**Chapter 24: Personalised Medicine and Microbial Diagnostics**

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**ABSTRACT**

This chapter discusses about the concept of personalized/precision medicine and explains how it evolved as a treatment strategy to cater to the individual characteristics of each patient, considering factors such as genetics, environment, and lifestyle. Microbial diagnostics, which involves identifying and characterizing microorganisms, plays a crucial role in this approach, particularly in infectious diseases. By precisely identifying the specific pathogens affecting a patient and understanding their resistance profiles, personalized treatment strategies can be developed, optimizing therapeutic efficacy and minimizing the development of antimicrobial resistance. With advancements in scientific research and technologies, this integration of microbial diagnostics into personalized medicine would enhance the ability to deliver targeted and effective treatments, improving patient outcomes and public health.

**Keywords** – personalised; microbiome; patient-specific; ethics; healthcare; diagnostics

1. **INTRODUCTION: HISTORY OF PERSONALIZED MEDICINE**

# **Overview**

Disease diagnosis and prognosis are two sides of the coin which are equally challenging to address in clinical practice. Correct diagnosis and treatment strategies are fundamental to effective healthcare. They involve a systematic approach to ensure accurate identification of a disease or condition, followed by implementation of the most appropriate therapy tailored to the requirements of the patient. The type of treatment plan adopted also plays a key role in disease prognosis. The efficacy of treatment decisions can be made based on the mechanism of action, therapeutic effect, body system or the condition they target. With scientific and technological advancements, the current treatment plans have narrowed down from generic to specific approach (Figure 1).

A diagram of a medical procedure

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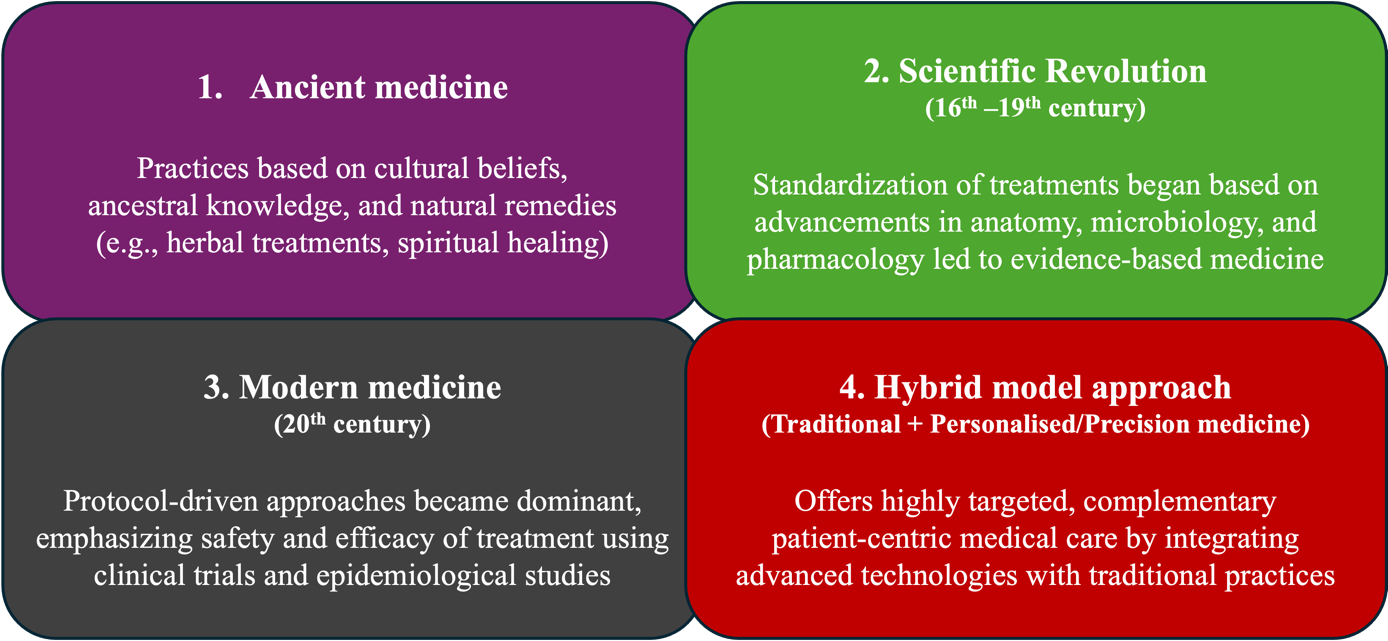
**Figure 1: Approach of treatment plans**

Deciding on treatment strategies for a disease is a comprehensive process requiring careful consideration of patient medical history, accurate diagnosis based on clinical symptoms, guidelines proposed based on the evidences of patient responses, involvement of a multidisciplinary team of consulting specialists - physicians, nurses, pharmacists, dieticians, etc., especially for complex diseases.​ How this transition occurred over a period of time highlights the evolution of treatment approaches in medicine as ‘one-size-fits-all’ and ‘targeted’ approaches.

* **‘One-size-fits-all’ approach**

Ancient medicine relied heavily on the use of herbal medicines, natural substances (Ayurveda, traditional Chinese medicine), with painful surgical procedures as anaesthesia and antiseptics were largely unknown alongwith treatments based on religious practices or superstitious beliefs. Illnesses were sometimes perceived as punishments from deities, leading to a lack of empirical methods in treatments. With limited understanding of the causes, on the downside, response to treatments were either prolonged or led to huge loss of lives.

Ground-breaking discoveries of microbes, antiseptics, and antibiotics, led to the development of modern medicine practices advancing our ability to prevent, diagnose, and treat diseases. With the discovery of microbes, germ theory of disease was established in the 19th century by Robert Koch who proved through experiments that specific pathogens were responsible for diseases/infections. The discovery of antiseptics like carbolic acid (phenol) by Joseph Lister led to the introduction of sterilization techniques and hygiene practices pioneering during the surgeries. The accidental discovery of penicillin by Alexander Fleming led to the use of antibiotics to control bacterial infections providing effective treatments for previously fatal diseases like pneumonia, tuberculosis, and sepsis. The widespread use of penicillin during World War II greatly reduced mortality from bacterial infections and set the stage for antibiotic therapy as a cornerstone of modern medicine. These discoveries led to a shift from empiricism to evidence-based practices with improved outcomes – a) diagnosis and treatment became more scientific, moving away from trial-and-error approaches, b) enhanced public health due to vaccines, antibiotics, and hygiene which reduced global disease burdens (pandemics) with drastic reduction in deaths from infections and diseases that were once considered untreatable. Thus, the roots of modern medicine can be traced back to the 19th century with significant advancements in germ theory, anaesthesia, and antiseptics.



**Figure 2: Evolution of treatment approaches**

The period spanning from 1950 to 1970s, was considered as the ‘golden era’ for the development of standardized treatment protocols based on extensive clinical trials with the introduction of chemotherapy for the treatment of several cancers. This era marked a transformative period in cancer treatment laying foundation for modern oncology with improved survival rates, better prognosis and long-term outcomes. Thus, for consistent approaches to chemotherapy administration, clinical trials became the cornerstone for evaluating new therapies and combinations. Substantial progress was made in understanding the cellular and molecular biology of cancer through extensive research which led to insights that drove further innovations in treatment. This era also popularized the concept of combination chemotherapy, where multiple agents were used concurrently to enhance effectiveness of the treatment. This approach not only increased the overall response rates to therapy but also diminished the likelihood of drug resistance, as different mechanisms targeted various aspects of cancer cell growth (Figure 2). Eventually, with the understanding of tumour biology, successes achieved during this period, set the stage for subsequent innovations in personalized medicine and targeted therapies.

* **Targeted approach**

The concept of targeted therapy emerged in the late 20th century, primarily in oncology. The evolution of chemotherapy from early experimental treatments to currently sophisticated, personalised therapies, has made a remarkable journey. While traditional chemotherapy affected all rapidly dividing cells causing increased side effects, targeted therapies focussed on specific molecular targets associated with cancer cells, improving efficacy and reducing side effects. The emergence of personalised and precision medicines can be attributed to the advent of scientific fields like molecular biology, biochemistry, microbiology, genetics, genomics and technology. This led to significant improvements in understanding diseases, developing diagnostic tools, and creating targeted treatment plans. The role of scientific research in clinical diagnosis and treatments gained significance when FDA approved Herceptin (Trastuzumab) in 1998, as a targeted therapy for treating HER2-protein-overexpressed breast cancer patients. Introduction of a tyrosine kinase inhibitor, imatinib (Gleevec), in 2001 represented another breakthrough in targeted cancer therapy, which specifically inhibited the BCR-ABL fusion protein in chronic myeloid leukaemia (CML) patients. Thus, the field of targeted therapy evolved when Human Genome Project (HGP) shed light on variations among individuals, including single nucleotide polymorphisms (SNPs) and other disease-linked genes as genetic markers in the year 2003. This monumental project revealed the role of 20,000 to 25,000 genes by developing a comprehensive genetic blueprint as a valuable source for researchers and clinicians. This paved way for developing tailored treatments for chronic diseases, including cancer, based on individual genetic profiles.

Knowing the genetics and genomics of diseases could help design specific treatment plans for an individual or a disease group based on the response to treatments. This signifies the role of personalised and precision medicines as targeted therapies. Though these terms are interchangeably used, there lies a subtle difference between them. Personalised medicine implies treatments specifically designed for each individual considering their unique characteristics, including their genetics, environment, and lifestyle. Recommending a treatment plan for diabetes or hypertension based on the lifestyle, family history, and preferences of the patient is a classic example of personalised treatment. On the other hand, precision medicine mainly focuses on the genomic information classifying the individuals into subpopulations based on their susceptibility to diseases and responses to treatments. Precision medicine might involve genetic testing of a tumour to identify specific mutations that can be targeted with particular therapies, such as using targeted drugs for patients whose tumours express certain biomarkers. So, this method classifies patients based on shared genetic features rather than customizing treatment solely based on personal preferences or history. While **personalised medicine is an individualized-treatment approach, precision medicine is a data-driven approach** integrating biological data from multi-omics research like genomics, transcriptomics, proteomics, and metabolomics in treatment decisions. Some examples of precision medicine approved for specific diseases through intensive research are listed in table 1. Thus, targeted therapies expanded beyond oncology to include immunotherapies, such as CAR-T cell therapy for certain blood cancers, and monoclonal antibodies for autoimmune diseases and other conditions.

**Table 1: Major breakthroughs in precision medicine**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **Name** | **Type** | **Mechanism** | **Reference** |
| 1. | Imatinib  (Gleevec) | Tyrosine kinase (TRK) inhibitor | Inhibits BCR-ABL tyrosine kinase in chronic myeloid leukaemia (CML), halting uncontrolled cell division | Druker, *et al.*, 2001 |
| 2. | Trastuzumab  (Herceptin) | Monoclonal antibody | Binds HER2 receptor in HER2-positive breast cancer, blocking cancer cell growth signals | Slamon, *et al.*, 2001 |
| 3. | Ipilimumab  (Yervoy) | Immune checkpoint inhibitor | Blocks CTLA-4, enhancing T-cell response against melanoma | Hodi, *et al.*, 2010 |
| 4. | Pembrolizumab  (Keytruda) | Immune checkpoint inhibitor | Blocks PD-1, restoring T-cell activity to fight cancers like melanoma and non-small-cell-lung-cancer (NSCLC) | Topalian, *et al.*, 2012 |
| 5. | Nivolumab  (Opdivo) | Immune checkpoint inhibitor | Inhibits PD-1, enabling T-cells to target and destroy cancer cells | Weber, *et al.*, 2015 |
| 6. | CAR-T Therapy  (Kymriah, Yescarta) | Cell therapy | Reprograms T-cells to target CD19-positive B-cell cancers | Maude, *et al.*, 2018 |
| 7. | Larotrectinib  (Vitrakvi) | TRK inhibitor | Targets NTRK gene fusions in solid tumours, independent of cancer type | Drilon, *et al.*, 2018 |
| 8. | Entrectinib  (Rozlytrek) | TRK/ROS1/ALK Inhibitor | Targets NTRK, ROS1, and ALK gene fusions in solid tumours | Doebele, *et al.*, 2020 |
| 9. | Sotorasib  (Lumakras) | KRAS inhibitor | Targets KRAS G12C mutations in NSCLC | Skoulidis, *et al.*, 2021 |
| 10. | Lecanemab | Monoclonal antibody | Targets amyloid-beta plaques in early Alzheimer’s disease | van Dyck, *et al.*, 2023 |

Modern medicine is thus underpinned by the principles of evidence-based medicine integrating rigorous scientific research through omics studies like genomics, proteomics and metabolomics. Technological advancements in providing sterile environment for surgical procedures, making the surgeries minimally invasive involving robotics, and enhanced imaging techniques have all led to the transformation of ‘one-size-fits-all’ approach to personalised or targeted approach. With growing knowledge of science and technology through digitalization, there has been a remarkable progress in treatment strategies incorporating artificial intelligence (AI) alongwith omics research. The landmark discoveries of the types of precision medicine in the last decade have led from microbial diagnostics, molecular diagnostics to targeted therapy, and now with AI-driven approaches (Figure 3).

**Figure 3: Breakthroughs in precision medicine**

1. **SCIENTIFIC AND TECHNOLOGICAL ADVANCEMENTS**

There has been a steep rise in technological advancements for increasing the likelihood of therapeutic efficacy highlighting the role of "omics" technologies in developing personalized treatment plans. Multi-omic approaches include:

1. **Genomics**

The **Human Genome Project (HGP)**, which was completed in 2003, provided a comprehensive map of the human genome,

fundamentally transforming the landscape of personalized medicine. Cataloguing genetic diversity illuminated variations among individuals, like single nucleotide polymorphisms (SNP), genetic variants associated with complex diseases, like diabetes, heart disease, cancers, etc. Understanding these variations allowed for customization of medical treatments based on individual genetic profiles. Mapping of the genome facilitated the development of ‘genetic testing’ as diagnostic tool for personalised screening and preventive strategies for cancers or hereditary conditions tailored to an individual’s genetic risks. Coupling counselling with genetic/carrier testing, individuals with conditions like cystic fibrosis, sickle cell anaemia or polycystic ovarian syndrome (PCOS) were able to make informed reproductive choices based on their genetic risks. Insights from the HGP have also driven the development of targeted therapies focussing on specific genetic mutations or pathways involved in diseases. It also laid the groundwork for pharmacogenomics; the study of how genetic variations affect individual responses to drugs. Not only these, HGP has also encouraged the establishment of biobanks, patient registries that collect genomic and clinical data, public genomic databases, such as dbSNP and GENE, which provide researchers and physicians fostering collaborative research efforts. These databases support large-scale studies that explore the genetic basis of diseases and the development of personalised therapies. Thus, the HGP has been instrumental in advancing personalised medicine by providing a foundational understanding of the human genome and its variations.​ The insights gained from the HGP have led to improved diagnostic tools, targeted therapies, and the integration of genetic data into clinical practice, ultimately enhancing the ability to deliver tailored healthcare solutions. As the field continues to evolve, the legacies of the HGP will continue to shape the future of personalised medicine, improving patient outcomes and redefining healthcare delivery.

The **Human Microbiome Project (HMP)** is an integral part of genomic studies, focusing on understanding the complex interactions between human hosts and their microbiota. The HMP aimed to characterise the microbial communities residing in the human body and understand their roles in health and disease. The findings from the HMP have led to significant advancements in clinical settings, transforming approaches to diagnosis, treatment, and prevention. One of the most significant advancements is the ability to profile the microbiome using advanced sequencing technologies. By analysing microbial communities, clinicians can identify specific microbial signatures associated with various diseases, such as inflammatory bowel disease (IBD), obesity, and diabetes. It has elucidated the complex interactions between the microbiome and host physiology, revealing how microbiota can influence not only physical health based on immune responses and metabolism, but also mental health conditions like depression and anxiety. HMP has even highlighted how diet influences microbiome composition, how microbial communities metabolize drugs, how microbial metabolites affect drug action and toxicity. Understanding the impact of antibiotics on microbiome through HMP, has led to more informed antibiotic stewardship practices for preserving microbial diversity and reducing the risk of antibiotic resistance.

With the completion of the **HGP and HMP**, emerged the high-throughput sequencing technologies with creation of advanced bioinformatics tools, which allowed rapid, cost-effective sequencing with analysis and interpretation of vast amounts of (meta)genomic data. These technologies revolutionized research by enabling large-scale (meta)genomic studies of populations, microbes and diseases (Figure 4).

A dna helix and a circular screen

AI-generated content may be incorrect. A human head with different microbes

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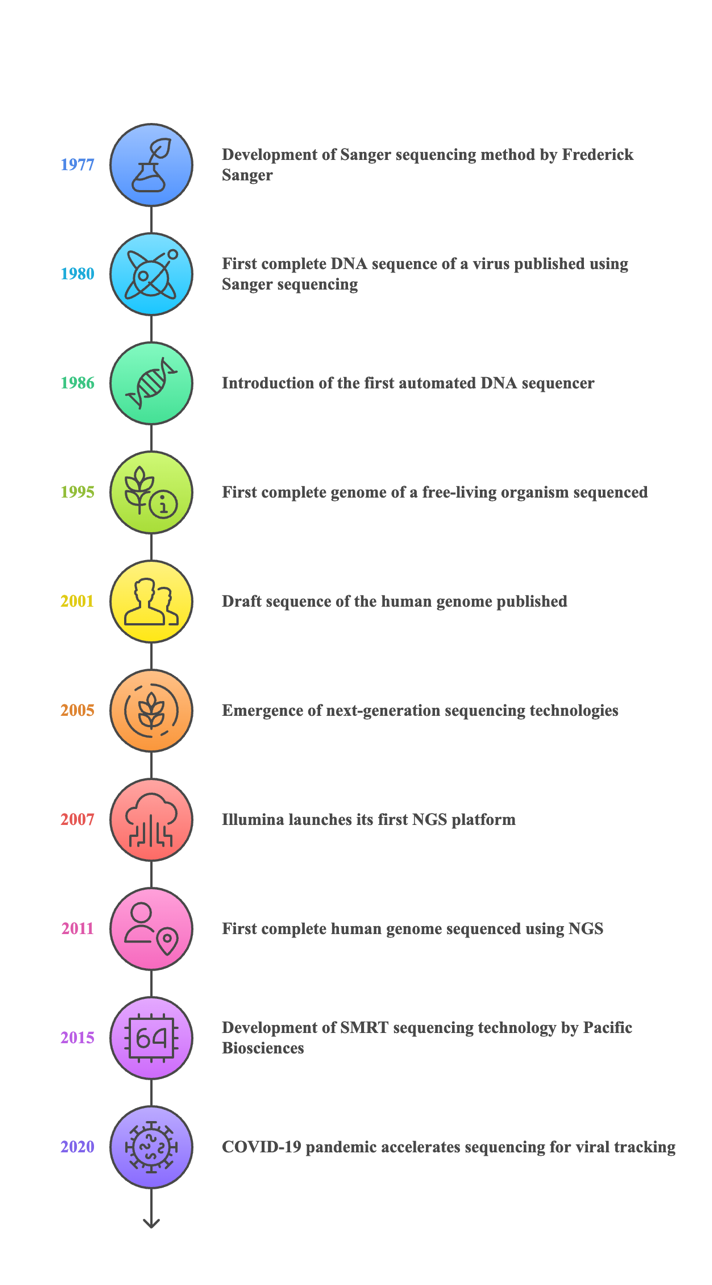
**PRECISION MEDICINE**

Clues from **Human Genome Project** through mapped human genes in health and disease

Clues from **Human Microbiome Project** through Metagenomics

**Figure 4: Emergence of precision medicine through genomic and metagenomic clues**

Rapid evolution of DNA sequencing methods (Figure 5) has had a profound impact on biological research and medicine, contributing to our understanding of genetics and the complexities of life. While genomic study involved one host or microorganism, **metagenomic** study involved community analysis purifying both human and microbial DNA from samples and analysing them using high-throughput sequencing technologies like Next generation Sequencing (NGS) providing a comprehensive view of the microbial diversity and functional capabilities present in the human microbiome.



**Figure 5: Revolutionizing milestones in the field of genomics**

HMP aimed to create a reference database of microbial genomes, including approximately 3,000 reference bacterial genomes isolated from the human body. This database serves as a valuable resource for future research and comparisons in microbiome studies. These findings contribute to a broader understanding of how gut microbiota interacts with human health and disease, linking microbial genomics to various health outcomes. From improved diagnostics and personalized treatments to preventive health strategies, the insights from the HMP are reshaping contemporary medical practices and paving way for innovative therapies in various fields of medicine.

The advancements in understanding human genetics from the HGP directly contributed to the development of **gene editing** technologies, particularly CRISPR-Cas9. The discovery of numerous genetic variations associated with diseases hinted as potential targets for gene editing, enabling repair of these defective genes. Gene editing, thus, allows for the correction of the mutations responsible for these conditions, offering potential cures rather than just symptomatic treatments. This approach enhances treatment effectiveness and minimizes side effects by tailoring therapies to the specific genetic makeup of patients. Creation of genetically modified organisms, such as mice with specific human disease mutations, became crucial for preclinical testing of gene editing techniques to evaluate the effects of potential therapies before advancing to human trials.

Though there is development of genome and microbiome assessment technologies, like gene editing, NGS, integrating these into precision medicine is still underway with discussions surrounding consent, access, and the long-term consequences of germline edits as they have become increasingly pertinent. Guidelines for safe application of gene editing technologies in human populations are being proposed to help ensure ethical standards are maintained as gene editing progresses in clinical settings.

1. **Transcriptomics**

Apart from identifying the defective genes, studying the expression patterns of these genes can throw insights into their role in health and disease, through transcriptomic analysis. Transcriptomics is the study of complete set of RNA transcripts produced by the genome under specific circumstances. It allows for comprehensive analysis of gene expression patterns associated with specific diseases. By comparing the transcriptomes of healthy and diseased tissues, researchers can identify genes that are upregulated or downregulated in various conditions, providing insights into disease mechanisms. It also helps to elucidate the molecular underpinnings of diseases, such as cancer or autoimmune disorders by mapping altered gene expression to biological pathways. Transcriptomic profiling has facilitated the discovery of diagnostic biomarkers that can help identify diseases at an early stage. For example, specific RNA signatures have been linked to various cancers, potentially allowing for earlier and more accurate diagnoses that were not possible with traditional methods. In addition to diagnostics, transcriptomics can provide prognostic information. The expression levels of certain oncogenes or tumour suppressor genes can inform the choice of targeted drugs in cancer treatment, resulting in more effective and personalized interventions. Transcriptomic data can also be used to predict responses to treatments. By understanding the expression profiles of genes associated with drug efficacy or resistance, clinicians can tailor therapies to individual patients, reducing trial-and-error approaches in treatment selection. Transcriptomics can thus, serve as a tool for monitoring therapeutic responses in real-time. By assessing changes in gene expression levels during treatment, clinicians can adapt strategies to improve patient outcomes, facilitating a more dynamic approach to managing diseases. Examples of employing transcriptomics in disease diagnosis and management - a) infection response profiling in the case of SARS-CoV-2 during COVID vaccine development, b) understanding gene expression profiles of neurological disorders like Alzheimer’s, c) in diabetes management to understand the molecular mechanisms of gene expression involved in insulin signalling and glucose metabolism, d) obesity research to understand the gene expression changes associated with obesity. Thus, transcriptomics is being employed across a diverse range of medical fields to enhance the treatment and management of various diseases.

1. **Proteomics**

Understanding how proteins involved in triggering a specific condition or disease interact leading to cascading effect through the display of symptoms, can be uncovered through proteomics. Proteomics allow for mapping of networks of interactions among proteins, providing insights into signalling pathways and cellular functions. It enables studying underlying disease mechanisms through protein-protein interactions or in identifying disrupted pathways, discovering novel targets for drug design, identifying protein biomarkers of diagnostic and prognostic values. Diagnostic biomarkers aid in diagnosing disease at an early stage while prognostic biomarkers aid in analysing changes in protein expression associated with disease progression, based on which researchers can predict outcomes and guide treatment decisions. For instance, in breast cancer, specific protein signatures can indicate the likelihood of recurrence, influencing therapy choices. By assessing proteins in patient samples during clinical trials, researchers can determine their utility in predicting treatment efficacy or patient survival, thereby enhancing clinical practice based on robust evidence. Advances in proteomics have encouraged its adoption in real-world clinical settings, allowing for the development of diagnostics and therapeutics tailored to specific populations or individual patients. This application promotes the transition from traditional one-size-fits-all therapies to more personalized approaches based on protein profiles. Thus, proteomics, the large-scale study of proteins and their functions, has become an essential component of precision medicine. By providing insights into protein expression, modifications, and interactions within biological systems, proteomics contributes significantly to understanding diseases and developing tailored therapeutic strategies.

1. **Metabolomics**

Disease mechanismcan be comprehended at genomic (to identify the genes involved), transcriptomic (to study gene expression), and proteomic (to discover the interacting proteins in disease condition) levels. But the mechanism can be elucidated by gaining insights into the metabolic pathways and their alterations in disease states through metabolomics approach. Metabolites produced in the body could result from food/drug/microbial metabolism. Metabolomics research thus provides a platform to understand the physiological condition of biological specimens (in this case, both the human and microbiota within), which together constitutes the metabolome. Metabolic indicators could suggest about the health status of an individual, either as positive responses or development of diseases. Some metabolites serve as - a) **Physiological markers** where the level of HbA1c is a well-established metabolic indicator for monitoring long-term glucose control in individuals with diabetes. Consistent monitoring of HbA1c levels can inform adjustments in diabetes management strategies, leading to improved overall glycaemic control; a study evaluated the decrease in LDL cholesterol levels in response to statin therapy (Minno, *et al.*, 2022) which was viewed as a positive response, as it is associated with reduced risk of atherosclerosis and cardiovascular events; b) **Disease markers** where elevated levels of CRP are indicative of systemic inflammation and have been associated with cardiovascular risk; measuring circulating tumour DNA can serve as tumour markers; c) **Nutritional markers** where specific metabolites like short-chain fatty acids (SCFA) produced by gut microbiota in the body could be indicative of the health status and nutritional deficiency; monitoring levels of vitamins and minerals (e.g., vitamin D, magnesium, and folate) can provide insights into nutritional status and disease risk; d) **Therapeutic markers** like those mentioned above could also serve as indicators for positive response following therapeutic interventions suggesting improved health conditions. Even with cancer treatment, one study found that specific metabolomic signatures predicted responses to 5-fluorouracil, allowing targeted therapies that maximized treatment effectiveness while minimizing side effects (Sasada, *et al.*, 2013). So, these indicators encompass a range of biochemical markers, metabolites, and metabolic pathways that can provide real-time insights into an individual's metabolic state. Monitoring these indicators can significantly enhance precision medicine by tailoring interventions based on objective biochemical data.

**A diagram of metabolites

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**Figure 6: Understanding disease mechanism using metabolomics**

Multi-omics approach allows for a more comprehensive understanding of biological processes aiding in uncovering the complex interactions between different molecular layers and how they collectively influence health and disease states. It not only promotes efficiency in diagnosis but prognosis too. Hence, multi-omics approach in medical field is gaining prominence as strategical method towards precision medicine for improved high-throughput screening of diseases, diagnostic efficiency, planning for systematic treatment regimens, and follow-ups for improved healthcare systems.

**Reasons for Personalized medicine**

Personalized and precision medicine approaches are emergent fields focusing on tailoring medical treatment to individual characteristics of patients and their genetic makeup resulting in better management of diseases, especially in complex conditions such as cancer and chronic illnesses, where standard approach may not be universally effective. Personalized medicine recognizes that individuals have unique genetic makeups, which influence their health, disease susceptibility, and response to treatments. By integrating genomic data into clinical practice, healthcare providers can make more informed decisions that align with a patient's genetic profile, optimizing therapeutic outcomes. By using genetic information to guide treatment decisions, personalized medicine can help identify which patients are likely to experience adverse effects from certain medications. This targeted approach minimizes the risks associated with trial-and-error prescribing and enhances patient safety. Precision medicine, on the other hand, encourages the development of therapies that target specific molecular pathways associated with diseases. By understanding the genetic variations influencing disease mechanisms, pharmaceutical companies can create more effective and targeted therapies, leading to a more efficient drug development process. Thus, when patients understand that their treatment is tailored to their specific needs and characteristics, they are more likely to participate actively in their healthcare, potentially leading to improved health outcomes.

The reasons for personalized and precision medicine stem from the need to enhance treatment outcomes, optimize drug development, reduce adverse effects, account for genetic variability, improve patient engagement, and address health disparities.​ These approaches represent a shift towards more personalized healthcare solutions that are better aligned with individual patient needs.

1. **INNOVATIVE ADVANCEMENTS: MICROBIAL DIAGNOSTICS**

Microbial diagnostics refers to the techniques or methods used to detect and identify microorganisms, including bacteria, viruses, fungi, and parasites. Exploiting microbes as diagnostic tool gained significance after Human Microbiome Project (HMP) underscored the importance of these microbes in health and disease. The importance of these diagnostics has surged in recent years due to several factors, including the emergence of new pathogens, the resurgence of old diseases, and the growing threat of antimicrobial resistance. Accurate and timely diagnosis can lead to better patient outcomes, informed treatment decisions, and enhanced public health responses.

The utilization of microbes as diagnostic tools is a significant scientific advancement that has transformed various fields of medicine (Khalid, *et al.*, 2024). Microbial diagnostics is becoming increasingly vital in the realm of healthcare and environmental science. As the world faces rising challenges from infectious diseases, antibiotic resistance, and environmental contamination, the ability to accurately identify and characterize microorganisms is crucial. Thus, this section explores the significance of microbial diagnostics, its advancements, and its implications for public health and safety.

1. **Nature of human microbiome**

The human microbiome consists of trillions of microorganisms, including bacteria, viruses, fungi, and archaea. The composition of the microbiome is unique to each individual and is influenced by factors such as genetics, diet, environment, and lifestyle. The microbiome is not static - it evolves over time, adapting to changes in the host's environment and health status. The majority of these microorganisms are symbiotic, meaning they coexist with the host without causing harm. In fact, they play essential roles in various bodily functions, including digestion, metabolism, and immune system regulation. The human microbiome is a complex and dynamic community of microorganisms that inhabit various parts of the human body, including the gut, skin, mouth, and other mucosal surfaces. The human microbiome is found in various habitats throughout the body, with the most significant populations residing in the gut.

**A diagram of different organs

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**Figure 7: Key habitats of human microbiome**

1. **Human microbiome in health and disease**

Human microbiome plays a key role in maintaining both physical and mental health of a person. Normal functioning of the body defines physical health while state of the mind refers to mental health that enables to cope up with the stresses in life, realise their potential to handle it and learning abilities. Both physical and mental health are closely connected to each other and constitute the total wellbeing of an individual. Adequacy and balance in nutrition, exercise and sleep are critical for proper functioning of the body. Likewise, balanced microbial composition i.e. microbiota is a vital component of health homeostasis, influencing the overall functioning of metabolic processes, immune function, and disease prevention.

Illustrating the significance of the microbiome in human health and disease, this section will delve into the diversity of microbiome, functional roles, and the factors influencing the condition of the health based on their habitat:

1. **Gut microbiome:**

The most extensively studied component of the human microbiome is that of the ‘gut’. The microbiome composition changes throughout life, with distinct profiles observed in infants, children, and adults (Yatsunenko, *et al.,* 2012). An adult is primarily composed of bacteria, with Firmicutes and Bacteroidetes being the two dominant phyla. Other notable phyla include Actinobacteria, Proteobacteria, and Verrucomicrobia (Huttenhower, *et al.,* 2012).

* **Firmicutes:** This phylum includes genera such as *Lactobacillus*, *Clostridium*, and *Faecalibacterium*. For instance, *Faecalibacterium prausnitzii* is known for its anti-inflammatory properties and is often found in higher abundance in healthy individuals (Sokol, *et al.,* 2008). Some bacteria like *Lactobacillus* spp., also contribute to the synthesis of essential vitamins, such as B vitamins and vitamin K.
* **Bacteroidetes:** This phylum includes genera like *Bacteroides* and *Prevotella*. *Bacteroides fragilis* plays a vital role in the metabolism of complex carbohydrates and the production of short-chain fatty acids (SCFAs), which are beneficial for gut health (Koh, *et al.,* 2016).
* **Actinobacteria:** In this phylum, *Bifidobacterium* species, particularly, play a crucial role in gut health by producing short-chain fatty acids (SCFAs) like acetate, which helps maintain intestinal homeostasis. But reduced levels of *Bifidobacterium* are associated with inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), and metabolic disorders like obesity and type 2 diabetes.
* **Proteobacteria:** The bacteria in this phylum are a diverse range of commensals, contributing to gut microbial diversity. They play a key role in nitrogen cycling and breaking down of complex molecules in the gut. An overabundance of Proteobacteria, especially *Escherichia coli*, *Salmonella*, and *Helicobacter pylori*, is linked to gut dysbiosis, inflammation, and diseases such as inflammatory bowel disease (IBD), colorectal cancer, and metabolic disorders. While elevated levels of Proteobacteria are often considered a biomarker of gut microbiome instability and increased intestinal permeability ("leaky gut").
* **Verrucomicrobia**: *Akkermansia muciniphila* is the most well-known member of Verrucomicrobia and plays a key role in maintaining gut barrier integrity by degrading mucin and promoting mucus layer renewal. It contributes to metabolic health by improving glucose metabolism, reducing inflammation, and regulating fat storage. But reduced Verrucomicrobia abundance is also linked to increased gut permeability and systemic inflammation.

A balanced presence of Actinobacteria, Proteobacteria, and Verrucomicrobia is essential for maintaining gut homeostasis. While beneficial species support digestion, immune function, and metabolic health, an imbalance can contribute to chronic diseases, inflammation, and gut disorders. Understanding their role helps in developing targeted probiotic and therapeutic strategies for gut microbiome modulation.

Human gut health is a multifaceted aspect of overall well-being that plays a critical role in various bodily functions. Research indicates that individuals with greater microbial diversity tend to experience better health outcomes. The composition and diversity of the microbiome can vary significantly between individuals, influenced by factors such as genetics, diet, environment, and lifestyle. One of the primary roles of the microbiome is its involvement in metabolic processes. The gut microbiota, in particular, aids in the digestion of fibres which are chemically complex carbohydrates as human enzymes cannot digest it. The digestion of these complex nutrients is carried out typically by anaerobic bacteria which ferment it to produce short-chain fatty acids (SCFAs) that serve as fuel for colon cell proliferation, regulating metabolism and inflammation.

Dysbiosis or changes in microbial diversity are often observed due to the interplay of dietary pattern changes and host metabolism increasing the complexity of these relationships. While fiber-rich diets promote the growth of beneficial bacteria, high-fat or high-sugar diets may lead to dysbiosis – an imbalance in the microbial community that can contribute to various diseases. A compromised microbiome can increase susceptibility to infections, as seen in conditions like *Clostridium difficile* infection, where antibiotic use disrupts the normal gut flora, leading to a decrease in the diversity with potential overgrowth of pathogenic species (Jernberg, et al., 2010). Various gut-related issues, including lactose intolerance, inflammatory bowel diseases, and metabolic disorders are also a result of dysbiosis. Lactose intolerance is characterized by gastrointestinal symptoms that arise from the inability to digest lactose due to lack of production of lactase, the enzyme responsible for breaking down lactose into glucose and galactose. Unabsorbed lactose can lead to osmotic changes in the intestine, promoting the growth of specific gut bacteria, particularly *Bifidobacterium* spp. that ferment lactose. This fermentation produces gases like hydrogen and methane, resulting in symptoms such as bloating and abdominal pain. A study involving 959 participants found that individuals with lactose intolerance had higher levels of *Bifidobacterium* compared to non-intolerant individuals, suggesting that these bacteria may both aid in lactose fermentation and exacerbate symptoms depending on their metabolic byproducts (Brandao Gois MF, *et al.*, 2022). Gastrointestinal disorders like inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) arise when certain pathogenic bacteria dominate inflaming the bowel with long-lasting abdominal pain and diarrhoea. With aggravating symptoms, it could also lead to bowel cancers like colorectal cancer. Other conditions like obesity and diabetes are linked to metabolic dysfunctions due to altered gut microbial composition. Emerging research suggests a strong connection between the gut and the brain, often referred to as ‘the gut-brain axis’. The associated microbiome may influence the mood, anxiety, depression, cognitive function and other mental health disorders through the production of neurotransmitters and signalling molecules.

1. **Skin Microbiome:**

Skin is the largest organ of human body which is home to millions of bacteria, fungi and viruses constituting the ‘skin microbiota or flora’. The skin microbiome is diverse and varies across different body sites. Dominated by bacterial population, the phyla on the skin include Actinobacteria, Firmicutes, and Proteobacteria (Grice and Segre, 2011). The composition of this microbial community governs the health of the skin and protects it from skin damage through defenses like sweat secretion, immune system regulation and shedding of skin. Sweat on the skin support the growth of acidophilic bacteria like Staphylococci, Micrococci, *Corynebacterium*, *Propionibacterium*, etc., due to the presence of lactic acid. While the acidic conditions of the skin enhance the secretion of antimicrobial substances to prevent the growth of pathogenic bacteria, alkaline conditions cause shedding of bacteria from the skin that fail to remain attached. The skin also provides protection from building up of dermatophytes (skin fungi) through activation of the skin’s immune system.

A structure of skin with hair growing out of it

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**In Health:**

*Staphylococcus epidermidis*

*Cutibacterium acnes*

*Corynebacterium* spp.

*Lactobacillus* spp.

*Acinetobacter* spp.

*Pseudomonas* spp.

*Methanobrevibacter smithii*

*Malassezia* spp.

Human papilloma viruses (HPV)

**In Disease:**

*Staphylococcus aureus*  - atopic dermatitis, wound infections

*Cutibacterium acnes* - acnes

*Corynebacterium* spp. - in immunocompromised patients

*Pseudomonas* spp. - wound infections, burns

*Malassezia* spp. - in dandruff, seborrheic dermatitis

*Candida* spp. - fungal infections/Candidiasis

HPV– oncogenic strains, warts

Herpes simplex virus – genital herpes

**Figure 8: Skin microbiota in health and disease**

Some common examples are *Staphylococcus epidermidis* which is an abundant commensal bacterium residing on skin and mucous membranes. Under altered skin environment, it becomes opportunistic causing infections of the wound, boils, endocarditis, etc. It is also the frequent cause of nosocomial infections. It can remain invisible in the body thereby escaping from the immune attacks. However, it plays a role in preventing colonization by pathogenic bacteria (Otto, 2009). On the other hand, the other key bacterium is *Propionibacterium acnes* which is known to be associated with acne but also contribute to the skin's immune defence (Kurokawa, *et al.*, 2009).

1. **Oral Microbiome:**

The oral microbiome is another critical component, consisting of over 700 species of bacteria, fungi, and viruses. The dominant phyla include Firmicutes, Bacteroidetes, and Fusobacteria (Dewhirst, *et al.,* 2010). The predominant inhabitant of oral microbiota are *Streptococcus mutans*, *Fusobacterium nucleatum*, *Candida albicans*, etc. *Streptococcus mutans* is a key player in dental caries, while *Streptococcus sanguinis* is associated with oral health (Kreth*, et al.*, 2008).

**A cartoon of a mouth

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**Figure 9: Oral microbiota in health and disease**

On the other hand, *Fusobacterium nucleatum* is linked to periodontal disease and has been shown to interact with other oral bacteria to promote inflammation (Kolenbrander, *et al.*, 2002).

1. **Vaginal Microbiome:**

The vaginal microbiome is predominantly composed of *Lactobacillus* species, which play a crucial role in maintaining vaginal health by producing lactic acid and preventing the growth of pathogenic organisms (Ravel, *et al.*, 2011). *Lactobacillus crispatus* and *Lactobacillus iners* are commonly found in healthy women and are associated with a lower risk of bacterial vaginosis (Brotman, *et al.,* 2014). Vaginal dysbiosis which is characterised by significant disruption in the microbiome leading to the dominance by complex and dynamic altered vaginal microbiota is the main cause of yeast infections, urinary tract infections (UTI) and bacterial vaginosis (BV). Below is a list of altered vaginal microbiota during infections:

**Table 2: List of altered bacteria leading to vaginal infections**

|  |  |
| --- | --- |
| **Vaginal microbiota in Health** | **Vaginal microbiota in Disease** |
| *Lactobacillus crispatus*  (found in healthy women and helps to maintain low pH levels, preventing infections) | *Gardnerella vaginalis*  (can lead to the risk of sexually transmitted infections or STI and complications during pregnancy) |
| *Lactobacillus iners*  (found in reproductive-age women) | *Bacteroides* sp.  (increases susceptibility to infections during pregnancy) |
| *Lactobacillus jenseii*  (helps in microbiota stability and protects against pathogens) | *Candida* sp.  (when predominantly present, can lead to yeast infections characterised by itching, discharge and irritation.) |
|  | *Staphylococcus aureus*  (causes infections including toxic shock syndrome) |
| *Escherichia coli*  (Some strains lead to urinary tract infections (UTIs)) |
| *Mobiluncus* sp.  (predominant presence increases infection risk) |

**Factors Influencing Microbiome Composition**

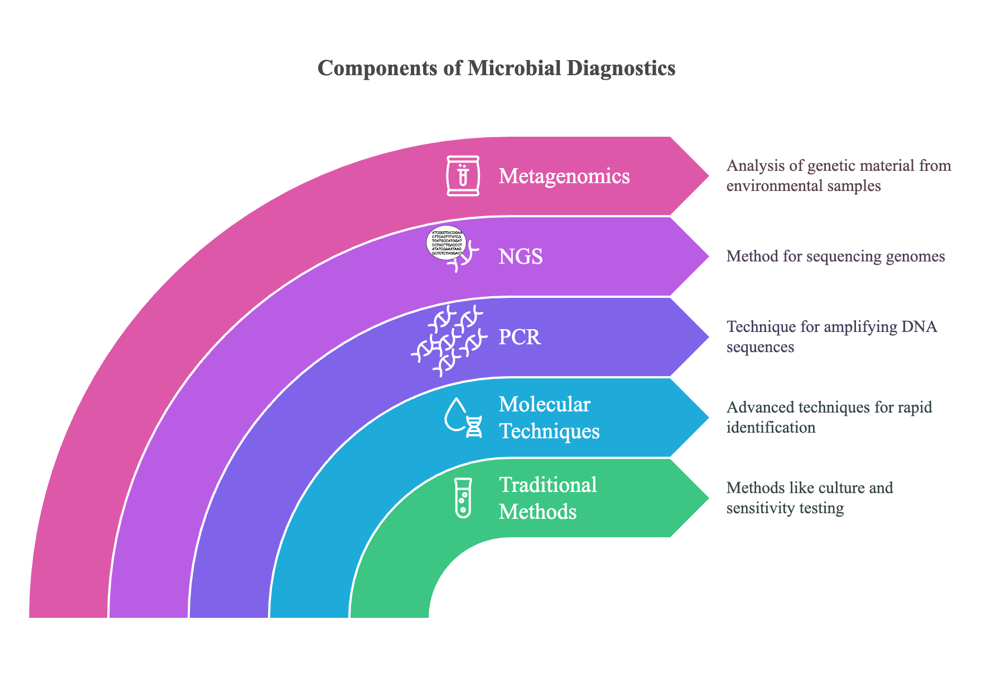
Several factors influence the composition of the human microbiome, like, 1) **diet**, where the types of food consumed can significantly affect microbiome diversity. Diets rich in fiber promote the growth of beneficial bacteria, while high-fat diets may lead to dysbiosis (David, *et al.*, 2014). 2) **antibiotic** use can disrupt the microbiome, leading to a decrease in the diversity while promoting potential overgrowth of pathogenic species (Jernberg, *et al.*, 2010) by developing resistance against these antibiotics. 3) The microbiome composition changes throughout life, with **age**, with distinct profiles observed in infants, children, and adults (Yatsunenko, *et al.*, 2012). 4) Further, **exposure to different environments**, such as urban versus rural settings, can shape the microbiome. Rural populations often have a more diverse microbiome compared to urban populations (Lloyd-Price, *et al.*, 2016).

1. **Microbes as diagnostic tool**

Microbes thrive in our body to help maintain ourselves in good health (homeostasis) or become responsible for infections/diseases when they become opportunistic. The knowledge about the occurrence, abundance or absence of microbial communities in some body sites, underscored by Human Microbiome Project (HMP) in 2017, enlightened us about their role in health maintenance. The microbiome is integral to the development and function of the immune system playing a vital role in preventing colonization of pathogens. It helps to train the immune system to distinguish between harmful pathogens and benign substances to avoid overreactions leading to allergies or autoimmune diseases. As research continues to uncover the complexities of these microbial communities, there is growing potential for harnessing the microbiome in therapeutic applications. Promoting a healthy microbiome through diet, lifestyle, and possibly probiotics could be a key to enhancing the overall health and preventing disease. Gut health has a high influence on physical, immune and mental wellbeing as majority of the microbiota reside in the gut. The composition and diversity of the microbiome can hint about the health status of an individual, which could serve as a biomarker or a diagnostic tool for detection of diseases.

The way, human genome project (HGP) generated a map of human genes with their location and role, HMP also generated a human microbiome map with information characterizing their metabolic capabilities in human health and disease states. The humongous data generated through both the phases of HMP bestowed on us with information on microbiome-associated diseases using robust computational tools which created integrated datasets of biological properties associated with host and microbiome interactions. Understanding these associations allows for the identification of microbial biomarkers that can aid in disease diagnosis, prognosis, and treatment. Both HGP and HMP have transformed the outlook of disease development/treatment by paving way for newer ways by integrating research for providing scientific solutions to resolve health issues.

Microbial diagnostics play a crucial role in the management of infectious diseases by providing detailed insights into the pathogens responsible for infections. This document explores how advanced microbial diagnostic techniques can inform personalized treatment strategies, enhancing patient outcomes through tailored therapeutic approaches. By identifying specific pathogens and their resistance profiles, healthcare providers can optimize treatment regimens, reduce the risk of adverse effects, and improve overall patient care. Microbial diagnostics encompass a range of laboratory techniques used to detect and identify microorganisms, including bacteria, viruses, fungi, and parasites. Traditional methods, such as culture and sensitivity testing, have been complemented by molecular techniques like polymerase chain reaction (PCR), next-generation sequencing (NGS), and metagenomics. These advanced methods allow for rapid and accurate identification of pathogens, even in complex clinical scenarios.



**Figure 10: Advancements in microbial diagnostics**

**Understanding Microbial Diagnostics**

Microbial analytical methods are reshaping diagnostic methods in clinical practice with advancements in technology promising better detection and prognosis. The most important developments are in the areas of targeted therapy or precision medicine through detailed understanding of the behaviour of the microbiome.

1. **Personalised/Precision medicine**

One of the primary benefits of microbial diagnostics is the ability to implement targeted therapy. By identifying the specific pathogen causing an infection, clinicians can select the most effective antimicrobial agents. For instance, in cases of bacterial infections, susceptibility testing can reveal which antibiotics are likely to be effective, minimizing the use of broad-spectrum antibiotics that can lead to resistance. Personalized or precision medicine aims to tailor medical treatment to the individual characteristics of each patient. The microbiome can play a pivotal role in this approach by:

* + - **Pharmacogenomics:** The microbiome can influence drug metabolism and efficacy. For example, certain gut bacteria can activate or deactivate medications, affecting their therapeutic outcomes. By analysing an individual's microbiome, healthcare providers can predict how a patient will respond to specific drugs, leading to more effective treatment plans.
    - **Disease Prediction and Prevention:** Microbial profiles can serve as biomarkers for disease risk. By monitoring changes in the microbiome, it may be possible to predict the onset of diseases and implement preventive measures tailored to the individual's microbiome composition.
    - **Targeted Therapies:** Understanding the role of microbiome in disease mechanisms can lead to the development of targeted therapies. For instance, faecal microbiota transplantation (FMT) has shown promise in treating recurrent *Clostridium difficile* infections by restoring a healthy microbiome balance.
    - **Dietary Interventions:** Personalized nutrition, guided by microbiome analysis, can optimize health outcomes. Specific dietary changes can promote beneficial microbial populations, enhancing overall health and potentially mitigating disease risk.

1. **Resistance Profiling**

Microbial diagnostics can also provide information on the antimicrobial resistance patterns. Understanding the resistance pattern of pathogens allows for the selection of appropriate treatments and helps avoid the use of ineffective antibiotics. This is particularly important in the context of rising antibiotic resistance, where inappropriate treatment can lead to treatment failure and prolonged illness.

1. **Monitoring Treatment Response**

Regular microbial diagnostics can be employed to monitor the effectiveness of treatment. By assessing changes in microbial load or resistance patterns during therapy, clinicians can make informed decisions about continuing, adjusting, or switching treatments. This adaptive approach ensures that patients receive the most effective care throughout their treatment journey.

1. **Predicting Outcomes**

Advanced microbial diagnostics can also aid in predicting patient outcomes. For example, certain biomarkers associated with specific pathogens can indicate the severity of an infection or the likelihood of complications. This information can guide clinicians in making proactive decisions regarding patient management, including the need for hospitalization or intensive care.

On a broader scale, microbial diagnostics contribute to public health by tracking outbreaks and understanding epidemiological trends. By identifying the strains of pathogens circulating in a community, health authorities can implement targeted interventions, such as vaccination campaigns or public health advisories, to mitigate the spread of infectious diseases.

1. **Advancements in Microbial Diagnostics**

In recent years, the field of microbial diagnostics has witnessed significant technological advancements that have transformed clinical practice. These innovations have enhanced the ability to detect, identify, and characterize microbial pathogens with greater accuracy and speed. It has helped to explore key advancements in microbial diagnostics, highlighting the importance of microbial biomarkers and the role of faecal microbiota transplantation (FMT) as a precision medicine approach. Traditional culture-based methods, which can be time-consuming and labour-intensive, are increasingly being supplemented or replaced by molecular techniques such as polymerase chain reaction (PCR), next-generation sequencing (NGS), and metagenomics. These methods allow for rapid and precise identification of pathogens, even in complex samples. As observed with the key example during COVID-19 detection:

**a) Rapid testing:** The development of rapid diagnostic tests has been a game-changer in clinical settings. These tests can provide results within hours, enabling healthcare providers to initiate appropriate treatment sooner. This is particularly crucial in cases of severe infections where time is of the essence.

**b) Point-of-Care Testing:** Point-of-care (POC) testing has gained traction, allowing for diagnostics to be performed at or near the site of patient care. This approach reduces the need for laboratory infrastructure and can be especially beneficial in remote or resource-limited settings.

**c) Implications for Public Health:** The implications of improved microbial diagnostics extend beyond individual patient care. Enhanced diagnostic capabilities contribute to better surveillance of infectious diseases, allowing public health officials to track outbreaks and implement control measures more effectively. This is particularly important in the context of global travel and trade, where pathogens can spread rapidly across borders.

**d) Antimicrobial Resistance:** One of the most pressing challenges in modern medicine is antimicrobial resistance (AMR). Microbial diagnostics play a crucial role in combating AMR by enabling the identification of resistant strains and guiding appropriate antibiotic use. This targeted approach can help preserve the efficacy of existing antibiotics and reduce the incidence of resistant infections.

**e) Environmental Applications:** Microbial diagnostics are not limited to human health; they also have significant applications in environmental science. Monitoring microbial communities in soil, water, and air can provide insights into ecosystem health, pollution levels, and the presence of pathogens in the environment. This information is essential for managing public health risks and ensuring environmental safety.

A diagram of steps with text and symbols

Description automatically generated with medium confidence

**Figure 11: Progression in microbial diagnostics**

1. **Microbial Biomarkers**

Derived from microorganisms, microbial biomarkers are specific indicators that can provide valuable insights into the presence, progression, or response to treatment of diseases. They are measurable indicators of microbial presence or activity that correlate with specific disease states. These biomarkers can be isolated from various sources, including blood, urine, stool, and tissue samples. Identification of microbial biomarkers has become crucial in diagnosing infections and understanding disease mechanisms. These methods allow for rapid and precise identification of pathogens, even in complex samples. NGS can reveal the presence of certain microbial communities in different niches of the body to identify the causative agents of complex infections, such as those caused by polymicrobial communities. These microbial signatures can be used for various purposes, like, diagnostic, prognostic, and therapeutic.

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**Figure 12: Highlighting the purpose of microbial biomarkers**

The degree of diagnosis has improved with multi-omic advancements enabling researchers and clinicians to dive deep into the cause of illness using microbes as biomarkers for disease diagnosis. Not only microorganisms, their biomolecules like DNA, RNA, proteins and metabolites can serve as biomarkers. Detecting microbial DNA and RNA is a core diagnostic tool in many clinical settings. Techniques like polymerase chain reaction (PCR) and next-generation sequencing (NGS) can identify specific pathogens in clinical samples. Plasma cell-free DNA, including microbial cfDNA, is another innovative biomarker increasingly used in clinical practice. It involves analysing fragments of microbial DNA circulating in the bloodstream, which can indicate the presence of infections like IBD with promising potential in diagnosing disease type and severity. Enzyme-linked immunosorbent assays (ELISA) and rapid diagnostic tests (RDTs) typically utilize microbial antigens as biomarkers that can indicate the presence of infections. The immune response to microbial infections involves the production of antibodies where serological tests measuring specific antibodies can indicate past or current infections. Microbial metabolites can serve as biomarkers which reflect the metabolic activity and provide insights into the microbial community composition indicative of disease or healthy status of an individual. Traditional culture techniques are still in practice, particularly in cases where pathogens are isolated from body fluids (like urine), essential for identifying antibiotic sensitivities. Serological markers, on the other hand, reflecting inflammatory responses to infections can serve as indirect indicators of microbial presence. Thus, microbial biomarkers show potential use as diagnostic markers (Figure 11).

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AI-generated content may be incorrect.

**Figure 13: Microbial components as diagnostic biomarkers**

**Next-Generation Sequencing (NGS)** testing has rapidly advanced and found a multitude of applications in clinical practice, fundamentally transforming the landscape of diagnosis, treatment, and disease management. Below are some prominent applications of NGS testing in clinical settings. It is increasingly applied in microbial genomics, allowing for extensive studies of pathogenic bacteria, viruses, and fungi. This capability is crucial for understanding microbial resistance and tracking outbreaks. With the pandemic of COVID-19, people became aware of **Reverse Transcription Polymerase Chain Reaction (RT-PCR)** techniques being used for viral load detection. RT-PCR was the gold standard for detecting SARS-CoV-2, the virus responsible for COVID-19. This technique was widely used due to its high sensitivity and specificity in detecting viral RNA. Since SARS-CoV-2 is an RNA virus, its RNA was converted into complementary DNA (cDNA) using an enzyme called reverse transcriptase. Specific primers and probes targeting SARS-CoV-2 genes (such as N (nucleocapsid), E (envelope), RdRp (RNA-dependent RNA polymerase), or S (spike protein) were used. DNA polymerase enzymes amplified the viral genetic material if present in the sample. It is still a widely accepted infection-detection tool despite its limitations like time-consuming process, requires trained personnel to handle this specialised instrument and can give false negative report due to improper sample collection or low viral load that could lead to missed detections.

**Mass spectrometry** also holds a standard position as a powerful analytical tool in microbial detection due to its ability to rapidly identify microorganisms based on their molecular composition. Particularly MALDI-TOF MS and LC-MS, have revolutionized microbial detection by providing rapid, accurate, and high-throughput identification of pathogens. Mass spectrometry finds wide application in clinical microbiology helping in rapid bacterial and fungal pathogen identification from blood, urine or respiratory samples. It also helps to identify resistance mechanisms by detecting bacterial enzymes (e.g., β-lactamase production). It also helps to profile gut, skin, and environmental microbiomes by detecting microbial metabolites and lipids. For instance, the presence of specific bacterial species, such as *Clostridium difficile*, can serve as a biomarker for antibiotic-associated diarrhoea. Additionally, the detection of microbial metabolites in bodily fluids can provide insights into the host microbiome and its association with various diseases.

Another vital microbial detection system used in clinical settings is the **VITEK** which is an automated microbial identification and antibiotic susceptibility testing (AST) platform. It is manufactured by bioMérieux and is known for its speed, accuracy, and ease of use in identifying bacteria and fungi. The prepared inoculum is loaded into a specific VITEK card for bacterial identification/yeast identification/antibiotic susceptibility testing (AST), which contains micro-wells with biochemical substrates. The VITEK instrument incubates the sample and monitors biochemical reactions (colour changes, turbidity) over time. The system analyses metabolic activities like carbohydrate fermentation, enzyme production, and other biochemical markers. The observed biochemical profile is compared against a large database of bacterial and fungal species. The system provides species-level identification in minutes to hours (faster than traditional culture methods). It helps guide precision antimicrobial therapy by identifying multidrug-resistant (MDR) organisms.

1. **BARRIERS IN BENCH-TO-BED TRANSITION OF MICROBIAL DIAGNOSTICS**

Implementing microbial diagnostics as personalized medicine faces several barriers, primarily due to technological, clinical, regulatory, and economic challenges. Below are the key barriers:

1. **Limited standardization and validation of diagnostic tools** due tolack of universally accepted protocols for microbial diagnostic methods. Different sequencing platforms and bioinformatics tools may yield **varying results**, making it difficult to ensure accuracy and reproducibility. Validation of new diagnostic techniques requires **large-scale clinical trials**, which are time-consuming and expensive.
2. **Complexity of the human microbiome as** it is highly **diverse and dynamic. Inter-individual variability** makes it difficult to establish microbial reference ranges for different health conditions. The microbiome is influenced by **diet, lifestyle, medications (e.g., antibiotics, probiotics), and geography**, adding layers of complexity.
3. **Challenges in identifying pathogenic vs commensal microbes due to** difficulty in distinguishing **harmful** from **beneficial** microbes. Many **opportunistic pathogens** (e.g., Escherichia coli, Klebsiella pneumoniae) exist as commensals in healthy individuals but can become pathogenic under certain circumstances. Traditional diagnostic cutoffs may not apply in personalized medicine, requiring **functional profiling** of microbes rather than just presence/absence detection.
4. **Data interpretation and clinical relevance** aslack of clear guidelines for translating microbial data into **actionable clinical decisions.** Physicians may struggle to interpret **complex microbiome sequencing reports** without standardized clinical guidelines. Predicting disease risk based on microbial profiles is still **in its early stages**, requiring integration with host genetics and other biomarkers.
5. **High cost and limited accessibility** asadvanced microbial diagnostics (e.g., **metagenomics, whole-genome sequencing**) are expensive. **Sequencing and bioinformatics analysis** require specialized infrastructure and trained personnel, increasing costs. These technologies are mainly available in **research settings** rather than routine clinical practice.
6. **Limited awareness and training** for healthcare providers. Many clinicians are unfamiliar with microbiome-based diagnostics and their potential applications. Resistance to adopting new methods due to preference for traditional culture-based diagnostics adds to the drawback.
7. **Influence of environmental and lifestyle factors** alter microbial profiles, complicating diagnostics.
8. **Regulatory and ethical challenges** likestrict regulations on **genomic and microbial data usage**. Regulatory bodies (e.g., **FDA, EMA**) require extensive **safety and efficacy validation** before approving microbial-based diagnostics.

Overcoming these barriers requires technological advancements, regulatory support, clinical training, and cost reduction to integrate microbial diagnostics into personalized medicine effectively.However, to ensure ethical application of microbial diagnostics in personalized or precision medicine, several key ethical principles must be integrated into research, clinical practice, and policy making. Ethical concerns arise regarding **data privacy, consent, and potential misuse of microbiome data** in insurance or employment.

1. **Informed consent** wherepatients are well informed about how their microbial data will be used, stored, shared, have control over their data while they can also opt out if they do not wish to share their data for any research or third part collaborations.
2. **Data privacy** should be ensured through **de-identification and encryption** to safeguard personal microbiome information. Data governance policies should be established to regulate who can access and use microbiome-based health data by ensuring compliance with global regulations like GDPR (General Data Protection Regulation) and HIPAA (Health Insurance Portability and Accountability Act).
3. **Clinical validity of microbiome data** should be provided through evidence-based guidelines to help physicians interpret microbiome diagnostics accurately. It should also be taken into consideration that these microbiome-based diagnoses will be used in complementary with other clinical and genetic data and not as standalone tests.
4. **Equitable access** by encouraging government and healthcare policies to subsidize microbiome-based diagnostic testing for underserved populations and responsible interpretation of results.
5. **Sharing microbiome surveillance data in public health** by anonymizing the data to track antimicrobial resistance and disease outbreaks.

Ethical considerations in microbial diagnostics for personalized and precision medicine require robust data privacy, informed consent, fair access, and responsibleuse of data. Regulatory frameworks, bioethics committees, and interdisciplinary collaboration between clinicians, researchers, policymakers, and patients are crucial to maintaining trust and fairness in microbial precision medicine.

1. **CASE STUDIES**

Public health safeguards are essential to ensure that innovation in healthcare, particularly with new technologies and practices, aligns with ethical responsibility. Below are case studies that illustrate how public health safeguards can balance these two vital aspects.

**Case Study 1: Microbiome-based kits**

Human Microbiome Project (HMP) which highlighted the importance of informed consent, data privacy and diversity in microbiome research. In this study, microbiome data was collected from healthy and diseased individuals to understand microbial roles in health and disease. Participants were given detailed consent form, explaining how their microbiome data might predict health risks. This paved way for the development of predictive microbiome testing like Direct-to-consumer (DTC) kits. Companies like uBiome, Viome and Thryve provided microbiome sequencing kits that allowed individuals to test their gut microbiota. But the results claimed to offer personalized diet recommendations, disease risk assessments, and probiotic suggestions. Some companies marketed their tests as diagnostic tools without scientific validation, leading to misinterpretation of results. Regulatory bodies (FDA, FTC) issued warnings against misleading health claims.

**Case Study 2: Gut Microbiome-based Therapies (Faecal Microbiota Transplantation (FMT) and Microbiome-Based Precision Medicine in Cancer Therapy)**

Faecal Microbiota Transplantation (FMT)involves transferring living microbes, raising risks of unintended pathogen transmission. Ethical protocols ensure rigorous donor screening for infectious diseases, antibiotic use, and gut health conditions. FMT is being used to treat *Clostridioides difficile* **(***C. difficile***)** infections by transferring gut bacteria from healthy donors to patients. It is also being researched for conditions like IBD, metabolic disorders, and autism. Some private clinics offered “unregulated” FMT treatments for non-approved conditions, raising ethical concerns. Therefore, regulatory agencies (FDA, EMA) have restricted FMT use to approved clinical trials.There is dire need for donor screening, informed consent, and regulation to prevent unethical commercialization. Scientific insights from HMP regarding the influence of gut microbiome in patients responding to immunotherapies which led to the exploration of probiotic interventions to enhance treatment response in cancers like melanoma and lung cancer. However, ethical frameworks ensured that probiotic-based therapies did not replace proven cancer treatments but were used as complementary interventions. Some biotech companies prematurely marketed microbiome-based cancer therapies without sufficient clinical evidence. Ethical guidelines now require peer-reviewed clinical trials before marketing such treatments.

**Case Study 3: Digital Contact Tracing Apps**

During the COVID-19 pandemic, many countries implemented digital contact tracing apps to manage outbreaks and keep populations safe. These apps were often introduced on a voluntary basis; however, ethical concerns arose regarding privacy, consent, and potential coercion. To protect individual voluntariness, recommendations were made that included a) Transparency: Clear communication regarding data usage and the benefits of participating, b) Opt-in Models: Allowing users to consciously choose to engage rather than mandating participation. These measures aimed to maintain public trust while leveraging innovative technology for public health.

**Case Study 4: AI Integration in Public Health Campaigns**

The incorporation of artificial intelligence (AI) and big data in public health campaigns has shown promising results in enhancing precision and personalization in healthcare delivery. A study highlights the transformative potential of AI in disease surveillance, diagnostics, and predictive modelling, emphasizing the need for ethical considerations in its deployment. Key challenges addressed include:

1. **Data Privacy**: Implementing robust frameworks to protect personal information collected through AI and big data systems.
2. **Equitable Access**: Ensuring that technological benefits are distributed fairly without exacerbating existing health disparities among different populations.

In Ghana, the integration of AI in automating vaccine distribution has raised questions about ethical governance. An evaluation of the Public Health Act revealed significant gaps in regulatory frameworks protecting against issues such as automated opacity and algorithmic bias. To balance innovation with ethical responsibility, recommendations included policy upgrades by adapting current legal frameworks to address technological challenges, ensuring accountability and transparency. Also, by involving stakeholders in decision-making, it would ensure that diverse perspectives inform public health policies. This approach would not only enhance operational efficiency but also build public confidence in health innovations.

**Case Study 5: Corporate Social Responsibility During the Pandemic**

Organizations in the healthcare sector adopted various Corporate Social Responsibility (CSR) initiatives during the COVID-19 pandemic, navigating the tension between public health needs and business interests. Some notable examples include:

1. **Mandatory Vaccination Policies:** Companies faced ethical dilemmas in enforcing vaccination among employees, balancing public health imperatives with individual rights.
2. **Support for Vulnerable Populations:** Many corporations redirected resources toward helping indigenous communities access healthcare during the pandemic.

Several businesses participated in CSR activities during COVID-19 pandemic to create awareness among public and promote social distancing. Few instances of the corporate world joined hands to combat the pandemic like, Amul, the king of dairy products in India, promoted the Indian method of greeting hands together (‘Namaste’). In the Bengal Beverage Company’s logo, a space was added between each letter to highlight the message ‘The only way to stay together is to stay apart’. India Health Alliance launched by State Bank of India (SBI) Foundation – a collaborative healthcare programme offered support to the Government of India in its efforts to combat the healthcare challenges during COVID-19 pandemic. These initiatives showcased how CSR can effectively align corporate practices with broader public health goals while addressing ethical implications. Apart from this, there are other giant companies like Adani group which is headquartered in India which operate CSR activities through ‘The Adani Foundation’. The foundation operates in 19 states and 5,753 villages through Mobile Health Care Units (MHCUs) nationwide, along with hospitals, clinics, both general and specialized health camps. Its initiatives align with the United Nations' Sustainable Development Goals.

1. **CONCLUSION**

Microbial diagnostics is emerging as a crucial tool in the advancement of personalized or precision medicine, enabling tailored treatments based on the unique microbiome composition and infection profile of an individual. By leveraging cutting-edge technologies such as next-generation sequencing (NGS), metagenomics, and advanced biomarker detection, microbial diagnostics can identify pathogenic microbes with high precision, facilitating targeted therapies and minimizing adverse effects. This approach enhances the efficacy of antimicrobial treatments, reduces antibiotic resistance, enables early detection of infections and dysbiosis-related conditions. Integrating microbial diagnostics into personalized medicine not only revolutionizes infectious disease management but also plays a vital role in conditions like gut disorders, cancer, and metabolic diseases by optimizing therapeutic interventions based on a patient’s microbial ecosystem. However, ethical considerations, data privacy concerns, and accessibility challenges must be addressed to ensure equitable implementation and widespread clinical adoption. The growing importance of microbial diagnostics cannot be overstated. As we face an increasingly complex landscape of infectious diseases and environmental challenges, the ability to accurately identify and respond to microbial threats is essential. Continued investment in research, technology, and infrastructure for microbial diagnostics will be crucial in safeguarding public health and ensuring a sustainable future. As research continues to evolve, the integration of microbiome data into clinical practice may revolutionize the way we approach health and disease management.

The human microbiome holds significant potential in the realm of personalised or precision medicine. By leveraging microbiome analysis, healthcare providers can develop patient-specific treatment regimens that improve patient outcomes and enhance the efficacy of medical interventions.

1. **CHALLENGES AND FUTURE DIRECTIONS**

Despite the promising role of the microbiome in personalized medicine, several challenges remain. The microbiome is highly diverse and dynamic, varying across individuals based on genetics, diet, lifestyle, and environmental exposure. Understanding its role in disease and therapy response requires extensive research. Different laboratories use varying methodologies for microbial diagnostics, making it difficult to compare results and establish universal guidelines for clinical applications. While microbial sequencing provides vast amounts of data, determining which microbial changes are clinically significant remains a challenge. Not all microbial variations have known implications for health or disease. The complexity and variability of the microbiome make it difficult to establish standardized protocols for analysis and interpretation. High costs of next-generation sequencing (NGS), metagenomics, and other advanced diagnostic tools limit accessibility, especially in low-resource settings. Affordable and scalable solutions are needed. The collection and storage of microbiome data raise concerns about patient privacy, consent, and potential misuse of genetic and microbial information. Robust regulations are needed to protect patient data. Regulatory frameworks for microbial diagnostics in precision medicine are still evolving. Ensuring the clinical validity and safety of microbiome-based therapies and interventions is crucial for wider adoption. Many healthcare providers lack training in microbiome-based diagnostics and treatments, leading to hesitancy in integrating these tools into routine clinical practice. Personalized microbial treatments must consider the risk of antibiotic resistance, ensuring that targeted interventions do not inadvertently contribute to the emergence of resistant pathogens.

Future research may lead to the development of personalized probiotics and microbiome-based interventions ideal to the microbial profile of an individual to restore gut health and prevent disease. The development of portable, cost-effective, and rapid microbial diagnostic tools will improve accessibility. This would help in setting up Point-of-care and rapid diagnostics for enabling real-time monitoring of microbial changes. should focus on large-scale studies to validate microbiome-based biomarkers and therapeutic interventions. Integrating microbiome data with genomic, proteomic, and metabolomic information will enhance our holistic understanding of individual health profiles and disease mechanisms. Precision medicine approaches will focus on developing targeted antimicrobial treatments that eliminate harmful microbes while preserving beneficial ones, reducing the impact on the overall microbiome. Future efforts will emphasize the establishment of ethical guidelines, data protection laws, and transparent policies to ensure responsible use of microbiome diagnostics in healthcare. By overcoming current challenges and leveraging technological advancements, microbial diagnostics in personalized medicine has the potential to revolutionize disease prevention, diagnosis, and treatment, leading to more precise and effective healthcare solutions.

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