Microbiome-based diagnostic biomarkers in pancreatic ductal adenocarcinoma: Current evidence and translational challenges (Review)

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Received March 19, 2026; Accepted July 3, 2026

DOI: 10.3892/ol.2026.15833

Abstract. Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies, as the majority of patients are diagnosed at an advanced disease stage. CA19-9, the biomarker most commonly used in clinical practice, lacks adequate sensitivity and specificity for early PDAC detection. Increasing evidence indicates that alterations in gut, oral and tumor-associated microbiota are associated with PDAC development and progression, supporting the potential diagnostic value of microbiome-based biomarkers in this disease. Multiple diagnostic models have been developed for PDAC using fecal, salivary or tissue-derived microbial profiles, and the combination of microbial signatures with CA19-9 or metabolomic markers has improved diagnostic performance in a number of cohorts. Despite these advancements, the clinical translation of microbiome-based models remains limited by methodological heterogeneity, patient-related variability, low levels of microbial biomass in pancreatic tissue and a lack of large-scale prospective validation. In addition, the majority of available evidence for microbiota-based alterations in PDAC is derived from retrospective case-control studies, and the reported diagnostic performance should therefore be interpreted cautiously. The present review summarizes current evidence on PDAC-associated microbial alterations and microbiome-based diagnostic models, and discusses the methodological, biological and regulatory challenges that must be addressed before microbiota-based approaches can be integrated into routine clinical practice.

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Key words: microbiome, pancreatic ductal adenocarcinoma, diagnostic biomarkers, microbiome-based models, clinical validation

Contents

1. Introduction

Pancreatic cancer (PC) exhibits one of the highest mortality rates among malignancies of the digestive system, and pancreatic ductal adenocarcinoma (PDAC) has been shown to account for 90-95% of all PC cases (1). Due to its aggressive biological behavior and late clinical presentation, PDAC remains a major global health challenge (1). Despite advances in cancer diagnosis and treatment, the incidence of PDAC has continued to increase over previous decades and this malignancy is projected to become the second leading cause of cancer-related mortality in the USA by 2030 (2). Early-stage PDAC is often asymptomatic or presents with non-specific symptoms, leading to delayed diagnosis in a large proportion of patients (3). Consequently, only a minority of patients with PDAC remain eligible for surgical resection (4). The overall 5-year survival rate for patients with PDAC is <12%, with limited improvement observed over previous decades (5,6). CA19-9 is a serum biomarker for PDAC (2). However, it has been shown to exhibit limited sensitivity and specificity (2), particularly in the early stages of PDAC development, which notably restricts its utility for PDAC screening and early diagnosis. In addition, elevated CA19-9 levels can also be observed in benign pancreatic diseases, while 5-10% of individuals are unable to express CA19-9 due to Lewis antigen negativity, further reducing the efficacy of this biomarker (7,8). These limitations highlight the notable necessity of novel, reliable and non-invasive biomarkers to improve early detection and risk stratification of PDAC (7,8).

Microbiome research has revealed important associations between microbial dysbiosis and multiple malignancies (9,10). It is estimated that 10-20% of cancers are attributable, at least in part, to infectious or microbial factors, underscoring the role of host-microbe interactions in carcinogenesis in specific cancer types (11). Advances in sequencing technologies have further facilitated the identification of disease-associated microbial signatures (12). Increasing evidence suggests that patients with PDAC exhibit distinct alterations in the gut, oral and tumor-associated microbiota (9,13). Dysbiosis of the oral and gut microbiota has been implicated in a number of mechanisms involved in pancreatic carcinogenesis, including chronic inflammation, immunomodulation and metabolic reprogramming (14). Intratumoral microbiome composition is associated with survival in patients with PDAC (15).

In parallel, several studies have developed microbiota-based diagnostic models of PDAC using fecal or oral microbial profiles (16-20), some of which have demonstrated improved performance when combined with conventional clinical markers (16). Given the challenge of early PDAC detection, such microbiota-based biomarkers hold promise as potentially non-invasive tools for screening and diagnosis (18,19). Fig. 1 summarizes the proposed association between microbial dysbiosis, microbiome-based functional pathways and the potential clinical applications of microbiome-based biomarkers in PDAC.

Additionally, accumulating evidence suggests that microbiome-associated alterations may be detectable before the clinical markers diagnose of PDAC, highlighting their potential as early diagnostic biomarkers (16,20,21). In a prospective cohort study, elevated circulating antibodies against oral pathogens, particularly *Porphyromonas gingivalis*, measured before the onset of PDAC were associated with an increased risk of PC (22). In a nested case-control study within the EPIC cohort, elevated pre-diagnostic levels of antibodies targeting *P. gingivalis* were markedly associated with future PC risk (22). The present review summarizes current evidence regarding microbiota-based alterations associated with PDAC, critically evaluates microbiota-based diagnostic approaches, and discusses the methodological limitations and translational challenges of such approaches that should be addressed before clinical implementation.

2. Search strategy

The present review was conducted as a narrative review. To improve transparency and reduce selection bias, a structured literature search was performed using PubMed (pubmed.ncbi.nlm.nih.gov), Web of Science (webofscience.com) and Embase (embase.com) to obtain studies published from the inception of these databases to January 31, 2026. The search terms included combinations of key words related to PC and the microbiome, such as 'pancreatic ductal adenocarcinoma', 'pancreatic cancer', 'microbiome', 'microbiota', 'gut microbiota', 'oral microbiota', 'tumor microbiome', 'diagnosis', 'biomarkers', 'early detection' and 'machine learning'. Only publications written in English were included. Original studies investigating microbiome alterations or microbiome-based diagnostic models in PDAC were included, and relevant review articles were screened to identify additional studies.

Studies were excluded if they: i) Lacked primary data; ii) focused exclusively on non-human models without translational relevance; iii) did not address diagnostic or biomarker-related outcomes; or iv) were case reports, conference abstracts or editorials. As the present study is a narrative review, study selection did not follow formal systematic review protocols. Instead, representative studies across different populations, sample types and analytical approaches were included in the present review to provide a balanced overview of current evidence supporting microbiome-based biomarkers in PDAC. Where available, studies reporting inconsistent or negative findings were also considered.

3. Microbial dysbiosis signatures associated with PDAC

Microbial dysbiosis has been increasingly reported in patients with PDAC across multiple anatomical sites, including the gut, tumor tissue and oral cavity (16,17,20). Although study designs and sampling strategies differ, recurrent alterations in microbial composition and diversity have been identified (9,20).

Gut microbiota alterations in PDAC. A cohort study analyzing 193 fecal samples from patients with PDAC and healthy controls (HCs) (156 patients with PDAC; 37 HCs) demonstrated that a number of notable alterations in gut-microbial composition were associated with pancreatic tumorigenesis. Notably, the inclusion of patients with pre-malignant pancreatic lesions enabled comparisons across different disease stages, and the results suggested that microbial alterations may progressively emerge during pancreatic carcinogenesis (17). A multicenter case-control study involving Spanish and German cohorts identified distinct fecal microbiota profiles in patients with PDAC compared with healthy individuals and patients with chronic pancreatitis (CP) (16,21); similarly, studies have shown that classifiers based on 27 microbial species have achieved area under the curve (AUC) values of 0.74-0.83 for detecting PDAC in independent cohorts (16,21).

α-diversity findings for PDAC remain inconsistent. Several studies have reported markedly reduced Shannon diversity, a commonly used measure of α-diversity, in patients with PDAC compared with HCs (23,24). Additionally, a meta-analysis including 12 studies (535 patients with PDAC; 677 controls) also supported an overall reduction in microbial α-diversity in patients with PC compared with controls (25). However, other studies have failed to detect notable differences in α-diversity between PDAC and control groups (26,27), highlighting notable levels of inter-study variability. By contrast, β-diversity analyses have more consistently shown a clear separation between microbial communities in PDAC and control groups (16,23,28), suggesting that the overall composition of microbial communities in patients with PDAC may be more reproducibly altered than within-sample diversity metrics.

At the taxonomic level, several recurrent patterns in bacterial community composition have been identified in PDAC. Enrichment of opportunistic and facultative anaerobic taxa, including members of the Enterobacteriaceae and Enterococcaceae families, has been reported in PDAC cohorts (29). Furthermore, an increased abundance of oral-associated genera, such as *Streptococcus* and *Veillonella*, has been consistently observed in fecal samples

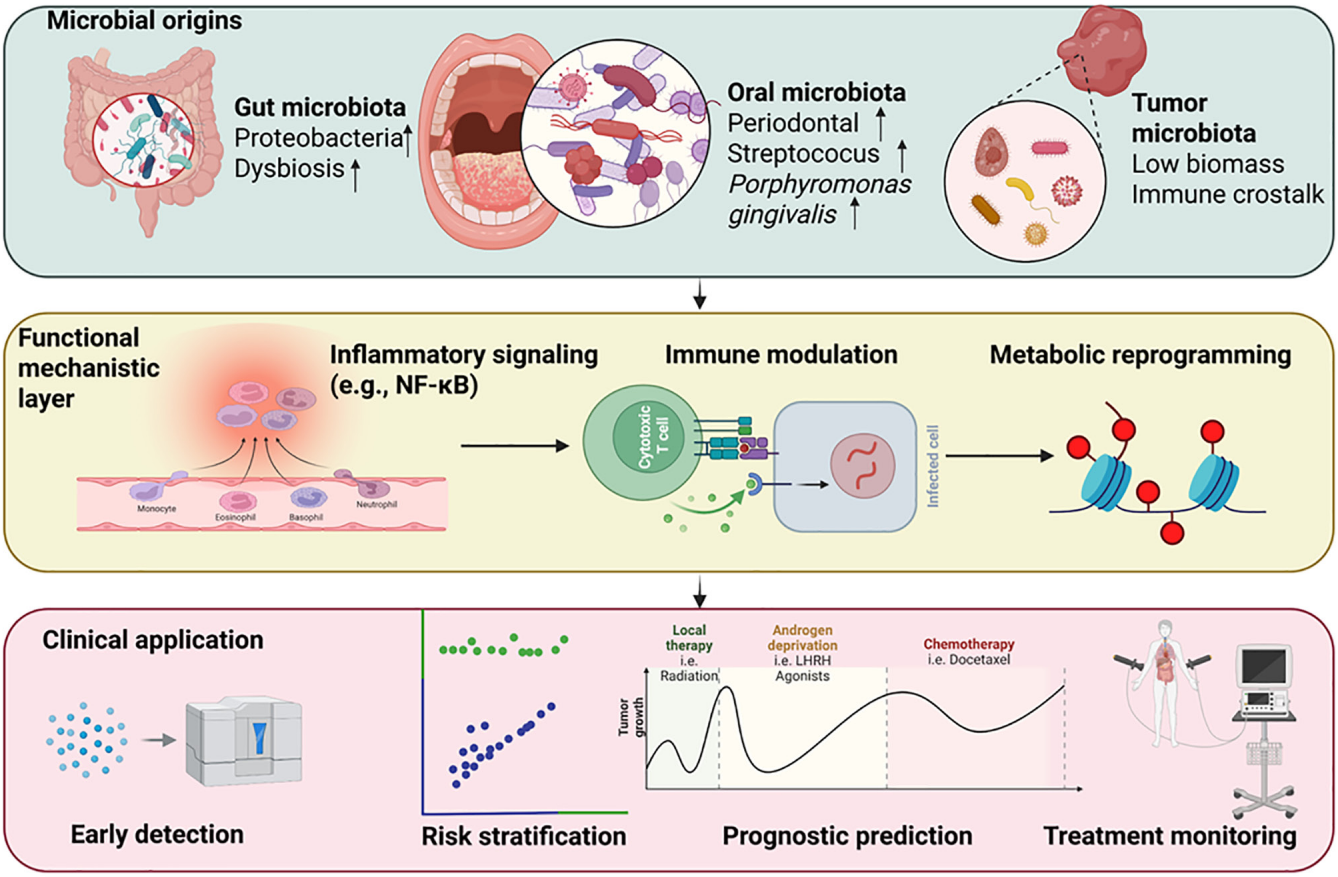

Figure 1. Conceptual overview of microbiome involvement in PDAC. Microbial dysbiosis in the gut, oral cavity and tissue- or tumor-associated microbial communities may contribute to PDAC initiation and progression through multiple interconnected mechanisms, including chronic inflammation, immune dysregulation, metabolic reprogramming and modulation of the tumor microenvironment. Potential microbiota-host interactions and downstream biological processes may influence tumor development and disease progression. These interactions support the potential of microbiota-based models for PDAC diagnosis and risk assessment in multiple clinical applications, including early disease detection, risk stratification, prognostic predictions and treatment monitoring. Created with BioRender.com. PDAC, pancreatic ductal adenocarcinoma; LHRH, luteinizing hormone-releasing hormone.

from patients with PDAC across multiple cohorts (30). By contrast, depletion of short-chain fatty acid-producing commensals, including *Lachnospiraceae*, *Ruminococcaceae* and *Faecalibacterium prausnitzii*, has also been consistently reported in independent cohorts of patients with PDAC, including multinational and Japanese cohorts (16-18,31). Notably, a cross-population analysis indicated that microbial signatures are influenced by ethnicity, as well as demographic, dietary and lifestyle factors, underscoring the importance of population-specific validation for microbial profiles (29).

In addition to indicating the presence of disease, gut microbiota composition may also be associated with clinical characteristics of PDAC. Compared with non-metastatic PDAC, metastatic PDAC exhibits an overall higher relative abundance of Gram-negative bacteria. Machine learning analysis has identified bacterial taxa that effectively distinguished metastatic from non-metastatic disease, such as *Anaerostipes hadrus* and *Porphyromonas* (32). Furthermore, an increased abundance of *Klebsiella pneumoniae* in the gut is a central component of dysbiotic microbial networks linking the gut, oral cavity and prognostic factors, and has also been associated with poor prognosis in patients with PDAC (33). In addition, long-term survivors of PDAC exhibit notable enrichment of *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* in the gut, suggesting potential associations between gut microbiota composition and patient prognosis (34). However, the aforementioned findings require validation in larger longitudinal cohorts.

Preclinical models have provided complementary insights into microbiome dynamics during PDAC development. In murine models of PDAC driven by the oncogenic *Kras*G12D mutation, disease progression is accompanied by time-dependent alterations in fecal microbiota composition and reduced α-diversity (35,36). Fecal microbiota transplantation (FMT) experiments have further suggested that fecal microbiota from patients with PC may transmit dysbiotic features to recipient mice (37). In addition, dietary and pharmacological interventions, including high-fat diet exposure and metformin treatment, have been shown to modulate the composition of the gut microbiota in preclinical PDAC models (38,39). Although these murine studies have provided mechanistic insights into microbiota-based alterations in PDAC, their direct relevance to human clinical translation remains ambiguous. Overall, current evidence supports the presence of gut microbiota dysbiosis in patients with PDAC. However, heterogeneity in sampling strategies, sequencing methods, including 16S ribosomal RNA (rRNA) sequencing and shotgun metagenomics, bioinformatics pipelines and cohort characteristics markedly

limits direct comparisons of findings across studies. Therefore, standardized methodologies and multicenter validation studies are required before gut-microbial signatures can be reliably translated into clinical diagnostic applications (40).

Tissue-associated microbiota in PDAC. A study analyzing 193 pancreatic tissue specimens, including PDAC, pre-malignant lesions and benign tumors, revealed distinct microbial compositions in tumor tissues compared with non-malignant pancreatic samples (19). Patients with PDAC have demonstrated markedly higher levels of α-diversity in tumor tissues compared with adjacent normal tissues, with Proteobacteria, Firmicutes and Actinobacteria identified as the dominant phyla found in tumor tissues (41). Enrichment of *Pseudomonas fluorescens* has also been associated with prolonged overall survival in patients with PDAC, and *in vitro* experiments have suggested that this bacterium may inhibit PC cell proliferation (41). However, tissue-based microbiome studies face notable methodological limitations. One study reported notably low levels of microbial biomass in PDAC resection specimens, which exhibited bacterial DNA levels comparable to negative controls, raising concerns regarding contamination and technical bias (42). In low bacterial biomass environments such as pancreatic tissue, the inclusion of negative controls, such as extraction blanks for detecting reagent- or kit-derived contamination or sequencing blanks, is key for distinguishing true microbial signals from environmental or reagent-derived contamination (43).

Anatomical location-associated differences in pancreatic tumor microbiota have been reported (19,36). Distinct microbial compositions have been observed between tumors located in the pancreatic head and those in the body or tail (44). In addition, a spatial analysis demonstrated associations between bacterial distribution patterns and immune cell niches, such as T cell-rich and T-cell-poor regions (45). Culture-based approaches enabled the isolation of γ-proteobacteria, including *Klebsiella pneumoniae*, from pancreatic cystic lesions associated with pancreatic cancer (46). However, these approaches may capture only a subset of the pancreatic microbiota, as culture-dependent methods can underestimate overall microbial diversity and remain vulnerable to contamination or selective growth bias (42,46).

Fungal components in pancreatic tissues have also been implicated in PDAC development. For example, enrichment of *Malassezia* species has been reported in PDAC tissues and discussed as a potential contributor to pancreatic carcinogenesis (47). A sequencing-based study identifies site-specific microbial signatures in pancreatic and duodenal tissues, suggesting potential microbial interactions or translocation along the gastrointestinal tract and the pancreas (48). Consistent with potential microbial interactions along the gastrointestinal-pancreatic axis, a multi-site microbiome study reported progressive microbial alterations during the transition from CP to advanced PDAC, including sequential enrichment of oral-associated taxa, such as *P. gingivalis* and *Filifactor alocis* (49). Although intratumoral and tissue-associated microbial profiles are increasingly recognized (50,51), their diagnostic value remains ambiguous. Low microbial biomass, spatial heterogeneity, differences in sampling and sequencing methods, and the risk of contamination further hinder clinical translation.

Oral and other body site microbiota in PDAC. In a large nested case-control study analyzing pre-diagnostic oral wash samples, the presence of *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* in samples was associated with a markedly increased risk of PDAC development, with adjusted odds ratio values of 1.60 and 2.20, respectively. However, the relative abundance of *Neisseria* was found to be inversely associated with PDAC risk (17). Notably, elevated levels of antibodies against *P. gingivalis* have also been associated with an ~2-fold increased risk of PC (52). A meta-analysis further supported an association between oral microbiota alterations and PC risk (53).

Salivary microbiome profiling has demonstrated potential discriminatory ability between patients with PDAC and controls (26), although fecal microbiota-based classifiers have generally shown improved performance compared with oral microbiota-based models (16). Notably, reduced oral-microbial diversity and enrichment of *Streptococcus* have been reported in PC cohorts (18).

Beyond the oral microbiota, an analysis of secretin-stimulated duodenal fluid has revealed markedly reduced α-diversity levels in patients with PDAC compared with controls and patients with pancreatic cysts. The enrichment of specific genera, including *Bifidobacterium* and *Fusobacterium*, has also been observed in duodenal fluid (28). Furthermore, studies of bile microbiota have demonstrated differences in β-diversity between patients with malignant and benign pancreaticobiliary diseases (54,55), although the low microbial biomass of bile samples requires cautious interpretation of these findings (56).

Notably, non-invasive biospecimens, including saliva, stool and duodenal fluid, may represent promising sources of microbial data for biomarker development (16). However, heterogeneity in study design, limited sample sizes and the lack of standardized predictive modeling approaches remain important limitations for the identification of such biomarkers (57). Large prospective studies with external validation are required to determine whether multi-site microbial signatures can demonstrate clinically robust diagnostic performance for PDAC. On the basis of these body site-specific microbial alterations, several studies have attempted to develop microbiota-based predictive models for PDAC diagnosis, as summarized in Tables I-III (16,17,30,41,58-65).

4. Diagnostic modeling strategies and performance evaluation

Previous evidence suggests that the microbiome may represent a promising source of non-invasive biomarkers for PDAC (16,66). Advancements in high-throughput sequencing technologies have enabled the comprehensive profiling of oral, fecal and tumor-associated microbial communities, and have revealed reproducible alterations in microbiota composition that are associated with pancreatic tumorigenesis (67,68). As such, microbiome-based classifiers are increasingly being developed for early detection, risk stratification and prognostic prediction for patients with PDAC (16,69). However, several challenges remain, including relatively small cohort size, geographic heterogeneity and insufficient external validation; additionally, robust clinically validated microbiome-based

Table I. Representative fecal microbiome-based diagnostic models for PDAC.

First author/s, year	Cohort (sample size)	Profiling method	Key microbial features	Modeling approach	Validation strategy	Reported AUC	Diagnostic task	(Refs.)
Kartal *et al*, 2022	Spanish cohort (n=136; 57 patients with PDAC, 50 HCs and 29 patients with CP) + German cohort (n=64; 32 patients with PDAC and 32 HCs) + 25 public datasets (n=5,792)	Shotgun metagenomics	27-species classifier ± CA19-9 biomarker	Random forest	Independent multi-cohort validation	0.840 (0.940 with CA19-9)	PDAC vs. HC	(16)
Wang *et al*, 2024	Chinese cohorts in a multicenter study (n=193; 156 patients with pancreatic tumors and 37 HCs)	16S rRNA sequencing	Abundance-based microbial features	Random forest	Internal validation (method not reported)	NR	PDAC vs. HC	(17)
Wei *et al*, 2020	PDAC/NPDAC cohort (n=110; 41 patients with PDAC and 69 HCs)	16S rRNA sequencing	Differential microbial taxa	Random forest	LOOCV	NR	PDAC vs. NPDAC	(65)
Yang *et al*, 2023	Chinese cohort (n=94; 44 patients with PDAC and 50 HCs)	16S rRNA sequencing	*Streptococcus* enrichment	Random forest	Internal validation (method not reported)	NR	PDAC occurrence and liver metastasis	(30)
Zhang *et al*, 2024	Multi-cohort (n=183; 101 patients with PDAC and 82 HCs)	Virome metagenomics	219 viral OTUs	Random forest	Multi-cohort validation	0.879	PDAC vs. HC	(63)
Li *et al*, 2025	Chinese cohort (n=187; 97 patients with PDAC and 90 HCs)	16S rRNA sequencing	Top 20 contributing genera	Random forest	Internal validation (7:3 split)	0.890	PDAC vs. HC	(61)
Gao *et al*, 2024	Chinese cohort (n=53)	16S rRNA sequencing	*Pseudomonas fluorescens* enrichment	N/A	Internal validation	NR	Mechanistic/ prognostic study	(41)
Zhou *et al*, 2021	Mixed cohort (n=96; 32 patients with PDAC, 32 patients with AIP and 32 HCs)	Shotgun metagenomics	SCFA depletion + Proteobacteria enrichment	ML classifier	External validation	0.907 (PDAC vs. HC); 0.889 (PDAC vs. AIP)	PDAC vs. HC or AIP	(62)

AIP, autoimmune pancreatitis; AUC, area under the curve; CP, chronic pancreatitis; HC, healthy control; LOOCV, leave-one-out cross-validation; ML, machine learning; NR, not reported; OTU, operational taxonomic unit; NPDAC, non-pancreatic ductal adenocarcinoma; rRNA, ribosomal RNA; SCFA, short-chain fatty acid.

Table II. Representative oral and salivary microbiome-based diagnostic models for PDAC.

First author/s, year	Cohort (sample size)	Sample type	Profiling method	Key microbial features	Modeling approach	Validation strategy	Reported AUC	Diagnostic task	(Refs.)
Li *et al*, 2025	Chinese cohort (n=187; 97 patients with PDAC and 90 HCs)	Oral	16S rRNA sequencing	Top 20 contributing genera; *Streptococcus* enriched	Random forest	Internal validation (7:3 split)	0.963	PDAC vs. HC	(61)
Wei *et al*, 2020	Chinese cohort (n=110; 41 patients with PDAC and 69 HCs)	Oral	16S rRNA sequencing	*Streptococcus* and *Leptotrichia* (risk); *Veillonella* and *Neisseria* (protective)	Logistic regression + ML classifier	Internal validation	0.963	PDAC vs. NPDAC	(65)
Wong *et al*, 2009	UCLA cohort (n=90; 3 0 patients with PDAC, 30 patients with CP and 30 HCs)	Saliva	qPCR and transcriptomics	*Neisseria elongata* + *Streptococcus mitis* + ACRV1 + DMXL2 + DPM1	Logistic regression	Internal validation	0.895 (microbial); 0.949 (combined)	PDAC vs. CP and HC	(64)
Farrell *et al*, 2012	UCLA cohort (n=83; 28 patients with PDAC, 28 HCs and 27 patients with CP)	Saliva	qPCR	*N. elongata* and *S. mitis*	Combined logistic model	Independent validation	0.900	PDAC vs. HC	(59)

ACRV1, acrosomal vesicle protein 1; AUC, area under the curve; CP, chronic pancreatitis; DMXL2, Dmx-like 2; DPM1, dolichyl-phosphate mannosyltransferase subunit 1; HC, healthy control; ML, machine learning; NPDAC, non-pancreatic ductal adenocarcinoma; NR, not reported; PDAC, pancreatic ductal adenocarcinoma; qPCR, quantitative PCR; rRNA, ribosomal RNA; UCLA, University of California, Los Angeles.

Table III. Representative multi-modal microbiome-based diagnostic strategies for PDAC.

First author/s, year	Biomarker combination	Specimen/data modality	Integration method	Modeling approach	Validation strategy	Reported AUC	Diagnostic task	(Refs.)
Wang *et al*, 2024	Fecal + tissue-associated microbiota integration	Feces + tumor tissue	Integrated microbial profiling	Multi-step classifier	External validation	NR	Early pancreatic tumor detection	(17)
Kartal *et al*, 2022	Fecal microbiota + CA19-9	Feces + serum biomarker	Shotgun metagenomics + CA19-9 integration	Random forest	External validation	0.940	Early PDAC detection	(58)
Guo *et al*, 2022	Fecal + metabolomics integration	Feces + metabolomics	Integrated microbiome profiling and metabolomics	ROC analysis	Exploratory study	NR	Resectable vs. unresectable PDAC	(60)
Wong *et al*, 2009	Microbiota + salivary mRNA	Saliva + transcriptomics	Microbiome and transcriptome i ntegration	Combined logistic regression model	Internal validation	0.949	PDAC vs. CP and HC	(64)

AUC, area under the curve; CP, chronic pancreatitis; ML, machine learning; NR, not reported; PDAC, pancreatic ductal adenocarcinoma.

diagnostic tools remain lacking (70). This section summarizes current microbiome-derived diagnostic models, evaluates their predictive performance and discusses emerging diagnostic strategies that integrate microbial, metabolic and conventional clinical biomarkers to improve diagnostic accuracy.

Fecal microbiota-based diagnostic models. Fecal microbiota profiling remains the most extensively studied approach for non-invasive PDAC detection. Machine learning-based approaches have been applied to develop microbiome-based classifiers for PDAC using shotgun metagenomics or 16S rRNA sequencing data (16). However, microbiome datasets are typically high-dimensional, increasing the risk of overfitting, especially in studies with small cohorts (71). Notably, a previous study has demonstrated that a fecal metagenomic classifier based on 27 microbial species achieved an AUC of 0.84 for distinguishing PDAC samples from controls, and this classifier exhibited similar levels of accuracy for identifying both early- and late-stage disease (16). The aforementioned model has also been shown to maintain high disease specificity across independent public datasets and external validation cohorts. Additionally, the integration of this model with serum CA19-9 levels led to further improvements in diagnostic performance, raising the AUC to 0.94 (16).

Using a penalized logistic regression and iterative random forest analysis, gut-microbial signatures have been able to discriminate metastatic from non-metastatic PDAC cases (32). The integration of fecal metabolomic profiles has been shown to further improve the discriminatory performance of these signatures and has revealed associations between microbial taxa and lipid metabolites, providing complementary metabolic information for PDAC detection (60). A previous study has demonstrated that a fecal microbiota classifier achieved an AUC of 0.856 (95% CI, 0.740-0.972) for distinguishing PDAC from controls (26), whereas another study has reported an AUC of 0.963 for identifying PDAC using oral microbiota profiled by 16S rRNA sequencing (61). An additional study has shown that a classifier based on discriminatory bacterial features exhibited for PDAC detection; however, considerable inter-individual microbial variability in the study cohort limited the applicability of this model for the early identification of pre-cancerous lesions (72). Notably, numerous studies have differed in how AUC values are reported, with inconsistent reporting of results from training datasets, internal cross-validation or independent external validation cohorts, which complicates direct comparisons of model performance (16,61).

Differential diagnostic performance in microbiota-based models has also been investigated. A previous study showed that a random forest classifier based on the relative abundance of the top 10 discriminatory fecal bacterial genera distinguished PC samples from non-malignant pancreatic lesions with an AUC of 0.894 and outperformed CA19-9-based diagnostic models in mucinous tumor subgroups (73). In addition, shotgun metagenomic profiling has demonstrated high accuracy for distinguishing PDAC from autoimmune pancreatitis (AIP), exhibiting AUC values of 0.907 for PDAC vs. HCs and 0.889 for PDAC vs. AIP (62).

Cross-population validation studies are particularly important for clinical translation. One such study involving

Finnish and Iranian cohorts achieved an AUC of 0.88 (95% CI, 0.78-0.97) in the independent Finnish cohort (29), although microbial signatures were partially influenced by demographic and lifestyle factors. Beyond bacterial taxa, the diagnostic potential of the gut virome has also been investigated. A previous study has demonstrated that a random forest classifier based on viral operational taxonomic units achieved an AUC of 0.879 for distinguishing patients with PDAC from healthy controls, and exhibited notable reproducibility across independent cohorts (63). Overall, fecal microbiota-based diagnostic models have frequently reported AUC values ranging from 0.80 to 0.90 (16,18); however, the majority of studies in this field employ a case-control design, and their performance in prospective screenings or real-world diagnostic settings remains to be established (57).

Oral and other body site microbiota-based diagnostic models. Oral microbiota-based diagnostic approaches have previously gained attention due to their convenience and high levels of patient compliance (74). One study has reported that a saliva-based random forest diagnostic model demonstrated an AUC of 0.916 (95% CI, 0.832-1.000) for PDAC detection, outperforming fecal classifiers in the same study (26). Furthermore, another study has demonstrated that an oral microbiota classifier achieved an AUC of 0.963 in differentiating PC from non-malignant pancreatic conditions, exceeding the performance of models based on CA19-9 levels (73). However, these high AUC values should be interpreted with caution, as they are frequently derived from analyses of small single-center cohorts and may reflect overfitting or cohort-specific microbial patterns.

Logistic regression models based on salivary taxa have also shown promising diagnostic performance for PDAC detection. A model incorporating *Neisseria elongata* and *Streptococcus mitis* has been shown to achieve an AUC value of 0.895 (64), and subsequent validation demonstrated that this model achieved an AUC of 0.90 (95% CI, 0.78-0.96) with a sensitivity of 96.4% and a of 82.1% specificity (59). Additionally, logistic regression analysis has identified *Streptococcus* and *Leptotrichia* as risk-associated taxa, whereas *Veillonella* and *Neisseria* have been shown to be inversely associated with PDAC (65).

Sex-stratified classifiers integrating oral and gut microbiota have been shown to achieve AUC values >0.90 in both male and female patients with PDAC (75), suggesting potential sex-specific microbial patterns. Beyond oral microbiota-based classifiers, a previous study has shown that bile microbiome-based classifiers exhibited notable discriminatory performance for distinguishing between PC and cholelithiasis, with an AUC of 0.966 (76), although the low microbial biomass of bile samples presents challenges for microbiome analysis and requires rigorous contamination control (55). One study demonstrated compositional differences in duodenal fluid microbiota between with PDAC compared with HCs (28), however, predictive modeling studies remain limited.

Overall, oral microbiota-based classifiers have frequently reported high AUC values (>0.85). However, sample sizes for these classifiers are typically modest, external validation remains limited and key clinical performance indicators are rarely reported.

Integrated multi-modal biomarker strategies. Given the heterogeneity of microbial signatures in PDAC, integrative approaches combining microbiota-based biomarkers with established biomarkers or metabolomic profiles may enhance diagnostic robustness. The integration of fecal and tissue-associated microbial features has been shown to exhibit improved classification performance compared with analyses based on either fecal or tissue-associated microbial features alone (17). Similarly, the combination of fecal microbiota signatures with serum CA19-9 levels has been shown to increase the AUC of the diagnostic model from 0.84 to 0.94 (16), demonstrating the potential value of multi-modal biomarker strategies for PDAC diagnosis.

Metabolomic integration further supports microbiome-based diagnostics. Associations between microbial taxa, including *Alistipes* and *Anaerostipes*, and lipid metabolites have previously been reported (60). One metabolomic analysis linked microbe-associated metabolites to inflammatory and oncogenic pathways, including the NF-κB and mTOR signaling pathways (77). However, integration these complementary data types introduces additional analytical challenges, including differences in data scale, normalization methods and feature selection (78), which may further increase model complexity and the risk of overfitting if not properly controlled.

Similarly, combined salivary mRNA and microbial biomarker models have demonstrated an AUC of 0.949 for distinguishing patients with PDAC from healthy controls and patients with CP (64). Beyond diagnosis, certain microbial signatures have also been associated with survival outcomes (36). Although one FMT study suggested that microbiome modulation may influence tumor growth and immune infiltration in experimental models (36), to the best of our knowledge, clinical validation of microbiome-based prognostic models remains preliminary. Overall, multi-modal integration strategies appear to exhibit improved diagnostic performance compared with single-marker approaches. However, to the best of our knowledge, no integrated microbiome-based diagnostic model has achieved the level of validation necessary for routine clinical use at present.

Critical reappraisal of reported diagnostic performance. The aforementioned studies have reported AUC values ranging from 0.80 to 0.96, which are often interpreted as evidence of strong diagnostic potential. However, several notable issues should be considered when interpreting these metrics.

Primarily, microbiome-based diagnostic models have been developed and evaluated using retrospective case-control designs, typically comparing established PDAC cases with HCs or selected benign disease controls (57,74). This design does not adequately reflect the complexity of real-world screening or early-detection settings, where the differential diagnosis for patients includes a broad spectrum of pancreatic and non-pancreatic diseases, and the worldwide incidence of PDAC was 10-15 cases per 100,000 population per year (79).

Additionally, reported AUC values alone are insufficient to determine the clinical utility of the aforementioned models. The positive predictive value (PPV) of a diagnostic test is dependent on disease prevalence. In low-prevalence screening settings, even tests with high sensitivity and specificity may

generate notably more false-positive results than true-positive results, resulting in a low PPV (80). For example, a theoretical test with 90% sensitivity and 90% specificity applied to a population with a PDAC prevalence of 0.01% would yield a PPV of <0.1%, indicating that the majority of positive results would represent false positives (81). This notable limitation is rarely addressed in the studies discussed in the present review, despite its relevance to assessing the potential applicability of microbiome-based diagnostic models.

Furthermore, a number of studies have failed to clearly distinguish between the training set performance, internal cross-validation and independent external validation of classifier models, despite their markedly different evidentiary strength. Training set AUC values are inherently optimistic due to overfitting, particularly in high-dimensional microbiome datasets where the number of microbial variables frequently exceeds the sample size (82). Although internal cross-validation of these classifiers may reduce optimism bias, it does not necessarily demonstrate robustness or generalizability across independent populations (83). Only independent external validation using a completely separate cohort can provide robust evidence that a classifier will perform reliably in new populations (84). In cases where external validation has been performed, diagnostic performance is typically decreased compared with initial reports (85).

Collectively, although the field has generated encouraging proof-of-principle evidence supporting the aforementioned classifiers, these models have not yet undergone sufficient clinical validation for routine clinical application. To the best of our knowledge, no microbiome-based diagnostic model for PDAC has yet undergone the rigorous prospective validation required for clinical implementation, and none have currently been recommended for routine clinical use in major clinical guidelines. The high AUC values frequently reported in the aforementioned studies should therefore be interpreted as hypothesis-generating findings rather than clinically validated performance metrics.

5. Translational challenges for microbiome-based biomarkers in PDAC

Despite the rapidly expanding evidence linking gut, oral and tissue-associated microbiota to PDAC, the clinical translation of microbiome-based biomarkers remains challenging. Although multiple studies have demonstrated the promising diagnostic performance and potential mechanistic relevance of microbiota-based diagnostic models, notable barriers to clinical translation persist, including methodological heterogeneity, low microbial biomass in pancreatic tissues, confounding host-related factors and insufficient prospective validation (57,86). Dysbiosis has been associated with carcinogenesis through chronic inflammation, immune evasion and microbial metabolite-mediated DNA damage or genomic instability (87); however, whether these microbial alterations represent causal drivers of carcinogenesis, secondary responses to tumor progression or parallel epiphenomena remains ambiguous (88).

A systematic review identified common microbial alterations across pancreatic diseases, including the depletion of beneficial microbial genera such as *Bifidobacterium* and *Lactobacillus* and the enrichment of oral-associated genera, including *Neisseria*, *Streptococcus* and *Porphyromonas*, in PDAC (89). However, the limited number of large-scale, prospective and independently validated human studies, together with notable inter-study variability highlight the need for standardized methodologies and multicenter validation (82). This section discusses the methodological, biological and clinical challenges that currently hinder the implementation of microbiome-based biomarkers in PDAC screening and risk stratification.

Methodological constraints and technical variability. Translating microbiome research into clinically useful diagnostic tools requires standardized and reproducible methodologies. However, the translation of PDAC microbiome studies is frequently limited by small sample sizes, batch effects, inconsistent sequencing platforms and heterogeneous bioinformatics pipelines (57,90). A Preferred Reporting Items for Systematic Reviews and Meta-Analyses-guided systematic review concluded that a universal 16S rRNA microbial signature for PDAC screening has not yet been established, largely due to heterogeneity in study design, sequencing methods and data reporting (57).

The pancreas represents a low-biomass tissue environment, which creates notable technical challenges for microbiome research (56). Investigation of tumor-associated bacteria have been hindered by poor reproducibility and potential contamination artifacts (91). Although modern high-resolution approaches, such as metagenomics, metatranscriptomics and metabolomics, have advanced PDAC-associated microbiome research, distinguishing true tumor-resident microbes from environmental or reagent contamination remains difficult (91). A previous study has demonstrated that PDAC resection specimens exhibit low microbial biomass, with bacterial DNA levels comparable to negative controls (42). Thus, some detected signals may reflect contamination rather than biologically meaningful intratumoral microbial communities (42).

Additionally, a pilot study has demonstrated that endoscopic ultrasound-guided fine needle aspiration samples can be used to characterize tumor-associated bacteria and fungi prior to surgical resection under stringent contamination control conditions (92). Therefore, similar approaches could facilitate preoperative microbiome-based detection of PDAC. However, standard operating procedures for sample collection, storage, DNA extraction and sequencing, and contamination assessment remain inconsistent across institutions (56).

The establishment of causal relationships between microbial alterations and tumor progression also remains challenging. One experimental study reported that antibiotic-mediated microbiome depletion reduce tumor growth and delay xenograft formation despite the absence of detectable intratumoral bacteria, suggesting that gut microbes may influence tumor progression through indirect systemic mechanisms (93). Transcriptomic analysis in this study further revealed changes in innate immune suppression and oncogenic pathways (93), highlighting the complexity of establishing direct causal relationships between gut microbiota alterations and tumor progression. Although these findings suggest a potential role for the microbiome in PDAC progression,

translating preclinical observations into validated diagnostic markers requires well-designed longitudinal human studies and standardized analytical frameworks.

Biological variability and host-related confounders. Microbiome composition is markedly influenced by host genetics, diet, metabolic status, medication use and environmental exposures. This biological variability complicates the reproducibility of microbiome-based biomarkers (94). High-fat diets have been shown to promote microbial dysbiosis and inflammatory signaling, potentially accelerating gastrointestinal tumorigenesis (95). Conversely, a low-protein diet has been shown to suppress tumor development and enhance antitumor immune activation in a microbiota-dependent manner (96). Furthermore, increased abundance of *Blautia coccoides* and activation of the P2Y purinoceptor 14/STAT1 axis have been implicated in macrophage polarization toward an immunostimulatory phenotype (96), although such microbiota-host interactions may be influenced by inter-individual biological variability. These observations suggest that dietary heterogeneity across populations may markedly influence microbiome-based biomarker profiles.

Metabolic comorbidities, such as obesity and type 2 diabetes, further complicate the interpretation of microbiome-associated biomarkers for PDAC. Obesity and diabetes are established risk factors for PDAC and have also been linked to gut-microbial alterations, immune suppression, oxidative stress and inflammatory signaling (97). Experimental models have demonstrated that hyperglycemia enriches drug-metabolizing bacterial populations and decreases sensitivity to gemcitabine-based chemotherapy (98). Thus, metabolic disturbances may confound microbial signatures identified in cross-sectional case-control studies.

Chemotherapy itself has also been shown to alter microbiome composition. In xenograft models, gemcitabine has been shown to reduce Firmicutes and Bacteroidetes levels and increase Proteobacteria and *Akkermansia* levels, which are changes linked to NF-κB pathway activation (99). Similarly, gut microbiota composition has been shown to modulate responses to immune checkpoint inhibitors, with taxa such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii* and *Bifidobacterium* spp. being associated with improved efficacy in anti-programmed cell death protein 1/programmed cell death 1 ligand 1 therapy (100). Although these findings are not specific to PDAC, they suggest that therapeutic interventions can markedly reshape microbial communities. Notably, a previous tissue-based microbiome study included surgically resected samples from patients who received neoadjuvant chemotherapy or chemoradiotherapy (101). As neoadjuvant treatment may alter microbial composition, its potential influence should be considered when interpreting microbiome profiling results (101,102).

Patients who use proton pump inhibitors (PPIs) have been shown to exhibit notable enrichment of oral-associated bacteria in fecal samples, and this microbial shift has been linked to poorer outcomes following immune checkpoint inhibitor therapy across solid tumors (103). These findings suggest that medication-induced alterations in the microbiome may produce non-specific or misleading microbial associations.

Tobacco smoking and alcohol consumption, both established PDAC risk factors, are key modulators of gut and oral microbiota. Smoking alters microbial diversity and enriches periodontal pathogens (20,53), whereas chronic alcohol consumption disrupts intestinal barrier integrity and promotes inflammatory dysbiosis (97). However, smoking and alcohol consumption are potential confounding factors. These variables have not been consistently considered or adjusted for, which may influence the interpretation of disease-associated microbial signatures (57).

Biliary obstruction, which is frequently present in patients with PDAC, represents an underrecognized source of bias in PDAC study (104). Obstructive jaundice alters bile flow, disrupts intestinal microbial ecology and promotes bacterial translocation (54,55). Furthermore, biliary stent placement and endoscopic biliary drainage procedures may independently influence the duodenal and gut microbiome, potentially resulting in microbial signals unrelated to the tumor microenvironment (TME) (101). However, biliary obstruction status and stent placement are not consistently reported or incorporated as covariates in microbiome-based PDAC studies (57,105).

The timing of biospecimen collection may also affect microbiome profiles. In a number of PDAC microbiome studies, the timing of stool, saliva or tissue collection relative to diagnosis, biliary stent placement, antibiotic or PPI exposure, and chemotherapy initiation remains poorly documented (16,28). This lack of temporal standardization makes it difficult to distinguish stable disease-associated microbial patterns from transient therapy- or procedure-induced alterations, thereby reducing the reproducibility of microbial profiles and limiting their clinical applicability.

Mendelian randomization study identified potential causal links between specific gut microbial taxa, such as *Collinsella* and *Ruminococcus torques*, and PDAC risk (106). Although these genetic approaches strengthen causal inferences, effect sizes remain modest and functional validation is still required. Furthermore, one epidemiological study reported a pooled relative risk of ~1.7, indicating an association between periodontal disease and PC risk (107), highlighting potential links between oral dysbiosis and PDAC. However, such associations alone do not establish causality, and residual confounding remains an important consideration in interpreting findings from observational study designs.

The complexity of the PDAC TME, characterized by dense desmoplasia and notable immunosuppression, further complicates the clinical translation of microbiome-based biomarkers (108). Microbiome profiles within the TME may be influenced by stromal composition, immune infiltration, exocrine dysfunction, biliary obstruction and prior treatment exposure (109). In surgical settings, the presence of *Enterococcus* in bile samples has been associated with postoperative complications and increased mortality (105), supporting the potential clinical relevance of bile-associated microbial profiles. However, to the best of our knowledge, there is currently no evidence that microbiome-targeted interventions improve survival or quality of life in patients with pancreatic diseases (110). This may partly reflect the complexity of the PDAC TME, in which microbial effects are likely influenced by multiple interacting tumor, immune and host factors. Furthermore, validated microbiome-based

biomarkers for early PDAC detection, particularly in high-risk groups such as patients with CP and intraductal papillary mucinous neoplasms (IPMNs), are lacking (70). Reverse causality inherent to case-control study designs further complicates the interpretation of microbiome-disease associations (111).

Table IV (16,20-21,29,38,53-55,58,62,73,95-100,103,105, 110), summarizes key host- and treatment-related confounding factors that may influence the findings of PDAC microbiome studies, including the potential effects of these factors on microbial composition and the extent to which current studies have controlled for them. Collectively, microbiome-based biomarkers must be interpreted within the broader context of metabolic, environmental and treatment-related variability within the patient. Without careful adjustment for confounders, reproducibility across populations may remain limited.

Clinical validation, regulatory considerations and implementation. Although a previous microbiome-based diagnostic model reported high diagnostic AUCs (16), another study relied on retrospective case-control designs and prospective, longitudinal, multicenter validation studies remain limited (57). A systematic review of 41 studies noted that although multiple predictive models have shown favorable diagnostic performance, the highest levels of diagnostic accuracy were often observed in classifiers based on blood- and tumor-derived microbial markers, which may be less practical for routine screening compared with non-invasive fecal- and oral-derived microbial markers (112). To the best of our knowledge, no microbiome-based biomarker has currently been incorporated into major clinical guidelines for PDAC screening or diagnosis, and microbiome-based assays have not been established as part of routine clinical diagnostic practice.

The clinical translation of microbiome-based diagnostics also faces notable methodological and regulatory challenges. Microbiome-derived disease signatures are notably sensitive to variability in sample collection, storage, DNA extraction, sequencing platforms and bioinformatics analysis. Without harmonized standard operating procedures, reproducibility across laboratories is challenging (40). For the translation of microbial signatures into *in vitro* diagnostic assays, standardization is needed throughout the entire workflow from sample acquisition to data interpretation, including the use of quality-controlled protocols under recognized standards, such as the International Organization for Standardization 13485 and Good Manufacturing Practice guidelines (113), as well as reproducible analytical pipelines (114). Furthermore, regulatory approval pathways represent a major obstacle for clinical translation (115). Any diagnostic test intended for clinical use must undergo a rigorous evaluation of analytical validity, clinical validity and clinical utility by agencies such as the Food and Drug Administration or the National Medical Products Administration. However, the complexity and dynamic nature of microbiome data create notable challenges for establishing standardized reference ranges, ensuring inter-laboratory reproducibility and validating machine learning-based algorithms (116).

Overcoming the remaining challenges, including methodological heterogeneity, technical variability, host-related confounding factors and insufficient prospective validation, requires large-scale, prospective, multi-ethnic cohort studies with standardized sampling protocols, comprehensive metadata collection and integrated multi-omics analyses. Robust validation of microbial signatures across diverse clinical settings is necessary for the development of clinically applicable microbiome-based screening strategies.

6. Future perspectives: Toward function-based and clinically validated biomarkers

An increasing body of evidence has indicated that the microbiome may influence PDAC risk, progression, therapeutic response and survival outcomes (6,117). Although findings remain largely exploratory, advances in mechanistic studies and multi-omics technologies have begun to improve the translational potential of microbiome research (118). Future clinical application of microbiome-based biomarkers will require methodological standardization, functional validation and targeted implementation in high-risk populations.

Variability in specimen type, sequencing platforms and bioinformatics pipelines has contributed to inconsistent microbial signatures across studies. Previous multi-omics approaches have enabled the functional characterization of microbial communities beyond taxonomic profiling (119). Notably, one integrated metagenomic-metabolomic study identified microbiota-derived metabolites, such as indole-3-acetic acid, which are associated with chemotherapy response and exert biological effects in experimentally-induced PDAC (120). Furthermore, microbial tryptophan metabolism and aryl hydrocarbon receptor signaling have been shown to be involved in PDAC progression and may serve as pathway-level biomarker candidates (77). These observations indicate that functional microbial outputs may provide biologically relevant diagnostic signals by reflecting microbial activity and metabolic consequences rather than compositional profiling alone. Fig. 2 summarizes the interactions between microbial dysbiosis, metabolites, host signaling pathways and TME remodeling.

The proposed immune-oncology-microbiome axis highlights the interconnected roles of gut and tumor-associated microbiota in regulating tumor metabolism and the immune microenvironment (121). An experimental study suggested that pancreatic tumors harbor distinct microbial communities, and that microbiome ablation can reprogram the TME toward an immunogenic phenotype and enhance responses to immunotherapy (122). Microbiota-derived metabolites, particularly short-chain fatty acids, have been shown to promote cytotoxic T-cell activity and modulate inflammatory signaling pathways (123,124). Furthermore, a reduction in butyrate levels may contribute to pancreatic carcinogenesis (125), whereas other metabolites, including trimethylamine N-oxide, may serve as potential biomarkers or therapeutic targets for PDAC (126).

Given the poor prognosis of PDAC and the lack of effective population-wide screening strategies, microbiome profiling may be particularly valuable in high-risk populations (127). Patients with CP, IPMNs, obesity, diabetes or a familial predisposition to PDAC may benefit from microbiome-based risk-stratification approaches. Microbiome analysis could also complement liquid biopsy and imaging approaches, particularly in early-stage disease where microbial and immune alterations may be more detectable (128). The enrichment of beneficial microbial taxa, such as *Faecalibacterium prausnitzii* and

Table IV. Major host-, disease-, procedure- and treatment-related confounding factors in PDAC microbiome studies.

Confounding factor	Potential effects on the microbiome and host biology	Relevance to PDAC microbiome studies	Current status in published PDAC microbiome studies	(Refs.)
Smoking	Alters oral and gut microbial diversity; enriches periodontal pathogens; depletes commensals; promotes chronic inflammation and oxidative stress.	Established PDAC risk factor; may independently affect microbial signatures in diagnostic models.	Often incompletely reported or inconsistently adjusted for; potential source of bias.	(20,53)
Alcohol consumption	Disrupts intestinal barrier; alters gut microbial diversity; promotes inflammatory dysbiosis; associated with oral microbiome shifts.	Linked to CP and PDAC risk; may independently contribute to observed microbiota alterations.	Inconsistently recorded; rarely incorporated into adjustment models.	(110)
Diet and nutritional status	Strongly shapes gut composition and metabolites (SCFAs, bile acids and tryptophan metabolites); influences inflammatory signaling.	Geographic and dietary variability may cause inconsistent signatures across cohorts; cachexia may influence the advanced PDAC microbiome.	Rarely standardized or longitudinally monitored; the majority of studies lack detailed dietary metadata.	(29,95,96)
Obesity and T2D	Associated with chronic inflammation, insulin resistance, altered gut permeability, dysbiosis and chemotherapy responses.	Both are established PDAC risk factors; may produce overlapping microbial signatures independent of cancer.	Adjustment for BMI, diabetes and metabolic parameters varies markedly; some studies exclude diabetic patients, others include mixed populations.	(97,98)
PPIs and other medications	PPIs enrich oral-associated taxa in gut; antibiotics and metformin reshape microbiome diversity and composition.	Medication-induced alterations may produce false-positive associations or obscure PDAC-specific signatures.	Medication history, including antibiotic, PPI and metformin treatments, is often incompletely reported.	(38,99,103)
Chemotherapy and neoadjuvant therapy	Alters microbial composition and immune-microbiome interactions; treatment-associated dysbiosis differs from treatment-naïve states.	Numerous tissue-based studies use resected specimens from patients who may have received neoadjuvant therapy, influencing microbial signatures.	Prior therapeutic exposure is inconsistently documented; treatment history is rarely analyzed separately in diagnostic models.	(99,100)
Biliary obstruction and stent placement	Alters bile flow and intestinal ecology; promotes bacterial translocation; drainage or stent placement procedures add selection pressure.	Obstructive jaundice common in patients with PDAC; may independently influence bile, duodenal and gut microbiota profiles.	Limited studies explicitly report obstruction status or timing of stent placement before sampling.	(54,55,105)
CP and inflammatory pancreatic disease	Alters gut permeability, microbial diversity and pancreatic microenvironment.	Microbial overlap between CP and PDAC limits diagnostic specificity and complicates differential diagnosis.	Some studies include CP controls, but sample sizes limited and overlap remains notable.	(62,73,110)
Geographical, ethnic and lifestyle variability	Regional diet, sanitation, ethnicity and lifestyle influence baseline microbiome composition.	Cross-population variability complicates the development of universal microbial biomarkers for PDAC.	External validation across independent geographical cohorts remains limited; population-specific signatures frequently observed.	(21,29,58)

PDAC, pancreatic ductal adenocarcinoma; CP, chronic pancreatitis; SCFA, short-chain fatty acid; T2D, type II diabetes; PPI, proton pump inhibitor.

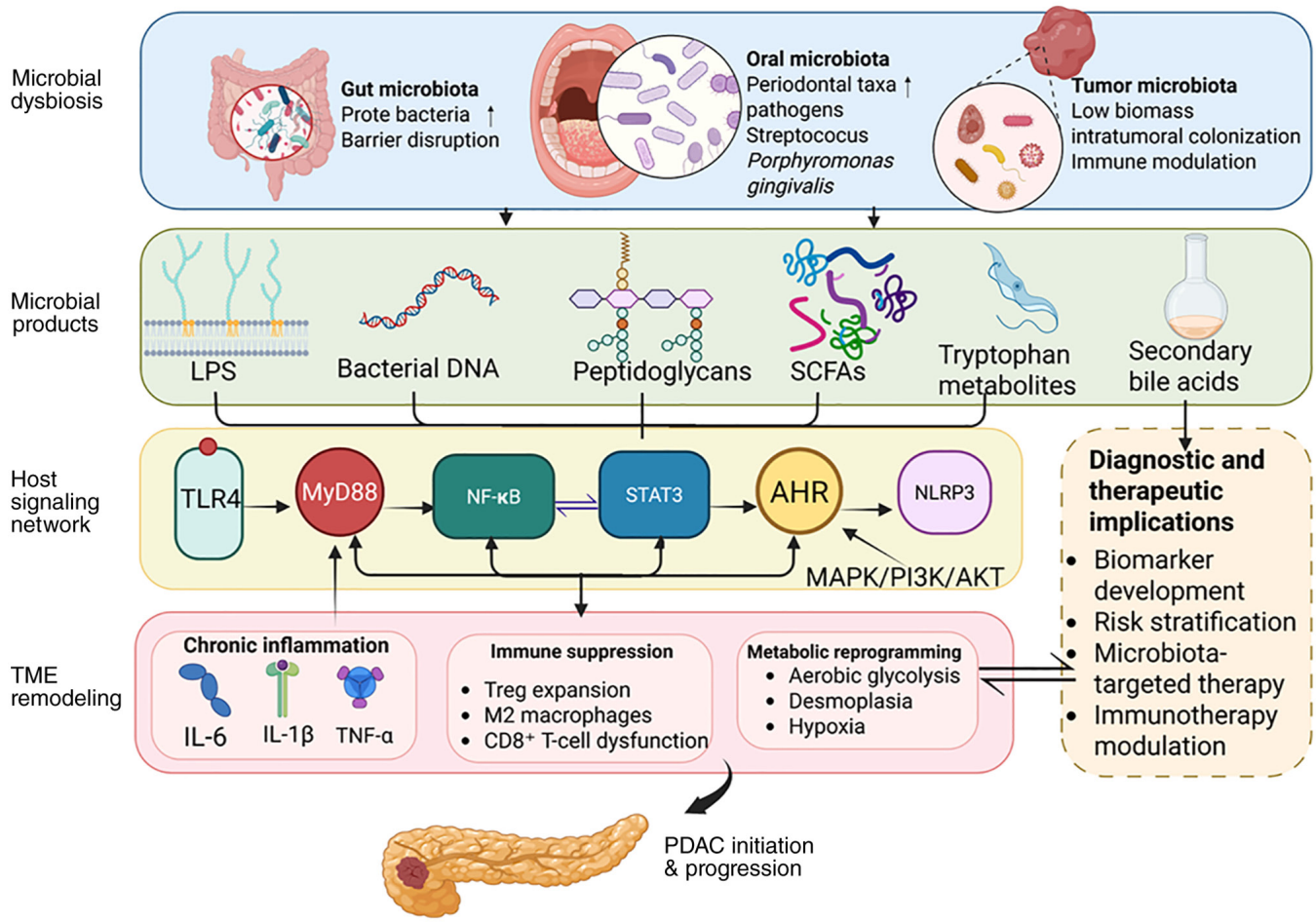

Figure 2. Mechanistic links between microbiota-derived products, PDAC progression and TME remodeling. Microbial dysbiosis in the gut, oral cavity and tumor- or tissue-associated microbial communities may contribute to PDAC progression through microbiota-derived molecules and metabolites, including LPS, bacterial DNA, peptidoglycans, SCFAs, tryptophan metabolites and secondary bile acids. These microbial products may engage or converge on host signaling pathways, including the TLR4, MyD88, NF-κB, STAT3, AHR, NLRP3 and MAPK/PI3K/AKT signaling pathways. Collectively, these pathways may promote chronic inflammation via IL-6, IL-1β and TNF-α production, immune suppression via mechanisms such as Treg expansion, M2 macrophage polarization and $CD8^+$ T-cell dysfunction; and iii) metabolic reprogramming , thereby facilitating TME remodeling and PDAC initiation and progression. Potential clinical implications of these microbiota-based mechanistic links in PDAC include biomarker development, microbial biomarker-based risk stratification, microbiota-targeted therapy and immunotherapy modulation. SCFA, short-chain fatty acid; LPS, lipopolysaccharide; TLR4, toll-like receptor 4; MyD88, myeloid differentiation primary response 88; AHR, aryl hydrocarbon receptor; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3; Treg, regulatory T cell; PDAC, pancreatic ductal adenocarcinoma; TME, tumor microenvironment. Created with BioRender.com.

Akkermansia muciniphila, observed in long-term survivors also supports a possible prognostic role of the microbiome in PDAC (34).

Beyond fecal biomarkers, multi-site microbial profiling may improve the diagnostic performance of microbiota-based classifiers. Although fecal samples remain attractive for non-invasive screening due to their accessibility, oral, biliary and tissue-associated microbiota have also been extensively investigated and may provide complementary diagnostic information related to pancreatic carcinogenesis (119,129). Saliva, in particular, represents a non-invasive diagnostic fluid with potential applications in transcriptomic and metabolomic profiling (130). However, the identification of consistent, disease-specific microbial signatures remains challenging regardless of body site, and the functions of individual microbial taxa in PDAC remain incompletely elucidated (131).

Preclinical studies have also indicated that microbiota-targeted interventions, including probiotic treatments and metabolite modulation, may influence tumor biology and therapeutic responses (132,133). Although the diagnostic utility of these interventions has not yet been established, these findings highlight the potential of precision microbiome approaches in PDAC. Future studies should focus on large-scale, prospective, multicenter cohorts with longitudinal sampling and integrated multi-omics analyses. Developing reproducible, function-based biomarker models rather than relying solely on descriptive taxonomic associations will be important for the clinical translation of microbiota-based biomarker models in PDAC. Furthermore, the validation of microbiome-based strategies in diverse clinical settings may improve early detection, risk stratification and personalized treatment management in patients with PDAC.

7. Conclusions

PDAC remains one of the most lethal malignancies, primarily due to delayed diagnosis and the lack of effective population-level screening strategies. Increasing evidence has

indicated that gut, oral and tissue-associated microbiota may be potential sources of non-invasive diagnostic biomarkers. Microbiome-based predictive models have demonstrated promising discriminatory performance, particularly when combined with established clinical markers of PDAC, such as CA19-9 or metabolomic signatures.

Mechanistic studies have further suggested that microbial dysbiosis may contribute to PDAC progression through immune modulation, inflammatory signaling and metabolic reprogramming. These findings provide biological support for microbiome-informed diagnostic approaches. However, clinical translation of these approaches remains limited by methodological heterogeneity, host-related variability, low microbial biomass in pancreatic tissues and a lack of large-scale prospective validation studies.

With further methodological standardization, functional validation and prospective clinical evaluation, microbiome-based diagnostic strategies may eventually complement existing diagnostic approaches and improve early detection and risk stratification in patients with PDAC.

Acknowledgements

Not applicable.

Funding

The present review was supported by the National Natural Science Foundation of China (grant no. 82102431), the Natural Science Foundation of Gansu Province, China (grant no. 26JRRA817), the Key Incubation Project Funds of The Second Hospital & Clinical Medical School, Lanzhou University (grant no. 2025-21-zdfy-004) and the Talent Introduction Plan of The Second Hospital & Clinical Medical School, Lanzhou University (grant no. yjrckyqdj-2021-03).

Availability of data and materials

Not applicable.

Authors' contributions

HJ and JY conceived the study and contributed to writing the original draft. HJ, HS and JY reviewed and edited the manuscript. HJ constructed figures. JY was also responsible for supervising the study and acquiring funding. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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