

Gut Microbiome Dysbiosis: An Ecological and Functional Framework for Detection, Prevention and Therapeutic Management (2)

PART II: DETECTION OF GUT MICROBIOME DYSBIOSIS

From Taxonomic Testing to Functional and Ecological Assessment

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ABSTRACT

Background: The clinical translation of gut microbiome science has been constrained by fundamental methodological and conceptual challenges. While next-generation sequencing technologies have enabled comprehensive characterization of the human gut microbiome, the translation of this knowledge into routine clinical practice remains limited. Current stool-based microbiome analyses are constrained by biological variability, methodological heterogeneity, limited standardization and insufficient clinical validation.

Objectives: This review examines the current state of gut microbiome detection and assessment, with emphasis on the limitations of taxonomic profiling and the potential for functional and ecological approaches. It critically evaluates available testing technologies, the problem of reference ranges, the distinction between stool and mucosal microbiomes, and the emerging role of artificial intelligence in microbiome diagnostics.

Methods: A narrative review was conducted based on synthesis of contemporary scientific literature addressing gut microbiome detection technologies, methodological challenges, biological variability, and clinical applications of microbiome testing.

Results: The clinical detection of gut microbiome dysbiosis presents a fundamental methodological challenge: the microbial ecosystem is intrinsically variable, whereas conventional clinical diagnostics are based on relatively stable deviations from reference ranges. A single stool sample provides only a snapshot of an ecosystem in motion. Current microbiome testing technologies, including qPCR, 16S rRNA sequencing, and shotgun metagenomics, provide different levels of information but all share limitations for clinical application. The authors propose that clinically meaningful assessment requires integration of microbial diversity, functional capacity, ecological stability, resistance and resilience. Disease-associated microbial signatures remain investigational, and artificial intelligence approaches require rigorous validation before clinical implementation.

Conclusions: Current microbiome diagnostics are better at describing microbial composition than at definitively diagnosing ecological dysfunction. The central limitations are not merely technological but arise from the biology of the ecosystem itself. A clinically meaningful understanding of dysbiosis requires a transition from predominantly taxonomic interpretation toward integrated assessment of microbial diversity, functional capacity, ecological stability and host-microbiome interactions. Routine microbiome testing should be interpreted cautiously and should not be used in isolation to determine therapeutic management.

Public Health Utility Statement: This review provides clinicians with a critical framework for understanding the current limitations of microbiome testing and the importance of functional and ecological assessment. It emphasizes that microbiome testing should be interpreted cautiously and should not replace clinical judgment in patient management.

Keywords: gut microbiome; dysbiosis; microbiome diagnostics; 16S rRNA sequencing; shotgun metagenomics; microbial diversity; artificial intelligence; clinical validation

Disbioza microbiomului intestinal: Un cadru ecologic și funcțional pentru depistare, prevenire și gestionare terapeutică

PARTEA A II-A: DETECTAREA DISBIOZEI MICROBIOMULUI INTESTINAL

De la testarea taxonomică la evaluarea funcțională și ecologică

REZUMAT

Context: Transpunerea clinică a științei microbiomului intestinal a fost constrânsă de provocări metodologice și conceptuale fundamentale. Deși tehnologiile de secvențiere de generație nouă au permis caracterizarea comprehensivă a microbiomului intestinal uman, transpunerea acestor cunoștințe în practica clinică de rutină rămâne limitată. Analizele actuale ale microbiomului pe bază de scaun sunt constrânse de variabilitatea biologică, eterogenitatea metodologică, standardizarea limitată și validarea clinică insuficientă.

Obiective: Acest review examinează starea actuală a detectării și evaluării microbiomului intestinal, cu accent pe limitările profilării taxonomice și potențialul abordărilor funcționale și ecologice. Evaluează critic tehnologiile de testare disponibile, problema intervalelor de referință, distincția dintre microbiomul fecal și cel mucozal și rolul emergent al inteligenței artificiale în diagnosticul microbiomului.

Metode: A fost realizat un review narativ bazat pe sinteza literaturii științifice contemporane privind tehnologiile de detectare a microbiomului, provocările metodologice, variabilitatea biologică și aplicațiile clinice ale testării microbiomului.

Rezultate: Detectarea clinică a disbiozei microbiomului intestinal prezintă o provocare metodologică fundamentală: ecosistemul microbial este intrinsec variabil, în timp ce diagnosticul clinic convențional se bazează pe abateri relativ stabile de la intervalele de referință. O probă unică de scaun oferă doar o imagine instantanee a unui ecosistem în mișcare. Tehnologiile actuale de testare a microbiomului, inclusiv qPCR, secvențierea 16S rRNA și metagenomica shotgun, oferă diferite niveluri de informații, dar toate prezintă limitări pentru aplicarea clinică. Autorii propun că evaluarea clinic semnificativă necesită integrarea diversității microbiene, capacității funcționale, stabilității ecologice, rezistenței și rezilienței. Semnăturile microbiene asociate bolilor rămân în stadii investigaționale, iar abordările bazate pe inteligență artificială necesită validare riguroasă înainte de implementarea clinică.

Concluzii: Diagnosticul actual al microbiomului este mai bun la descrierea compoziției microbiene decât la diagnosticarea definitivă a disfuncției ecologice. Limitările centrale nu sunt doar tehnologice, ci decurg din biologia ecosistemului însuși. O înțelegere clinic semnificativă a disbiozei necesită o tranziție de la interpretarea predominant taxonomică către evaluarea integrată a diversității microbiene, capacității funcționale, stabilității ecologice și interacțiunilor gazdă-microbiom. Testarea de rutină a microbiomului ar trebui interpretată cu prudență și nu ar trebui utilizată izolat pentru a determina managementul terapeutic.

Declarație privind utilitatea publică: Acest review oferă clinicienilor un cadru critic pentru înțelegerea limitărilor actuale ale testării microbiomului și importanța evaluării funcționale și ecologice. Subliniem că testarea microbiomului ar trebui interpretată cu prudență și nu ar trebui să înlocuiască judecata clinică în managementul pacientului.

Cuvinte cheie: microbiom intestinal; disbioză; diagnostic microbiom; secvențiere 16S rRNA; metagenomică shotgun; diversitate microbială; inteligență artificială; validare clinică

7. WHY IS DYSBIOSIS DIFFICULT TO DETECT?

7.1. The Fundamental Methodological Challenge

The clinical detection of gut microbiome dysbiosis presents a fundamental methodological challenge: the microbial ecosystem is intrinsically variable, whereas conventional clinical diagnostics are largely based on the identification of relatively stable deviations from established reference ranges¹ [1]. This distinction is particularly important when interpreting microbiome data. In conventional clinical medicine, parameters such as serum sodium, potassium, liver enzymes or other biochemical variables are generally interpreted against relatively well-defined physiological ranges [2]. The gut microbiome operates according to different ecological principles. It is an external ecosystem that

¹ The comparison between the internal milieu and the external ecosystem is crucial for understanding why microbiome testing differs fundamentally from conventional laboratory testing. While the internal milieu is characterized by narrow physiological ranges, the gut microbiome is a dynamic ecosystem whose composition can vary substantially without indicating pathology [1,4].

continuously interacts with food, medications, host physiology and environmental exposures [3]. Consequently, substantial variation in microbial composition may occur without necessarily representing pathology [4].

The authors emphasize that this variability has direct implications for the interpretation of microbiome tests. A single stool sample provides a snapshot of an ecosystem in motion [5]. It may describe what microorganisms are detectable at a particular time and under particular physiological and environmental conditions, but it does not necessarily establish whether the ecosystem is healthy, dysbiotic, resilient or functionally compromised [6].

7.2. The Problem of Reference Ranges

The concept of a universal reference microbiome is therefore problematic² [7]. The abundance of individual taxa may range widely among healthy individuals, and microbial communities can adapt to differences in diet, geographic environment, medication exposure and host physiology [8]. The authors consequently argue that taxonomy may have limited ability to discriminate between health and dysbiosis when stool samples are investigated [9].

This does not imply that microbial composition is clinically irrelevant. Rather, it suggests that composition must be interpreted within a broader ecological framework [10]. A clinically meaningful assessment should increasingly consider whether the microbial community possesses adequate:

- Functional competence [11]
- Microbial richness [12]
- Ecological stability [13]
- Resistance to perturbation [14]
- Resilience following perturbation [15]

These properties are presented as characteristics of an optimally functioning microbial community [16].

7.3. Temporal Variability of Fecal Microbial Composition

The recognition that gut microbiome composition exhibits substantial temporal variability represents a fundamental challenge to the clinical application of microbiome testing³ [17]. Longitudinal studies employing frequent sampling have revealed that the taxonomic composition of fecal samples can vary considerably from day to day within the same individual [18].

The authors describe longitudinal observations illustrating the magnitude of this variability. Even when the same individual is repeatedly sampled, microbial composition may change considerably at genus and species levels [19]. In one longitudinal observation, only a relatively small proportion of species remained consistently detectable across repeated

² The absence of universally applicable reference ranges for the gut microbiome reflects the fundamental ecological nature of this system. Unlike serum electrolytes or liver enzymes, which are maintained within narrow physiological ranges, the gut microbiome exhibits substantial variation between healthy individuals, making it difficult to define a single "normal" composition [7,9].

³ The temporal variability of the gut microbiome has important implications for clinical practice. A single stool sample may reflect transient effects of recent dietary intake or medication exposure rather than the stable ecological state of the microbiome. Studies have shown that up to 30% of bacterial taxa can fluctuate substantially within a single individual over days to weeks, suggesting that multiple samples over time may provide a more accurate assessment of the ecosystem's health [17,18].

samples, demonstrating that taxonomic composition is considerably more dynamic than would be expected from conventional internal physiological parameters [20].

The factors influencing this variability include:

- Dietary intake—the consumption of specific foods, particularly plant-derived substrates, can rapidly and reproducibly alter microbial community structure [21].
- Colonic transit time—variations in bowel motility affect the composition of fecal samples, with rapid transit yielding different microbial profiles compared to slower transit times [22].
- Medications—numerous commonly prescribed drugs, including antibiotics, proton pump inhibitors, and metformin, exert significant effects on gut microbial composition [23].
- Travel—exposure to different environments and dietary practices can transiently alter the gut microbiome [24].
- Age—the gut microbiome undergoes substantial changes across the lifespan, with particularly marked alterations in early childhood and older age [25].

This variability does not imply that the gut microbiome is chaotic or unregulated. Rather, it reflects the dynamic nature of the ecosystem and its responsiveness to environmental inputs [26].

7.4. Differences Between Fecal and Mucosa-Associated Microbiota

A further limitation of stool-based microbiome analysis is the distinction between the fecal microbiota and the mucosa-associated microbiota⁴ [27]. A fecal sample represents microorganisms and microbial material present within the intestinal lumen at the time of sampling. It does not necessarily reproduce the microbial communities associated with the intestinal mucosa [28].

The authors explicitly caution that fecal microbiota may not fully represent microorganisms attached to the intestinal mucosa [29]. This distinction matters because microorganisms occupying different intestinal niches may perform different functions and interact differently with host tissues [30].

Consequently, the absence of a particular organism from a fecal sample does not necessarily mean its complete absence from the gastrointestinal ecosystem [31]. Similarly, its detection in stool does not establish that it is functionally dominant within the mucosal environment [32].

The stool microbiome should therefore be regarded as a valuable but partial ecological window into the intestinal microbial ecosystem [33].

7.5. The Problem of Biological Variability

Beyond temporal variability, the gut microbiome exhibits substantial inter-individual variability⁵ [34]. As noted in Part I, the MetaHIT study of 214 European individuals identified

⁴ The distinction between luminal and mucosa-associated microbiota is particularly important because mucosa-associated communities are more directly involved in interactions with the host immune system and may be more relevant to disease pathogenesis. However, mucosal sampling is invasive and not suitable for routine clinical application [27,29].

⁵ Inter-individual variability in gut microbiome composition reflects the complex interplay of genetics, environment, and lifestyle factors. This variability is not merely a confounder but may itself be biologically

approximately 1,150 prevalent bacterial species in the population, with each individual harboring at least 160 species. Remarkably, only 18 species were shared among all individuals in the cohort [35]. Moreover, the abundance of the 57 most common species varied by one to four orders of magnitude across individuals [36].

This inter-individual variability arises from multiple factors:

- Genetics—host genetic variation influences the gut microbiome through effects on immune function, metabolism and barrier integrity [37].
- Early life exposures—mode of delivery, breastfeeding, and early antibiotic exposure shape the developing microbiome [38].
- Geography and environment—individuals from different geographic regions harbor distinct microbial communities [39].
- Dietary patterns—long-term dietary habits are among the strongest determinants of gut microbiome composition [40].
- Medication history—exposure to antibiotics, proton pump inhibitors, and other medications leaves lasting effects on the microbiome [41].
- Lifestyle factors—physical activity, sleep patterns, and stress influence microbial ecology [42].

The authors emphasize that this extensive inter-individual variability further complicates the establishment of universal reference ranges for microbiome health [43].

7.6. Influence of Diet, Medications and Intestinal Transit

The effects of diet, medications and intestinal transit on gut microbiome composition are particularly relevant to the clinical interpretation of microbiome tests⁶ [44]. These factors can produce substantial changes in microbial composition over short time periods, potentially confounding the interpretation of a single stool sample [45].

Diet is among the most powerful modulators of gut microbiome composition [46]. Even short-term dietary changes can produce measurable alterations in microbial community structure [47]. A diet rich in plant-derived fiber promotes the growth of SCFA-producing bacteria, while a diet high in processed foods and low in fiber is associated with reduced diversity [48].

Medications exert significant effects on the gut microbiome [49]. Antibiotics are among the most potent perturbators, capable of producing lasting reductions in microbial diversity [50]. Proton pump inhibitors alter gastric pH and have been associated with changes in gut microbial composition [51]. Metformin, a commonly prescribed medication for type 2 diabetes, has been shown to increase the abundance of specific beneficial bacteria [52].

meaningful, as different microbial configurations may be optimal for different individuals depending on their genetic background and environmental exposures [34,43].

⁶ The influence of diet on the gut microbiome is so profound that dietary history should be carefully documented when interpreting microbiome test results. Even short-term dietary changes (within 24-48 hours) can produce measurable alterations in microbial composition, potentially confounding the interpretation of a single stool sample. Studies have shown that a diet rich in fiber can increase SCFA production by up to 30% within days, while a high-fat diet can rapidly shift the microbial community toward a pro-inflammatory profile [46,48].

Intestinal transit time affects the composition of fecal samples [53]. Rapid transit results in different microbial profiles compared to slower transit times [54]. This is clinically relevant because transit time may be affected by diet, medications and disease states [55].

The authors conclude that these factors must be carefully considered when interpreting microbiome test results [56]. A single stool sample may reflect transient effects of recent dietary intake or medication exposure rather than the stable ecological state of the microbiome [57].

8. FROM TAXONOMIC PROFILING TO FUNCTIONAL ASSESSMENT

8.1. Why Taxonomy Alone Is Insufficient

Traditional microbiological thinking tends to organize microbial communities according to the identity and abundance of individual microorganisms⁷ [58]. Modern microbiome science has expanded this perspective by recognizing that the biological effect of a microbial community depends substantially on its collective functional capacity [59].

This distinction is central to dysbiosis assessment [60]. The authors identify three recurrent characteristics of dysbiotic microbiomes:

1. Reduced microbial richness or diversity [61].
2. Depletion of short-chain fatty acid-producing bacteria [62].
3. Instability of microbial composition over time [63].

Importantly, these characteristics are presented as properties of the ecosystem rather than as a specific list of pathogenic microorganisms [64].

Thus, the clinically relevant question becomes progressively less:

Which bacteria are present?

and increasingly:

What ecological and metabolic functions does this microbial community retain? [65]

This distinction is particularly important because different microbial taxa may possess overlapping functional capabilities, whereas the same taxon may behave differently depending on the ecological environment in which it exists [66].

8.2. The Ecological Assessment Framework

The authors propose an ecological assessment framework that integrates multiple dimensions of microbiome evaluation⁸ [67]. This framework moves beyond taxonomic profiling to consider:

1. Taxonomic composition—the identity and abundance of microbial taxa [68].
2. Microbial diversity—measures such as the Shannon index or species richness [69].

⁷ The shift from taxonomic to functional assessment represents a fundamental paradigm change in microbiome science. Rather than asking which bacteria are present, clinicians and researchers should increasingly ask what functions the microbial community can perform and whether these functions are being preserved [58,59].

⁸ The ecological assessment framework recognizes that a healthy microbiome is characterized not by a specific taxonomic composition but by functional competence, diversity, and ecological stability. This framework provides a more clinically useful approach to understanding and managing dysbiosis [67,74].

3. Functional capacity—the metabolic and regulatory functions encoded in the metagenome [70].
4. Ecological stability—the capacity of the ecosystem to resist perturbation [71].
5. Ecological resilience—the capacity to recover following disturbance [72].
6. Host-microbiome interactions—the bidirectional relationships between the microbial community and host physiology [73].

This framework is consistent with the ecological model developed in Part I [74]. It recognizes that the gut microbiome is not a static collection of microorganisms but a dynamic ecosystem whose health depends on functional competence and ecological stability [75].

9. CURRENT MICROBIOME TESTING TECHNOLOGIES

9.1. Culture-Independent Approaches

The advent of culture-independent approaches has revolutionized the study of the gut microbiome⁹ [76]. These methods allow characterization of microbial communities without the need for cultivation, providing access to the vast majority of microorganisms that cannot be cultured using standard laboratory techniques [77].

The authors note that culture-independent approaches have been instrumental in revealing the extraordinary diversity of the human gut microbiome and its variation across populations and disease states [78]. However, they also emphasize that the translation of these methods to clinical practice has been constrained by methodological and interpretive challenges [79].

9.2. Quantitative Polymerase Chain Reaction (qPCR)

Quantitative polymerase chain reaction (qPCR) can provide quantitative information regarding selected microorganisms or microbial targets¹⁰ [80]. Its major advantage is specificity. When the clinical question concerns a predefined organism or group of organisms, qPCR can provide useful information regarding their abundance [81].

However, the authors identify a major limitation: qPCR examines selected microbial targets and therefore cannot, by itself, determine whether the entire microbial ecosystem is dysbiotic [82]. It may potentially become useful for longitudinal monitoring of selected organisms—for example, *Faecalibacterium* in particular clinical contexts—but targeted measurement does not provide a comprehensive assessment of microbial diversity or ecosystem function [83].

Therefore:

⁹ Culture-independent approaches have revealed that the vast majority of gut microorganisms cannot be cultured using standard laboratory techniques—estimates suggest that up to 70-80% of gut bacteria are recalcitrant to cultivation. This has dramatically expanded our understanding of microbial diversity but has also introduced new challenges for clinical interpretation, including the difficulty of establishing causality and the lack of standardized protocols [76,77].

¹⁰ Quantitative PCR has the advantage of specificity and quantitative accuracy but the limitation of examining only preselected targets. It cannot provide a comprehensive assessment of microbial diversity or ecosystem function, making it unsuitable for diagnosing dysbiosis in isolation. Recent advances in multiplex qPCR have expanded its capabilities, but it remains a targeted rather than a discovery-based approach [80,84].

qPCR can answer a targeted taxonomic question, but it cannot independently answer the broader ecological question of whether the gut microbiome is dysbiotic [84].

9.3. 16S rRNA Sequencing

16S ribosomal RNA sequencing represents a major methodological advance because it allows the characterization of bacterial communities beyond the limitations of culture-dependent microbiology¹¹ [85]. The 16S rRNA gene is present in all bacteria and archaea, contains both conserved and variable regions, and can be amplified using universal primers [86].

In the context of dysbiosis, its particular relevance lies in the possibility of estimating microbial diversity and community composition [87]. The authors explicitly associate the assessment of diversity with 16S rRNA sequencing while also identifying an important limitation: this approach primarily characterizes bacteria and does not provide equivalent information regarding fungi and viruses [88].

Consequently, 16S rRNA sequencing represents an important tool for ecological characterization but cannot be considered a complete representation of the entire intestinal microbiome [89]. Its clinical value therefore depends on the question being asked.

If the objective is to describe:

- Bacterial richness [90]
- Bacterial community structure [91]
- Relative abundance [92]
- Bacterial diversity [93]

16S rRNA sequencing can provide valuable information [94].

If the objective is to determine:

- The entire microbial ecosystem [95]
- Its metabolic capacity [96]
- Functional gene repertoire [97]
- Its integrated bacterial, fungal and viral composition [98]

Additional methodological approaches are required [99].

9.4. Shotgun Metagenomic Sequencing

Shotgun metagenomic sequencing represents a broader approach because it interrogates microbial genetic material without restricting analysis to a particular phylogenetic marker¹²

¹¹ The 16S rRNA gene is a valuable phylogenetic marker that allows identification of bacteria and archaea. However, it does not provide information about fungi, viruses, or the functional capabilities of the microbial community. This limitation must be considered when interpreting 16S rRNA sequencing results. Additionally, the choice of primers and sequencing platform can significantly affect the results, making standardization challenging [85,89].

¹² Shotgun metagenomics provides a more comprehensive view of the microbial community than 16S rRNA sequencing, including information about functional potential. However, the presence of functional genes does not necessarily indicate active expression, and metabolomic approaches are needed to assess actual functional activity. The cost and computational complexity of shotgun metagenomics have decreased significantly, making it increasingly accessible for clinical applications [100,105].

[100]. Unlike 16S rRNA sequencing, which targets a single gene, shotgun metagenomics sequences all DNA present in a sample [101].

The authors identify shotgun metagenomic sequencing as capable of providing information extending beyond bacteria and, importantly, as potentially better suited to evaluating both diversity and functional capacities such as pathways associated with butyrate production [102].

This is particularly important for the ecological interpretation of dysbiosis [103]. If the defining problem is a loss of microbial function, then a methodology capable of identifying the genetic repertoire associated with metabolic pathways may ultimately be more informative than a purely taxonomic profile [104].

Nevertheless, the presence of a functional gene does not necessarily demonstrate that the corresponding metabolic pathway is actively expressed or that its products reach biologically relevant concentrations [105]. Therefore, even metagenomic information should not automatically be equated with functional activity [106].

This leads to an important conceptual hierarchy:

Taxonomic composition → *Functional potential* → *Actual functional activity* →
Clinical significance [107]

Each level provides different information, and movement from one level to the next requires additional data and inference [108].

9.5. Functional and Metabolic Profiling

Beyond taxonomic and metagenomic characterization, functional and metabolic profiling can provide information regarding the actual activity of the microbial ecosystem¹³ [109]. Metabolomics—the comprehensive analysis of metabolites in biological samples—can identify microbial products and their concentrations [110].

The authors note that microbial metabolites can be detected in blood, saliva, urine, cerebrospinal fluid, breast milk and bile [111]. This suggests that functional assessment of the gut microbiome may be possible through analysis of systemic metabolites [112].

However, metabolomic approaches face their own challenges, including:

- The complexity of the metabolome [113]
- The difficulty of distinguishing microbial from host-derived metabolites [114]
- The influence of diet on metabolite profiles [115]
- The need for large reference databases [116]
- Limited standardization across laboratories [117]

The authors suggest that future clinical applications may integrate metagenomic and metabolomic data for more comprehensive assessment of the gut microbiome [118].

¹³ Metabolomics offers the potential to assess actual functional activity of the gut microbiome, not just taxonomic composition or functional potential. However, metabolomic approaches face significant technical and interpretive challenges that must be addressed before clinical implementation. Recent advances in mass spectrometry and nuclear magnetic resonance spectroscopy have expanded the coverage of the metabolome, but standardization remains a challenge [109,113].

9.6. Stool Microbiome Versus Mucosal Microbiome

The distinction between stool-based and mucosa-based sampling is particularly important for clinical interpretation¹⁴ [119]. As noted earlier, fecal microbiota may not fully represent microorganisms attached to the intestinal mucosa [120].

The authors emphasize that microorganisms occupying different intestinal niches may perform different functions and interact differently with host tissues [121]. Mucosa-associated microbiota may be more relevant to immune interactions and barrier function, while luminal microbiota may be more relevant to metabolic functions [122].

However, mucosal sampling is invasive and not suitable for routine clinical application [123]. Stool samples remain the most practical approach for microbiome assessment [124].

The authors conclude that stool samples should be regarded as a valuable but incomplete representation of the intestinal microbial ecosystem [125]. Clinicians should be aware of this limitation when interpreting stool microbiome results [126].

10. CURRENT LIMITATIONS OF MICROBIOME TESTING IN CLINICAL PRACTICE

10.1. Lack of Methodological Standardization

The translation of microbiome science into routine clinical practice remains constrained by several methodological limitations¹⁵ [127]. The authors state explicitly that routine microbiome testing is currently limited by insufficient standardization and inadequate evidence for using test results to guide therapeutic decisions [128]. Fecal analyses therefore have limited capacity to guide treatment in contemporary clinical practice [129].

Several interconnected problems emerge. Differences in:

- Sample collection [130]
- Storage conditions [131]
- DNA extraction methods [132]
- Sequencing methodology [133]
- Bioinformatic processing [134]
- Taxonomic classification [135]
- Reporting procedures [136]

may influence the resulting microbiome profile [137]. Therefore, two laboratories may produce substantially different interpretations from biologically similar samples [138].

¹⁴ The stool microbiome provides valuable information but represents only a partial view of the intestinal microbial ecosystem. Mucosa-associated communities may differ substantially from luminal communities and may be more relevant to immune interactions and disease pathogenesis. Studies have shown that mucosa-associated communities are more stable over time and more closely associated with host immune responses, but their clinical utility is limited by the invasiveness of sampling [119,121].

¹⁵ The lack of methodological standardization across laboratories is a major barrier to the clinical implementation of microbiome testing. Differences in sample collection, processing, and analysis can produce substantially different results from the same biological sample, limiting the reproducibility and clinical utility of microbiome testing. Efforts such as the Microbiome Quality Control (MBQC) project have been initiated to address these challenges, but consensus standards are still emerging [127,137].

10.2. Biological Variability

As discussed in Section 7, microbial composition is influenced by¹⁶:

- Diet [139]
- Age [140]
- Intestinal transit [141]
- Medication exposure [142]
- Disease [143]
- Environmental conditions [144]

The authors emphasize that changes may occur even from day to day and that intestinal transit itself can alter the composition observed in fecal samples [145]. This makes interpretation of isolated measurements particularly difficult [146].

10.3. Taxonomic Overinterpretation

One of the principal risks in contemporary microbiome medicine is the tendency to interpret the presence or absence of individual organisms as evidence of disease¹⁷ [147]. Such an approach may be misleading because the microbiome is an ecosystem characterized by interactions, functional redundancy and contextual dependence [148].

The authors argue that the focus should shift from taxonomy toward broader properties such as diversity, functional capacity and ecological stability [149]. A clinically meaningful assessment should integrate multiple dimensions of microbiome evaluation rather than relying on the presence or absence of particular taxa [150].

10.4. Insufficient Evidence for Routine Therapeutic Decision-Making

Despite the substantial research progress in characterizing gut microbiome alterations in disease states, the translation of this knowledge into clinical practice remains limited¹⁸ [151]. The current clinical utility of microbiome testing is constrained by:

1. Insufficient evidence to guide therapeutic decisions—while research has established associations between gut microbiome composition and disease states, prospective interventional studies demonstrating that microbiome-directed interventions improve clinical outcomes are limited, particularly for conditions beyond recurrent *Clostridioides difficile* infection [152].

¹⁶ The substantial biological variability of the gut microbiome, both within and between individuals, poses a major challenge for clinical interpretation. Single stool samples may not provide a reliable assessment of the long-term ecological state of the microbiome. Recent studies have suggested that repeated sampling over time (e.g., weekly or monthly) may provide a more accurate picture of an individual's stable microbial composition, but this approach is not yet practical for routine clinical care [139,145].

¹⁷ Overinterpretation of taxonomic data is a significant risk in clinical microbiome testing. The presence or absence of a particular microorganism does not, by itself, establish a diagnosis of dysbiosis, as the functional consequences depend on the broader ecological context. For example, the presence of *Escherichia coli* in the gut is normal, but a relative increase in its abundance may be associated with dysbiosis depending on the overall community structure and host context [147,149].

¹⁸ The current evidence base is insufficient to support routine clinical use of microbiome testing for guiding therapeutic decisions. While research has established associations between microbial alterations and disease states, prospective interventional studies demonstrating improved clinical outcomes are limited. The field is currently at a stage similar to early genomics, where technological advances outpaced the development of clinical applications [151,152].

2. Limited predictive value—the ability of fecal microbiome profiles to predict treatment responses or disease outcomes has not been sufficiently validated to support routine clinical use [153].
3. Taxonomic focus of commercial tests—most currently available microbiome testing services emphasize taxonomic characterization, which may be less informative than functional and ecological assessments for clinical decision-making [154].

Nevertheless, promising research directions suggest potential future applications, including:

- Use of microbial signatures to predict response to biologic therapies in inflammatory bowel disease [155]
- Screening for colorectal cancer [156]
- Monitoring disease activity in chronic conditions [157]

However, the authors emphasize that these applications remain in early stages of development and have not yet achieved the validation required for clinical implementation [158].

10.5. Current and Emerging Prognostic Applications

Despite the limitations of current testing, the authors acknowledge that pilot studies have suggested potential prognostic applications¹⁹ [159]. For example:

- Inflammatory bowel disease—microbial signatures may help predict response to biological treatment [160].
- Colorectal cancer—fecal microbial signatures may contribute to screening [161].
- Metabolic disease—microbial features may correlate with treatment response [162].

However, the authors emphasize that these applications remain preliminary and require further validation before clinical implementation [163]. The current evidence does not support routine use of microbiome testing for prognostic purposes [164].

11. DISEASE-ASSOCIATED MICROBIAL SIGNATURES

11.1. Evidence for Disease-Specific Patterns

The identification of disease-associated microbial signatures represents one of the most attractive areas of microbiome research²⁰ [165]. The possibility of identifying a reproducible microbial pattern associated with a particular disease raises the prospect of microbiome-based diagnosis, prognosis and prediction of therapeutic response [166].

¹⁹ While pilot studies have suggested promising applications for microbiome testing in predicting treatment response and screening for disease, these applications remain in early stages and require rigorous validation before clinical implementation. Large-scale prospective studies with standardized protocols are needed to establish the clinical utility of microbiome-based prognostic markers [159,163].

²⁰ The distinction between disease-associated and disease-specific microbial signatures is crucial for clinical interpretation. A statistical association does not establish a diagnostic pattern with sufficient sensitivity and specificity for clinical use. The overlapping nature of dysbiotic signatures across diseases suggests that common ecological mechanisms may be at play, rather than disease-specific taxonomic shifts [165,168].

However, the authors adopt an appropriately cautious position. Although microbial differences have been reported across multiple chronic diseases, the observed alterations frequently overlap, suggesting a broad spectrum of dysbiosis rather than a unique taxonomic signature for each disease [167].

This distinction is crucial. A disease-associated microbiome is not necessarily equivalent to a disease-specific microbiome [168]. The former indicates statistical association. The latter would require a reproducible pattern with sufficient sensitivity, specificity and external validation to distinguish one clinical condition from other conditions and from health [169].

11.2. The Common Features of Dysbiotic Microbiomes

As described in Part I, the authors identify three recurrent characteristics of dysbiotic microbiomes²¹:

1. Loss of microbial richness—reduced species diversity [170].
2. Depletion of SCFA-producing bacteria—particularly butyrate producers [171].
3. Temporal instability—reduced ecological resilience [172].

These features are observed across multiple disease states, supporting the view that dysbiosis represents a general ecological pattern rather than a disease-specific fingerprint [173].

11.3. Disease-Specific Examples

The authors describe several disease-specific examples of microbiome alterations²²:

Atopic Dermatitis and Atopy: Longitudinal monitoring of European children over 10 years demonstrated that low Shannon index and reduced fermentative bacteria (*Faecalibacterium* and *Lachnobacterium*) in the first weeks of life predicted the subsequent development of atopic dermatitis [174]. This association was independent of delivery mode or breastfeeding duration, highlighting the importance of early-life microbial diversity [175].

Type 1 Diabetes: International studies have certified that diversity changes and loss of microbial genes involved in SCFA biosynthesis precede the appearance of autoantibodies against pancreatic Langerhans islets [176]. This offers a screening window before clinical onset of autoimmune disease [177].

Autism Spectrum Disorder: Studies have demonstrated severe reduction of *Coprococcus* (a key butyrate producer) and *Prevotella* in autistic children [178]. The reduction in microbial richness (OTUs) correlated directly with the severity of behavioral manifestations, including language disorders [179].

²¹ The concept of a "general spectrum of dysbiosis" has emerged from studies showing that the same microbial alterations—reduced diversity, depletion of SCFA producers, and temporal instability—are observed across multiple disease states, suggesting that dysbiosis represents a common ecological pattern rather than a disease-specific signature. This has important implications for therapeutic strategies, as interventions targeting these common features may have broad applicability [167,173].

²² The disease-specific examples illustrate that while there are common features of dysbiosis across conditions, there are also condition-specific patterns that may have diagnostic and prognostic value. However, these patterns require validation before clinical application. The predictive value of early-life microbial diversity for atopic disease, for example, has been replicated in multiple cohorts, making it one of the most robust findings in microbiome epidemiology [174,178,180].

Inflammatory Bowel Disease: In ulcerative colitis and Crohn's disease, acute flares are characterized by a collapse of Chao1 and Shannon indices [180]. Maintenance of high instability during remission phases represents a major negative prognostic marker, indicating an imminent risk of relapse [181]. This is correlated with collapse of *Faecalibacterium* populations and increases in oxygen-resistant bacteria (*Fusobacterium*, *Escherichia*) [182].

11.4. Association Versus Causality

The authors emphasize that the observation of disease-associated microbial alterations does not establish causality²³ [183]. The observed changes could represent:

- Causal factors—microbial alterations that contribute to disease development [184]
- Consequences of disease—changes that result from the disease process [185]
- Responses to treatment—alterations induced by pharmacological interventions [186]
- Consequences of disease-related changes in diet or physiology—secondary changes resulting from the disease state [187]

The authors suggest that the answer is likely to be "both"—that microbial alterations may contribute to disease processes while the disease itself may further modify the microbiome [188].

12. ARTIFICIAL INTELLIGENCE AND THE FUTURE OF MICROBIOME DIAGNOSTICS

12.1. The Promise of AI-Assisted Microbiome Analysis

The increasing complexity of microbiome data creates a natural role for computational approaches, including artificial intelligence (AI) and machine learning²⁴ [189]. The authors explicitly identify AI as a future direction for improving the accuracy of microbiome-related diagnosis and prognosis [190].

The rationale is straightforward: microbiome datasets are multidimensional [191]. They may contain thousands of microbial features together with clinical, metabolic, dietary and demographic variables [192]. Conventional statistical approaches may therefore have difficulty capturing nonlinear interactions among these variables [193].

AI-based models could potentially integrate:

- Microbial composition [194]
- Microbial diversity [195]
- Functional gene profiles [196]

²³ The bidirectional relationship between microbial alterations and disease has important implications for clinical management. Interventions addressing both the microbiome and the disease process may be necessary, as microbial changes can be both causes and consequences of disease. This complexity underscores the need for integrated approaches that consider the dynamic interplay between the microbiome and host physiology [183,188].

²⁴ Artificial intelligence offers the potential to integrate multidimensional microbiome data with clinical information, identifying patterns that may not be apparent from taxonomic analysis alone. However, AI models require rigorous validation before clinical implementation. The "black box" nature of many AI algorithms raises concerns about interpretability and clinical trust, which must be addressed through the development of explainable AI approaches [189,193].

- Microbial metabolites [197]
- Clinical phenotype [198]
- Medication exposure [199]
- Dietary characteristics [200]
- Longitudinal changes [201]

Such integration could eventually permit the identification of clinically meaningful patterns that cannot be recognized from isolated microbial taxa [202].

12.2. Potential Applications

The authors describe several potential applications of AI-assisted microbiome analysis²⁵:

Pattern Recognition: Advanced AI algorithms can process massive heterogeneous multi-omic datasets (metagenomic, metabolomic, transcriptomic) generated through whole genome shotgun sequencing [203]. Computerized systems can go beyond simple identification of bacterial species, analyzing complex networks of metabolic interactions and enzymatic kinetics [204].

Clinical Prediction: By correlating complex microbial signatures with patients' clinical, imaging and biochemical data, AI-based predictive models can generate individualized risk scores [205]. This tool could enable not only precision diagnosis of functional dysbiosis but also anticipation of therapeutic response to biologics in IBD or non-invasive early screening for colorectal carcinoma [206].

Treatment Optimization: Machine learning algorithms applied to large-scale microbiome datasets may enable prediction of treatment responses and optimization of intervention strategies [207].

12.3. Challenges and Limitations

However, computational complexity should not be confused with clinical validity²⁶ [208]. An AI model may identify a statistical association without establishing biological causality [209]. Furthermore, models trained on one population may perform poorly in another because microbiome composition is strongly influenced by geographic, dietary, cultural and environmental factors [210].

Consequently, future microbiome AI systems will require:

1. Large and diverse datasets [211]
2. Longitudinal validation [212]

²⁵ AI-assisted microbiome analysis has the potential to enable precision diagnosis, prediction of therapeutic response, and early screening for disease. However, these applications require rigorous validation and demonstration of clinical benefit. Recent studies have shown that machine learning models can predict inflammatory bowel disease status with high accuracy from fecal metagenomic data, but these models have yet to be validated in diverse populations and clinical settings [203,206].

²⁶ Computational models, including AI, must be rigorously validated against clinical outcomes. Statistical associations do not establish causality, and models trained on one population may not generalize to others due to geographic, dietary, and cultural differences in microbiome composition. The phenomenon of "population-specific" microbial signatures highlights the need for diverse training datasets and careful external validation [208,210].

3. External validation across populations [213]
4. Standardized microbiome measurements [214]
5. Transparent performance metrics [215]
6. Clinical interpretability [216]
7. Demonstration of actual benefit in patient management [217]

The authors conclude that AI should be considered a potential integrative layer of microbiome medicine, rather than a substitute for biological validation [218].

12.4. The Path to Clinical Implementation

The translation of AI-based microbiome diagnostics into clinical practice will require²⁷:

- Rigorous validation of candidate biomarkers and interventions through prospective clinical trials demonstrating improved patient outcomes [219]
- Addressing the current limitations of microbiome testing—including lack of standardization, insufficient evidence for clinical decision-making, and the need for functional rather than merely taxonomic assessment [220]
- Development of user-friendly interfaces for clinicians [221]
- Integration with electronic health records [222]
- Demonstration of cost-effectiveness [223]

The authors emphasize that these requirements must be met before AI-based microbiome diagnostics can be widely implemented [224].

13. TOWARD AN ECOLOGICAL DEFINITION OF MICROBIOME HEALTH

13.1. The Ecological Model of Microbiome Health

The preceding discussion suggests that the assessment of the gut microbiome should move toward an ecological model²⁸ [225]. According to the framework presented in the source material, the optimal microbial community is characterized by functional competence and ecological stability, while microbial diversity and richness may serve as surrogate indicators of functional fitness and stability [226].

This model changes the clinical question [227]. Instead of asking:

Is this bacterium present? [228]

the assessment should progressively ask:

²⁷ The path to clinical implementation of AI-based microbiome diagnostics requires prospective clinical validation, demonstration of improved outcomes, integration with clinical workflows, and cost-effectiveness analysis. As with any new diagnostic technology, the burden of proof lies with demonstrating that AI-based approaches improve patient outcomes compared to current standards of care [219,223].

²⁸ The ecological model shifts the clinical question from "which bacteria are present?" to "does the ecosystem possess sufficient diversity, functional resources, and resilience to maintain host-supportive functions?" This represents a fundamental paradigm shift in microbiome assessment. It aligns with the recognition that the gut microbiome is an ecosystem whose health depends on functional competence and ecological stability rather than taxonomic identity per se [225,229].

Does the microbial ecosystem possess sufficient diversity, functional resources and resilience to maintain host-supportive functions under perturbation? [229]

Such an approach is more consistent with the biological nature of the gut microbiome [230].

13.2. Implications for Clinical Assessment

The ecological model has several implications for clinical assessment²⁹:

1. Interpretation of microbiome tests should consider context—including diet, medication exposure and disease status [231].
2. Multiple dimensions should be assessed—including diversity, functional capacity and stability, not just taxonomic composition [232].
3. Longitudinal assessment may be more informative than isolated measurements—though this is not yet practical for routine clinical care [233].
4. Reference ranges should be population-specific—and may need to account for geographic, dietary and lifestyle factors [234].
5. Functional assessment should complement taxonomic characterization—through metagenomic, metabolomic or other approaches [235].

13.3. The Therapeutic Implications of the Ecological Model

The ecological model also explains why adding microorganisms to the gastrointestinal tract does not necessarily result in durable ecological change³⁰ [236]. The source material describes the microbiome as an internally regulated ecosystem and notes that microorganisms introduced through interventions such as fecal transplantation may not necessarily persist over time [237].

This observation becomes particularly important for the therapeutic discussion in Part III [238]. If the microbiome is an internally regulated ecosystem, interventions must address the ecological conditions that support the desired microbial community, not merely introduce selected organisms [239].

14. CLINICAL APPLICATIONS AND RECOMMENDATIONS

14.1. When Should Microbiome Testing Be Considered?

Based on the current state of evidence, the authors identify limited clinical scenarios in which microbiome testing may be considered³¹:

²⁹ The ecological model has multiple implications for clinical assessment, including the need for context-aware interpretation, multidimensional assessment, and population-specific reference ranges. This approach recognizes that the "normal" microbiome varies substantially across populations and that clinical decision-making should be guided by functional and ecological parameters rather than a fixed taxonomic standard [231,234].

³⁰ The ecological model has important therapeutic implications: interventions must address the ecological conditions that support the desired microbial community, not merely introduce selected organisms. This explains why fecal transplantation without dietary modification may have limited durability [236,239].

³¹ Routine clinical use of microbiome testing is not currently supported by sufficient evidence. Testing should be limited to research settings or specific clinical questions where validated markers are available. Clinicians

1. When validated markers would be available—an international expert consensus strongly emphasizes that routine clinical use of microbiome testing should be supported by dedicated studies and validated markers [240].
2. In research settings—microbiome testing remains primarily a research tool, with limited evidence to support routine clinical application [241].
3. For specific clinical questions—such as monitoring selected organisms in particular contexts (e.g., *Faecalibacterium* in Crohn's disease) [242].

The authors do not recommend routine microbiome testing for:

- Patients after a course of antibiotics [243]
- Patients with a BMI of 35 or more [244]
- Crohn's disease patients not responding to biologicals [245]

While these scenarios are associated with microbiome alterations, the evidence does not yet support using microbiome testing to guide clinical decisions in these contexts [246].

14.2. The Current Role of Probiotics, Prebiotics and Symbiotics

The authors address the role of probiotics, prebiotics and symbiotics in the context of microbiome management³² [247].

Probiotics—defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"—represent a complementary approach to dietary modification for improving gut microbiome health [248]. Current evidence supports the use of specific probiotic strains for particular indications, including:

- Prevention and treatment of antibiotic-associated diarrhea [249]
- Management of IBD [250]
- Management of IBS [251]
- Prevention of allergic disease in high-risk populations [252]

However, the authors caution that the indiscriminate use of probiotics without consideration of strain-specific effects, appropriate indications, and individual patient characteristics is not supported by evidence [253]. Moreover, the gut microbiome's intrinsic ecological forces of resistance and resilience may limit the persistence of exogenously administered strains in the absence of supportive dietary conditions [254].

Prebiotics—non-digestible food ingredients that selectively stimulate the growth and activity of beneficial gut microorganisms—offer another approach to modulating the gut microbiome [255]. Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and inulin are among the most extensively studied prebiotics [256].

Symbiotics—combining probiotics with prebiotic substrates that support their growth—represent a rational integration of these approaches [257].

should be cautious about interpreting results from commercial microbiome tests, as the clinical utility of these tests has not been established for most indications [240,246].

³² Probiotics, prebiotics, and symbiotics offer complementary approaches to microbiome modulation. However, their use should be evidence-based and strain-specific, considering individual patient characteristics and the ecological context of the recipient microbiome. The indiscriminate use of probiotics is not supported by evidence and may even be harmful in certain populations, such as immunocompromised patients [247,253].

14.3. The Role of Diet in the Absence of Validated Tests

In the absence of validated, clinically actionable microbiome tests, the authors recommend dietary approaches to increase microbial production of SCFAs³³ [258]. This recommendation is based on the recognition that diet is the primary modulator of the gut microbiome and represents the most evidence-based approach to improving microbiome health [259].

The authors recommend:

- Progressive increase in consumption of vegetables, legumes, nuts, fruits and fermented foods [260]
- Careful attention to digestive tolerance and gradual adaptation to higher fiber intake [261]
- Consideration of dietary diversity as a principle rather than specific food prescriptions [262]

The symptoms of gas and bloating that often accompany increased fiber intake should not be grounds for immediate exclusion of plant-based foods [263]. Rather, a progressive, stepped approach to increasing fiber intake, allowing time for microbial adaptation and adjustment of the gut ecosystem, is recommended [264].

15. INTERIM SYNTHESIS AND TRANSITION TO PART III

15.1. Summary of Key Findings

Part II has examined the detection and assessment of gut microbiome dysbiosis, revealing several key findings³⁴:

1. Detection is fundamentally challenging—the gut microbiome is a dynamic ecosystem, not a static collection of microorganisms [265].
2. Current testing has significant limitations—including lack of standardization, biological variability, and insufficient clinical validation [266].
3. Taxonomy alone is insufficient—clinically meaningful assessment requires integration of diversity, functional capacity and stability [267].
4. Disease-associated signatures are not necessarily disease-specific—overlapping patterns are observed across multiple conditions [268].
5. AI offers promise but requires validation—computational approaches may enhance pattern recognition but must be validated against clinical outcomes [269].

³³ In the absence of validated clinical tests, dietary approaches to increase SCFA production represent the most evidence-based strategy for improving microbiome health. Dietary diversity and gradual adaptation to higher fiber intake are key principles. The recommended daily fiber intake of 25-35 grams for adults can be achieved through a diet rich in whole grains, legumes, fruits, and vegetables [258,261].

³⁴ The key findings of Part II emphasize the fundamental challenges in detecting dysbiosis, the limitations of current testing approaches, and the need for integrated ecological assessment. These findings underscore the importance of moving beyond taxonomic profiling toward functional and ecological evaluation of the gut microbiome [265,267].

15.2. The Clinical Implications

The authors conclude that current microbiome diagnostics are better at describing microbial composition than at definitively diagnosing ecological dysfunction [270]. The central limitations are not merely technological. They arise from the biology of the ecosystem itself³⁵ [271]:

- The microbiome is dynamic [272]
- Its composition varies between and within individuals [273]
- Stool provides only a partial representation [274]
- Taxonomy does not necessarily reflect function [275]
- Functional potential does not necessarily equal functional activity [276]
- Disease-associated microbial patterns are not necessarily disease-specific [277]

15.3. Transition to Prevention and Therapeutic Modulation

The authors emphasize that prevention and therapeutic modulation should be considered within an ecological framework³⁶ [278]. The recognition that dietary exposures, microbial substrates, medications, host factors and the gut-microbiome interaction collectively influence ecosystem stability provides the basis for a comprehensive approach to managing dysbiosis [279].

The source material consequently places emphasis on maintaining microbial diversity and ecological functionality, while identifying diet as an important modulator and antibiotics as major perturbing factors [280].

This leads naturally to the third part of the review: prevention and therapeutic modulation of gut microbiome dysbiosis [281].

16. CONCLUSIONS

16.1. Summary of Key Points

Part II of this review has examined the detection of gut microbiome dysbiosis, revealing several key insights³⁷:

1. The clinical detection of dysbiosis is fundamentally challenging because the gut microbiome is a dynamic ecosystem, not a static collection of microorganisms [282]. A single stool sample provides only a snapshot of an ecosystem in motion [283].

³⁵ The limitations of current microbiome diagnostics are not merely technological but arise from the biology of the ecosystem itself. This fundamental constraint must be recognized in clinical interpretation. Clinicians should be aware that current microbiome tests provide a snapshot of a dynamic system and should not be overinterpreted [270,271].

³⁶ The ecological framework developed in Parts I and II provides the foundation for a comprehensive approach to prevention and therapeutic modulation, which will be addressed in Part III. The key principle is that interventions should target the ecological conditions that support a healthy microbiome, not just the microbial taxa themselves [278,281].

³⁷ The key insights from Part II emphasize that current microbiome diagnostics are better at describing microbial composition than at definitively diagnosing ecological dysfunction, and that a transition to integrated ecological assessment is needed. The path forward requires standardized methodologies, longitudinal assessment, and integration of taxonomic and functional data [282,287].

2. Current microbiome testing technologies—including qPCR, 16S rRNA sequencing, and shotgun metagenomics—provide different levels of information but all share limitations for clinical application [284]. These include lack of standardization, biological variability, and insufficient clinical validation [285].
3. Taxonomy alone is insufficient for diagnosing dysbiosis [286]. A clinically meaningful assessment requires integration of multiple dimensions, including microbial diversity, functional capacity, ecological stability, resistance and resilience [287].
4. Disease-associated microbial signatures remain investigational [288]. While pilot studies have suggested potential applications in predicting treatment response or screening for disease, these require further validation before clinical implementation [289].
5. Artificial intelligence offers promise for enhancing microbiome diagnostics but requires rigorous validation of predictive models against clinical outcomes [290].

16.2. Implications for Clinical Practice

The authors conclude that current microbiome diagnostics are better at describing microbial composition than at definitively diagnosing ecological dysfunction [291]. The central limitations are not merely technological but arise from the biology of the ecosystem itself [292].

Clinicians should therefore interpret microbiome testing cautiously and should not use such testing in isolation to determine therapeutic management³⁸ [293]. In the absence of validated, clinically actionable microbiome tests, dietary approaches to increase microbial production of SCFAs represent the most evidence-based approach to improving microbiome health [294].

16.3. The Path Forward

The path forward requires³⁹:

- Standardized methodologies for sample collection, processing and analysis [295]
- Longitudinal assessment rather than isolated measurements [296]
- Integration of taxonomic and functional data [297]
- Clinically validated biomarkers [298]
- External validation of predictive models [299]
- Carefully designed randomized trials of microbiome-directed interventions [300]

The authors emphasize that the development of AI-based approaches may facilitate integration of multidimensional datasets, but computational prediction will require rigorous biological and clinical validation before routine implementation [301].

³⁸ Clinicians should interpret microbiome testing cautiously and should not use such testing in isolation to determine therapeutic management. Dietary approaches to increase SCFA production represent the most evidence-based strategy in the absence of validated tests. The focus should be on supporting the ecological conditions that promote a healthy microbiome rather than on manipulating specific microbial taxa [291,294].

³⁹ The path forward requires standardized methodologies, longitudinal assessment, integration of taxonomic and functional data, clinically validated biomarkers, and rigorous validation of predictive models. The development of AI-based approaches should be pursued, but these must be validated against clinical outcomes before implementation [295,300].

16.4. Key Takeaway

The central message of Part II is that⁴⁰:

Current microbiome diagnostics are better at describing microbial composition than at definitively diagnosing ecological dysfunction [302].

A clinically meaningful understanding of dysbiosis requires a transition from predominantly taxonomic interpretation toward integrated assessment of microbial diversity, functional capacity, ecological stability and host-microbiome interactions [303].

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⁴⁰ The central message of Part II is that current microbiome diagnostics are better at describing microbial composition than at definitively diagnosing ecological dysfunction, and that a transition to integrated ecological assessment is needed. This transition represents a paradigm shift in how clinicians approach the gut microbiome, moving from a taxonomic to an ecological and functional perspective [302,303].

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