bowel movements and gas passage, as well as inability to tolerate solid foods.

Probiotic – a dietary supplement containing live microorganisms that helps sustain the equilibrium of the intestinal microbiota, leading to beneficial health outcomes.

Surgical site infection – a superficial, deep or organ space infection that happens within 30 days after an operation.

7. INTRODUCTION

Colorectal surgery serves as a cornerstone technique for the treatment of a variety of illnesses including colorectal cancer (CRC), diverticular disease, inflammatory bowel disease (IBD), intestinal obstructions, proctological disorders and ischemic pathologies, among others [1]. As a result, efforts to mitigate the risk factors for complications following colorectal surgery have been an area of continuous focus of research and clinical practice over time. Improvements in antiseptic practices, standardized surgical methods and centralisation of colorectal surgeries within tertiary-level hospitals have collectively helped reduce many of the short-term adverse outcomes linked to colorectal surgery [2]. However, despite accounting for patient-specific and unchangeable risk factors (i.e., age, gender, pre-existing comorbidities), a significant number of patients undergoing colorectal procedures continue to experience postoperative morbidity due to anastomotic complications, primarily in the form of colorectal anastomotic leakage (CAL), surgical-site infections (SSIs) or post-operative ileus (POI) [3].

Hence, many theories have been put forth to identify the "missing piece" that could explain the origins of these complications. With recent leaps in molecular biology enabling culture-independent, rapid and reliable microbial analysis via genomic sequencing, proteomics and metagenomic analysis of DNA and RNA, a novel train-of-thought has emerged in the scientific community presenting the gut microbiota (GM) as one of the determining factors for anastomosis complication development [4].

The intestinal microbiome comprised mostly of diverse communities of bacteria, but also viruses, fungi and archaea, has been well-established as the key contributor to gastrointestinal homeostasis and intestinal epithelial cell wellness [5]. It has been shown to play a crucial role in facilitating epithelial repair and wound healing, with a strong correlation observed between the specific microbial communities present in the wound and the eventual healing outcome [6]. It is, therefore, very likely that specific microbial dysbiosis, resulting from a combination of the underlying disease, surgery trauma and perioperative management involving mechanical bowel preparation (MBP) and gut modulation with antibiotics/probiotics, may contribute to local and systemic inflammation as well as overall immune dysregulation, leading to repressed wound healing and consequently, playing a significant role in the onset of anastomosis complications following primary colorectal surgery [7].

Therefore, this systematic literature review will explore how GM composition influences intestinal inflammation and healing, and how it relates to the development of CAL, SSIs and POI. Additionally, it will touch upon aspects of perioperative management that might influence the gut flora to either mitigate or prevent these adverse outcomes.

8. AIM AND OBJECTIVES

Aim: The primary aim of this systematic literature review is to analyse and synthesise existing literature linking the hosts GM with the occurrence of intestinal anastomotic injuries following primary colorectal surgery. The overarching goal is to better understand how bacteria in the colon affect inflammation and healing in the gut, and how this insight can help shape both prevention and treatment approaches in colorectal surgery practice.

Objectives:

1. Investigate the impact of GM diversity and composition on the development of CAL, SSIs or POI in patients after primary colorectal resection, without preoperative bowel preparation.
2. Determine the impact of GM diversity and composition on the development of CAL, SSIs or POI in patients after primary colorectal resection, where the bowel underwent preoperative preparation with or without antibiotic therapy.
3. Assess the effects of GM alterations induced by preoperative peroral antibiotic administration on the development of CAL, SSIs or POI following primary colorectal resection.
4. Consider how incorporation of gut flora modulators like probiotics, can cause shifts in GM diversity capable of both facilitating and impairing the anastomotic healing process.

9. LITERATURE REVIEW

9.1 Definition and physiology of the human GM

The intestinal microbiome is an ensemble of microorganisms and their metabolic byproducts residing within the gastrointestinal tract, which are essential for maintaining immune system homeostasis and modulating the host's susceptibility towards numerous immune-related diseases and disorders [8]. The composition and functional characteristics of the GM are shaped by a variety of pleiotropic factors, including dietary preferences, medication use, hormonal profiles, gastrointestinal surgical interventions, harmful habits (i.e., smoking), age and gender, geographical location, as well as stress levels, physical activity patterns, and other environmental conditions [9]. These complex influences contribute to substantial fluctuations in the GM, which have been recorded even over the course of a single day, despite the GM's inherent tendency to revert to a stable baseline state [10]. Additionally, the GM composition of healthy individuals exhibits pronounced subject-specific heterogeneity and significant interpersonal diversity [11]. Hence, a universal agreement on the composition and characteristics of a "healthy/normal microbiota" has not yet been established.

However, the sequencing of bacterial 16S ribosomal RNA genes of the human microbiota as well as animal model-based *in vivo* and translational research has facilitated the identification of resident microbial taxa that tends to be consistently present, despite exhibiting significant variations in their relative abundances across different individuals [10, 12]. Studies demonstrate that healthy individuals typically harbour a GM composed of one archaeal phylum, *Euryarchaeota*, and five predominant bacterial phyla: *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* [13].

Subsequent research has also distinguished two spatially distinct compartments of the GM. Firstly, the mucosal-associated microbial community (MAMC), which was characterised by higher representation of *Bacteroidetes* and *Proteobacteria* and exhibited greater permanence and resilience to extrinsic factors like diet and stress. Secondly, the intra-luminal microbial community (ILMC) that exhibited a greater abundance of *Firmicutes* and *Actinobacteria* but was more dynamic and responsive to environmental fluctuations [14, 15]. Interestingly, the bacterial species present in the ILMC were also found in the MAMC, while some were exclusively present only in the MAMC, likely a reflection of their distinct environmental preferences and functional roles [14]. Nonetheless, the precise implications of the distinct microbial compositions of the MAMC and ILMC still remain poorly understood. However, mounting evidence suggests that the dysregulation of the balance between these bacterial communities, a state referred to as dysbiosis, is frequently linked not only with the emergence of

conditions like CRC and IBD, but also with the incidence of anastomotic complications following surgical resection procedures implemented to manage these pathologies [16].

9.2 GM-associated complications after primary colorectal surgery

Some researchers hypothesize that surgical trauma resulting from colorectal resection, in combination with perioperative interventions, induces a delay in the physiological reestablishment of baseline GM homeostasis [4]. This delay allows pathogenic bacteria to take advantage of the disruption and also causes commensal species to shift towards a more virulent state. The deleterious effects of the aforementioned factors culminate in an overall reduction of beneficial members of the microbiota, effectively recalibrating the intestinal flora into a pathobiome. This pathobiome compromises the immune system and provokes an inflammatory response that can impair proper restoration of the epithelial barrier and hinder normal wound healing, ultimately contributing to the development of anastomotic complications such as CAL, SSIs or POI [17].

CAL and SSIs are major primary colorectal surgery complications, occurring in approximately 5–20% and 30% of patients, respectively [18, 19]. Among the first to recognise the direct influence of the GM on the healing of surgical anastomoses was the pioneering work of Cohn and Rives in 1955 [20], which found that administering intraluminal antibiotics directly at the level of the anastomosis could allow for proper healing of the bowel tissue despite local ischaemia. More recently *in vivo* investigations have revealed that specific environmental factors, such as surgical trauma induced stress, exposure to oxygen and ischaemia, along with bowel cleansing and antibiotics, can induce members of the GM like *Enterococcus faecalis*, *Serratia marcescens* and *Pseudomonas aeruginosa* to exhibit a collagenase-producing proinflammatory phenotype [21]. Inoculation with these collagenolytic bacteria leads to the direct degradation of collagen I and III fibrils within the healing tissue [21, 22]. Type I collagen and Type III collagen comprise 68% and 20% of the total collagen content, respectively, and when there is a deficiency in these key collagen types, the surgical connection (anastomosis) is unable to heal properly, leading to the occurrence of CAL and incisional SSIs [23, 24]. Beyond the direct impact of bacterial collagenase on tissue, these collagenolytic strains can also bind to and overstimulate components of the fibrinolytic system, such as matrix metalloproteinase 9 (MMP9) [25]. This leads to a cascade of supraphysiological collagenolysis, potentially resulting in excessive degradation of the collagen structure [25, 26, 27]. Similarly, another study found that rodents colonized by a mutant strain of *P. aeruginosa* exhibited an increased prevalence of CAL [28]. This particular strain demonstrated altered pyocyanin synthesis and heightened collagenase activity, along with elevated swarming motility and

destructive effects on cultured intestinal epithelial cells (i.e., apoptosis, cytolysis) [28]. As a result, the intestine's ability to defend against the virulent characteristics of this mutated phenotype was diminished.

To summarise, the studies have collectively highlighted that the *in vivo* transformation of commensal microbes into a tissue-destroying phenotype due to surgical disruption of the GM may have important implications in the pathogenesis of CAL and SSIs following colorectal surgery. Therefore, elevated preoperative levels of certain bacterial species may serve as a predictive biomarker for anastomotic complications. For example, a nationwide prospective cohort study by Julien et al. [29] demonstrated that a gut microbiome-based predictive model utilizing six bacterial genera (*Hungatella*, *Epulopiscium*, *Fusobacterium*, *Ruminococcaceae_ucg_009*, *Actinomyces*, and *Ralstonia*) can be effectively employed to forecast the risk of SSIs in Crohn's disease patients undergoing ileocolonic resection. This would represent an improvement over the current diagnostic approach, which relies on on-demand CT imaging but often fails to identify anastomotic complications at an early enough stage, which is very important as early detection is crucial to minimize postoperative morbidity and mortality due to reoperation, which can increase up to 40% if the diagnosis is delayed by 2.5 days [30].

Unlike CAL and SSIs, evidence elucidating the association between the GM and POI in human patients remains limited. Currently, the prevailing view among surgeons is that despite the initial phase of postoperative ileus being neurogenic in origin, it is actually an inflammatory condition, as even minimal surgical manipulation of the intestines can trigger an acute inflammatory response in the intestinal muscular layer [31]. Given the involvement of the intestinal microbiota in the activation of most immune-mediated intestinal inflammatory processes, it stands to reason that it may also play a pivotal causative role in the development of POI. For example, one study discovered that surgical manipulation of the colon can facilitate the translocation of lipopolysaccharide (LPS), a structural component of gram-negative bacteria cell wall, from the intestinal lumen to the muscularis externa [32]. They also demonstrated that LPS can trigger inflammation in the muscular layer of the small intestine in mice, directly impacting gastrointestinal motility [32].

Nonetheless, prior to April of 2020, the research by Shogan et al. [33] represented one of the few studies that demonstrated significant differences in the rectal microbiome composition of CRC patients who developed prolonged POI compared with those who did not, prompting a surge of interest in investigating the precise mechanisms by which the GM or its metabolites contribute to the onset of POI. The prevailing hypothesis was that following intestinal manipulation, LPS triggers the phosphorylation of p38 mitogen-activated protein kinases (p38–MAPK), instigating an inflammatory cascade in resident macrophages within the intestinal muscularis, ultimately resulting in the exacerbation of POI [34]. Building upon this hypothesis, Wehner et al. [35] had previously demonstrated that inhibiting the p38-MAPK signalling pathway with the experimental drug semapimod, limits the

production of nitric oxide (NO) in macrophages, thereby reducing inflammation and alleviating dysmotility, suggesting a promising approach for the future prevention of POI.

9.3 The effects of GM modulation on anastomotic complications after primary colorectal surgery

Given the intestinal microbiota's potential to serve as a source of microbial infection following colorectal surgery, surgeons have implemented mechanical bowel preparation (MBP) as a strategy to reduce the bacterial load by diminishing faecal content, typically through the administration of oral laxative agents and warm saline enemas [36]. However, this approach has been called into question, as existing research investigating the clinical efficacy of MBP has suggested that instead of preventing postoperative surgical complications associated with infection, it may potentially be exacerbating these adverse outcomes [37, 38].

Studies have found that bowel cleansing can cause notable short-term alterations in GM composition and diversity, potentially enabling the proliferation of opportunistic pathogens, which may subsequently impact the development of anastomotic complications [38]. Shobar et al. [39] contributed to the discussion by demonstrating that MBP not only immediately impacted the composition of the GM but also altered the quality and production of the protective mucous layer, thereby permitting bacterial translocation [39]. However, other contradictory studies were unable to establish this link between MBP and occurrence of anastomotic complications [40]. At this point in time, the establishment of the enhanced recovery after surgery (ERAS) protocol has formalised guidelines for conducting colorectal procedures in patients with unprepared colons without substantially compromising surgical outcomes [41]. Nevertheless, preoperative MBP use still remains under the discretion of the operating surgeon. Many colorectal surgeons in Europe and the United States still require bowel washout as a rule of thumb for left hemicolectomy, mainly with the exception of low anterior resection with coloanal anastomosis, as faecal contamination could complicate elemental steps of the surgical technique [42]. Thus, the benefits of preoperative use of MBP still remains a matter of debate in the context of elective colorectal surgery.

Nonetheless, an idea has been put forward that MBP should not be evaluated in isolation, as its primary advantage is to enable enhanced administration of oral antibiotic formulation to the bowel mucosa [38]. Scarborough et al.'s study [43], which analysed data from over 4,000 patients within the ACS NSQIP database, showed that the combined use of preoperative oral antibiotics and mechanical bowel preparation led to a reduction in the rate of anastomotic leakage from 5.7% to 2.8% in colorectal

surgery, compared to patients who did not receive any form of preparation. In contrast, the use of oral antibiotics or mechanical bowel preparation alone did not result in a lowered CAL rate [43]. However, more recent studies analysing the same database have only found an effect with oral antibiotics alone, indicating that the combination with mechanical bowel preparation provides no additional benefit [44, 45].

In contrast to MBP, the administration of antibiotics results in a near-complete reduction of the total bacterial population. While the overall number of bacteria recovers rapidly, the diversity of the microbial community experiences a very slow and incomplete regeneration, persisting for months or even years after the initial treatment [46]. This disruption substantially compromises the normal intestinal barrier, enabling increased pathogenic invasion and commonly contributing to impaired anastomotic healing. However, at the same time, preoperative prophylactic application of selective antibiotics, such as tobramycin, colistin, and amphotericin B, helps reduce CAL by acting on pathogens detrimental to intestinal recovery and anastomotic healing [47]. Therefore, it appears that the disturbance of the microbiota goes hand-in-hand with the well-proven positive effects of antibiotics on anastomotic healing. For example, the findings of a 2021 meta-analysis involving 6 randomized controlled trials suggests that the concurrent administration of oral selective antibiotics and intravenous broad-spectrum antibiotics can significantly lower the incidence of SSIs and CAL in patients undergoing laparoscopic CRC surgery [48]. The underlying rationale is that broad-spectrum antibiotics induce more extensive disruption of the patient's intestinal microbiome, whereas targeted antibiotics selectively eliminate key pathogens such as aerobic gram-negative bacteria, *Staphylococcus aureus*, and fungi, ultimately leading to a more favourable clinical outcome [47]. In conclusion, to decrease the incidence of anastomotic complications following colorectal resection, patients are advised to receive preoperative selective oral and intravenous antibiotic prophylaxis, while routine administration of MBP remains controversial and needs to be supported by more evidence.

On the other hand, systematic literature reviews and meta-analyses show that the administration of probiotic supplements favourably impact clinical outcomes following abdominal surgery, with *Bifidobacteria* (the dominant members of the *Actinobacteria* phylum) and lactic acid bacteria being the primary genera of beneficial microorganisms [49, 50]. A randomized, double-blind, placebo-controlled study conducted by Kotzampassi et al. revealed that intake of a probiotic formulation containing *L. acidophilus* LA-5, *L. plantarum*, *B. lactis* BB-12 and *S. boulardii,* initiated one day prior to major primary colorectal surgery and maintained for 15 days after the procedure, stabilised the GM and significantly decreased the incidence of SSIs and CAL after colorectal surgery. The primary mechanism underlying these clinical benefits was associated with modulation of gene expression of suppressor of cytokine stimulation-3 (SOCS3), a regulatory protein known to suppress excessive cytokine responses involving TNF and IL-6 [51, 52]. Similarly, a related investigation by Reis et al. [53] found that certain

probiotics can modulate the balance between T helper type 1 (Th1) and T helper type 2 (Th2) cells, stimulating the secretion of anti-inflammatory cytokines while also suppressing pro-inflammatory cytokine production, potentially helping to manage complications associated with infections after colon resection operations. Another investigation by Mizuta et al. [54] utilising microbial 16S rRNA MiSeq sequencing revealed that probiotics had a beneficial effect on postoperative outcomes, specifically CAL, which were mainly associated with increased *Actinobacteria* and decreased blood inflammatory parameters, whereas the absence of probiotics was associated with elevated *Bacteroidetes* and *Proteobacteria* along with heightened inflammatory blood markers. Moreover, another animal study also recognised that preoperative administration of probiotic supplements ameliorates gastrointestinal motility in guinea pigs with POI, likely by reestablishing the beneficial gut microbial species *Bifidobacterium bifidum* and *Bifidobacterium longum* and increasing production of the short-chain fatty acid butyrate [55]. However, according to other limited reports compiled by Zheng at al. [7], apart from reducing first flatus and defecation time, in most clinical cases the effects of preoperative or postoperative probiotic administration on infectious complications, anastomotic leakage and GI motility after primary colorectal surgery were not satisfactory. This suggests that at this point in time results may still be too conflictual and too heterogeneous to support any formal clinical recommendations. However, there are indications that probiotic microorganisms will most likely comprise an alternative approach to non-selective antibiotic therapy in the near future.

10. RESEARCH METHODOLOGY AND METHODS

10.1 Research question

The research question formulation was guided by the **PICO** framework, as follows:

P, population: adult patients who had undergone primary colorectal surgical operations with the creation of an intestinal anastomosis

I, intervention: analysis of the variations in ILMC or MAMC composition and diversity through the application of culture-based or genome sequencing techniques, using samples derived from faecal matter, mucosal biopsies, resected surgical specimens, or drain fluid

C, comparison: analysis of bacterial abundances in the microbiome specimens of patients undergoing colorectal surgery with and without modifications to gut microbiota composition

O, outcome: GM-associated postoperative complications including CAL, SSIs and POI

In patients undergoing primary colorectal surgery (**P**), how do variations in GM composition, influenced by preoperative bowel preparation methods and gut flora modulators (**I**), compared to no preparation or modulation (**C**), affect the occurrence of CAL, SSIs or POI (**O**)?

10.2 Systematic literature review protocol

The systematic literature review was prepared following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [56].

Table 1. Eligibility criteria

Inclusion	Exclusion
Human studies	Animal studies
Research published no earlier than 10 years ago from the initial search date (2014-2024)	Studies published before 2014
Studies published in English	Studies published in other languages than English
Studies with full text available	Studies without access to the full text
Observational studies (prospective cohort and retrospective case-control examinations),	Case reports, conference abstracts, systematic literature reviews, meta-analyses, book excerpts,

interventional studies (RCTs, controlled clinical trials, prospective study)	mechanical (laboratory) studies, unpublished material
Clinical studies that examined short-term postoperative complications (CAL, SSI, POI) after primary colorectal surgery in relation to the microbiome of the gut	Studies focusing on short-term complications following other types of complex gastrointestinal surgery involving anastomosis (e.g. oesophagojejunostomy) or studies examining other types of complications (e.g. diversion colitis, post-op adhesions)
Studies with obligatory examination of MAMC or ILMC from faecal samples, colorectal tissue or drain fluid done via culture or sequencing	Studies with no analysis of the GM
Studies with data on interventions like MBP, antibiotics or probiotics	Studies without data on interventions like MBP, antibiotics or probiotics
Adult patients of all ages and genders	Underage patients

10.3 Information sources and search strategy

A systematic search for clinical studies evaluating the potential impact of intestinal dysbiosis on postoperative course by comparing microbiota diversity and composition between patients with and without complications post colorectal surgery was carried out in electronic databases subscribed to by the Lithuanian University of Health Sciences (LSMU): *PubMed* and *ScienceDirect*. All sources were last consulted on the 27th of July 2024.

The following keywords were employed for each database: microbiome, microbiota, flora, colorectal surgery, colorectal resection, anastomosis, complications, outcomes, anastomotic leak, surgical site infection, ileus.

For the most accurate results all the search terms were connected into concepts using Boolean operators (AND, OR), and parentheses were used to group the concepts together, forming a single search string for all the databases: (microbiota OR microbiome OR flora) AND (colorectal surgery OR colorectal resection OR anastomosis) AND (complications OR outcomes OR anastomotic leak OR surgical site infection OR ileus). A filter was then applied to limit the search to human-focused studies published in English within the past 10 years since the initial search date (2014-2024), and with the full text freely available.

10.4 Selection process

The search results were imported into the free reference management software *Mendeley* to automatically remove any duplicates. During the initial stage of selection, the titles and abstracts of the articles were independently screened by the author, and those that potentially corresponded to the topic and content of this systematic literature review progressed to the second stage for full text evaluation. After the full text of the selected publications was read and analyzed, the articles meeting the inclusion criteria described in ***Table 1.*** underwent further systematic literature review and those that did not were removed from further analysis. However, to ensure a thorough approach, the reference lists of the articles selected for inclusion were double checked for any other eligible records and pertinent review articles to identify any potential additions that could also meet the inclusion criteria. In addition, the "similar articles" and "cited by" function was used in *PubMed*. The full process is visually depicted in the PRISMA flow diagram (Fig. 1.), which is presented in subsection 11.1 titled "Literature search and selection".

10.5 Data collection process

The author independently extracted data from the selected studies and organized the information into a dedicated spreadsheet for analysis. The extracted data included the following elements: first author's name, year and country of publication, study design, type of postoperative outcome (CAL, SSI, POI), number of analyzed patients and outcomes, perioperative interventions, microbiome type and sample material, time of sample collection, method used to detect microbiome, bacteria specifically associated with analysed outcome.

10.6 Study risk of bias assessment

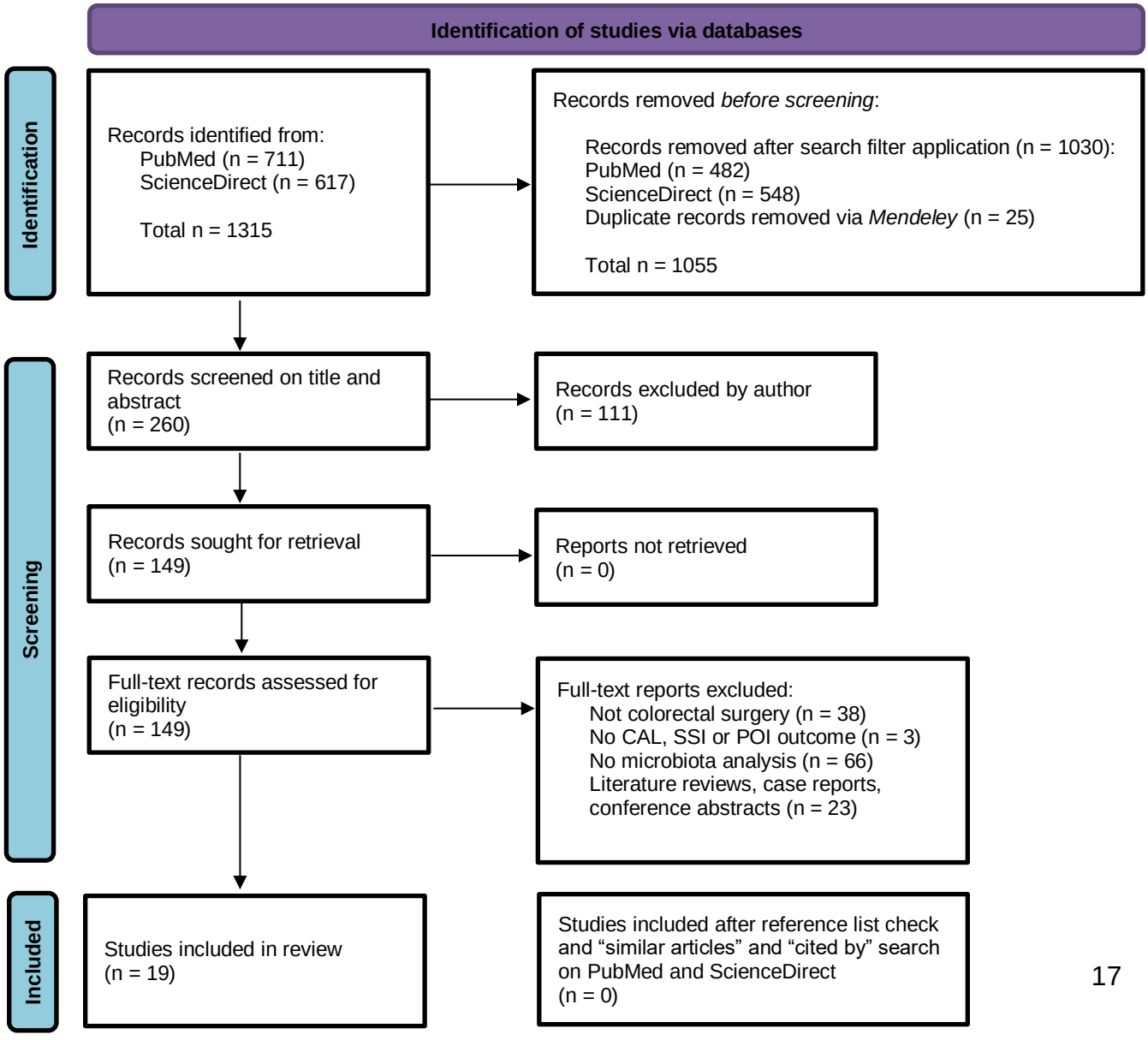
Assessing the risk of bias is an essential step in any systematic literature review, as it helps determine whether the credibility and trustworthiness of the included studies might be affected. This could occur due to incomplete or insufficient information that fails to address the objectives of the systematic literature review, or due to information presented in an unstructured manner that is potentially influenced by the researchers' biases, which could have impacted the findings of the analysed studies. In this systematic literature review the author carried out the risk of bias assessment for each study individually using either the Cochrane *RoB 2* or *ROBINS-I* tools. The results of this assessment are presented in ***Table 2.*** and ***Table 3.*** in subsection 11.2.

11. DATA ORGANIZATION AND ANALYSIS

11.1 Literature search and selection

The initial literature search yielded a total of 1,315 potentially relevant publications, with 711 results retrieved from the *PubMed* database and the remaining 617 sourced through *ScienceDirect*. Subsequently, 1,030 papers were excluded through the filtering process, and an additional 25 were identified as duplicates by the *Mendeley* reference management tool, which the author then manually verified. Ultimately, a total of 260 publications were evaluated for suitability based on their titles and abstracts, and the full texts of 149 of these articles were obtained for thorough systematic literature review. Of these, 19 articles were deemed to be relevant and included in the final analysis, while 130 met the exclusion criteria established for this study and were not considered further.

The overall process of database screening and selection is depicted in the below PRISMA flow diagram Fig. 1.



11.2 Risk of bias evaluation

The author independently assessed the risk of bias for the studies according to study type. The Cochrane Risk of Bias (Rob 2) tool was used to assess the risk of bias for randomized and prospective trials [57]. While the risk of bias evaluation for observational cohort and case-control studies was based on the ROBINS-I tool [58].

The overall risk of bias across all the reviewed studies was low. Each study demonstrated rigorous methodology, clear definitions, and consistent application of interventions, minimizing risks in most domains. However, 14 out of 19 studies showed some concerns of bias due to potential confounding from unmeasured variables, non-randomized assignments, and small sample sizes. High risk of bias was identified in one study due to lack of randomization. Overall, the findings are robust and reliable despite the inherent limitations of observational and pilot study designs.

Table 2. Result of risk of bias assessment for each randomized study using the RoB 2 tool

First author's name, year	D1	D2	D3	D4	D5	Overall risk
Sadahiro et al. (2014) [59]	+	+	+	+	+	+
Du et al. (2019) [60]	!	?	+	+	+	!
Ye Jin et al. (2020) [61]	+	+	+	+	+	+

Table 3. Result of risk of bias assessment for each observational study using ROBINS-I tool

First author's name, year	D1	D2	D3	D4	D5	D6	D7	Overall risk
Komen et al. (2014) [62]	?	+	+	+	+	?	+	?
Aisu et al. (2015) [63]	?	?	+	+	+	+	+	?
Dubinsky-Pertzov et al. (2018) [64]	?	+	+	+	+	+	+	+
van Praagh et al. (2019) [65]	?	+	+	+	?	?	+	?
Sparreboom et al. (2019) [66]	?	+	+	+	?	?	+	?
Dong Li et al. (2019) [67]	?	+	+	+	?	?	+	?
Xu et al. (2019) [68]	?	+	+	+	+	+	+	+
Palmisano et al. (2020) [69]	+	+	+	+	+	+	+	+
Mima et al. (2020) [70]	?	+	+	+	+	+	+	+
Anderson et al. (2021) [71]	?	+	+	+	+	+	+	+
Jin et al. (2022) [72]	?	+	+	+	+	+	?	?
Nalluri-Butz et al. (2022) [73]	+	+	+	+	+	+	+	+
Shi et al. (2022) [74]	?	+	+	+	+	+	+	+
Reuvers et al. (2022) [75]	?	+	+	+	+	+	+	+
Liu et al. (2022) [76]	?	+	+	+	+	+	+	+
Vishnoi et al. (2023) [77]	?	+	+	+	+	+	+	+

Judgement: ! – high risk of bias; ? – some concerns of bias; + – low risk of bias

Table 2. Risk of bias domains [57]:
D1: the randomization process.
D2: deviation from planned intervention.
D3: incomplete outcome data.
D4: outcome measurement.
D5: selective reporting of results.

Table 3. Risk of bias domains [58]:
D1: confounding variables in the data.
D2: participant selection.
D3: intervention categorization.
D4: deviations from intended interventions.
D5: incomplete outcome data.
D6: outcome measurement.
D7: selective reporting of results

11.3 Descriptive study characteristics

The 19 selected studies spanned the time period from 2014 to 2023, with 6 investigations conducted in Europe (Germany, Netherlands, Belgium, Italy, Serbia, Switzerland and Israel), 10 carried out in Asia (Japan and China), 2 performed in North America and 1 undertaken in Australia.

The majority of the studies were observational in design, comprising 14 case-control studies [62, 63, 65, 67, 68, 69, 70, 71, 72, 73, 75, 74, 76, 77] and 2 cohort studies [64, 66] while the remaining 3 investigations [59, 60, 61] employed randomized trial methodology. All the selected research studies focused on analyzing short-term outcomes, with 10 providing data on incidence of CAL [62, 65, 66, 67, 69, 70, 71, 72, 73, 74], 7 reporting on SSIs [59, 60, 63, 64, 68, 75, 77] and 2 investigating POI [61, 76]. The studies enrolled a highly variable number of participants, ranging from as few as 11 individuals to over 5,000 patients. Nonetheless, the cumulative number of patients across all studies totaled 8,299, of whom 155 were diagnosed with CAL, 421 had SSIs and 39 presented with POI after undergoing primary colorectal resection with anastomosis due to either colorectal malignancy, IBD, diverticular disease, ischemic colitis or other etiologies. The use of specific i/v or enteral antibiotic regimens was documented in a total of 9 studies [59, 62, 64, 67, 70, 72, 73, 76, 77] and data concerning specific bowel cleansing procedures was presented in 6 studies [59, 64, 66, 70, 73, 76]. In addition, in 3 of the studies [59, 63, 68] probiotic strains were administered for varying durations to investigate their effects on the participants. Most of the articles included in this systematic literature review predominantly utilized the ILMC as the sample material, with 7 studies reporting the use of fecal samples [59, 63, 68, 69, 73, 76, 72], 3 studies analyzing drain fluid [62, 66, 71], and another 3 studies employing rectal or subcutaneous tissue swabs [64, 75, 77]. The remaining studies used MAMC as their sample material, specifically examining mucosal tissue samples obtained through biopsies or scrapings [60, 61, 65, 67, 70, 74]. While microbiota quantification from the aforementioned samples was done via culture in 3 studies [60, 64, 71], the remaining articles employed real-time quantitative PCR (qPCR) to detect DNA and quantify bacterial populations, targeting the bacterial 16S ribosomal RNA gene DNA sequence. The general characteristics and main results of the selected studies are summarized in **Annex No. 2.**

12. RESULTS AND THEIR DISCUSSION

12.1 Impact of the GM on anastomotic complications in the absence of bowel preparation

In this systematic literature review, 3 studies [61, 71, 74] have explored the relationship between the composition and diversity of the intestinal microbiota and the development of anastomotic complications in patients without any prior bowel preparation. Firstly, Anderson et al. [71] reported that single- or multi-drug antibiotic-resistant collagenolytic bacteria were linked to the occurrence of anastomotic leaks in 14 out of 19 observed cases. The collagenase-producing phenotype of *Enterococcus faecalis* was the most abundant organism isolated from the patients' ILMC samples, with an incidence ratio 36.8%, closely followed by a high prevalence of collagenolytic *Streptococcus* spp. Similarly, owing to the high levels of *Fusobacterium nucleatum* identified through 16S-rRNA sequencing in the colorectal samples from a CAL cohort of 10 patients, Shi et al. [74] also hypothesized that the degradation of collagen by specific members of the gut flora was the underlying mechanism driving the pathogenesis of anastomotic leakage post colorectal surgery. It was explained that akin to *E. faecalis, F. nucleatum* potentially binds to and then induces the expression of MMP9 in colonic epithelial cells through the E-cadherin/β-catenin signalling pathway, thus contributing to the breakdown of collagen in the intestinal tissue and ultimately resulting in anastomotic leakage [66, 74]. However, unlike *E. fecalis,* which is also consistently found in the GM of healthy individuals, *F. nucleatum* is a native species of the oral cavity and is exclusively linked to pathological changes if detected in the microbiotal composition of the colon and rectum, rendering it a potential marker of heightened anastomotic complication risk [78].

In contrast to the 2 case-control studies discussed above that focused on evaluating the impact of the GM on the development of anastomotic leaks, the clinical trial by Ye Jin et al. [61] sought to clarify the potential primary role of the gut flora in the etiology of postoperative ileus. Up to this point this topic has received very limited research attention, as a temporary disruption in gastrointestinal motility (<72h in the large intestine) following surgical intervention, particularly without any prior bowel preparation, is often regarded as a normal physiological response to the intraoperative physical manipulation of the intestines [14, 79]. However, recent advances in DNA sequencing techniques enabled Ye Jin et al. to identify significant differences in the diversity of microbial communities within the mucosal samples of 9 patients who experienced POI following CRC resection surgery, compared to patients who had an uncomplicated post-operative recovery. Specifically, patients with ileus presented with around twice the proportions of *Proteobacteria* and *Actinobacteria*, while simultaneously showing

nearly a four-fold reduction in the abundance of *Fusobacteria* and *Bacteroidetes*, relative to patients without ileus. Most importantly, those with ileus exhibited greatly diminished levels of *Faecalibacterium* (namely, *F. prausnitzi*), the most abundant and significant commensal bacteria within the human GM responsible for the synthesis of butyrate and other anti-inflammatory molecules via fermentation of undigested dietary fiber [80, 81]. Given that butyrate has been reported to enhance colonic motility, either directly through stimulating the release of serotonin (5- HT) or indirectly by promoting cholinergic pathways, it is not unlikely that individuals with diminished levels of gut bacteria who produce this specific compound may be predisposed to developing POI [82]. Thereby, there seems to be a scientific basis to utilise *Faecalibacterium* colonisation levels as a biomarker for predicting the risk of POI.

In general, the findings from the above studies collectively underscore the critical role of GM composition in shaping postoperative outcomes, particularly in patients who undergo colorectal surgery without prior bowel preparation. The presence of collagen-degrading bacteria appears to compromise anastomotic integrity. At the same time, imbalances in microbial diversity, such as reduced levels of anti-inflammatory butyrate-producing species, may predispose patients to postoperative ileus, suggesting that the GM, when left completely unmodulated, may both reflect and contribute to the risk of postoperative complications.

12.2 Impact of the GM on anastomotic complications after MBP alone versus with antibiotics

3 studies [66, 68, 69] investigated in this systematic literature review explored the alterations in gut biome diversity and composition resulting from mechanical bowel cleansing without concomitant antibioticotherapy prior to colorectal resection, and the impact such bacterial flora changes had on the development of anastomotic complications.

Xu et al. [68] reported an association between the 7 instances of SSIs in their case-control study and increased copy numbers of *E. coli* and *E. faecalis*, coupled with lower numbers of the beneficial *Bifidobacterium* and *Lactobacillus* species within the examined faecal ILMC samples. On the other hand, the 292-patient cohort study by Sparreboom et al. [66] showed no significant difference in positive cultures for *E.coli* and *E. faecalis* among their CAL and non-CAL patients during the first three post operative days (POD), suggesting that the utility of these microbes as biomarkers for anastomotic complications following primary colorectal surgery may be limited.

In contrast to the two aforementioned studies, but in concordance with the previously presented research findings by Ye Jin et al., Palmisano et al. [69] highlighted that their comparative DNA sequencing of faecal samples revealed that all 21 patients who went on to develop CAL following hemicolectomy had significantly reduced abundances of the protective bacterium *F. prausnitzii*, accompanied by an increase in dysbiosis-associated bacteria belonging to the *Proteobacteria* phylum, specifically *Acinetobacter lwoffii* and *Acinetobacter johnsonii*. As a result, the aforementioned beneficial members of the intestinal flora could no longer sustain pro-regenerative signalling cascades or modulate epithelial cell proliferation and migration, thereby severely impeding the normal wound healing process. Interestingly, *A. lwo*ffi, analogous to *F. nucleatum* discussed previously in the study by Shi et al., is present in the typical oropharyngeal microbiome of 25% of individuals, possibly further substantiating the hypothesis of orally-driven intestinal dysbiosis playing a role both in the pathogenesis of CRC and anastomotic complications after its surgical treatment [78].

Although MBP has been a surgical dogma for more than a century, the combined efficacy of MBP and antibiotic prophylaxis in improving colorectal surgery outcomes was established back in the 1980s by Baum et al. and further validated by an updated Cochrane review in 2014 [83, 84]. Therefore, an additional 6 scientific research articles [59, 64, 65, 70 73, 76] were compared in this systematic literature review to assess whether the composition of the intestinal bacterial community in patients who underwent surgical bowel preparation, involving both mechanical bowel cleansing and antibiotic administration, contributed to the development of CAL, SSIs or POI.

Overall, the shared consensus between 5 of the 6 [59, 64, 65, 70, 73] studies was that the bowel cleansing solution flushed out the superficial mucus and luminal bacteria, introduced oxygen into the normally anaerobic colonic environment, and reduced the nutritional supply for the intestinal bacteria. Concurrently, the antimicrobial properties of the different antibiotics facilitated the ecological proliferation of pathogens, enabling their translocation to anastomotic sites and consequently causing inflammation and anastomotic complications. Namely, 3 studies [59, 64, 73] found bacterial translocation of antibiotic-resistant *Enterococcus* spp. into the surgical anastomosis site as the primary mechanism underlying the SSI and CAL cases described in their research. These findings may lend support to the "Trojan-horse hypothesis", which proposes that one mechanism of microbiota-mediated SSIs involves certain low-abundance microbes or microbes from distinct body sites, translocating inside the host’s immune cells like macrophages or neutrophils and subsequently being directly delivered into the surgical anastomosis site, leading to infection [31, 85].

1 article [70] also reported a nearly fourfold higher incidence of CAL among patients with elevated levels of *Bifidobacterium* spp. within the mucosal tissue adjacent to the anastomosis. There is a theory that *Bifidobacterium* may contribute to the development of CAL by impacting the blood flow and oxygen delivery to the anastomosis or other components of the GM, leading to reduced perfusion

and tissue hypoxia [86]. However, this indirect speculation needs to be supported by more direct evidence, as *Bifidobacteria* is often classified as a beneficial and health-promoting bacterial group known to downregulate the pro-inflammatory response of the gut epithelium [63]. The findings from the 4 studies discussed above are not unexpected, as even a brief 5-day course of fluoroquinolone, nitroimidazole or aminoglycoside antibiotics is known to provoke substantial taxonomic disruptions, particularly evident in an increased *Bacteroidetes/Firmicute*s ratio and a 25% reduction in overall microbial richness and diversity, which is not fully restorable even after 6 months [87].

However, in opposition to the above, Liu et al. [76] concluded that because the MBP reduced the faecal bulk and improved antibiotic delivery to the bowel mucosa, butyrate-producing *Lachnospiraceae* genera, such as *Roseburia,* were able to fester. These species were found to have the capacity to downregulate LPS-induced pro-inflammatory factors, including NO and IL-12, which are associated with smooth muscle contraction inhibition and the occurrence of POI [88]. Interestingly, van Praagh et al. [65] also reported that *Lachnospiraceae* accounted for 40% of the bacterial community in anastomotic tissue samples from the 10 patients who developed CAL. However, instead of *Roseburia,* the *Lachnospiraceae* family was observed to have a substantially increased representation of *Blautia obeum,* a genus that degrades mucin, the major component of the intestinal mucus layer necessary for intestinal protection, and whose disruption leads to impaired anastomotic healing [89]. This underscores the need for a more refined understanding of the microbiota at the strain level, as different species within the same family can exert opposing effects on anastomotic recovery.

In summary, while bowel preparation combined with antibiotic prophylaxis has historically been considered a protective strategy in colorectal surgery, evidence does suggest it may also inadvertently disrupt the GM in ways that promote anastomotic complications.

12.3 Effects of GM alterations induced by peroral antibiotic administration on the development of anastomotic complications

In 6 studies [60, 62, 67, 72, 75, 77] addressed in this systematic literature review, the researchers deviated from a complete surgical bowel preparation routine, instead opting to administer their patients solely prophylactic oral antibiotics during the preoperative period. Subsequently, it was assessed whether the intestinal flora profiles of the patients who experienced postoperative complications differed from those with an uneventful postoperative recovery following the surgery.

5 studies [60, 62, 72, 75, 77] detected a substantial disparity in the postoperative gut microbial profiles between patients who did not develop anastomotic complications and those who did, notwithstanding the provision of enteral β-lactam antibiotics before the surgical procedure. Their

investigations indicated that the proportions of *Enterococcus*, extended spectrum beta-lactamase (ESBL)/AmpC beta-lactamase producing *Escherichia/Shigella* as well as *Proteobacteria* (mainly *P. aeruginosa)* strains demonstrated a positive association with increased bacterial invasion of intestinal epithelial cells and were markedly higher in the 43 individuals diagnosed with CAL and 227 with SSIs. This was especially apparent on POD 2-4 and 4-5, respectively, with a negative *E. faecalis* test on the 3rd POD virtually ruling out CAL with a 92.9% sensitivity [62]. Based on available clinical data, it is likely that the combined effects of oral antibiotics, surgical trauma-induced stress, oxygen exposure, and ischemia directly correlate with an overgrowth and increased virulence of the aforementioned bacterial species. The effects of these factors culminate in virulent bacterial invasion through disruption of epithelial tight junctions, promotion of collagenolysis through MMP9 activation, and induction of cytotoxicity [30, 32], leading to further injury at the anastomotic site and ultimately impairing the body's ability to achieve effective anastomotic healing.

Unlike the previous studies, Dong Li et al. [67] were the first to demonstrate that *Corynebacterium suicordis*, detected exclusively in the mucosal samples of CAL patients with a history of presurgical enteral antibiotic use, was positively associated with impaired anastomotic healing. This correlation was attributed to the bacterium's invasive capabilities and inflammatory mechanisms, similar to how the previously examined *E. faecalis* contributed to the pathogenesis of impaired anastomotic healing by enhancing collagen-degrading activity and activating intestinal tissue MMP9. The investigation additionally revealed that a regular member of the GM known as *Alistipes shahii* was substantially more abundant solely in the non-CAL group, implying its potential contribution to the healing of the anastomosis. A comparable notion was also brought up in the study discussed earlier by Nalluri-Butz et al. [73], which proposed a potential post-operative compensatory mechanism whereby the microbial communities redirect the butyrate-producing function to members of the *Bacteroidetes* phylum, including *Alistipes*, to enhance anastomotic repair. Nonetheless, some animal models have found that distinct *Alistipes* strains, specifically *Onderdonkii kh35*, can exhibit an opposing influence by increasing the faecal concentrations of pro-inflammatory cytokines such as macrophage inhibitory protein-1α, MIP-2, and IL-17A/F, subsequently leading to impaired surgical anastomosis recovery [90]. This further highlights that the functional characteristics of individual bacterial strains, rather than their mere taxonomic presence, are the critical determinants of anastomotic integrity.

To conclude, the reviewed studies insinuate that the simplified strategy of using only oral antibiotics, without full bowel preparation, affects the gut microbial environment in ways that may promote the overgrowth of pathogenic species, thus potentially compromising postoperative outcomes in colorectal surgery. Overall, the findings underscore the complexity of the GM's role in surgical recovery, and while certain taxa may promote healing, others, depending on their functional traits, can hinder it. This, in turn, highlights the need to move beyond taxonomy to understand the metabolic and

pathogenic potential of specific microbes and collectively points to the GM as a modifiable factor that may play a key role in post-surgical outcomes and warrants further targeted investigation.

12.4 Effect of probiotics on the GM and post-op anastomotic complications

3 studies [59, 63, 68] investigated in this systematic literature review offered insights into how modulation of the GM with probiotics has an influence on the incidence of anastomotic complications.

The study by Sadahiro et al. [59] was unable to corroborate the prophylactic effect of orally administered probiotics on postoperative infection, as the rate of SSI was almost 3 times lower (6.1% vs 18%) in the oral antibiotics-treated group compared to the *Bifidobacteria*-treated group. Surprisingly, even the group of patients to which neither probiotics nor oral antibiotics were administered reported a slightly lower rate of SSIs (17.9%), which suggests that probiotics may have actually had a negative impact on the overall post-operative outcome.

On the other hand, the studies by Aisu et al. [63] and Xu et al. [68] arrived at a similar conclusion that consecutive preoperative and postoperative probiotic treatment can improve the intestinal barrier function and support recovery from the early inflammatory response after colorectal surgery, as it was observed that the incidence rate of SSIs was almost thrice lower in the probiotic-treated cohort compared to controls (6.7% vs 19.7%) among patients that have undergone CRC surgery with anastomosis [63]. These achievements were attributed to the measurable increase in the mean proportion of beneficial bacteria in the probiotic-treated groups, such as *Bifidobacteria* and *Lactobacilli*. Because some *Bifidobacteria* species produce SCFA's like acetate, which can enhance the immune response mediated by epithelial cells [17], the probiotic cohort may have benefited from this reinforced defence, which in turn increased their resistance to SSIS [55, 63]. Similarly, studies have also demonstrated that *Lactobacillus* taxa can contribute to wound healing and repair via mechanisms promoting the secretion of reactive oxygen species (ROS) by NADPH oxidase 1 (Nox1), thus enhancing the multiplication and migration of epithelial cells and helping maintain the intestinal barrier [91, 92].

In addition, Aisu et al.'s study also established a potential connection between the administration of probiotics and reduced inflammation associated with the surgical stress following CRC surgery. By using a special assay to measure the activation of the cytokine cascade (ATP activity in CD4+ lymphocytes), which serves as an inflammation marker, they found that the magnitude of inflammation was elevated significantly compared to the preoperative value in the group that did not receive probiotics. In contrast, the value of the first POD in the probiotic cohort remained stable, suggesting that systemic inflammatory responses can be favourably modified by probiotics, resulting in reduced incidence of complications following colorectal surgery [63].

Overall, the reviewed studies suggest that the impact of GM modulation with probiotics on the incidence of postoperative outcomes is variable, highlighting both potential benefits via improved inflammatory response as well as limitations, suggesting possible harm. These differences may be due to variations in probiotic strains, timing, or patient factors. However, despite the conflicting results, the potential of probiotics to improve colorectal surgery outcomes via microbiome modulation remains a promising avenue for further research.

12.5 Limitations of this systematic literature review

1. As microbiome studies generate vast amounts of output data, most studies included in this systematic literature review do not provide a complete presentation of the data on the species level. Therefore, it is very likely that to control the scope of their research papers the authors only described a selection of the most relevant bacterial findings, making the studies difficult to compare equally and draw informed conclusions about the specific type of GM involved in the occurrence of different anastomotic complications.
2. The systematic literature review was informed by a limited number of studies, each with a relatively small patient cohort, so to validate the reported findings, further research involving larger participant groups would be necessary.
3. Due to regulatory constraints, the process of identifying eligible publications for inclusion in the systematic literature review was done solely by the author, rather than two independent reviewers, which may have limited the objectivity of the assessment.

13. CONCLUSIONS

1. In patients without presurgical bowel preparation, most anastomotic complications can be attributed to intestinal mucosal collagen degradation induced by collagenase-producing phenotypes of *E. faecalis* and *F. nucleatum*. Microbial imbalances, specifically reduced *Faecalibacterium* abundance, may also cause these complications via impaired butyrate production and disrupted colonic motility.
2. An overgrowth of *E. coli* and *E. faecalis* is inconsistently associated with anastomotic complications in patients who underwent only MBP before primary colorectal resection, while reduced *F. prausnitzii* and increased dysbiosis-associated taxa like *Acinetobacter spp.* are more consistently linked to these adverse outcomes.
3. Preoperative MBP combined with antibiotics, as well as antibiotic use alone, induces a statistically significant dysbiosis marked by an overgrowth of pathogenic (*E. faecalis, F. nucleatum, C. suicordis, P. aeruginosa*) and a reduction in protective strains (*F. prausnitzii, Roseburia, and Bifidobacterium*), which disrupts tissue repair and promotes collagen degradation, directly contributing to the development of anastomotic complications.
4. Probiotics help maintain a favourable ratio of *Bifidobacteria* and *Lactobacillus* spp. to *Enterobacteriaceae* spp., thus limiting intestinal leakiness and bacterial translocation. This effect is achieved by increasing the levels of short-chain fatty acids and triggering the secretion of helpful molecules like reactive oxygen species, both of which strengthen the gut lining, lowering the risk of anastomotic complications.

14. PRACTICAL RECOMMENDATIONS

From the perspective of personalized surgical care, analysis of the preoperative GM, particularly the detection of an unfavourable GM composition that could negatively impact anastomotic healing, should be taken into account in the decision-making process regarding the surgical strategy for each patient. For example, in the presence of pathogens expressing collagenase, like *E. faecalis* or *P. aeruginosa*, oral phosphate and phosphate carrier compounds could be introduced before low colorectal anastomosis to potentially prevent CAL. Similarly, administering oral or rectal butyrate, or oral pectin, during the perioperative period of an intestinal resection is associated with higher levels of intestinal SCFAs that are likely produced through fermentation of these compounds, contributing thus to better intestinal healing and resulting in stronger anastomoses. Overall, a targeted approach that would ensure the preservation of the microbiota in a way that can selectively inhibit the pathobionts and simultaneously preserve the good bacteria should be implemented. Such a targeted treatment prior to surgery would create the optimal ecosystem and minimise the risk of complications.

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16. ANNEXES

Annex No. 1. Approval by the Ethics Committee

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETO
BIOETIKOS CENTRAS

Medicinos akademija (MA)
Vientisųjų studijų programa - Medicina
5 k. studentė: Sandra Ruseckaitė
Darbo vadovas: Docentas Saulius Švagždys
Chirurgijos klinika

Nr. 2024-BEC2-172

DĖL PRITARIMO TYRIMUI

LSMU Bioetikos centras, įvertinęs pateiktus dokumentus, moksliniam-tiriamajam darbui tema „The Influence of the Microbiota on Anastomosis Complications after Primary Colorectal Surgery – A literature review“ P R I T A R I A .

dr. Aušra Urbonienė 2024-02-09 12:56:43

** Pastaba: šis pritarimas neatleidžia tiriamąjį mokslinį darbą vykdančių asmenų nuo prievolės laikytis Bendrojo duomenų apsaugos reglamento nuostatų ir nuo atsakomybės gauti nacionalinio arba regioninio bioetikos komiteto leidimą, jei toks leidimas būtinas pagal LR Biomedicininių tyrimų etikos įstatyme numatytus reikalavimus.*

Annex No. 2. Characteristics and findings of studies examining the role of GM in determining CAL, SSI and POI.

First author's name. (year) (country)	**Study design**	**No. of analyzed participants[1] (outcomes)**	**Outcome**	**Interventions MBP[2] EABs[3] IVABs[4] PRBs[5]**	**Microbiome type (sample material)**	**Timing of sample acquisition**	**Microbiota quantification method**	**GM profile deemed high risk for occurrence of analysed outcome**
1. No bowel preparation								
Ye Jin et al. (2020) (China) [61]	Prospective clinical trial	51 (9)	POI	-	MAMC[8] (colorectal mucosa tissue)	During surgery	16S rRNA Seq[10]	↑*Proteobacteria (↑Escherichia/Shigella)* ↑*Actinobacteria* ↓*Bacteroidetes* ↓*Fusobacteria* ↓*F. prausnitzii*
Anderson et al. (2021) (USA) [71]	Case-control	42 (19)	CAL	-	ILMC[9] (pelvic drain fluid)	During + After surgery	Culture	↑*E. faecalis* ↑*Streptococcus* spp.
Shi et al. (2022) (China) [74]	Case-control	20 (10)	CAL	-	MAMC (colorectal mucosa tissue)	After surgery	RT-PCR[11] 16S rRNA Seq	↑*F. nucleatum*
2. MBP+AB								
Sadahiro et al. (2014) (Japan) [59]	Randomized controlled trial	294 (56)	SSI	MBP (PEG[7] + Sodium picosulphate) EABs (kanamycin + metronidazole) IVABs (flomoxef)	ILMC (faecal matter)	Before + After surgery	RT-PCR	↑ *Enterococcus* spp. ↓*Bifidobacterium*

Dubinsky-Pertzov et al. (2018) (Israel, Switzerland, Serbia) [64]	Cohort study	662 (104)	SSI	MBP (PEG) IVABs (3rd gen cephalosporin + metronidazole)	ILMC (rectal swab)	Before + During surgery	Culture	↑ *Klebsiella* spp. ↑ *Enterococcus* spp. ↑ *P. aeruginosa*.
Xu et al. (2019) (China) [68]	Case-control study	60 (7)	SSI	MBP (n/s) + 12.5% glucose solution	ILMC (faecal matter)	Before + After surgery	RT-PCR	↑*E. faecalis* ↑*E. coli* ↓*Bifidobacterium* ↓*Lactobacillus*
van Praagh et al. (2019) (Netherlands) [65]	Case-control	58 (10)	CAL	n/s[6]	MAMC (colorectal mucosa tissue)	During surgery	16S rRNA Seq	*Lachnospiraceae (*↑*B. obeum* + ↓*Roseburia)* ↑*Bacteroidaceae* ↓ overall diversity
Sparreboom et al. (2019) (Netherlands, Belgium) [66]	Cohort	292 (38)	CAL	MBP (PEG)	ILMC (pelvic drain fluid)	After surgery	RT-PCR	↑→ *E.faecalis, E.coli*
Palmisano et al. (2020) (Italy) [69]	Case-control	21 (5)	CAL	MBP (n/s)	ILMC (faecal matter)	Before surgery	16S rRNA Seq	↑*A. lwoffi* ↑*A. jhonsonii* ↓*F. prausnitzii*
Mima et al. (2020) (Japan) [70]	Case-control	256 (29)	CAL	MBP (Magnesium citrate) IVABs (cefmetazole)	MAMC (colorectal mucosa tissue)	During surgery	RT-PCR	↑*Bifidobacterium* ↑→ *E.faecalis, E.coli, F. nucleatum*

Nalluri-Butz et al. (2022) (USA) [73]	Case-control	11 (1)	CAL	MBP (Miralax + Magnesium citrate) EABs (neomycin + metronidazole) IVABs (ciprofloxacin or Trimethoprim/sulfamethoxazole + metronidazole, cefotetan)	ILMC (faecal matter)	Before + During + After surgery	16S rRNA Seq	↑*Streptococcus* ↑*Enterococcus* ↓→*Faecalibacterium*
Liu et al. (2022) (China) [76]	Case-control study	60 (30)	POI	MBP (PEG) EABs (neomycin + metronidazole)	ILMC (faecal matter)	Before + After surgery	RT-PCR 16S rRNA Seq	↑*Lachnospiraceae (*↑*Ruminococcus torques* + ↑*Roseburia)* ↓*Lactobacillus*
3. EABs								
Komen et al. (2014) (Germany) [62]	Case-control	243 (19)	CAL	EABs (cefuroxime)	ILMC (pelvic drain fluid)	After surgery	RT-PCR[10]	↑*E. faecalis* ↑*E. coli*
Du et al. (2019) (China) [60]	Prospective surveillance study	5729 (206)	SSI	EABs (n/s)	MAMC (wound secretions)	After surgery	Culture	↑*E. coli (*55.26% colonisation in the colon resection group and 36.92% in the rectal resection cohort) ↑ *P. aeruginosa (20% incidence rate)*. ↑ *Enterococcus* spp. (29.43% incidence rate in the rectal surgical cohort)

Dong Li et al. (2019) (China) [67]	Case-control	37 (7)	CAL	EABs (neomycin + metronidazole)	MAMC (mucosa adjacent to the surgical margins)	During + After surgery	16S rRNA Seq	↑*C. suicordis* ↓*Alistipes shahii*
Jin et al. (2022) (China) [72]	Case-control	34 (17)	CAL	EABs (cefuroxime, aztreonam)	ILMC (faecal matter)	After surgery	16S rRNA Seq	↑*E. faecalis* ↑*Escherichia/Shigella* ↑→ *B. obeum, Bacteroides*
Reuvers et al. (2022) (Netherlands) [75]	Case-control study	223 (13)	SSI	EABs (n/s)	ILMC (rectal swab)	During surgery	16S rRNA Seq	↑*Proteobacteria* (mainly *P. aeruginosa)*
Vishnoi et al. (2023) (Australia) [77]	Case-control study	50 (8)	SSI	EABs (2nd gen cephalosporin + metronidazole)	ILMC (subcutaneous tissue swab)	During surgery	16S rRNA Seq + Culture	↑*Bacteroides* ↑*Enterobacteriaceae (E. coli, K. pneumoniae and P. mirabilia)*
4. PRBs								
Sadahiro et al. (2014) (Japan) [59]	Randomized controlled trial	294 (56)	SSI	PRBs (*Bifidobacteria)* consumed orally x3 daily after each meal for 7 days pre-op, and from the fifth post-op day for an additional 10 days.	ILMC (faecal matter)	Before + After surgery	RT-PCR	↑ *Enterococcus* spp. ↓*Bifidobacterium*
Aisu et al. (2015) (Japan)	Case-control	156 (27)	SSI	PRBs (*Enterococcus faecalis, Clostridium butyricum, Bacillus mesentericus)* start	ILMC (faecal matter)	Before + After	RT-PCR 16S rRNA Seq	↑*E. faecalis* ↑*E. coli* ↓*Bifidobacterium*

[63]				3-15 d. pre-op, restart post-op on the same day oral hydration resumes.		surgery		↓*Lactobacillus*
Xu et al. (2019) (China) [68]	Case-control study	60 (7)	SSI	PRBs (*Bifidobacterium longum*, *Lactobacillus acidophilus*, *Enterococcus faecalis* combined with a pre-operative 12.5% glucose solution) for 7 days post-op.	ILMC (faecal matter)	Before + After surgery	RT-PCR	↑*E. faecalis* ↑*E. coli* ↓→*Bifidobacterium* ↑*Lactobacillus*

[1] All participants included in the analysis underwent primary colorectal resection with anastomosis due to either colorectal cancer (malignancy), inflammatory bowel disease (IBD), diverticular disease, ischemic colitis or other causes.
[2]MBP – mechanical bowel preparation
[3]EABs – enteral antibiotics
[4]IVABs – IV antibiotics
[5]PRBs – probiotics
[6]n/s – the use of preoperative mechanical oral bowel preparation with an osmotic laxative and/or enteral wide spectrum antibiotic prophylaxis is stated but not specified.
[7]PEG – Polyethylene glycol
[8]MAMC – mucosa-associated microbial community
[9]ILMC – intra-luminal microbial community
[10]16S rRNA Seq – 16S ribosomal RNA sequencing
[11]RT-PCR - real-time polymerase chain reaction

↑: increased abundance; ↓: decreased abundance; ↑↓→: statistically insignificant increased or decreased abundance.