Thesis

Bacterial Cultures in Pediatric Appendicitis – A Literature Overview

Submitted by

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1 Declaration of Academic Integrity

I solemnly declare that I have written the present work independently and without external assistance, have not used any sources other than those specified, and have clearly identified passages that have been taken verbatim or in essence from the sources used.
Furthermore, I hereby declare that if artificial intelligence (AI) tools were used to generate and/or revise certain text passages in the preparation of this work, their use complied with ethical principles, academic integrity, and the guidelines of my university. Such use has been transparently disclosed and appropriately labeled.

Graz, am 02.04.2025 Kamel Abd El Hai m.p.

2 Acknowledgment

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3 Zusammenfassung

Hintergrund

Die genaue Pathogenese der akuten Appendizitis ist noch weitgehend ungeklärt. Verschiedene Theorien und Modelle deuten auf bakterielle Infektionen als primären Auslöser der pädiatrischen akuten Appendizitis hin. Diese Übersichtsarbeit zielt darauf ab, aktuelle Forschungsergebnisse zu bakteriellen Pathogenen, die an der Pathogenese der akuten Appendizitis beteiligt sind, zusammenzufassen und zu analysieren. Eine detaillierte Auswertung von Bakterien, die durch perioperative Abstrichkulturen aus dem Appendikallumen, der Oberfläche, der Peritonealhöhle oder der Peritonealflüssigkeit identifiziert wurden, kann wertvolle Einblicke in den Zusammenhang zwischen diesen Mikrobiomen und der Entstehung, dem Verlauf, dem Schweregrad und den Komplikationen der Erkrankung liefern.

Methoden

Die Literaturrecherche wurde in den Datenbanken PubMed, Medline, Google Scholar und Cochrane Library durchgeführt. Die ausgewählten Publikationen wurden analysiert und zusammengefasst.

Ergebnisse

Eine zentrale Rolle bei der akuten Appendizitis spielt die mikrobielle Dysbiose, wobei *Fusobacterium nucleatum* und *Fusobacterium necrophorum* in Mukosaläsionen, insbesondere bei perforierten oder gangränösen Fällen, überrepräsentiert sind. Komplizierte Appendizitiden zeigen eine erhöhte Häufigkeit von oralen Pathogenen (*Fusobacterium, Parvimonas micra*) und eine Reduktion kommensaler Bakterien (*Bacteroides, Faecalibacterium*). Polymikrobielle Infektionen mit *Escherichia coli*, *Bacteroides fragilis* und *Pseudomonas aeruginosa* treten häufiger bei schweren Verläufen auf, wobei *Pseudomonas* mit längeren Krankenhausaufenthalten assoziiert ist. Die mikrobielle Diversität ist bei komplizierter Appendizitis höher, angetrieben durch proinflammatorische Gattungen wie *Prevotella* und *Sutterella*. Kulturbasierte Methoden identifizieren dominante Spezies, während die 16S-rRNA-Sequenzierung zusätzliche Pathogene wie *Porphyromonas endodontalis* aufdeckt, was den Bedarf an komplementären diagnostischen Ansätzen unterstreicht.

Schlussfolgerung

Die Expansion oraler Pathogene wie *Fusobacterium* und *Parvimonas* sowie die Abnahme kommensaler Bakterien wie *Bacteroides* und *Faecalibacterium* tragen maßgeblich zum Krankheitsverlauf und -schweregrad bei. Polymikrobielle Infektionen mit *Escherichia coli, Bacteroides fragilis* und *Pseudomonas aeruginosa* erschweren das klinische Bild, insbesondere bei perforierten oder gangränösen Fällen.

4 Abstract

Background

The exact underlying pathogenesis of acute appendicitis is still largely unknown. Several theories and models have been suggested to explain the role of bacterial infections as a primary precipitating factor of pediatric acute appendicitis. This article aims to review and synthesize current research on bacterial pathogens implicated in the pathogenesis of acute appendicitis. A detailed analysis of bacteria identified through perioperative swab cultures from the appendiceal lumen, surface, peritoneal cavity, or peritoneal fluid may provide valuable insights into the correlation between these microbiomes and the development, progression, severity, and complications of the disease.

Methods

This literature review was carried out by analyzing and summarizing the searched publications in the PubMed, Medline, Google Scholar and Cochrane Library databases.

Results

Microbial dysbiosis plays a central role in acute appendicitis, with *Fusobacterium nucleatum* and *Fusobacterium necrophorum* being overrepresented in mucosal lesions, particularly in perforated or gangrenous cases. Complicated appendicitis shows an increase in abundance of oral pathogens (*Fusobacterium, Parvimonas micra*) and reduction of commensal bacteria (*Bacteroides, Faecalibacterium*). Polymicrobial infections involving *Escherichia coli, Bacteroides fragilis, and Pseudomonas aeruginosa* are more prevalent in severe cases, with *Pseudomonas* being linked to prolonged hospital stays. Microbial diversity is higher in complicated appendicitis, driven by pro-inflammatory genera like *Prevotella* and *Sutterella*. Culture-based methods identify dominant species, while 16S rRNA sequencing reveals additional pathogens like *Porphyromonas endodontalis*, highlighting the need for complementary diagnostic approaches.

Conclusion

The expansion of oral pathogens like *Fusobacterium* and *Parvimonas* and the depletion of commensal bacteria such as *Bacteroides* and *Faecalibacterium* contributes significantly to disease progression and severity. Polymicrobial infections involving *Escherichia*

coli, Bacteroides fragilis, and *Pseudomonas aeruginosa* further complicate the clinical picture, particularly in perforated or gangrenous cases.

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6 Abbreviations

AA	Acute Appendicitis
AIR	Appendicitis Inflammatory Response score
CA	Complicated Appendicitis
CT	Computed Tomography
EAES	European Association of Endoscopic Surgery
ESBL	Extended-Spectrum Beta-Lactamase
FISH	Fluorescence in Situ Hybridization
GALT	Gut-Associated Lymphoid Tissue
GPAC	Gram-Positive Anaerobic Cocci
HCs	Healthy Controls
IAA	Intra-Abdominal Abscess
IA	Incidental Appendectomy
IBD	Inflammatory Bowel Disease
IntA	Interval Appendectomy
LPS	Lipopolysaccharide
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
MRI	Magnetic Resonance Imaging
NOM	Non-Operative Management
NOS	Newcastle-Ottawa Scale
PAS	Pediatric Appendicitis Score
PA	Perforated Appendicitis
pARC	Pediatric Appendicitis Risk Calculator
POCUS	Point-of-Care Ultrasonography
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSA	Pseudomonas aeruginosa
SA	Simple Appendicitis
SAG	Streptococcus anginosus Group
SCFA	Short-Chain Fatty Acids

SDI	Socio-Demographic Index
SSI	Surgical Site Infection
Th17	T-Helper 17 Cells
UA	Uncomplicated Appendicitis
US	Ultrasonography

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9 Introduction

9.1 Pediatric Appendicitis

9.1.1 Definition, Epidemiology,Clinical Presentation

Pediatric appendicitis is the most frequent surgical emergency and a leading cause of acute abdomen among children (1). Pediatric appendicitis represents a significant health burden in children, leading to considerable healthcare costs and resource utilization (2). Approximately 1- 8% of pediatric patients presenting to the emergency department with symptoms indicative of an acute abdomen are ultimately diagnosed with acute appendicitis (AA) (3). Delayed diagnosis and treatment may lead to serious complications such as perforation, abscess formation and peritonitis and is associated with significant morbidity and mortality (4). The global annual incidence of AA varies geographically and ranges between 100-151 cases per 100,000 individuals (5). An excess of 30% of the affected are children with a peak incidence at 11-12 years of age (6). A decline in incidence has been observed in countries with high Socio-Demographic Index (SDI) scores, whereas countries with middle and low SDI scores are experiencing a steady increase (7). The lifetime incidence of AA is approximately 7%, with a male-to-female ratio of 3:2 up to the fourth decade of life, although the risk of undergoing an appendectomy is 50% lower in men (8). The clinical presentation of AA exhibits broad differences between patients and up to half of the affected children experience indistinct symptoms (9). The typical presenting symptoms such as nausea, vomiting, fever, anorexia and abdominal pain with migration to the right lower quadrant have been proven to be unreliable in predicting the diagnosis (10). It has been reported that many "classic" symptoms were absent in children with pathologically confirmed appendicitis, with 40% lacking anorexia, 29% presenting without nausea or vomiting, 50% without pain migration, and 50% without rebound tenderness (11). Patients may present with inability to walk, rebound tenderness, local or generalized peritoneal signs, Rovsing sign, obturator sign and iliopsoas sign (12). Prior research assessing the diagnostic value of these clinical signs has shown that the Rovsing, obturator, and iliopsoas signs exhibit low sensitivity (16%–44%) but high specificity (86%–98%) for appendicitis detection (2). Approximately one-third of children exhibit atypical symptoms,

including irritability, periumbilical pain, and diarrhea, with conditions such as mesenteric adenitis or gastroenteritis potentially mimicking appendicitis (13).

9.1.2 Pathogenesis

AA demonstrates hallmark features of transmural inflammation, beginning with early changes such as serosal vessel dilation, wall edema, and progressing to intramural abscess formation with mesoappendix involvement. In catarrhal appendicitis, inflammation is confined to the intraluminal space, mucosa, and submucosa, representing an early and localized stage. Fluid accumulation and elevated intraluminal pressure may result in tissue distention, mucosal ulceration, and bacterial translocation across the epithelial barrier. In contrast, phlegmonous appendicitis is distinguished by diffuse neutrophilic infiltration of the muscularis propria, which may be accompanied by ulceration, vascular thrombosis and the formation of intramural abscesses, reflecting a more advanced and severe inflammatory state. The progressive increase in intraluminal pressure and thrombosis further facilitates bacterial invasion into the tissue. The gangrenous stage is marked by large areas of tissue necrosis due to sustained bacterial penetration and compromised blood supply. The combination of persistent intraluminal pressure and thrombosis exacerbates tissue damage and the ongoing tissue necrosis and damage may culminate in perforation of the appendix, posing a significant risk of peritonitis and systemic infection (14).

The pathogenesis of AA remains predominantly equivocal and requires further clarification despite being broadly researched. Long-standing assumptions suggest that the development of AA begins with obstruction of the appendix lumen by factors such as fecaliths, impacted stool, foreign bodies, lymphoid hyperplasia, or tumors. This blockage initiates a cascade involving luminal distension, impaired circulation, ischemia, and secondary bacterial invasion of the appendiceal wall, ultimately leading to acute inflammation and perforation (15). This hypothesis is supported by limited evidence, as obstruction by a fecalith and increased luminal pressure are observed in only approximately 34% of appendicitis cases (16, 17). Appendiceal colic, resulting from the presence of appendiceal worms or faecoliths, represents a clinically significant entity. It should be considered a potential cause of abdominal pain, even in cases where microscopic evidence of appendicitis is

absent (18). There is increasing evidence and growing interest in the role of viral infections as a potential trigger, which may lead to secondary bacterial infection and subsequent inflammation (19). Superficial mucosal ulceration was observed to manifest earlier in the disease course, suggesting a potential role for viral infection such as enteroviruses as an initiating factor, followed by secondary bacterial invasion, in the underlying pathogenesis (15).

Despite the lack of genetic evidence the relative risk of developing appendicitis is three times higher in families with positive history of appendicitis than families without any history (20). Children presenting with blunt abdominal trauma were found to have a higher incidence of appendicitis compared to the general population. It has been hypothesized that the presence of edema, hematoma, bruising, or rupture of the mesoappendix, commonly observed in such cases, may lead to vascular compromise. This compromise could subsequently facilitate bacterial invasion of the appendiceal wall, contributing to the development of appendicitis (15).

Recent studies have been exploring the theory that primary bacterial infections play a crucial role in the pathogenesis of AA. This infectious etiology is supported by the evidence of temporo-spatial interaction (21), seasonal variation (22) and low incidence in rural areas of the developing world (23). During the COVID-19 pandemic, the incidence of pediatric appendicitis decreased by 40% in 2020 compared to the previous year. This decline may be attributed to the implementation of social distancing measures and enhanced hygiene practices, which likely reduced exposure to infectious pathogens (24).

9.1.3 Classification

AA can be clinically, pathophysiologically and epidemiologically divided into two different forms: uncomplicated and complicated acute appendicitis. The classification of AA in either uncomplicated or complicated according to the European Association of Endoscopic Surgery (EAES) is based on the presence of inflammation with or without periappendiceal phlegmon, perforation, gangrene or a perityphlitic abscess (25).

The distinction between both forms is essential for the stratified management approach (26). Accurately identifying cases of uncomplicated appendicitis (UA) is crucial, as it significantly influences treatment decisions. Most patients with UA can be effectively managed with an initial antibiotic-based approach (8). Approximately 60% of adult

patients with UA can be successfully treated with antibiotics. However, a significant proportion will eventually require a subsequent appendectomy (27).
A universally accepted classification system for differentiating complicated from uncomplicated appendicitis remains lacking, despite its critical importance for guiding appropriate treatment strategies. Uncomplicated and complicated appendicitis exhibit substantial differences in both mortality and morbidity. While the morbidity risk for uncomplicated appendicitis is approximately 6.9%, it escalates to 20.1% in complicated cases (28). Reliance on clinical judgment alone has led to negative appendectomy rates of up to 36%, with diagnostic accuracy in the absence of imaging reported to be as low as 75–80% (28, 29). In children under six years of age, clinical signs and symptoms are often unreliable, making it particularly challenging to distinguish complicated appendicitis (CA) from conditions such as gastroenteritis (13). Imaging techniques are considered superior to patient history, physical examination, laboratory findings, and clinical scoring systems in the evaluation of suspected AA (30).
The traditional teaching that AA without early appendicectomy always progresses to perforation seems to be relevant to CA and the conservative management has proven to be plausible and safe (31). Recent evidence indicates that non-perforated appendicitis often resolves spontaneously without treatment, highlighting the need to shift the clinical focus from preventing perforation to the early identification and management of advanced appendicitis. To reduce mortality, morbidity, and healthcare costs, minimizing unnecessary appendectomies is more crucial than solely aiming to prevent perforation (32)

9.1.4 Diagnosis

The diagnosis usually relies on combination of the history taking, clinical examination, laboratory results and radiological imaging which are partially being used in the AA clinical scoring systems. Scoring systems have been developed to differentiate between uncomplicated and complicated appendicitis, as well as to stratify patients based on their risk of appendicitis, distinguishing those at low risk from those at high risk (33).
The Alvarado score, Pediatric appendicitis score (PAS), Appendicitis Inflammatory Response score (AIR) and pediatric Appendicitis Risk Calculator (pARC) are sufficiently useful in risk stratification of patients with suspected AA and in the management to reduce hospital admissions, radiation exposure and negative surgical explorations (8). Clinical scoring systems demonstrate sufficient sensitivity to identify low-risk patients, thereby

reducing the reliance on imaging and minimizing unnecessary surgical interventions, such as diagnostic laparoscopy, in cases of suspected AA (8). The use of the AIR score algorithm in low-risk patients resulted in less imaging, fewer admissions, fewer negative explorations and fewer operations for non-perforated AA (34). Among the various clinical prediction models available for the diagnosis of AA, the AIR demonstrates the highest diagnostic performance, with a sensitivity of 92% and a specificity of 63% (35). The Alvarado score and PAS are among the most widely used clinical scoring systems in pediatric populations, both incorporating similar clinical parameters for the diagnosis of acute appendicitis (8). Additionally, biomarkers have proven to be valuable when integrated with the systematic application of these scoring systems (36). The diagnostic accuracy of various biomarker panels has been prospectively validated, demonstrating high sensitivity and negative predictive values for AA in large cohorts of patients presenting with right iliac fossa pain (37). Point-of-care ultrasonography (POCUS) has emerged as a valuable diagnostic tool for AA, significantly enhancing clinical decision-making. The overall sensitivity and specificity of ultrasonography (US) are 76% and 95%, respectively, while computed tomography (CT) demonstrates sensitivity and specificity of 99% and 84%, respectively (38). The increased radiation exposure associated with CT scans is a significant concern, particularly in the pediatric population. Additionally, the limited availability of MRI in many facilities may restrict its practicality as an imaging modality. The need for anesthesia to perform an adequate pediatric MRI further complicates its use, creating additional barriers. While ultrasound avoids radiation exposure and is widely accessible in most hospitals, its operator-dependent nature can reduce diagnostic accuracy, potentially leading to bias, the need for further imaging, and delays in care. Ultrasound is also limited in its ability to identify complicated appendicitis and is associated with a high rate of non-visualization of the appendix, particularly in patients with obesity. However, evidence suggests that the reliability of ultrasound can be enhanced through the implementation of a standardized reporting structure, which may improve its diagnostic utility (39, 40).

9.1.5 Management of pediatric appendicitis

The promising outcomes associated with antibiotic treatment for uncomplicated AA further emphasize the critical importance of accurately distinguishing between uncomplicated and complicated forms of the disease prior to initiating therapeutic interventions. The majority of acute appendicitis cases are of an uncomplicated nature, which may not necessitate surgical intervention and can, in some instances, resolve spontaneously without progressing to perforation (41).

9.1.5.1 Uncomplicated appendicitis

The management of acute UA in children offers two primary treatment pathways: non-operative management (NOM) with antibiotics alone or operative management with appendectomy. While laparoscopic appendectomy is now the current standard of care with an average length of stay of 1 day (2). The NOM with antibiotics has been proven as safe and effective in treating AA without increase in treatment-associated complications compared to surgery (42). The most recent meta-analysis evaluated the outcomes of non-operative versus operative management of AA revealed that 8% of patients experienced early treatment failure, necessitating appendectomy during their initial hospital stay, while 16% of patients required appendectomy after discharge. These findings suggest that NOM is a viable alternative to surgery, with comparable hospitalization durations and acceptable rates of treatment failure (43). Additional studies have identified several clinical and imaging factors significantly associated with recurrence of appendicitis following NOM. These factors include the presence of rebound tenderness, muscle guarding, an appendiceal diameter greater than 9 mm, intraluminal appendiceal fluid, higher pain scores, and a longer duration of pain prior to presentation (2). The operative management of UA is associated with complication rates ranging from 5% to 15%. These complications include intra-abdominal abscesses, superficial and organ space surgical site infections, small bowel obstruction, and postoperative ileus (44).
The presence of an appendicolith has been identified as an independent prognostic risk factor for treatment failure in the NOM of UA. When an appendicolith is present in conjunction with AA, it is associated with an increased risk of perforation, further complicating the clinical course (45). The success of NOM approach in pediatric AA

hinges on careful patient selection, with the exclusion of cases involving gangrenous AA, abscess formation, or diffuse peritonitis (46). Evidence suggests that NOM can effectively avoid appendectomy in a significant majority of children after a 1-year follow-up period. However, current data remain insufficient to recommend NOM as a universal treatment for all children with UA (42). Notably, at the 1-year mark, children managed nonoperatively demonstrated fewer disability days and lower appendicitis-related healthcare costs compared to those who underwent appendectomy (47).

9.1.5.2 Complicated appendicitis

CA is defined as appendicitis accompanied by either a grossly identifiable perforation in the appendix, the presence of a fecalith in the abdominal cavity, or the formation of a well-defined abscess or frank pus within the abdomen (48). CA accounts for less than 19% of hospital admissions related to appendicitis (49). However, it is associated with significantly worse clinical outcomes and increased resource utilization. Specifically, patients with CA experience a three-fold increase in length of hospital stay and a 50% rise in total healthcare costs compared to those with uncomplicated cases (2, 49). The management of CA can be approached through one of three primary strategies: antibiotics alone, antibiotics followed by interval appendectomy (IntA), or early appendectomy at the time of initial presentation (2).

This condition can be further categorized into two distinct groups: perforation without abscess/phlegmon and perforation with abscess/phlegmon. Management strategies vary based on the clinical presentation; children with CA and abscess/phlegmon tend to have better outcomes with initial NOM followed by IntA. In contrast, those presenting with free perforation without abscess/phlegmon generally benefit from early appendectomy during the initial hospitalization (50). A survey conducted by the European Pediatric Surgeons' Association revealed that 96% of surgeons initiate preoperative antibiotic therapy in cases of (CA). For stable patients with perforated appendicitis, the presence of a phlegmon or abscess was identified as the primary contraindication to immediate surgery by 96% of respondents and length of clinical history for 36% (51). **Table 1** presents the criteria for distinguishing UA and CA, adapted from the European Association for Endoscopic Surgery (EAES) (1). Although inflammation occurs in both forms, gangrene, phlegmon,

perityphlitic abscess, free fluid, and perforation are exclusive to complicated appendicitis (8, 25).

	Uncomplicated Appendicitis	Complicated Appendicitis
Inflammation	+	+
Gangrene	–	+
Phlegmon	–	+
Perityphlitic abscess	–	+
Free fluid	–	+
Perforation	–	+

Table 1: Criteria for uncomplicated vs. complicated appendicitis, adapted from EAES (25)

10 Materials and Methods

This thesis is based on a comprehensive literature review of online databases such as Pubmed, Cochrane library and Google Scholar with emphasis on studies from the last 15 years. The following keywords and MeSH terms were used in various combinations: "pediatric appendicitis", "bacterial cultures", "microbiology of appendicitis", "antibiotic resistance in appendicitis", "pediatric surgical infections", "anaerobic bacteria in appendicitis", "aerobic bacteria in appendicitis", "perforated appendicitis microbiology", “appendix microbiome”, “appendix dysbiosis”. Boolean operators (AND, OR) were used to refine the search, and filters were applied to include studies published between 2009 and 2024 to ensure relevance to current clinical practices.

Studies focusing on pediatric populations (ages 0-18 years), reporting bacterial culture results from intraoperative specimens and published in English were included in the review. Studies including comparisons between UA and CA were considered. Studies involving adult population only, not reporting bacterial culture data, case reports, editorials and non-peer-reviewed articles were excluded. The study selection process followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines **(Figure 1)**. By the initial screening, titles and abstracts of retrieved articles were screened for relevance followed by full-text review where articles meeting the inclusion criteria were reviewed in full to assess eligibility and studies that aligned with the research objectives were included in the review **(Table 2)**. As illustrated in **Figure 1**, the initial database search yielded 3295 records. After removing duplicates (n = 46), studies were screened based on titles and abstracts, excluding 3088 irrelevant articles. The remaining 161 full-text articles were assessed for eligibility, with 135 excluded due to lacking microbiological data, focusing on adults, case reports or conference abstracts. Ultimately, 26 studies were included in the final analysis.

All references were meticulously organized and managed using EndNote to ensure accuracy and consistency in citation.

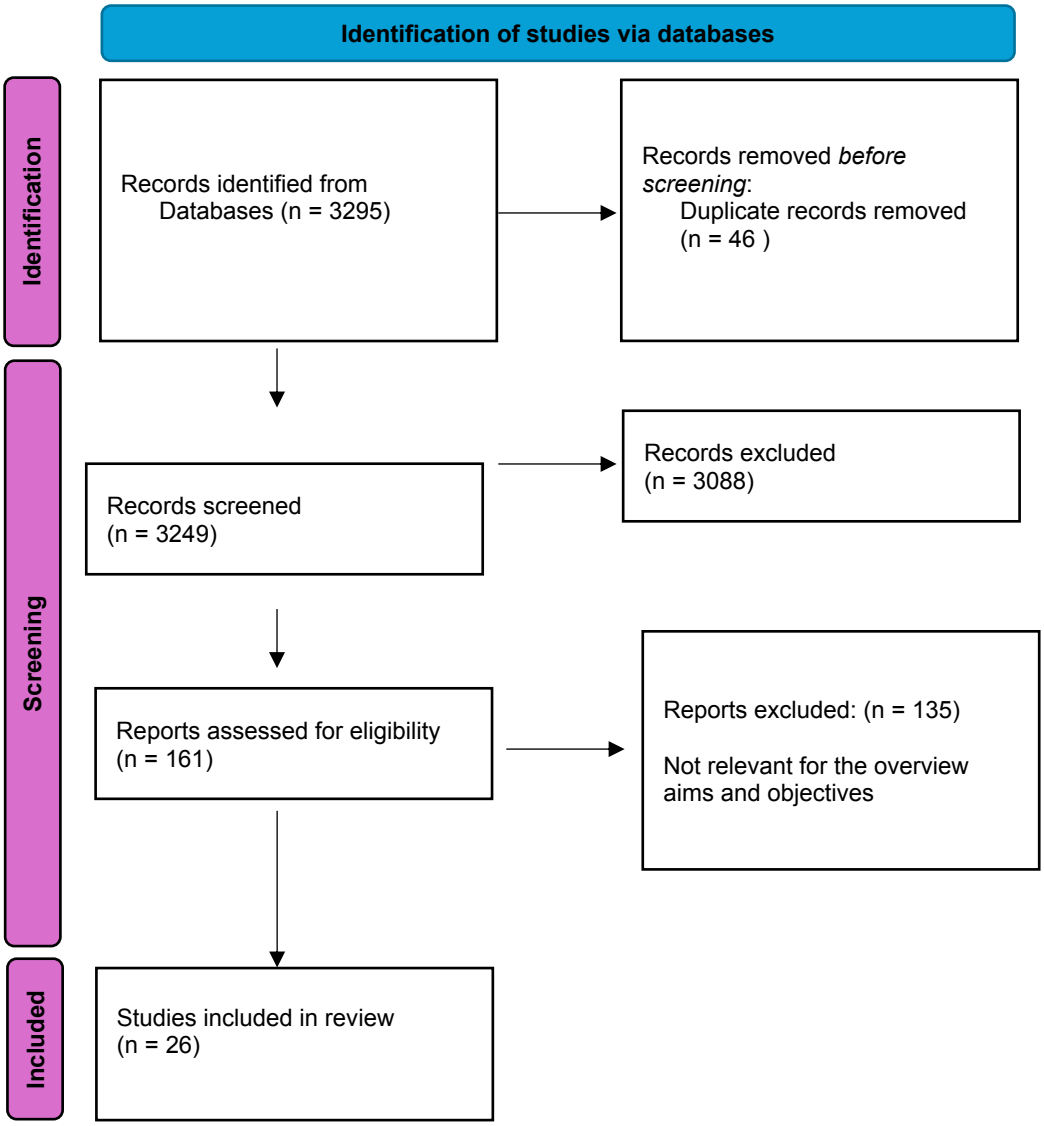

Figure 1: PRISMA flow chart

10.1 Data Extraction and Synthesis

Data from the selected studies **(Table 2)** were systematically extracted using a standardized template, encompassing study characteristics, patient demographics, bacterial culture methodologies, identified bacterial pathogens, and their respective prevalence rates. The extracted data were thematically synthesized to align with the research objectives. The analysis focused on characterizing bacterial pathogen profiles in pediatric appendicitis, examining how microbial distributions differ between uncomplicated and complicated presentations. Particular emphasis was placed on comparing detection methodologies, evaluating how traditional culture-based techniques contrast with molecular approaches

like gene sequencing and Fluorescence In Situ Hybridization (FISH) in identifying pathogenic organisms. These microbiological findings were further contextualized within their clinical relevance, exploring implications for diagnosis and treatment paradigms.

Study	Purpose of Study	Method Used
Swidsinski et al. (2011) (52)	Investigate microbial composition in complicated vs. uncomplicated appendicitis	16S rRNA sequencing and FISH of appendiceal tissue
Zhong et al. (2014) (53)	To survey microbial communities using a culture-independent approach	16S rRNA sequencing of appendiceal swabs
Jackson et al. (2014) (54)	Alterations in microbiota may contribute to appendicitis. Comparing microbial profiles of normal appendices vs. rectal samples and perforated cases	16S rRNA sequencing of appendiceal and rectal samples
Rogers et al. (2016) (55)	Investigate Fusobacterium abundance in appendices from patients with AA	16S rRNA sequencing of appendiceal swabs
Salö et al. (2017) (56)	To evaluate the microbiome in the normal appendix and in appendicitis divided into the three grades of inflammation	16S rRNA sequencing of appendiceal tissue.
Schülin et al. (2017) (57)	To characterize the microbiome in AA comparing extraluminal and intraluminal samples	Cultures and 16S rRNA sequencing of appendiceal tissue

Blod et al. (2018) (58)	To investigate the oral and appendiceal microbiome of affected children compared to healthy controls.	16S rRNA sequencing of appendiceal- and gingival sulcus swabs
Andrey et al. (2019) (59)	To describe the bacteriology of complicated appendicitis and to determine the risk of infectious complications	Culture-based analysis of peritoneal swabs
Viel-Thériault et al. (2019) (60)	Microbiology and antimicrobial treatment of complicated appendicitis	Culture-based analysis of peritoneal fluid
The et al. (2019) (61)	Identify microbial clusters in simple vs. complex appendicitis	16S rRNA sequencing of appendiceal tissue.
Dahlberg et al. (2019) (62) .	Compare intraoperative and abscess culture results in complicated appendicitis	Culture-based analysis of intraoperative and abscess samples
Plattner et al. (2021) (63)	To assess the current epidemiology and microbiology of perforated appendicitis, how antibiotic choice and duration correlate with meaningful clinical outcomes	Culture-based analysis of preop. drains, intra-op cultures, post-op-abscess and blood
Theodorou et al. (2021) (64)	To investigate if PSA infection associated with worse outcomes in perforated appendicitis	Culture-based analysis of appendiceal swabs

Kakar et al. (2022) (65)	To determine the prevailing microbiota as well as evaluate the antibacterial sensitivity of the isolated microorganisms	Culture-based analysis of appendiceal -and peritoneal swabs
Gerber et al. (2022) (66)	Analyse the Microbiology and resistance profiles of pathogens of com- plicated appendicitis	Culture-based analysis of peritoneal swabs
Bi et al. (2022) (67)	Analyze dysbiosis in acute appendicitis.	16S rRNA sequencing of fecal and blood samples
Tamura et al. (2022) (68)	To compare bacterial floras in ascites culture between perforated and non-perforated appendicitis	Culture-based analysis of peritoneal fluid
Naji et al. (2023) (69)	Identify the predominant bacteria cultured and determine the appropriate choice of antibiotics for preoperative and postoperative management	Culture-based analysis of appendiceal swabs
Garzon-González et al. (2023) (70)	To determine the association between microorganism resistant to the antibiotic used in empirical therapy and the development of intra-abdominal abscesses in children with perforated appendicitis	Culture-based analysis of peritoneal fluid

Bhaskar et al. (2023) (71)	Investigate the bacterial pathogens to guide empirical surgical antimicrobial prophylaxis options	Culture-based analysis of Intra-operative swabs
Felber et al. (2023) (72)	Assess bacterial presence and diversity and their antibiotic resistances in relation to appendicitis severity	Culture-based analysis of appendiceal swabs and peritoneal fluid
Zachos et al. (2023) (6)	Investigate microbial profiles in perforated appendicitis and peritoneal fluid	Culture-based analysis of appendiceal and peritoneal swabs
Blohs et al. (2023) (14)	Examine oral pathogen expansion in complicated appendicitis	16S rRNA sequencing of appendiceal tissue, rectal- and peritoneal swabs
Aiyoshi et al. (2023) (73)	To determine the optimal first-line antibiotic treatment for pediatric complicated appendicitis	16S rRNA sequencing of appendiceal lumen- , saliva-, faecal swabs
Nasrallah et al. (2024) (74)	To investigate the incidence, predictors, and outcomes of PSA-associated acute appendicitis in children	Culture-based analysis of peritoneal fluid
Yu et al. (2024) (75)	To investigate the microbiological characteristics of acute appendicitis to permit accurate empirical antibiotic use for uncomplicated appendicitis	Culture-based analysis of blood, ascites, abscess, drain, wound swabs

Table 2: Studies included in the Review

10.2 Quality Assessment

The methodological quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS) **(Table 3).** The NOS is a widely recognized and validated tool for assessing the methodological quality of non-randomized studies, particularly in observational research. Its structured framework evaluates studies across three key domains: selection of study groups, comparability of groups, and ascertainment of outcomes or exposures. By assigning a star-based rating system within each domain, the NOS provides a quantitative measure of study quality, enabling researchers to identify potential biases and assess the reliability of findings in systematic reviews and meta-analyses.

The NOS is particularly valuable in medical research, where it helps distinguish high-quality studies from those with methodological limitations. By systematically evaluating the representativeness of study populations, the adequacy of control groups, and the rigor of outcome or exposure assessment, the scale ensures a robust synthesis of evidence. This is especially critical when analyzing heterogeneous datasets or drawing conclusions from diverse clinical settings and patient populations. One of the key strengths of the NOS lies in its ability to control for confounding variables, which is essential for minimizing bias in observational studies. The comparability domain, for instance, allows researchers to assess whether studies have adequately adjusted for critical factors such as age, sex, or comorbidities. This ensures that the observed associations are more likely to reflect true relationships rather than confounding effects. Moreover, the NOS enhances the transparency and reproducibility of quality assessments, which are fundamental to evidence-based medicine. Its standardized criteria facilitate the comparison of studies across different contexts, enabling researchers to draw more reliable conclusions. This is particularly important in fields like pediatric appendicitis, where variations in study design, sampling methods, and diagnostic criteria can significantly impact the validity of findings. By employing the NOS, we can systematically identify and address potential sources of bias, thereby strengthening the credibility of their conclusions. This, in turn, supports more informed clinical decision-making and contributes to the development of evidence-based guidelines.

Study	Selection (Max 4)	Comparability (Max 2)	Outcome (Max 3)	Total Score (Max 9)	Quality
Swidsinski et al. (2011) (52)	4	2	3	9	High
Zhong et al. (2014) (53)	4	2	3	9	High
Jackson et al. (2014) (54)	4	2	3	9	High
Rogers et al. (2016) (55)	4	2	3	9	High
Salö et al. (2016) (56)	3	1	2	6	Moderate
Schülin et al. (2017) (57)	4	2	3	9	High
Blod et al. (2018) (58)	4	2	3	9	High
Andrey et al. (2019) (59)	4	2	3	9	High
Viel-Thériault et al. (2019) (60)	3	1	2	6	Moderate
The et al. (2019) (61)	4	2	3	9	High
Dahlberg et al. (2019) (62)	3	1	2	6	Moderate
Plattner et al. (2021) (63)	4	2	3	9	High
Theodorou et al. (2022) (64)	3	1	2	6	Moderate
Kakar et al. (2022) (65)	3	1	2	6	Moderate

Study	Selection (Max 4)	Comparability (Max 2)	Outcome (Max 3)	Total Score (Max 9)	Quality
Gerber et al. (2022) (66)	3	1	2	6	Moderate
Bi, et al. (2022) (67)	3	1	2	6	Moderate
Tamura et al. (2022) (68)	4	2	3	9	High
Naji et al. (2023) (69)	3	1	2	6	Moderate
Garzon-González et al. (2023) (70)	3	1	2	6	Moderate
Bhaskar et al. (2023) (71)	3	1	2	6	Moderate
Felber et al. (2023) (72)	3	1	2	6	Moderate
Zachos et al. (2023) (6)	3	1	2	6	Moderate
Blohs et al. (2023) (14)	4	2	3	9	High
Aiyoshi et al. (2023) (73)	4	2	3	9	High
Nasrallah et al. (2024) (74)	4	2	3	9	High
Yu et al. (2024) (75)	4	2	3	9	High

Table 3: NOS Assessment Table

11 Results

11.1 The Appendiceal Microbiota

The human gut microbiota plays a pivotal role in regulating immune responses, providing protection against pathogens, facilitating digestion, and contributing to neurological signaling and vascularization (76). The pediatric gut microbiome is predominantly composed of bacteria from the phyla Bacteroidetes and Firmicutes. However, in contrast to adults, the gut microbiota of healthy children is characterized by a significantly reduced relative abundance of Bacteroidetes and a markedly higher prevalence of Firmicutes and Actinobacteria (77). At the genus level, *Bacteroides* represents nearly 40% of the gut microbiome in healthy children, with the remaining microbial community comprising genera such as *Faecalibacterium, Alistipes, Ruminococcus, Roseburia*, and others (77) .
The microbial composition of the healthy appendix is characterized by a greater abundance of *Selenomonas*, *Fusobacterium*, *Parvimonas*, and *Peptostreptococcus* compared to the rectal microbiota. In contrast, the normal rectum demonstrates higher levels of *Frankineae*, *Dyadobacter*, *Curvibacter*, *Melissococcus*, *Variovorax*, *Larkinella*, and *Actinomycineae* relative to the appendix (54).
It has been postulated that the human appendix serves as a reservoir of beneficial microbes with the potential to repopulate the gut after antibiotic treatment, diarrhea or pathogen colonization (78). Its tube-like structure is well adapted to maintain biofilms with mutualistic intestinal flora and the secretion of IgA and mucin supports this growth (79). Its protected position away from the pathogen containing fecal stream makes it ideal for maintaining beneficial microbiota.
The presence of Gut-Associated Lymphoid Tissue (GALT) and the immune system's role in supporting mutualistic biofilms in the gut suggest that the appendix may serve as a potential site for maintaining these biofilms (79). The appendix plays a vital role in the production of IgA, the predominant antibody in the GALT system. IgA plays a dual role in host defense by neutralizing bacteria and viruses. It binds pathogenic microbes and toxins with high affinity to facilitate their elimination while also binding to commensal microbiota with low affinity, regulating their size and composition (80, 81).
In the assessment of the microbiome, numerous potential confounding factors must be considered. Current research conducted within the general population identifies key

covariates, including medication use, hematologic parameters, bowel movement patterns, dietary habits, overall health status, anthropometric measures, and lifestyle factors, as the most significant variables influencing microbiome composition and function. These covariates should be carefully accounted for in the design and interpretation of microbiome-related studies (56).

Studies examining the microbiome in healthy controls have provided valuable insights into the baseline composition of gut and appendiceal microbiota, allowing for comparisons with diseased states such as appendicitis. Research utilizing both culture-based and gene sequencing methods has consistently demonstrated that healthy individuals harbor a more diverse and stable microbial community compared to those with appendicitis.

Swidsinski et al. analyzed appendix tissue samples from healthy controls and found that the microbiota was predominantly composed of *Bacteroides* spp., *Faecalibacterium prausnitzii*, and *Clostridium* spp., with a significantly lower presence of *Fusobacterium* compared to those with appendicitis (52). The stability of the microbiota in healthy individuals suggests that a disruption in this balance may contribute to the onset of inflammation. Similarly, *Salö et al.* reported that healthy controls exhibited a high relative abundance of *Bacteroides* spp. and Firmicutes, with an absence or very low levels of *Fusobacterium* spp. and *Parvimonas* spp., which were strongly associated with acute appendicitis in their study (56).

Fecal microbiome studies, such as those by *Bi et al.*, further reinforced these findings by demonstrating that healthy individuals had a balanced composition of Firmicutes and Bacteroidetes, whereas appendicitis patients exhibited an increase in Proteobacteria and Fusobacteria. The study also noted that specific beneficial bacteria, including *Bifidobacterium* and *Faecalibacterium,* were more prevalent in healthy individuals, potentially playing a role in maintaining gut homeostasis and preventing opportunistic infections (67).

Zhong et al. investigated the microbial composition of the appendiceal lumen in both healthy individuals and those with appendicitis. Their findings indicated that healthy controls had a diverse microbiota dominated by *Bacteroides* spp. and *Prevotella* spp., whereas diseased appendices showed an overrepresentation of *Fusobacterium* spp. and *Parvimonas* spp. (53). This shift in microbial composition supports the hypothesis that appendicitis may result from dysbiosis rather than the presence of a single pathogenic organism.

Jackson et al. examined the rectal and appendiceal microbiome in healthy controls, further confirming that *Parvimonas, Porphyromonas*, and *Bulleidia* were found at much lower levels in healthy individuals compared to those with perforated appendicitis. Their study also identified that healthy controls had a relatively stable core microbiota with minimal fluctuations, in contrast to the disrupted and inflammation-associated microbial shifts observed in appendicitis cases (54).

Overall, the microbiome in healthy controls is characterized by a high diversity and predominance of beneficial commensal bacteria, particularly *Bacteroides, Faecalibacterium, and Clostridium* spp., with low levels of *Fusobacterium* spp. and other opportunistic pathogens. In contrast, appendicitis is associated with a loss of microbial diversity and an overgrowth of specific anaerobic pathogens, particularly *Fusobacterium nucleatum, Parvimonas micra,* and *Bacteroides fragilis*. These findings highlight the importance of microbial homeostasis in preventing inflammation and suggest that dysbiosis, rather than a single pathogen, may be a key driver of appendicitis.

11.2 Sampling methods

The detection of pathogens in clinical studies is heavily influenced by the sampling method used, as different techniques yield varying microbial profiles based on anatomical site, oxygen exposure, and microbial abundance **(Table 4). Figure 2** further illustrates these disparities, showing the relative prevalence of *Escherichia coli (E. coli)*, *Fusobacterium*, *Bacteroides*, and *Enterococcus* across sampling methods (e.g., peritoneal fluid culture vs. appendiceal tissue samples).

The utility of obtaining intraoperative swabs during appendectomy remains a subject of debate in the surgical and infectious disease communities. Proponents argue that intraoperative swabs enable targeted antibiotic therapy in the postoperative period, which may optimize treatment outcomes and reduce the risk of complications. The procedure itself is quick, technically straightforward, and associated with minimal additional costs per patient, making it an attractive option for routine practice (82). However, critics highlight several limitations to this approach. The clinical impact of intraoperative swabs is often limited, as culture results rarely lead to significant changes in antibiotic management. Furthermore, evidence suggests that the collection of swabs and subsequent antibiotic adjustments do not substantially reduce the incidence of intraabdominal abscesses or other

postoperative complications (83). When considering the cumulative costs of routine swab collection across a large patient population, the financial burden becomes non-negligible, raising questions about cost-effectiveness (84)**.**

The appendiceal lumen is the primary site of infection in appendicitis. Samples collected from this location, either through swabbing or fluid aspiration, are highly representative of the pathogens involved in the disease. In cases of perforated appendicitis, the peritoneal cavity often contains pus or inflammatory exudate, making it a critical site for sample collection. Aspiration of peritoneal fluid or abscess contents is another common method and effective technique for isolating pathogens in liquid environments. Fluid samples are suitable for both culture-based and molecular analyses. Although less commonly used, blood cultures can be valuable in cases of sepsis or systemic infection secondary to appendicitis. Stool samples can provide insights into the gut microbiota and its potential role in appendicitis. The localized proliferation of opportunistic pathogens within the appendix appeared to exert no significant influence on the composition of the rectal microbiome. This observation may potentially be attributed to the inherent microbial diversity of the large intestine, coupled with a "dilution effect" of microbial signatures or organisms originating from the appendix as they transit through the colonic lumen. While rectal samples do not demonstrate sufficient discriminatory power to differentiate between complicated and uncomplicated appendicitis, they may hold utility in distinguishing patients with AA from healthy individuals (14) .

In contrast, peritoneal samples may serve as a more reliable indicator of AA severity. Invasive pathogens, such as Fusobacteria, were detected not only in cases of perforated appendicitis but also in some instances of phlegmonous and gangrenous appendicitis. Although the diagnostic utility of peritoneal samples is limited by the necessity of invasive procedures for sample collection (14).

As illustrated in **Table 4**, peritoneal fluid culture has been commonly employed to identify pathogens associated with peritonitis and appendicitis, as seen in studies by *Tamura et al., Felber et al*., and *Nasrallah et al.* (68, 72, 74). These studies frequently identified *E.* coli*, Enterococcus spp., and Pseudomonas aeruginosa*, consistent with known enteric flora implicated in intra-abdominal infections. However, due to exposure to oxygen and limitations in culture media, these methods tend to underrepresent anaerobes, which are now increasingly recognized as playing a critical role in appendicitis.

In contrast, **Table 4** highlights that appendiceal tissue sampling has provided a more detailed view of the microbial environment of the appendix. Studies such as those conducted by *Swidsinski et al., Salö et al., and Blohs et al.* utilized gene sequencing to uncover a much higher prevalence of anaerobic bacteria, including *Fusobacterium nucleatum, Bacteroides fragilis, and Clostridium spp.* These organisms were largely absent in studies relying solely on culture-based methods, suggesting that appendicitis may be driven by a more complex and diverse microbiome than previously understood.

Appendiceal lumen swabs, examined in studies by *Zhong et al., Aiyoshi et al., and Zachos et al*., further demonstrated the presence of a highly diverse bacterial community, including *Prevotella, Parvimonas, and Porphyromonas spp.* gene sequencing results from these samples consistently detected a broader spectrum of microbes than those obtained from peritoneal fluid cultures, indicating that sampling directly from the appendix provides a more accurate representation of the disease-associated microbiota.

Comparative insights from **Figure 2** and **Table 4** reveal that appendiceal lumen swabs and tissue samples capture a broader microbial diversity, including *Prevotella* and *Parvimonas*, compared to peritoneal fluid cultures. For example, *Fusobacterium* was detected in 15–30% of appendiceal samples but <5% of peritoneal fluid cultures (**Figure 2**), aligning with studies by *Swidsinski et al.* and *Salö et al.*

The use of peritoneal swabs, as reported by *Zachos et al. and Gerber et al.,* also provided insights into microbial invasion during peritoneal infections. While these methods detected *E. coli* and *Enterococcus* in most cases, consistent with peritoneal fluid culture results, gene sequencing methods revealed additional anaerobic species such as *Clostridium* and *Veillonella*, further highlighting the limitations of traditional culture techniques. Fecal sampling studies by *Aiyoshi et al.* and *Bi et al.* aimed to identify shifts in gut microbiota associated with appendicitis. The results indicated high microbial diversity, with sequencing detecting Bacteroidetes, Firmicutes, and Proteobacteria, as well as significantly higher levels of *Fusobacterium* spp. in appendicitis patients compared to controls, suggesting a possible role for this organism in disease progression.

Blood sampling, investigated in studies by *Bi et al*. and *Plattner et al*., has been used to assess bacteremia in appendicitis cases. While culture-based methods commonly detected *E. coli* and *Streptococcus* spp., gene sequencing results revealed low-level bacteremia involving anaerobes such as *Bacteroides* and *Fusobacterium*, organisms that were often missed in traditional blood cultures. Rectal and gingival swabs provided additional insights

into the possible microbial sources associated with appendicitis. *Jackson et al.* and *Blohs et al.* found that rectal swabs contained high levels of *Fusobacterium* and *Bacteroides*, further supporting the hypothesis that gut microbiota translocate to the appendix, potentially contributing to inflammation. *Blod et al*. examined gingival sulcus samples and identified *Porphyromonas endodontalis* exclusively in appendicitis patients, suggesting a potential oral-gut microbial connection (58).

Sampling Method	Studies
Peritoneal Fluid Culture	*Tamura et al. (2022), Felber et al. (2023), Garzon-González et al. (2023), Nasrallah et al. (2024), Viel-Thériault et al. (2019)*
Appendiceal Tissue Samples	*Swidsinski et al. (2011), Salö et al. (2017), The et al. (2019), Blohs et al. (2023)*
Serosa Swabs	*Felber et al., 2023*
Appendiceal Lumen Swabs	*Zhong, D., et al. (2014), Aiyoshi, T., et al. (2023), Zachos, K., et al. (2023), Rogers, M. B., et al. (2016), Schülin, S., et al. (2017), Blod, C., et al. (2018)*
Peritoneal Swabs	*Zachos, K., et al. (2023), Gerber, F., et al. (2022), Blohs, M., et al. (2023), Andrey, V., et al. (2019)*
Fecal Sampling	*Aiyoshi, T., et al. (2023), Bi, Y., et al. (2022)*
Gingival Sulcus	*Blod, C., et al. (2018)*
Drain Sampling	*Plattner et al., 2021; Dahlberg et al., 2019*
Rectal Swabs	*Jackson, H. T., et al. (2014), Blohs, M., et al. (2023)*
Blood Sampling	*Bi, Y., et al. (2022), Plattner, A. S., et al. (2021), Yu, C.-H., et al. (2024)*

Table 4: Sample Collection Methods

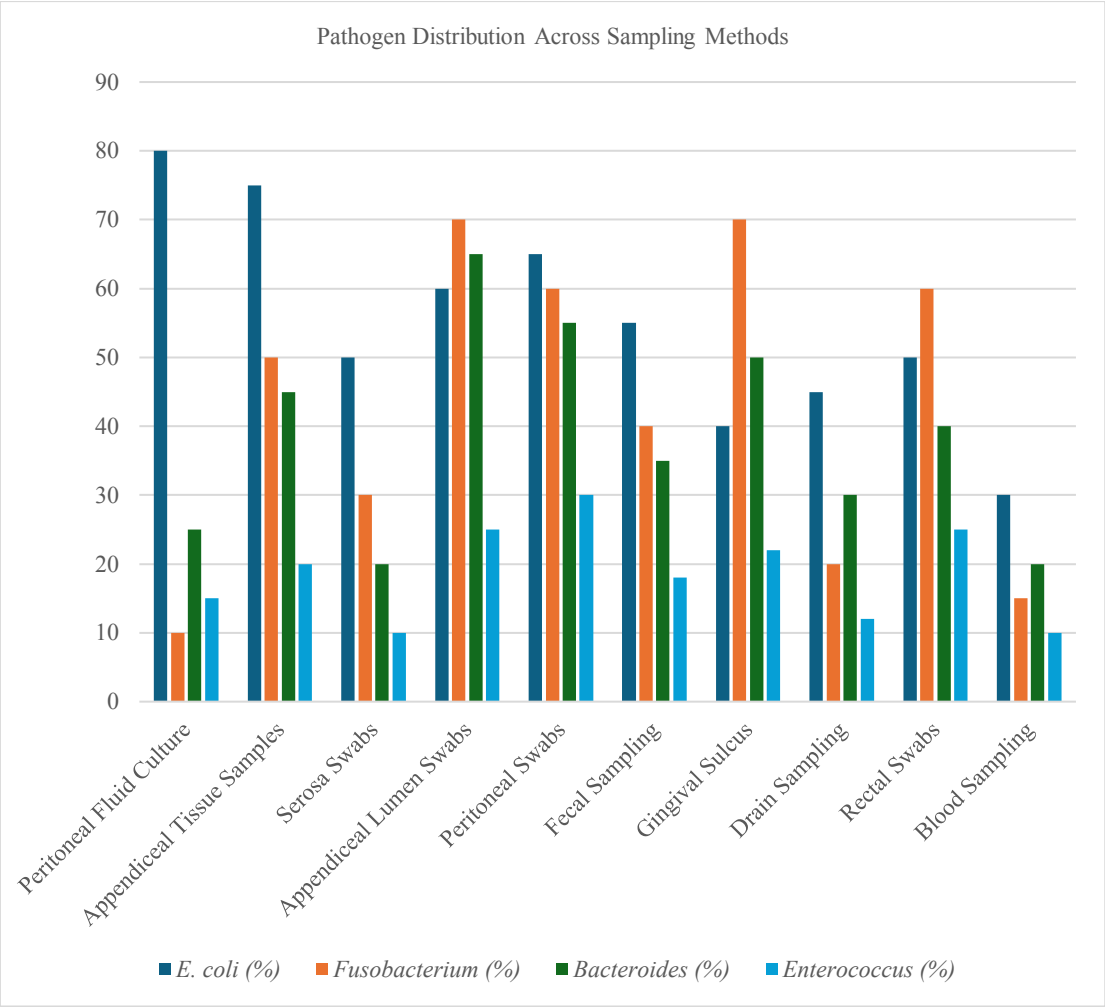

Figure 2: Pathogen Distribution Across Sampling Methods

11.3 Bacterial cultures versus microbiome profiling

In clinical practice, bacterial cultures are commonly utilized to identify microorganisms present in samples obtained during appendectomy. **Figure 3** comparing Culture-based and Gene sequencing and summarizes key studies demonstrating that culture-based methods predominantly detect aerobic and facultative anaerobic pathogens like *E. coli*, *Pseudomonas aeruginosa*, and *Enterococcus* spp., while underrepresenting strict anaerobes.These samples, typically collected from the peritoneal cavity or appendix fossa, are analyzed to determine bacterial profiles and assess antibiotic susceptibility. This approach is essential for guiding appropriate antimicrobial therapy in cases where there is no improvement with empirical treatment and for monitoring changes in organism susceptibility to antibiotics (**Table 5**) (85). However, **Figure 4** reveals a critical limitation:

culture-based detection rates for anaerobes like *Fusobacterium* (≤20%) are significantly lower than those identified via gene sequencing (60–80%), highlighting a diagnostic gap. The isolation of a pathogen through culturing at the time of appendectomy may not necessarily represent the causative organism involved in the pathogenesis. Additionally, the selective culturing of more complex cases, such as those with intra-operative identification of pus, could bias the findings toward severe presentations **(Figure 4),** potentially failing to capture the broader microbiological profile across all patients (64). The relationship between the predominant organisms identified during initial sampling and those implicated in the development of intra-abdominal abscesses (IAA) remains unclear. Several smaller studies have reported considerable discrepancies between organisms cultured intraoperatively and those isolated from IAAs (62, 86). Preoperative administration of intravenous broad-spectrum antibiotics is standard practice. This has raised questions about the utility of intraoperative culturing, as studies have shown that most causative organisms are highly sensitive to empirically prescribed antibiotics, with sensitivity rates between 68% and 97% (87). The routine perioperative administration of antibiotics significantly impacts bacterial culture results. Therefore, culture findings alone may not be sufficient for guiding postoperative antimicrobial therapy in appendicitis and should be considered alongside clinical assessment and other diagnostic modalities (88). The detection of bacterial species via 16S rRNA gene analysis depends on the amplification of the small subunit ribosomal RNA gene, which plays an essential role in cellular function (57). The 16S rRNA gene contains nine hypervariable regions that exhibit significant sequence diversity among different bacterial species. These hypervariable regions are instrumental in identifying individual bacterial species or distinguishing between a limited number of closely related species (89). The resulting sequences after processing through this method are then compared against a 16S ribosomal database to identify the species. However, using the 16S rRNA gene as a phylogenetic marker has its limitations, such as intragenomic redundancy. Bacterial genomes can carry multiple copies (up to 15) of this gene and that can lead to inaccuracies and distort estimates of microbial abundance when relying solely on gene counts (90). This redundancy tends to result in an underestimation of taxa with a low number of 16S rRNA gene copies, while taxa with a higher number of copies may be overrepresented (90). While most variable regions of the 16S rRNA gene are sufficient for genus-level identification, they generally lack the resolution necessary for reliable species-level discrimination. As a result, Analysis

targeting these regions could fail to capture the true species diversity within the examined sample (91). As shown in Figure 5, Swidsinski et al. and Salö et al. demonstrated that 16S rRNA sequencing and Fluorescence in situ hybridization (FISH) could detect a variety of difficult to culture organisms including *Alloprevotella*, *Fusobacterium*, and *Parvimonas* (52, 56) .

FISH could serve as a complementary approach to identify bacterial species within tissue specimens. In comparison to culture-based methods, FISH makes it possible to visualize the bacteria in their native environment and sheds light on their distribution and interactions with the infected host. Despite the less common employment of FISH in appendicitis research compared to culture-based methods and 16S rRNA sequencing, it provides certain advantages, such as the possibility to visualize unculturable organisms and biofilm-associated pathogens. However, its implementation in research settings is limited by technical demands such as the need for specialized equipment and expertise and its lower processing rate compared to sequencing-based methods (52).

Feature	Culture-Based Methods	16S rRNA Sequencing / FISH
Bacterial Diversity	Detects facultative anaerobes, but limited anaerobe detection	Identifies broad bacterial communities, including unculturable bacteria
Key Pathogens Detected	*E. coli*, *P. aeruginosa*, *B. fragilis*	*Fusobacterium*, *Parvimonas*, *Clostridium, Porphyromonas*
Detection in Different Appendicitis Types	Detects dominant bacteria in SA/CA	Shows polymicrobial complexity, more oral/gut microbiota in CA/PA
Antibiotic Sensitivity	Provides direct antibiotic susceptibility testing	Identifies resistance genes but not direct susceptibility
Anaerobe Detection	Underestimates strict anaerobes	Better identification of anaerobes
Clinical Relevance	Useful for guiding immediate antibiotic therapy	Better for understanding microbial ecology and resistance evolution

Table 5: Cultures vs 16S rRNA Sequencing / FISH

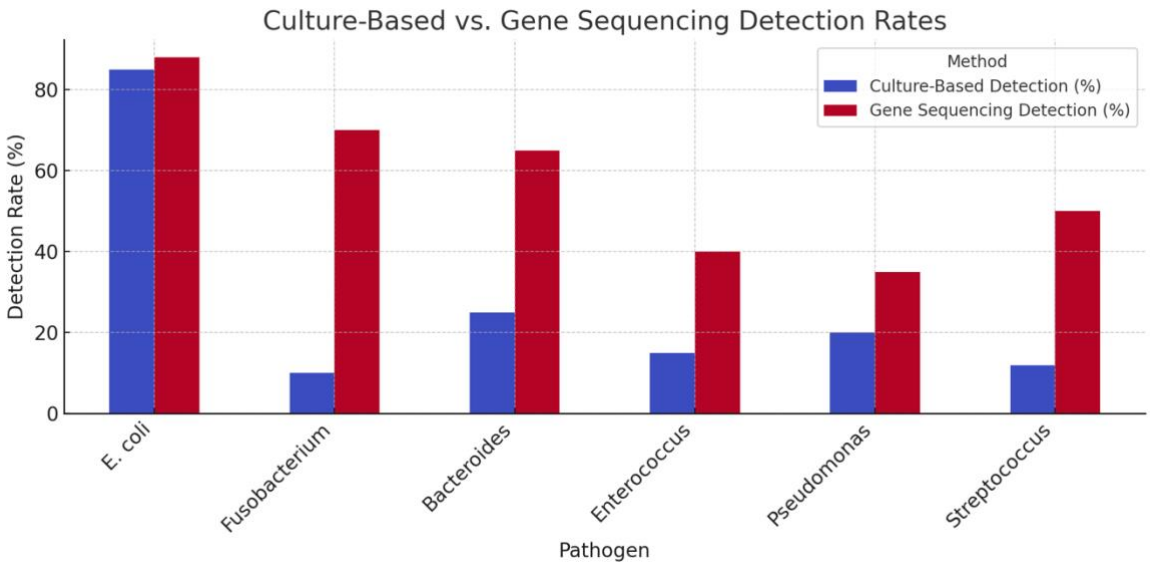

Figure 3: Culture-Based vs. Gene Sequencing Analysis

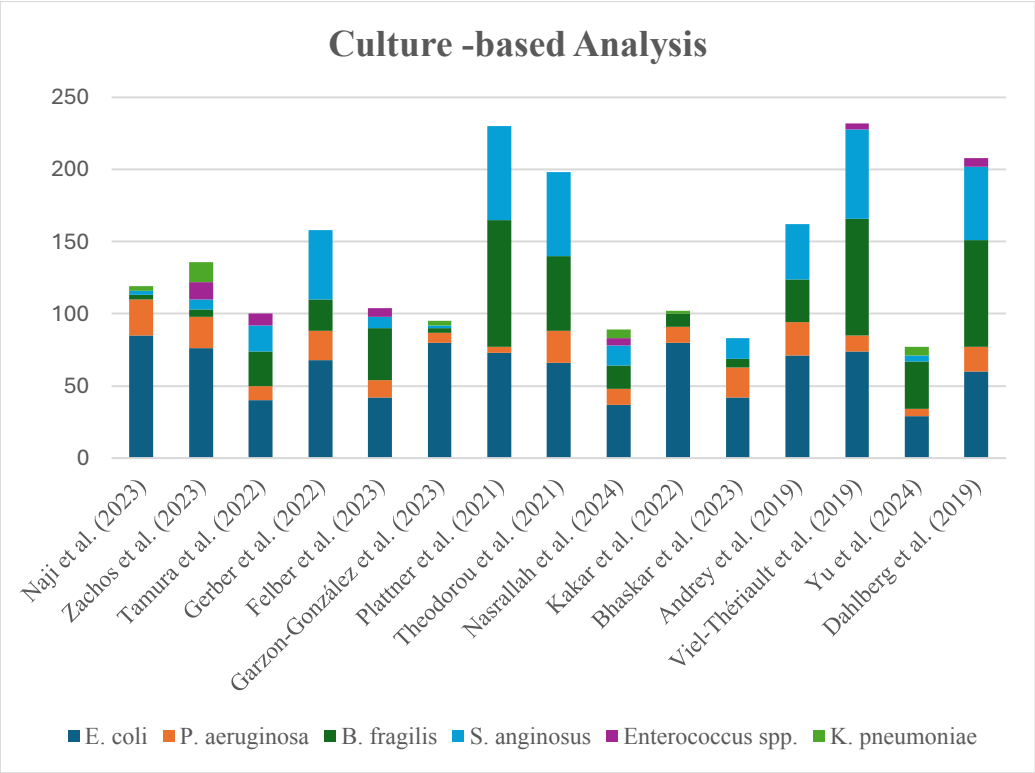

Figure 4: Culture-Based Analysis

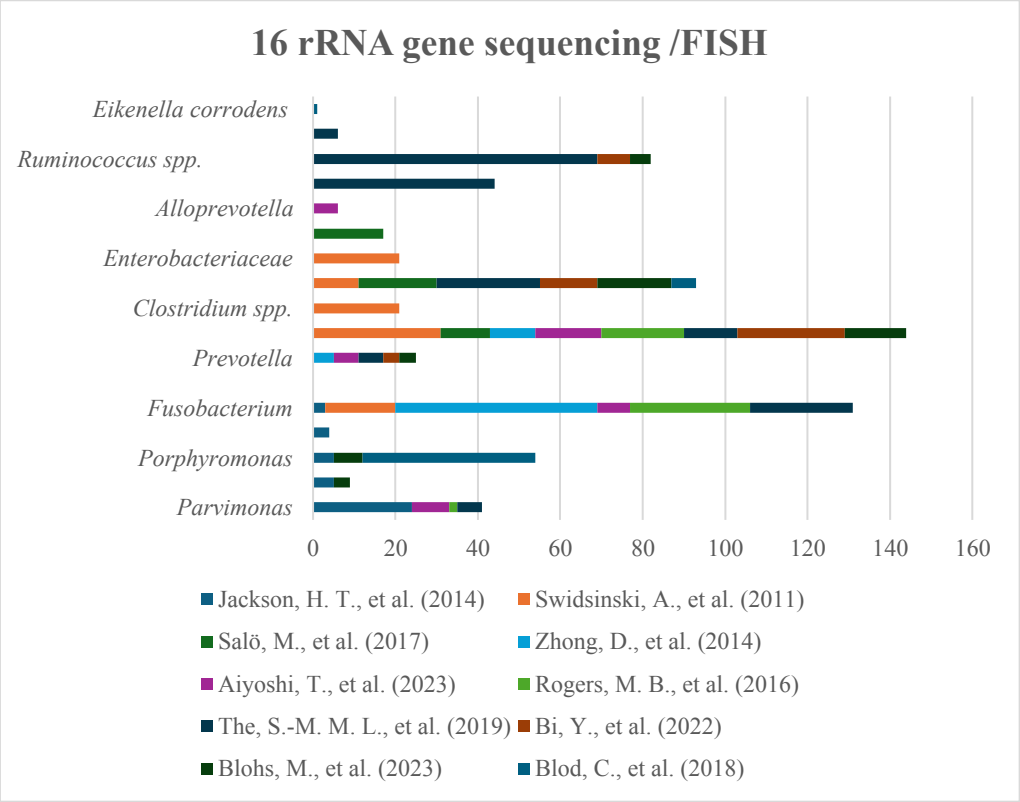

Figure 5: 16 rRNA gene sequencing / FISH

11.4 Common Bacterial Pathogens

11.4.1 The Role of Oral Pathogens in Pediatric Appendicitis

The oral microbiome has been associated with several extra-oral diseases and systemic inflammatory processes (53, 56). New evidence and studies have indicated high levels of mouth dwelling bacteria such as *Fusobacterium, Peptostreptococcus, Porphyromonas*, and *Gemella* within the appendiceal tissues and lumen. From these, Firmicutes appeared as the major phylum and *Streptococcus* as the main genus discovered in the gingival sulci. The abundance of *Porphyromonas stomatis* were much higher in the gingival sulci of pediatric patients with AA compared to HCs. However, in inflamed appendiceal tissue, *Eikenella corrodens* and *Fusobacterium nucleatum* prevalence was lower than the prevalence in the gingival sulci of appendicitis patients (58). The route of infection is still unknown and as transmission via blood seems unlikely given the negative blood cultures and the delayed onset of fever, excluding a systemic inflammatory response (55, 92). The digestive tract may act as a potential route for mouth bacteria to travel from the oral cavity to the appendix following meals. After food intake the gastric pH rises to approximately 5.5 allowing oral bacteria such as *Porphyromonas stomatis, Eikenella corrodens,* and *Fusobacterium nucleatum,* , as they've been shown to tolerate pH levels as low as 4 (58).

11.4.1.1 Fusobacterium

Among anaerobic Gram-negative bacteria*, Fusobacterium* spp. have been implicated as a key pathogen in appendiceal and intra-abdominal infections. These organisms appear particularly prevalent in advanced disease states like perforated or gangrenous appendicitis. Its contribution to the progression of the disease, polymicrobial infections, and biofilm formation became more apparent through both culture-based and gene sequencing methods.

High presence of *Fusobacterium* in the cultures obtained from appendiceal lumen of pediatric AA were found. Moreover, its presence in the saliva and feces of these patients was notably higher compared to that observed in healthy controls (HCs) (73).
It is among the widely detected bacterial species in the oral cavity and implicated in the pathogenesis of various diseases (93). *F. nucleatum*, a commensal of the oral cavity, is frequently identified at extra-oral sites due to systematic dissemination and colonization in association with pathological conditions (94). There are four suggested subspecies within the *F. nucleatum* species with varying prevelance under disease conditions *ss. animalis, ss. fusiforme, ss. polymorphum* and *ss. vincentii* (95). Vital mechanisms for colonization, dissemination, evasion of host defenses and induction of host response are adherence and invasion (93). *F. nucleatum* encodes FadA an adhesin and invasin that binds to host cells and is required for invasion through binding to cadherins in various cells and tissues. *F. nucleatum* binds to epithelial and endothelial cells, monocytes , erythrocytes, fibroblasts and NK cells (96). This microbe causes root canal infections or periodontitis and was detected in some extra oral diseases such as colorectal cancer, atherosclerotic disease, rheumatoid arthritis and inflammatory bowel disease (55).
Studies have consistently demonstrated that *Fusobacterium* spp., particularly *Fusobacterium nucleatum* and *Fusobacterium necrophorum*, are markedly more prevalent in complicated appendicitis compared to uncomplicated cases or HCs. *Swidsinski et al*. reported that *Fusobacterium* was present in 17% of UA and 21% of CA but was hardly detected in HCs. In Addition, it was identified in just two out of 400 caecal biopsies and was negative in the 400 stool samples analyzed (52). This supports the notion of a correlation between *Fusobacterium* and disease severity in cases involving mucosal lesions and submucosal infiltration (52). Supporting this further, *Salö et al.* established a higher level of *Fusobacterium* in phlegmonous appendicitis (19%) and perforated appendicitis (32%) and its abundance was less than 5% in gangrenous appendicitis and healthy controls (56). An increased level of *Fusobacterium* in patients with appendicolith obstruction highlights a possible role in the pathogenesis of some appendicitis cases and is most likely in a polymicrobial setting (56). *Fusobacterium* was detected by *Zhong et al*. as a dominant genus in the luminal fluid of patients with suppurative appendicitis and perforated appendicitis and witnessed a reduction in *Bacteroides* spp., revealing a shift in microbial composition during AA (53). These findings were supported by *Aiyoshi et al.*, who found elevated levels of *Fusobacterium* in

both fecal and appendiceal lumen samples of patients with acute AA. Furthermore *Bacteroides* abundance was higher in cases of CA compared to UA (73). Additionally the study revealed the interactions in biofilm formation between *Fusobacterium* and other oral pathogens, such as *Parvimonas micra*, which possibly contributes to mucosal inflammation and disease progression (73).
The persistence of *Fusobacterium* even after antibiotic treatment demonstrates its Adaptive capacity and its potential role as a source for recurrent infections (55). *Rogers et al*. reported that *Fusobacterium* was detected in four out of eight interval appendectomy patients who had received 10–14 days of broad-spectrum antibiotic therapy, suggesting that the appendix may serve as a microbial reservoir during acute disturbances. This persistence, alongside its ability to form biofilms, may explain its resistance to conventional treatments and its association with prolonged or recurrent infections (55). *The et al.* concluded that *Fusobacterium nucleatum* and *Parvimonas micra* are more abundant in CA compared to UA leading to the assumption that these pathogens contribute to disease severity through pro-inflammatory mechanisms (61).

16S rRNA gene sequencing is an example of a molecular technique that has improved the detection and understanding of *Fusobacterium* in appendicitis. This technique was employed by *Schülin et al*. to identify *Fusobacterium necrophorum* and *Fusobacterium nucleatum* in appendiceal samples, which were not detectable by culture-based methods (57). *Fusobacterium* appears to drive dysbiosis by disrupting the microbial homeostasis, contributing to inflammation and tissue damage. *Blohs et al.* documented a concerning microbial pattern in CA, while oral bacteria such as *Fusobacterium, Porphyromonas,* and *Parvimonas* expanded beneficial *Bacteroides,* and other commensal bacteria diminished (14). These findings suggest that *Fusobacterium* may play a key role in appendicitis pathogenesis, especially in CA and the significant clinical implications. This necessitates the anaerobic coverage in antibiotic regimens, with agents such as metronidazole or clindamycin often recommended (14).

11.4.1.2 Streptococcus anginosus

The *Streptococcus anginosus* group (SAG), historically classified as the Milleri group streptococci, is a commensal organism found on mucosal surfaces, including the oral

cavity, gastrointestinal tract, respiratory tract, and urogenital tract (97, 98). This group is comprised of three distinct species: *S. anginosus, S. constellatus, and S. intermedius* (99). Members of the *S. anginosus* group are now established as threatening pathogens and are often detected in blood cultures and abscess cultures, highlighting their clinical importance in invasive infections (99). SAG is often involved in pyogenic infections throughout the body. Typical infection sites range from the central nervous system, oral and dental structures, thoracic cavity, pleura, skin, and intra-abdominal regions (99, 100). This highlights their pathogenic potential with their commensal functions in mucosal membranes (100). SAG thrive as part of polymicrobial infections and most commonly isolated with the predominant pathogens *E. coli* and *B. fragilis (Bacteroides fragilis)* (101). Evidence indicates that *Eikenella corrodens, Fusobacterium nucleatum, Prevotella intermedia,* and organisms within the SAG may act synergistically to facilitate the development of pyogenic infections (102).

The pathogenic mechanisms of *S. anginosus* include its ability to form abscesses and its synergistic interactions with other pathogens. *S. anginosus* can worsen abscess formation in partnership with *Fusobacterium nucleatum*, a common anaerobic pathogen in appendicitis. This partnership and cooperation is possibly due to secretion of virulence factors such as hyaluronidase and DNase, causing tissue invasion and immune evasion (102). *S. anginosus* shows the ability to form biofilms in association with *Parvimonas micra*, leading to its persistence in infections and resistance to antibiotic therapy (103). Various studies have shown the prevalence of *S. anginosus* in appendicitis. *Theodorou et al.* found *S. anginosus* in 58.4% of intraoperative cultures, establishing it as a major pathogen and comparable to the most frequently isolated pathogens such as *E. coli* and *B. fragilis* (64). The role it plays in severe disease was reported by *Gerber et al.* as it was abundant in 48% of CA cases (66). *Dahlberg et al.* found that *S. anginosus* was often isolated in intraoperative and intra-abdominal abscess (IAA) cultures, leads to the conclusion that it is involved in postoperative complications and abscess formation (62). *S. anginosus* exhibits higher detection rate in polymicrobial infections and in perforated appendicitis. *Tamura et al.* detected *S. anginosus* in almost 50% of perforated appendicitis cases and 35.8% of non-perforated cases. This difference in prevalence between both groups shows its role in driving severe disease outcomes (68). Its systemic impact was mentioned by *Zachos et al.* as S. anginosus was often isolated from both the appendiceal lumen and the peritoneal cavity in CA (6).

The pro-inflammatory nature of *S. anginosus* contributes to its pathogenic role in appendicitis. *The et al.* discussed the contribution of *S. anginosus* in severe inflammation and tissue damage associated with Th17-mediated immune response. This inflammatory response and the ability to form abscesses makes it a main factor in causing complications such as intra-abdominal abscesses and postoperative infections (61).
S. anginosus is mostly susceptible to antibiotics and the great majority of strains responding to first-line antibiotics. *Yu et al.* reported that *S. anginosus* isolates shows consistent susceptibility to ampicillin (100%) and it would respond well to a monotherapy. However, its coexistence in polymicrobial infections could make treatment more difficult, as co-infecting pathogens may exhibit varying resistance patterns (75) .
In conclusion, *S. anginosus* plays a major role in AA and especially in complicated and perforated cases. Its clinical significance arises from its ability to form abscesses, cause severe inflammation, and its involvement in postoperative complications. Despite its antibiotic susceptibility, its role in polymicrobial infections necessitates comprehensive treatment strategies that address co-infecting pathogens to optimize patient outcomes.

11.4.1.3 ***Porphyromonas spp.***

Porphyromonas spp. such as *Porphyromonas endodontalis* and *Porphyromonas gingivalis* are anaerobic, gram-negative bacteria strongly implicated in the pathogenesis of periodontitis. These bacteria show the ability to evade and modulate the host immune response. Specific strategies like the secretion of various virulence factors, including lipopolysaccharide (LPS) and extracellular proteases could be employed. Recent evidence supports the suggestion that *Porphyromonas* spp. could affect systemic health by causing gut dysbiosis and compromising gut barrier integrity, leading to inflammation and dysregulation of host metabolism (104).
Porphyromonas spp. influence the oral and gut microbiota in completely different ways (105). Some studies reveled inverse shift in microbial dynamics between gut and oral microbiota during *Porphyromonas* infection. The diversity increases orally while the gut ecosystems undergo dysbiosis and decrease in diversity (106, 107). There is emerging evidence that *Porphyromonas* spp. has also been implicated in the etiology of rheumatoid arthritis, cardiovascular disease, Alzheimer's disease and orodigestive cancers (108).

The presence of *Porphyromonas* in appendicitis is often linked to its role in biofilm formation and polymicrobial interactions. *Aiyoshi et al.* brought to our attention the high relative abundance of *Porphyromonas* in the appendiceal lumen of pediatric AA patients (73). The study discusses the interactions between *Porphyromonas* and a host of other oral pathogens, including *Fusobacterium* and *Parvimonas,* contributing to mucosal inflammation and biofilm formation (73). This biofilm formation could complicate and add to disease severity by increasing bacterial survival and resistance to host immune responses.

Porphyromonas has been linked to the progression of appendicitis to more severe forms. *Schülin et al.* identified *Porphyromonas endodontalis* as a dominant species in phlegmonous appendicitis, suggesting its potential role in driving early inflammatory responses (57). Yet its absence in gangrenous cases in the same study raises questions about its specific role in disease progression (57). *Blod et al*. further supported the involvement of *Porphyromonas* in appendicitis, noting a significant increase in its abundance in inflamed appendices compared to HCs (58).

The same study found that during the detection of *Porphyromonas* by 16S rDNA sequencing there was no confirmed presence of viable bacteria using RT-qPCR, leading to the suggestion that the detected *Porphyromonas* may be bacterial fragments rather than active infections (58).

The detection of *Porphyromonas* alongside other oral pathogens supports it involvement in the oral-appendiceal axis of infection. *Blod et al*. identified *Porphyromonas* in both gingival sulcus and appendiceal samples and questioning a possible migration route from the oral cavity to the appendix. This migration may be assessed by postprandial retrograde transport and could explain the presence of oral pathogens in the appendiceal lumen and their contribution to appendicitis (58).

In terms of clinical implications, the presence of *Porphyromonas* in polymicrobial infections seems to complicate treatment strategies. *The et al.* reported a higher level of *Porphyromonas* in CA compared to UA, further highlighting its role in severe disease. The partnership with other pathogens, such as *Fusobacterium* and *Parvimonas*, demands broad-spectrum antibiotic regimens that effectively target anaerobic bacteria (61).

In conclusion, *Porphyromonas* is a key pathogen in AA, demonstrating an important role in complicated and severe disease. Its contribution in biofilm formation, polymicrobial interactions, and potential oral-appendiceal migration positions it as a significant driver of

inflammatory progression in appendicitis. While its detection and viability remain areas of further research, its presence in severe cases highlights the need for comprehensive treatment strategies that address anaerobic pathogens and their synergistic interactions.

11.4.1.4 ***Prevotella spp.***

Prevotella spp. are Gram-negative, anaerobic, non-spore-forming, non-motile bacilli within the Bacteroidetes phylum (109). They make up the second most abundant genus in the human oral cavity and show high abundance across various sites of the body (110). The study of *Prevotella* species was historically limited due to challenges associated with culturing and the Phenotypic ambiguity in traditional classification system. However, advances in culture-independent microbial profiling such as 16S rRNA sequencing have revolutionised our understanding of *Prevotella* spp. and its wide distribution in the skin, oral cavity, vagina, and gastrointestinal tract (109). *Prevotella* spp. dominate the members of the gut microbiome in developing societies while showing marked depletion in industrialized societiese. This reduced abundance is often associated with a significant compensatory increase in the abundance of *Bacteroides* spp.. This inverse correlation represents distinct microbiome profiles associated with different lifestyles and dietary habits (109). The role of Prevotella spp. in health and disease still largely unclear, despite their prevalence in healthy microbiomes. They have been implicated in a wide range of conditions like inflammatory and autoimmune diseases, oral diseases, and bacterial vaginosis While a well-established direct causal link require further investigation (111). The pathogenic potential of *Prevotella* spp. arises from its collection of virulence factors, including adhesins, hemolysins, secretion systems, exopolysaccharides, lipopolysaccharide (LPS), and proteases (112). The abilities of *Prevotella* spp. to modulate the body inflammatory responses and their contribution to the mixed microbial environments allow their participation in appendiceal inflammation. Prevotella role in driving severe disease outcomes was reported by *Jackson et al, highlighting* the increased levels of the bacteria in perforated appendicitis compared to non-perforated cases (54). The same study mentioned the possibility of systematic involvement of *Prevotella* in the disease process as it was more abundant in rectal swabs from appendicitis patients compared to HCs (54). The association of *Prevotella* with other oral and gut pathogens in a polymicrobial environment highlights its important role in the microbial dynamics of

appendicitis. *Aiyoshi et al.* findings places *Prevotella* as a keystone bacterial genera in the appendiceal lumen of pediatric appendicitis patients, often coexisting with *Fusobacterium* and *Parvimonas* (73). The result from *Tamura et al*. study enforces the knowledge that *Prevotella* spp. were highly susceptible to metronidazole and the anaerobic coverage in empirical antibiotic regimens should be a sufficient treatment (68). The polymicrobial environment of *Prevotella* with other resistant pathogens, such as *Pseudomonas aeruginosa (P. aeruginosa)* and *E. coli* could complicate matters and increase the need for alternatives (68).

11.4.1.5 ***Gram-Positive Anaerobic Cocci (GPAC)***

Gram-positive anaerobic cocci (GPAC) make up 25–30% of all anaerobic bacteria isolated from clinical specimens and are a vital members of the human microbiota (113). They could be found in a wide variety of anatomical sites, including the skin, mucosal surfaces of the oral cavity and upper respiratory tract, the gastrointestinal tract, and the female genitourinary tract (114). GPAC are generally function as opportunistic microbes and are commonly identified in polymicrobial infections alongside known pathogenic species, which has historically led to masked pathogenic potential and clinical significance of individual GPAC species. These bacteria have presented laboratory challenges due to their prolonged cultivation times and demanding nutritional requirements resulting in complex isolation protocols (113). A meaningful improvement in the identification and classification of GPAC because of the developments in molecular techniques, such as 16S rRNA gene sequencing, pyrosequencing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). These advancements have opened new avenues for understanding the clinical and pathogenic roles of individual GPAC species in human health and disease.
This diverse group of bacterial genera include *Anaerococcus, Anaerosphaera, Atopobium, Blautia, Coprococcus, Finegoldia, Gallicola, Murdochiella, Parvimonas, Peptoniphilus, Peptostreptococcus, Ruminococcus,* and *Sarcinia* (115).

11.4.1.6 Parvimonas micra

Parvimonas micra is a Gram-positive anaerobic coccus and an oral pathogen. It has been often cultured in endoperiodontal lesions, apical abscesses, and periodontitis. In addition to its role in oral infections, *P. micra* has been involved in a variety of systemic infections, including meningitis, cervical and brain abscesses, infective endocarditis (IE), and spondylodiscitis (116). This highlights its potential role as an opportunistic pathogen with contribution abilities to both localized and systemic disease processes. The historical difficulties of identification and cultivation of *P. micra* are the reason for the lack of better understanding of its clinical characteristics. *Parvimonas* was found by *Aiyoshi et al.* to have the highest relative abundance among oral pathogens identified in the appendiceal lumen of patients with AA (73). Several studies including *Horiuchi et al*. support the idea that *P. micra* and *F. nucleatum* engage in synergistic interactions during biofilm formation (103). These findings conclude that oral bacteria, such as *Fusobacterium* and *Parvimonas*, may contribute to biofilm development within the appendiceal lumen of pediatric AA patients and play a key role in mucosal inflammation (14, 103). In cases of perforation or abscess formation, *Parvimonas* seems to drive severe disease outcomes as its abundance was higher in cases of CA compared to UA (14, 61). *Blohs et al*. observed a local expansion of oral pathogens with the involvement of *P. micra* within the appendix during CA (14). A shift in the microbiome favoring pathogenic to beneficial species was noticed by *Blohs et al*. as the high levels of *P. micra* was accompanied by a depletion of beneficial bacteria such as *Bacteroides* (14). This shift may be a factor driving the progression of appendicitis to more severe forms, such as gangrenous or perforated appendicitis. *Blod, C., et al*. detection of *P. micra* in both the gingival sulcus and inflamed appendices of pediatric patients supports the claims of the existence of a migratory route from the oral cavity to the gastrointestinal tract (58). The translocation hypothesis of oral pathogens, including *P. micra*, to the appendix and the initiation or exacerbation of inflammation may be supported by these findings. The molecular techniques such as 16S rRNA sequencing have been critical in the identification of *P. micra* in polymicrobial infections in peritoneal fluid cultures, showing its significance in intra-abdominal infections and the limitations of conventional culture methods (57, 74).

11.4.1.7 Peptostreptococcus

Peptostreptococcus spp. are gram-positive anaerobic cocci and consists of just four species *P. anaerobius, P. stomatis, P. russellii* and *P. cani* (117). *P. anaerobius* is recognized as one of the most prevalent GPAC implicated in infections of the abdominal cavity and the female urogenital tract (118). *P. anaerobius* is most frequently encountered in polymicrobial infections where it enhances virulence through cooperative interactions with other anaerobic and facultative organisms. However, it has also been rarely detected as a sole infective agent. *P. anaerobius* was found in a variety of clinical sites, including the abdominal cavity, soft tissues, bones, brain, female urogenital tract, implant-related infections, and the respiratory tract (117).
Recent evidence from multiple studies analyses clarifies the role of *Peptostreptococcus spp*. in the microbial shifts and polymicrobial interactions in pediatric appendicitis.
As a vital member of an anaerobic microbial community, *Peptostreptococcus* spp. has been consistently detected in both UA and CA cases. *Peptostreptococcus spp*. are more present in inflamed appendices compared to normal controls. *Jackson et al*. demonstrated that *Peptostreptococcus* accounted for 0.32% of the microbial community in normal appendices but increased to 5.07% in cases of appendicitis (54). In severe infection like in CA, *Peptostreptococcus* spp. are often present with other anaerobes like *Fusobacterium* and *Parvimonas* (14, 27, 54). *Blod et al*. detected P. *stomatis* alongside other oral pathogens in both the gingival sulcus and inflamed appendices of pediatric patients leading to the assumption that a migratory route from oral cavity to the appendix exists (58)

11.4.2 Bacteroides spp.

Bacteroides species are Gram- negative, anaerobic bacilli that makes up a large section of the normal human colonic microbiota and up to 25% of the anaerobic organisms in the colon. They tend to develop and maintain a complex and equally beneficial relationship with the host (119). However, *Bacteroides* species can act as opportunistic pathogens and are frequently implicated in anaerobic infections, which are associated with a mortality rate of approximately 19% (120). Species of note in these species are *B. caccae, B.*

eggerthii, B. fragilis, B. ovatus, B. stercoris, B. thetaiotaomicron, B. uniformis, and *B. vulgatus* (121).

The clinical significance of infections caused by *Bacteroides* develop from the translocation of bacteria from the intestine to extraintestinal sites following inflammation, perforation, trauma, or surgery. The consequences of such translocation could be severe due to localized tissue spread or hematogenous dissemination (122). *Bacteroides* are not only restricted to their pathogenic potential but could also play a vital role in immunomodulation and the maintenance of stability of the immune system. Several secreted metabolites, including short-chain fatty acids (SCFAs) such as acetate and propionate, as well as capsular polysaccharide A (122). Especially SCFAs show potent anti-inflammatory effects by inhibiting the release of pro-inflammatory cytokines from neutrophils and macrophages (123). *Bacteroides* species play a significant role in preventing the intestinal colonization and translocation of potential pathogens, such as *Clostridium difficile* (120). Among these species, *B. fragilis* is of clinical relevance. Although it constitutes only 0.5% of the human colonic microbiota, it is the most frequently isolated anaerobic pathogen and it enhances its pathogenic potential through the expression of potent virulence factors (121).

B. thetaiotaomicron contributes critically to the host health and physiology by facilitating nutrient absorption and supporting the maturation and maintenance of epithelial cells. It represents approximately 12% of all *Bacteroides* species in the human intestinal microbiota (124). *Bacteroides spp.* has been linked to the pathogenesis and show a positive correlation to disease severity and complications. These bacteria tend to be detected in the appendiceal lumen in CA and are often part of a polymicrobial infection. *Theodorou et al*. reported *B. fragilis* in 52% of intraoperative cultures, while *Gerber et al.* observed it in 22% of CA cases (64, 66).

The relationship between *Bacteroides spp*. and the severity or progression of appendicitis demonstrates context-dependent patterns. *Swidsinski et al*. observed an inverse correlation between *Bacteroides* abundance and appendicitis severity, with a depletion from 87% in UA cases to 51% in CA (52). This suggests that while *Bacteroides spp.* are prevalent in the appendiceal microbiome, their beneficial role may change into more pathogenic as the disease progresses to more severe forms and translocation becomes more apparent. On the other hand, *Salö et al.,* have reported increased *Bacteroides* abundance in gangrenous

appendicitis compared to phlegmonous cases and giving the indication that its role may change to adopt to a specific clinical and histopathological context (56).

Bacteroides spp. have been found to be linked to postoperative complications such as postoperative collections, hospital readmissions, and length of stay. *Bhaskar et al*. demonstrated clinically significant associations of positive intraoperative cultures for *B. fragilis* with an increased risk of postoperative collections and higher hospital readmission rates (71). *Andrey et al.* supported these observations and found that *B. fragilis* was present in 30% of peritoneal fluid cultures from patients with CA but couldn't find any strong evidence linking its presence alone to any infectious complications (59). However, the study pointed that insufficient antibiotic coverage for Bacteroides spp. may lead to exacerbation of postoperative outcomes, emphasizing the importance of appropriate anaerobic coverage in empirical antibiotic regimens (59).
Bacteroides spp. remains largely susceptible to key anti-anaerobics, with most strains remaining sensitive to first-line antibiotics such as metronidazole. *Tamura et al.* reported that 78.1% of *Bacteroides spp.* were susceptible to cefazolin and cefmetazole, while *Yu et al.* found that *Bacteroides spp.* were highly susceptible to metronidazole (100%) but had limited susceptibility to penicillin G (1.3%) (68, 75). This doesn't contradict with the continued use of metronidazole as first-line anti-anaerobic therapy for appendicitis.
In conclusion, *Bacteroides spp.* and especially *B. fragilis* are vital actors in the microbial composition of AA. Their clinical importance is highlighted through the obvious prevalence in both UA and CA samples, along with their association with postoperative complications. Their antibiotic susceptibility, role in polymicrobial infections and disease progression emphasizes the need for comprehensive antimicrobial strategies that effectively target anaerobic pathogens.

11.4.3 Escherichia coli

E. coli plays a vital role in the development and complications of pediatric acute appendicitis and is the most isolated pathogen in acute appendicitis. It is the most prevalent bacteria in culture specimen across all stages of appendicitis and some studies even reporting a detection rate of up to 96% of perforated cases (72). *E. coli* is found to be dominant in the appendiceal lumen as the primary pathogen or part of polymicrobial infections. *Garzon-González et al*. identified *E. coli* in 80.14% of positive cultures, making

it the most abundant bacteria in appendicitis (70). *Kakar et al.* reported the high levels of *E. coli* in 79 samples of both uncomplicated and complicated appendicitis cases strengthening the claim of its relative abundance (65).

The presence of *E. coli* is positively correlated to the disease severity and complications. Its clinical significance was mentioned by *Bhaskar et al.*, observing that *E. coli*-positive intraoperative cultures were linked to prolonged hospital stays and extended antibiotic therapy (71). In cases of perforated appendicitis, *E. coli* remains a key pathogen and often was cultured with other bacteria such as *S. anginosus* and *P. aeruginosa* (6). The inflammatory response is then exacerbated by this polymicrobial environment and leads to an increase in the risk of complications, such as intra-abdominal abscesses and postoperative infections (59).

Growing evidence reporting the antibiotic resistance to commonly used antibiotics among *E. coli* strains seems to complicate treatment strategies. *Kakar et al.* observed that 54.2% of *E. coli* strains were resistant to ampicillin, while 30.5% were resistant to amoxicillin/clavulanic acid (65). *Garzon-González et al.* added to these observations and found that 26.99% of *E. coli* isolates were resistant to ampicillin/sulbactam, and 5.31% were extended-spectrum beta-lactamase (ESBL)-producing strains (70). In the environments with high resistance rates the need for tailored antibiotic regimens because of this pattern appears to be necessary.

The clinical implications of antibiotic-resistant *E. coli* are of an important magnitude. *Andrey et al.* found that co-amoxicillin-resistant *E. coli* was evidently linked with postoperative infectious complications, including intra-abdominal abscesses and wound infections (59). Despite the lack of evidence linking ESBL-producing *E. coli* strains to postoperative complications in the study of *Gerber et al.* they pose a challenge due to their resistance to cephalosporins, a common class of antibiotics used in empirical therapy (66).

In conclusion, *E. coli* is a key pathogenic actor in acute appendicitis and its prevalence and resistance patterns affect the disease outcomes. Its increasing antibiotic resistance and with its demonstrated pathogenicity in both uncomplicated and complicated appendicitis, this bacteria requires continued surveillance and the development of targeted therapeutic strategies to neutralize disease burden in patient care.

11.4.4 Pseudomonas aeruginosa

P. aeruginosa is an aerobic, Gram-negative bacilli and opportunistic pathogen involved in a wide range of infections, spanning multiple organ systems such as the skin, ears, eyes, urinary tract, heart, and respiratory system, especially the lungs (125). Over the last few decades , *P. aeruginosa* has become one of the most prevalent causative agents of nosocomial infections and is directly linked to increased morbidity and mortality rates in healthcare settings (126). This pathogen is characterized by a variety of disease associated virulence factors and a regulatory network of intracellular and intercellular signaling mechanisms, leading to the enhancement of its adaptability and pathogenic potential (127). *P. aeruginosa* pathogenicity stems from the production of diverse virulence factors, the ability to form biofilms, and developing antibiotic resistance (127). *P. aeruginosa* is a common colonizer of the human intestine upon hospitalization, immunosuppression, antibiotic treatment, surgery, severe trauma and intestinal carriage increases from ~3% in normal people to ~20% in hospitalized patients (128). The pathogen employs a variety of virulence mechanisms to disrupt epithelial integrity, including the secretion of enzymes (e.g., proteases and elastases), toxins, adhesins, flagella, and specialized protein secretion systems. The impairment of the barrier function of tight junctions due to these factors facilitates bacterial invasion and systemic dissemination (129). *P. aeruginosa* increases its virulence and worsens systemic inflammation in immunocompromised patients in both acute and chronic infections (128).

The reported prevalence rates of *P. aeruginosa* across studies in the appendiceal and intra-abdominal infections is relevant but less abundant in comparison to *E. coli* with rates from 4% to 29% (6, 68, 69). It is more frequently associated with CA (e.g., perforated or gangrenous cases) and intra-abdominal infections, particularly in the peritoneal cavity (65, 66). *P. aeruginosa* often coexists with other pathogens, such as *E. coli, B. fragilis, and S. anginosus*, contributing to polymicrobial infections that complicate treatment strategies (57, 71). The need for tailored antibiotic regimens due to the pathogen's resistance to first-line antibiotics, such as Cefazolin involves the therapy with broader-spectrum agents like gentamicin or amoxicillin/clavulanic acid (59, 60). Culture-independent techniques, such as 16S rRNA gene sequencing and shotgun metagenomics, have revolutionized our capacity to understand its role within complex microbial communities, showing its interactions with anaerobic bacteria and its contribution to systemic inflammation under

host stress conditions (52, 54). These observations highlight that the presence of *P. aeruginosa in* complicated intra-abdominal infections in hospitalized or immunocompromised patients necessitates the consideration of combination therapy to address both aerobic and anaerobic components of the infection (53, 56).

11.5 Antibiotic Resistance and Sensitivity

The data from multiple studies highlight the critical role of antibiotic resistance and sensitivity in the management of AA, particularly in complicated cases. The findings underscore the importance of tailored antibiotic regimens to mitigate complications and improve patient outcomes. As detailed in **Table 6**, *E. coli* is the most abundant organism in both UA and CA, with a various resistance rate in numerous studies.
Resistance to ampicillin (54.2%), amoxicillin/clavulanic acid (30.5%), and cefazolin (0.6% in children) is notable, though susceptibility to gentamicin (76.3%) remains high (65, 75). Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* strains are increasingly reported, particularly in complicated appendicitis, and are associated with higher rates of postoperative complications (59, 66). **Table 6** shows these strains are resistant to cephalosporins (e.g., cefazolin, cefotaxime) is common, but susceptibility to penicillin-based regimens (e.g., ampicillin) and carbapenems (e.g., meropenem) remains high (68, 75).
P. aeruginosa is more prevalent in CA, particularly perforated cases, and is associated with prolonged hospital stays and antibiotic durations (65, 74). The resistance profile in **Table 6** reveals notable resistance to ceftazidime (26.3%) and cefotaxime (63.2%) is notable, but susceptibility to gentamicin, meropenem, and piperacillin/tazobactam remains high (64, 65). The lack of consideration and insufficient antibiotics coverage of *P. aeruginosa* in empiric regimens increases the risk of postoperative abscess formation (59). *B. fragilis* is sensitive to metronidazole (100%) and shows resistance to penicillin G (1.3%) and amoxicillin/clavulanic acid (10%) (59, 75). In 21.9% of cases the resistance to cephalosporins (e.g., cefazolin) and sensitivity to penicillin-based regimens is observed (68). *S. anginosus* presence is strongly associated with abscess formation and CA with the observation of sensitivity to ampicillin (100%) and penicillin-based regimens, but resistance to clindamycin (50%) is reported (62, 75).

A combination therapy of ampicillin, gentamicin, and metronidazole has proven to be effective against the most abundant pathogens in AA, such as *E. coli, B. fragilis,* and *S. anginosus* (75). Penicillin-based regimens (e.g., ampicillin) are superior to cephalosporins (e.g., cefazolin) in treating perforated appendicitis due to the resistance of *Enterococcus* spp. and *P. aeruginosa* to cephalosporins (68).

Meropenem and imipenem are effective against ESBL-producing *E. coli* and *P. aeruginosa* and are suitable for complicated cases with resistant pathogens (65, 66). The inclusion of piperacillin/tazobactam or ceftazidime in empiric regimens is critical for covering *P. aeruginosa*, particularly in perforated appendicitis (59, 74). Polymicrobial infections and resistant pathogens (e.g., ESBL-producing *E. coli, P. aeruginosa*) are strongly associated with postoperative abscess formation (62, 71). The data presented in **Table 6** demonstrate that an inadequate initial antibiotic coverage targeting *P. aeruginosa* and *Enterococcus* spp. increases the risk of abscess development (59, 60). Resistance profiles impact clinical courses, with resistance to first-line antibiotics (e.g., cephalosporins, ampicillin) correlates with longer hospital stays and extended antibiotic therapy (65, 74). Patients with *P. aeruginosa* or ESBL-producing *E. coli* infections are at higher risk of readmission and often require longer treatment time (59, 66). Resistant pathogens such as *P. aeruginosa* and *Enterococcus* spp. are strongly linked to increased rates of surgical site infections (SSI) and wound complications (64, 66).

For UA , ampicillin, gentamicin, and metronidazole remain effective first-line options (75). For CA, particularly perforated cases, piperacillin/tazobactam or carbapenems should be considered to cover *P. aeruginosa* and ESBL-producing *E. coli* (68, 74). Intraoperative cultures should be obtained in complicated cases to guide antibiotic selection, particularly for resistant pathogens (62, 71). The cultures from postoperative abscesses reveal pathogens not detected previously in intraoperative samples and demanding the adjustments in antibiotic therapy (62). To reduce complications, the regular checks of antibiotic resistance patterns and the following adaption of the empiric are required (59, 66).

In conclusion, antibiotic resistance in *E. coli, P. aeruginosa,* and *Enterococcus* spp., is reported to be a relevant causative factor for complications in AA. These clinical complications include postoperative abscesses, prolonged hospital stays, and surgical site infections. Empiric antibiotic regimens must account for local resistance patterns, with a preference for penicillin-based therapies and antipseudomonal coverage in complicated

cases. Culture-guided therapy and ongoing resistance monitoring are critical to optimizing treatment outcomes and reducing the burden of complications in AA.

Pathogen	Resistance	Sensitivity
E. coli	Ampicillin (54.2%), Amoxicillin/Clavulanic Acid (30.5%), Cefazolin (0.6%)	Gentamicin (76.3%)
E. coli (ESBL+)	Cephalosporins (e.g., Cefazolin, Cefotaxime), Penicillin-based Regimens	Carbapenems (e.g., Meropenem, Imipenem)
P. aeruginosa	Ceftazidime (26.3%), Cefotaxime (63.2%)	Gentamicin, Meropenem, Piperacillin/Tazobactam
B. fragilis	Penicillin G (1.3%), Amoxicillin/Clavulanic Acid (10%), Cephalosporins (21.9%)	Metronidazole (100%), Penicillin-based Regimens
S. anginosus	Clindamycin (50%)	Ampicillin (100%), Penicillin-based Regimens

Table 6: Antibiotic Resistance and Sensitivity

11.6 Uncomplicated vs. Complicated Appendicitis

Both Culture-based and molecular approaches provide a complementary insight into the distinct similarities and differences in the microbial profiles in the UA, CA and HC. Culture-based studies demonstrate a less diverse bacteriological profile in UA and the prevalence of aerobic bacteria, with *E. coli* detected in 36–85% of cases and *P. aeruginosa* in 5–25% of cases (64, 70). In contrast, CA is characterized by more varied microbial growth and higher levels of both aerobic and anaerobic pathogens, with *E. coli* isolates recovered in 80–96% of cases and *P. aeruginosa* in 7–30% of cases. Anaerobic bacteria represented by *B. fragilis* and *Fusobacterium* spp., are also more prevalent in CA, with *B. fragilis* identified in 30–52% and *Fusobacterium* spp. in 27–32%

of cases (52, 56). This suggests that CA is more associated to a polymicrobial infection with the involvement of both aerobic and anaerobic pathogens, whereas UA tends to be aerobic dominated and has less varied growth.

The 16S rRNA sequencing analyses have both confirmed and expanded these culture-derived observations and have revealed variations in bacterial population in health and disease. *Fusobacterium, Parvimonas, and Porphyromonas* are evidently more abundant in CA compared to HC and UA. *Jackson et al.* found that *Fusobacterium* showed a higher presence levels in UA (3.21%) compared to HC (1.04%), while *Swidsinski et al*. reported that Fusobacteria were more abundant in CA (27%) compared to HC (0.5%) (52, 54). While *Bacteroides* spp. are often identified in culture-based studies of CA, 16S rRNA sequencing analyses on the other hand show their reduced abundance in UA and CA compared to HC (26, 52, 53). This obvious discrepancy may reflect differences in detection methods, as culture-based techniques may favor the growth of certain anaerobic species like *Bacteroides,* while sequencing provides a broader, more comprehensive view of the microbial community.

The increased bacteriological complexity of CA results mainly from sequencing data, which shows simultaneous enrichment of multiple anaerobic genera alongside depletion of typical enteric organisms. *Swidsinski et al.* found
that Fusobacteria and *Bacteroides* showed high abundance levels in CA, with rates of 27% and 34%, respectively, while Enterobacteriaceae were more prevalent in UA (21%) compared to HC (4%) and CA (4.6%) (52). These findings align with the clinical observation that complicated appendicitis often involves synergistic polymicrobial infections, whereas uncomplicated cases tend toward more limited bacterial profiles. Additionally, the presence of S. anginosus, which is associated with abscess formation in CA, is consistent with the increased severity and complexity of CA infections observed in both culture-based and sequencing studies (60, 64).

These microbiological differences carry a direct therapeutic consequence. UA responds well to targeted antibiotic therapy such as antibiotics effective against *E. coli* and *P. aeruginosa* because of its lower microbial diversity and predominance of aerobic pathogens. Conversely, CA requires broader-spectrum antibiotics to cover both aerobic and anaerobic pathogens due to the polymicrobial nature of the infection. Anaerobes like *B. fragilis* and *Fusobacterium* spp. higher levels in CA also increases the risk of complications, such as intra-abdominal abscesses and sepsis (52, 56). The presence of *S.*

anginosus in CA is associated with more severe outcomes such as abscess formation and prolonged antibiotic therapy (60, 64).

In summary, both culture-based and 16S rRNA sequencing studies highlight the distinct microbial profiles of UA and CA, with CA characterized by higher microbial diversity and a greater prevalence of anaerobic pathogens. These findings underscore the need for tailored antibiotic regimens and highlight the potential role of specific bacterial taxa in the pathogenesis and clinical outcomes of appendicitis.

11.7 Clinical implications

The observed microbial changes in AA carry a relevant clinical implication for understanding pathogenesis, optimising treatment strategies, and predicting complications. As summarised in **Table 7**, the overrepresentation of *Fusobacterium* species, such as *Fusobacterium nucleatum* and *Fusobacterium necrophorum*, in CA suggests their potential role as key pathogens driving mucosal inflammation and disease severity (52, 56). The depletion of commensal bacteria like *Bacteroides* and *Faecalibacterium prausnitzii* appears to worsen inflammation through compromised epithelial barrier function and disrupted immune regulation (52, 67). This dysbiosis creates a more suitable environment for pathogenic bacteria to thrive and contributes to the progression from UA to CA.

The persistence of *Fusobacterium* even following an antibiotic therapy with broad-spectrums supports the hypothesis that the appendix serves as a reservoir for these pathogens that predisposes and increases the risk of disease recurrence (55). The cooperative interaction between *Fusobacterium* and other oral pathogens, such as *P. micra*, in biofilm formation within the appendiceal lumen may worsen mucosal inflammation and clinical severity (61, 73). These findings highlight the importance of targeting biofilm-forming pathogens in treatment strategies.

Table 7 highlights that Polymicrobial infections, involving *E. coli, B. fragilis,* and *P. aeruginosa*, are linked with higher complication rates, such as postoperative abscess formation, prolonged hospital stays, and surgical site infections (64, 72, 74). The need for tailored antibiotic regimens arises from the fact that these pathogens are Resistant to first-line antibiotics, such as cephalosporins. While *P. aeruginosa* shows clear associations with

perforated appendicitis and prolonged treatment courses, its precise pathogenic role remains uncertain due to widespread empirical antipseudomonal use.

S. anginosus and *Enterococcus* spp. in complicated cases show resistance to non-empiric cephalosporins highlighting the importance of penicillin-based therapies (62, 68).

The distinction between UA and CA with the help of microbial profiling reveals distinct microbial clustering patterns, with CA has been shown to exhibit a greater species diversity and an increased abundance of oral pathogens such as *F. nucleatum* and *P. micra* (14, 61). These findings bring to the conclusion that appendicitis may represent two distinct phenotypes, with complex cases driven by an interplay between oral pathogens and local immune dysregulation. The pro-inflammatory role of *Sutterella* spp., which is more prevalent in CA, further supports this hypothesis, as it may contribute to a Th17-mediated immune response (61).

Empiric antibiotic regimens must account for local resistance patterns, with a preference for penicillin-based therapies and antipseudomonal coverage in complicated cases (68, 74). Intraoperative cultures should be obtained in complicated cases to guide antibiotic selection, particularly for resistant pathogens (62, 71). Microbiological analysis of postoperative abscess specimens frequently uncovers bacterial species not detected during initial surgical sampling, requiring subsequent modification of antimicrobial treatment protocols (62). Regular review of resistance trends allows for timely adjustments to treatment algorithms to reduce complications (59, 66).

In conclusion, the microbial changes in AA are represented by the expansion of pathogenic bacteria and the depletion of commensal species show a significant clinical implication. These changes lead to disease progression, complicate treatment, and increase the risk of postoperative complications. Tailored antibiotic regimens adjusted to culture results and resistance patterns are vital to the purpose of the optimization of treatment outcomes and reducing the burden of complications in AA.

Study	Key Findings	Microbial Changes	Clinical Implications
Swidsinski et al. (2011) (52)	*Fusobacterium* species (*F. nucleatum*, *F. necrophorum*) were markedly higher in CA. Commensal bacteria (*Bacteroides*, *Faecalibacterium prausnitzii*) inversely correlated with severity	↑ *Fusobacterium;* ↓ *Bacteroides, Faecalibacterium prausnitzii*	Suggests *Fusobacterium* as a key pathogen in CA; commensal depletion may exacerbate inflammation
Zhong et al. (2014) (53)	AA linked to overrepresentation of Fusobacteria and oral pathogens, with a reduction in *Bacteroides*	↑ Fusobacteria, oral pathogens. ↓ *Bacteroides*	Highlights the role of oral pathogens and dysbiosis in appendicitis
Jackson et al. (2014) (54)	Normal appendices had higher *Fusobacterium*, *Selenomonas* , and *Peptostreptococcus* compared to rectal samples. Perforated cases had elevated *Bulleidia*, *Fusobacterium*, and *Porphyromonas*	↑ *Fusobacterium, Selenomonas, Peptostreptococcus* in UA; ↑ *Bulleidia, Fusobacterium* in perforated cases	Suggests unique microbial profiles in the appendix and their role in perforation
Rogers et al. (2016) (55)	*Fusobacterium* persisted after antibiotic therapy, suggesting the appendix as a reservoir	Persistent ↑ *Fusobacterium*	Supports the appendix as a microbial reservoir, increasing recurrence risk
Salö et al. (2016) (56)	Fusobacteria abundance increased in phlegmonous and perforated appendicitis but was low in gangrenous cases. No significant correlation between microbiome profiles and disease severity	↑ Fusobacteria in phlegmonous/perforated cases; ↓ in gangrenous cases	Fusobacteria may contribute to pathogenesis but is unlikely to be the sole causative agent

Study	Key Findings	Microbial Changes	Clinical Implications
Schülin et al. (2017) (57)	*F. necrophorum* was associated with catarrhal appendicitis. *P. endodontalis* with phlegmonous appendicitis; *F. nucleatum* with gangrenous appendicitis	↑ *F. necrophorum, P. endodontalis, F. nucleatum*	Suggests distinct microbial profiles at different stages of appendicitis
Blod et al. (2018) (58)	Oral pathogens (*Fusobacterium*, *Peptostreptococcus*, *Porphyromonas*) were abundant in the appendiceal microbiome of pediatric AA patients	↑ *Fusoacterium, Peptostreptococcus, Porphyromonas*	Suggests a potential oral-gut axis in the pathogenesis of appendicitis
Andrey et al. (2019) (59)	*E. coli* was the most common bacterium in peritoneal fluid cultures, followed by *S. anginosus* and *P. aeruginosa*.	↑ *E. coli, S. anginosus, P. aeruginosa*	*P. aeruginosa* increases the risk of infectious complications if not adequately covered by antibiotics
Viel-Thériault et al. (2019) (60)	Anaerobes were the most frequently isolated pathogens in peritoneal fluid cultures, followed by *E. coli* and *S. anginosus*	↑ Anaerobes, *E. coli, S. anginosus*	Polymicrobial infections increase the risk of postoperative complications

Study	Key Findings	Microbial Changes	Clinical Implications
The. et al. (2019) (61)	Two distinct bacterial clusters identified in UA vs. CA. *F. nucleatum* and *P. micra* were more abundant in complex cases	↑ *F. nucleatum, P. micra in CA*	Suggests appendicitis may represent two distinct phenotypes with different microbial drivers
Dahlberg et al. (2019) (62)	Limited concordance between intraoperative and abscess cultures. *B. fragilis*, *E. coli*, and *S. anginosus* were most common intraoperatively	↑ *B. fragilis, E. coli, S. anginosus*	Abscess formation is often driven by resistant organisms not detected in intraoperative cultures
Plattner et al. (2021) (63)	*B. fragilis*, *E. coli*, and Viridans group streptococci were the most prevalent bacteria isolated, aligning with findings reported in previous literature	↑ *B. fragilis, E. coli,* Viridans group streptococci	Supports the role of polymicrobial infections in CA
Theodorou et al. (2022) (64)	*E. coli*, *S. anginosus*, and *B. fragilis* were the most common species identified. *P. aeruginosa* was present in 22% of perforated cases	↑ *E. coli, S. anginosus, B. fragilis, P. aeruginosa*	*P. aeruginosa* is associated with prolonged antibiotic use and hospital stays

Study	Key Findings	Microbial Changes	Clinical Implications
Kakar et al. (2022) (65)	*E. coli* was the most prevalent organism in both UA and CA. *P. aeruginosa* was more frequent in complicated cases	↑ *E. coli, P. aeruginosa*	*P. aeruginosa* is more common in CA and associated with resistance
Gerber et al. (2022) (66)	*E. coli*, *S. anginosus*, and *B. fragilis* were the most frequent pathogens in CA. *P. aeruginosa* was present in 22% of cases	↑ *E. coli, S. anginosus, B. fragilis, P. aeruginosa*	Resistant pathogens increase the risk of postoperative complications
Bi et al. (2022) (67)	↓ *Bacteroides, Faecalibacterium,* and *Bifidobacterium;* ↑ *Clostridium,* Ruminococcaceae, and *Prevotella* in AA	↓ *Bacteroides, Faecalibacterium, Bifidobacterium* ↑ *Clostridium,* Ruminococcaceae, *Prevotella*	Implicates dysbiosis in inflammatory progression and immune modulation
Tamura et al. (2022) (68)	*P. aeruginosa*, *Enterococcus* spp., and *S. anginosus* were more common in perforated appendicitis and resistant to cephalosporins	↑ *P. aeruginosa, Enterococcus spp., S. anginosus*	Supports the use of penicillin-based antibiotics for perforated appendicitis

Study	Key Findings	Microbial Changes	Clinical Implications
Naji et al. (2023) (69)	*E. coli* was the most common pathogen in inflamed appendices, followed by *P. aeruginosa* and *K. pneumoniae*	↑ *E. coli, P. aeruginosa, K. pneumoniae*	No significant difference in pathogens between perforated and non-perforated appendicitis
Garzon-González et al. (2023) (70)	*E. coli* was the most frequently isolated organism in peritoneal fluid cultures, followed by *P. aeruginosa* and *K. pneumoniae*	↑ *E. coli, P. aeruginosa, K. pneumoniae*	Polymicrobial infections increase complication risk; highlights the need for targeted antibiotic therapy
Bhaskar et al. (2023) (71)	*E. coli* was the most prevalent organism in intraoperative cultures, followed by *P. aeruginosa* and *S. milleri*	↑ E. *coli, P. aeruginosa, S. milleri*	Polymicrobial infections increase the risk of postoperative complications
Felber et al. (2023) (72)	Bacterial presence increased with disease severity. *E. coli*, *B. fragilis*, *P. aeruginosa*, and *S. anginosus* were most prevalent	↑ *E. coli, B. fragilis, P. aeruginosa, S. anginosus*	Polymicrobial infections are associated with higher complication rates

Study	Key Findings	Microbial Changes	Clinical Implications
Zachos et al. (2023) (6)	*E. coli*, *P. aeruginosa*, and *Streptococcus* spp. were the most frequent pathogens in perforated appendicitis. Polymicrobial cultures correlated with higher complication risk	↑ *E. coli, P. aeruginosa, Streptococcus* spp.	Polymicrobial infections increase complication risk; highlights the need for targeted antibiotic therapy
Blohs et al. (2023) (14)	Local expansion of oral pathogens (*Fusobacterium*, *Parvimonas*) in CA; decline in *Bacteroides*, Ruminococcaceae	↑ Oral pathogens; ↓ *Bacteroides,* Ruminococcaceae	Highlights the role of oral pathogens in disease severity and progression
Aiyoshi et al. (2023) (73)	*Fusobacterium* and *Parvimonas* were predominant in appendiceal lumen samples. *Bacteroides* abundance decreased in CA	↑ *Fusobacterium, Parvimonas;* ↓ *Bacteroides*	Suggests synergistic biofilm formation by oral pathogens contributing to mucosal inflammation

Study	Key Findings	Microbial Changes	Clinical Implications
Nasrallah et al. (2024) (74)	*E. coli* and *S. milleri* were the most frequently isolated organisms in peritoneal fluid cultures. *P. aeruginosa* was detected in 23% of positive cultures	↑ *E. coli, S. milleri, P. aeruginosa*	Polymicrobial cultures correlate with higher complication risk
Yu et al. (2024) (75)	*E. coli*, *Bacteroides* spp., and Viridans group streptococci were the predominant pathogens in AA, with no significant differences between adults and children	↑ *E. coli, Bacteroides spp.,* Viridans group streptococci	Highlights consistent microbial profiles across age groups

Table 7: Key Findings of Microbial Changes

12 Discussion

The pathogenesis of AA is increasingly understood to involve significant alterations in the gut microbiome, particularly the overrepresentation of pathogenic bacteria and the depletion of commensal species. A consistent finding across multiple studies is the prominent role of *Fusobacterium* species, such as *Fusobacterium nucleatum* and *Fusobacterium necrophorum*, in the development and progression of appendicitis. These bacteria are frequently identified in mucosal lesions of patients with acute and complicated appendicitis, particularly in cases of perforation or gangrenous appendicitis, and are notably absent in healthy controls (52, 53, 55, 56). The persistence of *Fusobacterium* even after broad-spectrum antibiotic therapy suggests that the appendix may act as a reservoir for these pathogens, potentially contributing to disease recurrence (55). Furthermore, the synergistic interaction between *Fusobacterium* and other oral pathogens, such as *P. micra*, in biofilm formation within the appendiceal lumen may exacerbate mucosal inflammation and disease severity (61, 73). In contrast, the depletion of commensal bacteria, particularly *Bacteroides, Faecalibacterium prausnitzii,* and *Bifidobacterium*, is a hallmark of appendicitis. These genera, which are typically abundant in healthy gut microbiomes, exhibit an inverse correlation with disease severity, with their relative abundance declining significantly in CA (52, 67). This microbial shift may disrupt mucosal barrier integrity and immune homeostasis, creating a permissive environment for pathogenic bacteria to thrive. For instance, the reduction in *Bacteroides* and *Faecalibacterium* has been linked to diminished anti-inflammatory signaling, while the expansion of pro-inflammatory genera such as *Prevotella* and *Klebsiella* may exacerbate local inflammation (14, 67).

The microbial landscape of appendicitis is further complicated by the presence of polymicrobial infections, particularly in complicated cases. *E. coli, B. fragilis, and P. aeruginosa* are frequently isolated in both intraoperative and peritoneal fluid cultures, with their prevalence increasing with disease severity (64, 72, 74). Notably, *P. aeruginosa* is more commonly associated with perforated appendicitis and is linked to prolonged antibiotic use and hospital stays, although its role in driving complications remains unclear due to the widespread use of antipseudomonal antibiotics (68, 74). Similarly, *S. anginosus* and *Enterococcus* species are frequently identified in complicated cases, with

their resistance to non-empiric cephalosporins underscoring the importance of tailored antibiotic regimens (62, 68).

The distinction between UA and CA is further supported by microbial clustering patterns. CA is characterized by greater species diversity within the phyla Bacteroidetes and Proteobacteria, as well as an increased abundance of oral pathogens such as *F. nucleatum* and *P. micra* (14, 61). These findings suggest that appendicitis may represent two distinct phenotypes, with complex cases driven by a synergistic interplay between oral pathogens and local immune dysregulation. The pro-inflammatory role of *Sutterella* spp., which is more prevalent in CA, further supports this hypothesis, as it may contribute to a Th17-mediated immune response (61).

Despite these insights, methodological limitations in microbial profiling, particularly the reliance on conventional culture techniques, have hindered a comprehensive understanding of the appendiceal microbiome. While culture-based methods have identified *E. coli, Bacteroides* spp*.,* and *P. aeruginosa* as dominant species, 16S rRNA sequencing has revealed a more diverse microbial community, including species such as *P. endodontalis* and *F. necrophorum*, which are not detectable through culturing (57). However, the failure of 16S rRNA sequencing to detect *P. aeruginosa* highlights the need for complementary approaches to fully characterize the microbial landscape of appendicitis (57, 62).

The findings from this literature overview highlight the significant differences in microbiota composition between healthy individuals and those with appendicitis, reinforcing the growing understanding that appendicitis is not solely caused by a single pathogen but rather a result of microbial dysbiosis. Culture-based methods have historically identified *E. coli*, *Enterococcus* spp*.,* and *P. aeruginosa* as the primary causative agents, particularly in peritoneal fluid cultures. However, advancements in gene sequencing have revealed a much broader microbial community associated with appendicitis, including anaerobic bacteria such as *F. nucleatum, B. fragilis, P. micra, and Prevotella* spp., which were largely undetected using traditional culture techniques. These findings suggest that the microbial environment of the appendix is more complex than previously understood, and the shift in bacterial populations may contribute to inflammation and disease progression.

Comparing different sampling methods has further emphasized the role of anaerobes in appendicitis. Appendiceal lumen and tissue samples analyzed using sequencing methods

consistently showed a higher abundance of *Fusobacterium* and *Parvimonas* in diseased cases, whereas peritoneal fluid cultures detected primarily facultative anaerobes and aerobes. This discrepancy indicates that peritoneal fluid may not fully capture the microbial dynamics within the appendix and highlights the limitations of culture-based diagnostics. Additionally, fecal and rectal microbiome studies have suggested a potential gut-appendix axis, with shifts in gut microbiota composition correlating with appendicitis, particularly the increased presence of *Fusobacterium* in both appendicitis patients and those with gut dysbiosis.

The microbiome in healthy controls has been characterized by a high diversity of beneficial bacteria, including *Bacteroides, Faecalibacterium, and Clostridium spp*., with low levels of *Fusobacterium* and other inflammation-associated pathogens. This supports the hypothesis that appendicitis may result from an imbalance in the microbial ecosystem rather than a direct infection by a single bacterium. The significant increase in *Fusobacterium* and other anaerobic pathogens in diseased appendices suggests that microbial shifts may be a driving factor in the pathogenesis of appendicitis. This aligns with previous research on microbial dysbiosis in other inflammatory conditions, such as inflammatory bowel disease (IBD), where Fusobacterium has been implicated in mucosal inflammation.

Although gene sequencing has provided valuable insights into the microbiota of the appendix, there are still limitations to consider. While sequencing allows for the identification of a broader range of microbes, it does not differentiate between live and dead bacteria, which may impact the interpretation of results. Additionally, culture-based methods remain essential for determining antimicrobial susceptibility, which is critical for clinical management. Therefore, an integrated approach combining both culture and sequencing methods may provide the most comprehensive understanding of the microbiota associated with appendicitis and improve diagnostic accuracy.

This literature review reinforces the evolving concept that appendicitis is a polymicrobial disease influenced by microbial dysbiosis rather than a single bacterial infection. Culture-based methods, while valuable for identifying common enteric pathogens, fail to capture the full spectrum of microorganisms present in the appendix. Gene sequencing has significantly expanded our understanding of the microbial communities involved in appendicitis, revealing a prominent role for anaerobic bacteria such as *F. nucleatum* and *B. fragilis*, which were largely undetectable using traditional culture techniques. The

comparison of different sampling methods has further demonstrated the importance of tissue and lumen-based sampling for a more accurate representation of the appendix microbiome.

The microbiota of healthy individuals is characterized by high microbial diversity and the presence of beneficial bacteria, which may play a protective role against inflammation. In contrast, appendicitis is associated with a loss of microbial balance and an overgrowth of pathogenic anaerobes, suggesting that microbial shifts, rather than the presence of a single pathogen, may be the key driver of disease. These findings have significant clinical implications, as they support the need for a more comprehensive diagnostic approach that includes both culture and sequencing methods to improve accuracy in detecting pathogens and guiding treatment strategies. Future research should focus on further elucidating the mechanisms through which microbial dysbiosis contributes to appendicitis and exploring potential microbiome-targeted therapies to prevent or manage the condition.

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