CLINICAL ALERT

Statement on analysis and interpretation of clinical human gastrointestinal microbiome testing using next-generation sequencing in South Africa

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Advances in DNA sequencing technologies and computational tools over the past few years have led to vast improvements in the metagenomic analysis of the human microbiota. While this has also significantly improved our understanding of the role of the host-microbiome interaction in health and disease, the current clinical expectation is that testing, particularly of the gastrointestinal biome, can be used to diagnose, manage and treat patients. The authors outline the available technologies and highlight current limitations of these techniques to address this clinical demand. Through understanding the limitations of and need for more research and data collection, one can improve the appropriate utilisation and interpretation, as well as the current rational clinical application of these techniques.

S Afr Med J 2021;111(3):203-205. https://doi.org/10.7196/SAMJ.2021.v111i3.15336

The evolution of humans has occurred in association with many forms of microbial life.[1] The human body is home to a variety of microbes, including bacteria, archaea, fungi, parasites and viruses. This microbial community is referred to as the microbiota. The collective genome content of the microbiota or the microbial metagenome is known as the microbiome.[2]

Microbiota can be found throughout the human body, which contains about as many microbial cells as there are cells in the human body.[3]

Technical advances have led to an exponential rise in microbiome research and consequently to clinician and patient interest in the human microbiome, which has increased the clinical demand for laboratory microbiome testing and diagnostics. It is anticipated that these tests will provide enough information to diagnose and/or manage patients with chronic/complex conditions, where existing diagnostic and management tools might have been deficient.

It has been well established that the microbiota play an important role in human biology and that the microbe-human interaction may be significant in determining disease and health.[4]

Many factors, such as diet, age and medication, are known to change the composition and function of gastrointestinal tract (GIT) microbiota.[5] As an example, urbanisation, which involves, e.g. changes in diet, environmental exposures, antibiotics and caesarean section delivery, has been associated with changes and/or an imbalance in the gut microbiota and an increased risk of chronic diseases, such as asthma, inflammatory bowel disease and other systemic conditions, e.g. obesity, atherosclerosis, type 2 diabetes and Alzheimer's disease.[5-8]

Although there are clear associations between the composition and functions of certain microbiota and specific metabolic/neurological/autoimmune disorders, many of these relationships may not be causal. Establishing causation, albeit more challenging, is important to inform accurate diagnostic and safe management practices.[9]

The role of microbiome testing and how to use the results of these tests in an appropriate manner, also need to be established. The immense challenge of microbiota assessments is the standardisation of microbiota testing and reporting, as there are a variety of laboratory techniques. Even though new diagnostic approaches for microbiome analysis are more advanced and accessible, these are variable.

There is much interest in precision medicine and the tailoring of therapy according to individual microbiota profiles/functions based on accurate microbiota assessments. The future aim would be to manipulate the microbiota to affect function and composition – thereby treating disease. The fundamental notion of *primum non nocere*,[10] – 'first, do no harm' – should be inherent in such treatment. Any manipulation of the microbiota using strategies as dramatic as faecal microbiota transplantation, or potentially simple or 'harmless' interventions, such as probiotic administration, may potentially have a deleterious effect. Therefore, a case for a donor stool bank in South Africa (SA) is proposed.[11] As an example, even probiotics may have an effect on metabolic activities, cause systemic infection, such as endocarditis in susceptible populations, and have inappropriate effects on host immune responses.[12]

How are the human microbiota assessed?

The current standard 'microbial analysis' in clinical practice involves pathogen-specific diagnostic procedures, where clinicians suspect that a specific infectious aetiology causes a certain infectious clinical syndrome. These pathogen-specific tests, e.g. stool or sputum microscopy, culture and sensitivity, and standard molecular tests, e.g. multiplex polymerase chain reaction (PCR), focus on the differentiation and isolation of pathogens from normal flora. This testing does not specifically address the detection of the 'microbiome'

in that particular environment, i.e. all of the uncultivable normal commensals or transient colonisers or pathobionts.

Assessment of the microbiome requires the use of robust protocols. The standardisation of each step from sample collection to data analysis is empirical, but still emerging. An important step is the method used to profile the microbial community, which gives varying results. For example, 16S rRNA next-generation sequencing uses primers that target one or more of the variable regions within the 16S rRNA gene, giving a high-level, low-resolution overview of the bacterial and archaeal community.[13] This method is well tested and applicable to a wide range of sample types and study designs. It is also quick and cost effective. Whole metagenomic sequencing, however, provides a deeper insight into all the microbial genomes present in a sample, including viral and eukaryotic, narrowing down to strain-level resolution and allowing for functional assessment of genes present.[13] This method is more expensive to perform and the analysis is more complex than the targeted gene approach mentioned above.

The technical challenges include standardising of protocols for specimen collection, laboratory processing, testing/analysis and storage, reporting, interpretation, as well as various computational methods. This may result in a variety of test results.[14] Taking into account the dynamic nature of the microbiota and the ability of the composition to change quite rapidly, a single snapshot in addition to the technical variation may produce a result that is very difficult to interpret.

Normal v. abnormal microbiomes

Microbiota analysis to assess taxonomic content and abundance of organisms has been used to assess organisms at a particular site. The composition and abundance of organisms based on taxonomic grouping are assessed, e.g. at phylum, genus or species level. The organisms may be grouped into operational taxonomic units, which are working definitions to classify groups of related organisms.

Once again, there is much variation in composition and abundance of organisms in and between individuals. Because of temporal variation/dynamics, the taxonomic content of the microbiota of various individuals may differ significantly and is highly personalised, and thus a normal that is context dependent may be more appropriate to consider.

Based on taxonomy, the assessment of a healthy core of organism in people without disease has been proposed. Furthermore, the possibility of finding organisms/biomarkers/disease signatures, where their presence is always indicative of a disease state, has also been suggested.[15]

Defining context-specific 'normal' and 'abnormal' microbiota for a specific region may be important. To define 'unhealthy/abnormal', one needs to describe normal, or normal core of organisms for a specific area, i.e. normal vaginal, normal GIT. We know that each region is vastly different in terms of taxonomic composition and function of the microbes[16] and thus each region has its own normal.

How do we then characterise a normal healthy anatomical site-specific microbiome? A couple of important factors need to be considered. These include physiological intra-individual variation (at the individual level) of the microbiota, i.e. circadian rhythm, sex, age, genetic factors, menstrual cycle and normal interindividual variation (at the population level) that may have an effect on the microbiota, such as geographical location and race/ethnicity.[5]

If a patient sample is therefore tested at a certain time point, one must consider that this snapshot of the microbiota at one point in time may not be appropriate.

Once a context-dependent normal is established, can one more easily define imbalanced flora? The term dysbiosis is used in the literature to describe an unhealthy imbalanced ecosystem that results in disease or an unhealthy phenotype. This may be due to imbalance in physiological/metabolic functions of the microbes to the detriment of the host.

Various methods have been used to assess dysbiosis. Some indicators include diversity and resilience.[15]

Diversity

Diversity in a microbial community refers to richness (number of taxa present) and evenness (abundance of many microbial constituents). Tools have been developed to compare patient cohorts. These include alpha diversity (variation of microbes in a single sample), e.g. the Shannon diversity index, which combines richness and diversity and measures the number of species and inequality between species abundances. Beta diversity measures variation of microbial communities between samples, e.g. the Bray-Curtis dissimilarity. It has been postulated that high diversity has generally been associated with health. Although this principle has mainly referred to the GIT, where a relative lack of diversity has been noted in many disease states and the skin where a low diversity is associated with, e.g. atopic dermatitis,[17] diversity may not be the only marker of a healthy ecosystem.[18] This is because the principle of a low diversity only being present with disease, and a high diversity only being present with health, is not always true.[18] Also, there are other anatomical sites where an increased diversity approximating health may not necessarily apply, e.g. the vaginal microbiome, where increased diversity may be associated with bacterial vaginosis and therefore local inflammation.[19]

The notion of a diverse community being healthy is in part due to the concept of temporal stability (ability to withstand external perturbations and rapidly return to a healthy state). This is coined 'resistance'/'resilience', e.g. 'colonisation resistance', which is the ability to withstand colonisation with a pathogen, thus potentially preventing disease.

It is pivotal to consider that there is no standard normal definition and testing protocol to assess diversity and that it is dependent on context.[18] Lastly, and perhaps most importantly, we need to take into account that the analytical variability/lack of standardisation of testing methods has a major impact on the assessment of microbiota.

Beyond the microbiome

We have, however, noted that industrialisation has had an impact on microbiota and the 'disappearing microbe' hypothesis, where certain microbes are no longer present in modern civilizations compared with primitive societies, with a loss of diversity as a result of industrialisation.[20]

Researchers have argued that analysis based purely on taxonomic content may not be appropriate, as it does not factor in the metabolic activity/core functions of the population assessed.

The concept of functional redundancy occurs when different taxonomic profiles lead to ecosystems with similar behaviour/function. Integration of both metabolomic and metagenomic data with standardised computational data analysis tools for the assessment of the functions/metabolites of a particular community may be more accurate.[13] Multi-omic analysis is therefore a new approach, where data sets of different omic groups are combined during analysis. The different omic strategies employed during multi-omics are genome, proteome, transcriptome, epigenome, exome and microbiome, which may provide better clinical applications in the future.

Microbiome analysis in routine patient management

Advances in the microbiome testing pipelines, especially the bioinformatics tools and applications in analysing the data, have been valuable in finding associations with disease in humans and making one aware of the importance of the microbiota.

Although the microbiome and health and disease are very topical and microbiome testing is becoming more accessible locally and internationally, it is important to note that testing of the microbiota (microbiome) is currently not part of routine clinical diagnostics and it may take some time before microbiome-based tests become routine practice.

The results of these tests should not be used for diagnostic purposes or to inform treatment and management decisions. If testing is performed, the results should be used for informative purposes only. Although there is an abundance of literature on the role of the microbiota in human health and disease, this field is evolving and the use of these results and their clinical relevance need to be elucidated further. Associations with health and disease are often merely associations and causality may/may not have been established.

Standard medical protocols for diagnosis and treatment of patients are advised as per local/national guidelines.

Another confounder is that very few data on the composition and functions of the microbiota of SA patients are available as a comparable reference range. What may be considered normal in Europe or the USA, may not necessarily be the case locally. Growing our own SA microbiome database and approved biorepositories for various clinical conditions may assist with research and answering many questions in the future. This may potentially improve diagnostics and information for future clinical microbiota testing so that clinical relevance can be elucidated further.

The authors encourage clinicians to consult experts in the field with regard to limitations of the available testing and to educate patients regarding the current heterogeneity and lack of diagnostic value. A collaborative approach where universities, researchers, public and private entities, such as laboratories, hospitals and biotechnology experts, can all work together is required to achieve this goal.

In future, using microbiome methods for risk assessment to prevent or reduce disease and enhance/personalise treatment may become a reality. To implement these into clinical practice, the limitations need to be addressed first. Personalised medicine is also being explored for drug therapy to reduce side-effects or improve efficacy of treatment and personalised nutrition.[21]

Conclusions

Systematic analysis and cataloging of biome multi-omics in defined patient populations and understanding of the potential protective, metabolic and immune functions and hazards associated with its manipulation, are required to facilitate multiple potential novel diagnostic, treatment and preventive interventions and tools. A myriad of proof-of-concept or principle studies are imminent to unlock the multitude of functions and confirm cause and effect in disease states before routine analysis is recommended.

Declaration. None.

Acknowledgements. We acknowledge Prof. Mark Nicol for motivation with initiation of this article.

Author contributions. JMW and EdT contributed to the writing of the article. AD and AB checked the article for clinical relevance and applicability.

Funding. None.

Conflicts of interest. JMW and EdT have research interests in personalised medicine and clinical applicability of personalised GIT microbiome reports.

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Accepted 23 November 2020.