

Application of Sustainable Microbial Techniques in Biomonitoring for Environmental Sustainability

Archita Tiwari¹, Bharat Mishra^{2*}, Bhupendra Singh³, Shrishti Mishra⁴

¹Faculty of Pharmacy, Khwaja Moinuddin Chishti Language University, Lucknow, UP, India

²Department of Pharmacology, Dr. Shakuntala Misra National Rehabilitation University, Lucknow, Uttar Pradesh, India

³Department of Pharmaceutical Sciences, CT Group of Institutions, Jalandhar, Punjab, India

⁴Department of Pharmaceutics, Central Drug Research Institute, Lucknow, UP, India

Received: 4th Feb, 2025; Revised: 21st Mar, 2025; Accepted: 16th Apr, 2025; Available Online: 25th Jun, 2025

ABSTRACT

The growing rates of pollution have been driving global warming, rising sea levels, and species extinction, impacting both developed and developing nations alike. Traditionally, chemical analysis of air, water, and soil was employed to detect pollutants. However, by the time harmful chemicals were identified in these environments, significant damage had already occurred, making remediation efforts challenging. Bioindicators refer to living organisms like plants, animals or microbes which mirror the changes in environment composition due to pollution. Monitoring species or organisms which are very sensitive to such environmental changes constitute biomonitoring. For these techniques such as PCR, microscopy, immunological assays, biosensors, and microfluidics are crucial for environmental sustainability. PCR detects microbial contaminants and assesses biodiversity, while microscopy identifies and monitors microorganisms. Immunological assays like ELISA and EIA detect specific toxins and track environmental changes. Biosensors offer real-time pollutant data, lateral flow assays provide rapid on-site testing, and microfluidics enable efficient, high-throughput analysis. ELFA offers high sensitivity for trace pollutants, and other serological assays help identify pathogens and assess pollution impacts, collectively improving pollution monitoring and ecosystem health. The effect of environmental pollutants reflects in the lower levels before it occurs in higher levels. Lower levels include cellular, molecular and physiological changes that occur as a result of pollution and known as biomarkers, which act as an early warning system for toxic pollutants. Microbial communities present in air, water and soil are one of the early bioindicators used for assessing the pollution levels. Evaluation methods include detecting, tracking and quantifying microbes; monitoring diversity indices of microbial communities and analysing specific metabolic pathways. Biomonitoring techniques include high-throughput sequencing, quantitative Polymerase Chain Technology, metaproteomics, metatranscriptomics and metagenomics. Cultivable microbes are commonly used but cultivation independent methods like pathogen tracking and toxin tracking needs to be included for an accurate picture. Integrating biological methods with technology like bioinformatics and machine learning tools could increase the efficiency of biomonitoring using microbes.

Keywords: Microbes, Techniques, Pollution, Biomonitoring, Biomarkers, Environmental Sustainability

How to cite this article: Archita Tiwari, Bharat Mishra, Bhupendra Singh, Shrishti Mishra. Application of Sustainable Microbial Techniques in Biomonitoring for Environmental Sustainability. International Journal of Drug Delivery Technology. 2025;15(2):578-91. doi: 10.25258/ijddt.15.2.27

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Microbes, including yeasts, moulds, and bacteria, play a crucial role in human survival and have both beneficial and harmful impacts on human health. Many areas of life have been increasingly intertwined with microbes in the last decade, including genetic engineering, medicine, biotechnology, and the food sciences. Their one-of-a-kind properties mark them valued constituents in several medicinal ingredients, including hormones, antibiotics, amino acids, and other. The infection of aquaculture structures by pathogens such as bacteria, viruses, and protozoa has arisen as a severe concern in current years. In an aquaculture setting, an increase in bacterial pathogen loads has two major outcomes. To start, aquaculture diseases can grow when raised up fish contract infections instigated by harmful microorganisms. Second, these

destructive bacteria can cause stern health problems in humans if they develop into seafood. Microorganisms and genetically engineered microbes (GEMs) are being used for bioremediation due to the greater than before pollution. Yeast, bacteria, and fungi have the ability to break down harmful chemicals into less harmful ones. This includes compounds like benzene, toluene, PCBs, and dioxins. For bioremediation, it is important to use the following microorganisms: Actinobacteria, Alcaligenes, Flavobacterium, Mycrococcus, Phanerochaete, Nitrosomonas, Pseudomonas, Serratia, Trametes, and Xanthobacter¹. The first way to cultivate and isolate bacteria for the purpose of studying human microbiota was the invention of bacterial culture, an artificial medium. Microbiologists have recently prioritised molecular techniques—including PCR, sequencing, and especially

*Author for Correspondence: bharatekansh@gmail.com

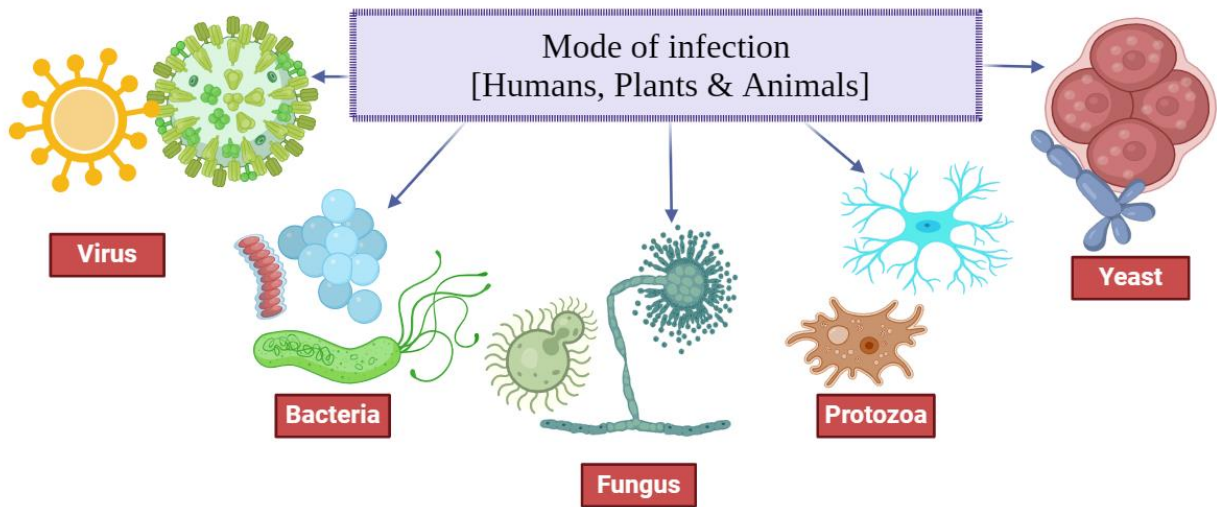


Figure 1: Types of microorganism

metagenomics—over traditional cultural practices, since their advent in the 1970s. Microorganisms can multiply rapidly and have been exploited to produce different product and by-products which may cause damage and unfortunate effect on biological system. Due to this property of microorganisms and advancement in the technology of genetic engineering is not sufficient to ruled out the harmful microbes that cause serious damage to the life. Microbes can contribute to environmental pollution through the degradation and transformation of pollutants, production of harmful byproducts, and alteration of soil and water quality.

Extensive biomonitoring using advanced techniques is crucial for understanding these effects, managing pollution, and protecting ecosystem health. Techniques like high-throughput sequencing, quantitative PCR, biosensors, and bioinformatics tools provide critical insights into microbial roles and environmental impacts, enabling more effective pollution management and remediation strategies. To rapidly detect presence and extent of microorganism in any environment biosensors that provide us with a tool the best emerging technology is used that counteract the problem with genetic engineering². Over the past decade, microbial detection has been based on the culturing of solid media. From the naturally occurring microbial population, microbes without culturing identification and quantification by polymerase chain reaction (PCR) microscopic analysis, and immunological assays. Colony morphology characterization has been used to study microbial variety, adaptation, and evolution. The use of colorimetric measurements with ATP bioluminescence has the potential to quickly and accurately identify mould, bacteria, and pathogens by their metabolites and related toxins in food, thus replacing more laborious and time-consuming traditional methods of microbiological detection in milk and food. Recently developed bio-recognition ligands have the potential to enhance the selectivity of detection methods used in biosensors, lateral flow assays, microfluidic

devices, and other similar technologies for identifying cells within complicated food matrices. Microscopic analysis is also employed but not effective for identification. Although molecular method like PCR which is very sensitive and specific, this technique is time-intensive. Furthermore, some other speedy and high throughput capacity immunological assays comprises ELISA[enzyme-linked immunosorbent assay], EIA[enzyme immunoassay], ELFA[enzyme-linked fluorescent assay] and other serological assays are used that have better possibility for precised quantification of the targeted organisms³. To understand ecosystem structure and function, quantifying microorganisms within a community is essential. Microbial morphology is often too simple to differentiate species, and many microorganisms resist cultivation. In spite of setbacks experienced by conventional methods of culture, the intricacy of microbial communities has been better understood thanks to recombinant DNA technology. Analysis of 16S rRNA sequences is one molecular technique that permits direct examination of diversity, community structure, and phylogeny. Additionally, nucleic acid hybridization techniques can quantify individual microorganisms or entire microbial communities. The following culture-independent molecular based methods are useful for obtaining general information about the structures and genetic diversity of microorganisms: next generation sequencing (NGS) technology, fatty acid methyl esters (FAME) analysis, 16S rDNA clone library preparation, denaturing and temperature gradient gel electrophoresis (DGGE/TGGE), and terminal restriction fragment length polymorphism (T-RFLP). Applied molecular microbiology develops molecular methods to monitor and identify microorganisms in natural ecosystems, offering a valid alternative to traditional microbiology. Since the early 1990s, identifying bacterial communities in environmental samples using molecular methods, especially 16S rDNA sequencing, has become crucial⁴. These tools effectively study bacterial interactions with

their environment and are known for their speed and reliability. In bioinformatics, genetic fingerprinting shows how diverse microbial communities are genetically. One popular culture-independent fingerprinting method is denaturing gradient gel electrophoresis (DGGE), which separates identically sized PCR amplicons with distinct sequences. For the purpose of microbe identification, mass spectrometry—and MALDI-TOF MS in particular—provides precise, fast, and sensitive spectra of bio-analytes by employing positively charged peptides and proteins with molecular weights ranging from 2000 to 20,000 m/z. Individual mass peaks offer valuable information for microbial fingerprinting⁵.

Microbial infection in Human, animal and plants

Microbial infections caused by fungi, bacteria, parasites, viruses, and prions result in a diverse array of illnesses. Once in the body, these microbes can cause mild to life-threatening disruptions in normal body functions, triggering a spectrum of diseases. Microbial infections are diagnosed through lab tests such as cultures, molecular techniques, and serological assays to identify the specific microbes causing the disruption. Treatment varies by infection type and causative agent, using antifungals, antibiotics, antivirals, or antiparasitic drugs. There are some most important types of microbes and their significance, type of microbial infections (Figure 1).

Table 1: The impact of bacteria on disease development in plants, animals, and humans

Class of bacteria	Host	Bacteria	Disease	Mode of transmission
Gram negative bacteria	Plants	<i>Xanthomonas campestris</i>	black root in cruciferous plants, including cabbage and cauliflower	enters plants through natural openings like stomata or wounds caused by insects, mechanical injuries, or cultural practices
		<i>Pseudomonas syringae</i>	break down the plant's cell walls, aiding invasion	infects through wounds or natural openings in the plant
		<i>Erwinia amylovora</i>	Fire blight, a devastating disease	natural openings, such as flowers, nectarhodes, or wounds on floral structures
	Animals	<i>Escherichia coli</i>	Enteric diseases	ingestion of contaminated food or water
		<i>Salmonella</i>	Gastroenteritis	transmit Salmonella to their offspring, particularly in poultry and certain reptiles
		<i>Mannheimia haemolytica</i>	Bovine respiratory disease in cattle	Through direct or indirect contact by inhalation or ingestion
		<i>Bordetella bronchiseptica</i>	Kennel cough in dogs	spreads through direct contact (licking, nuzzling) and through the air (coughing or sneezing), or via contaminated fomites.
	Humans	<i>Pseudomonas aeruginosa</i>	respiratory, urinary, and gastrointestinal tracts infections	Through contracted from heavily infested surfaces such as tubing or water tubs
		<i>Escherichia coli</i>	UTIs and gastrointestinal infections	Ingesting food contaminated with pathogenic E. coli
		<i>Mycobacterium tuberculosis</i>	Tuberculosis (TB)	airborne transmission
		<i>Neisseria gonorrhoeae</i>	gonorrhea	sexual contact Transmission occurs through vaginal, anal, or oral sex with an infected individual
		<i>Vibrio cholerae</i>	cholera	contaminated water and inadequate sanitation
		<i>Borrelia burgdorferi</i> <i>Helicobacter pylori</i>	Lyme disease gastric ulcers and stomach inflammation, as well as an increased risk of stomach cancer	bite of infected black-legged ticks oral-oral or fecal-oral transmission
	<i>Salmonella species</i>	salmonellosis	Improper food handling, such as using the same cutting board or utensils for raw meat and ready-	

Table 1: The impact of bacteria on disease development in plants, animals, and humans

Class of bacteria	Host	Bacteria	Disease	Mode of transmission
Gram positive bacteria	Animal	<i>Legionella pneumophila</i>	Legionnaires and Pontiac fever	to-eat foods without proper cleaning inhalation of contaminated water droplets
		<i>Chlamydia trachomatis</i>	sexually transmitted infections (STIs) and ocular infections	transmitted through unprotected vaginal, anal, or oral sex with an infected individual
	Human	<i>Clostridium perfringens</i>	enterotoxemia in livestock, enteritis especially in sheep and goats	contaminated feed, or even through the fecal-oral route
		<i>Staphylococcus aureus</i>	bloodstream infections, pneumonia, or bone and joint infections	direct skin contact with an infected person
		<i>Streptococcus pyogenes</i>	streptococcal infections, including strep throat and skin infections.	infected person coughs or sneezes, respiratory droplets containing <i>Streptococcus pyogenes</i> can spread
		<i>Streptococcus pneumoniae</i>	Pneumonia, respiratory and invasive infections	respiratory secretions
		<i>Clostridium difficile</i>	diarrhea and pseudomembranous colitis	spread through several avenues
		<i>Listeria monocytogenes</i>	listeriosis	contaminated food is the most common route of infection
<i>Bacillus anthracis</i>	anthrax	be transmitted through contact with contaminated animal products		
		<i>Clostridium botulinum</i>	spores can germinate in the infant's intestines, producing the toxin.	Inadequate food processing or storage can allow the spores to germinate and produce toxin.

Table:2 The impact of virus on disease development in plants, animals, and humans

Host	Virus	Disease	Mode of transmission
Plant	Tobacco Mosaic Virus (TMV)	infection creates a mosaic like pattern, mottling and discoloration of the leaves	spread through contaminated hands, tools, clothing, and even through infected plant sap
	Potato Virus Y (PVY)	potato tuber necrotic ringspot disease	transmitted mechanically through contaminated tools, equipment, infected seeds or hands
	Cucumber Mosaic Virus (CMV)	stunted growth, and deformities in fruit	transmitted mechanically through contact. This includes contaminated hands, tools, and equipment used in agricultural practices
	Citrus Tristeza Virus (CTV)	Tristeza disease	through insect vectors, particularly aphids, CTV can also spread through grafting or budding practices in citrus tree propagation.
	Gemini viruses	cauliflower mosaic disease	transmitted by insect vectors like whiteflies or leafhoppers
	Tospo viruses	diseases like tomato spotted wilt and can lead to necrosis, mottling, or ring spots in plants	Transmitted by thrips, small insects that feed on plant sap

Table:2 The impact of virus on disease development in plants, animals, and humans

Host	Virus	Disease	Mode of transmission
Animal	Feline Immunodeficiency Virus (FIV)	can lead to immune deficiency in cats, develop cancer and immune-mediated blood disorders than healthy catsmaking them more susceptible to other infections	The primary mode of transmission is through bite wounds inflicted during fights between infected and uninfected cats
	Rabies virus	Furious rabies Paralytic rabies	transmitted through the bite of an infected animal and can lead to a fatal viral encephalitis
	Canine Parvovirus (CPV)	severe gastrointestinal disease	Transmission occurs when uninfected dogs come into contact with the feces of infected dogs or contaminated environments, such as shared water bowls and kennels.
	Bovine Viral Diarrheavirus (BVD)	Bovine Viral Diarrhea	primary mode of transmission is through direct contact between infected and susceptible animals
	Herpesviruses	causing diseases like equine herpesvirus in horses, canine herpesvirus in dogs, feline herpesvirus in cats, and various other diseases in different species	spread through direct contact with infected animals, their bodily fluids (like saliva, nasal discharge, or genital secretions), or through respiratory droplets when infected animals sneeze or cough
Human	Variola virus	Small pox	
	Hepadnaviruses,	Hepatitis B	Direct contact with infected blood through direct skin-to-skin contact, particularly mucous membranes or areas with micro-abrasions.
	Human Papillomaviruses	cervical cancer, genital warts, and some oral and throat cancers	

Table 3: The impact of fungus on disease development in plants, animals, and humans

Host	Fungus	Disease	Mode of transmission
Animal	<i>Phytophthora</i> <i>infestans</i>	Late blight of potatoes and tomatoes	from infected tubers to plant tissues of the next season's crop may occur during seed-tuber handling, cutting, and planting
	<i>Fusarium</i> <i>spp.</i>	Fusarium wilt.	pathogen moves up the plant through the vascular system and can spread through the air
	<i>Ustilagomaydis</i>	Corn smut	facultative biotrophic fungus that depends on sexual reproduction to cause disease
	<i>Botrytis</i> <i>cinerea</i>	Graymold	dispersed by wind or water
	<i>Alternaria</i> <i>spp</i>	Alternaria leaf spot	spread by spores as they become airborne and land on plants
	<i>Aspergillus</i> <i>spp</i>	Aspergillosis	Transmission occurs through inhalation of airborne conidia
	<i>Microsporum</i> <i>spp.</i> and <i>Trichophyton</i> <i>spp</i>	Dermatophyte infections	Transmission occurs via direct contact with an infected animal or person, or by contact with infected hair and scale on fomites or in the environment
	<i>Blastomyces</i> <i>dermatitidis</i>	Blastomycosis	The primary mode of transmission is through inhalation
	<i>Candida</i> <i>spp</i>	Candidiasis	transmission include person-to-person contact, sexual contact, and contact with contaminated objects or surfaces.
	<i>Malassezia</i> <i>pachydermatis</i>	Malassezia dermatitis	Through direct contact.

Table 3: The impact of fungus on disease development in plants, animals, and humans

Host	Fungus	Disease	Mode of transmission
Human	<i>Candida albicans</i>	Candidiasis	transmission include person-to-person contact, sexual contact, and contact with contaminated objects or surfaces
	<i>Aspergillus</i> spp	Aspergillosis	through inhalation of airborne conidia.
	<i>Cryptococcus neoformans</i>	Cryptococcosis	inhalation of basidiospores or desiccated yeast cells from the environment
	<i>Trichophyton</i> spp	Dermatophyte infections	Warm and humid environments, sharing personal items (towels, combs), and close contact with infected individuals.

Bacterial infections

Prokaryotic single-celled bacteria thrive in diverse environments, from the human gut to deep-sea vents. They participate in nitrogen fixation, bioremediation, decomposition, and can be pathogens. Gut bacteria aid digestion, produce vital vitamins, and support the immune system. Bacteria are crucial in producing vaccines, antibiotics, insulin, and fermented foods like cheese and yogurt. They are also used in brewing, baking, biofuel production, biomaterial synthesis, and as biocatalysts. In biotechnology, bacteria serve as model organisms. Bacterial infections can cause diseases such as tuberculosis, strep throat, and foodborne illnesses. Antibiotic-resistant bacteria pose significant treatment challenges, emphasizing the need for prudent antibiotic use and innovative therapies.

The environment in which humans live is delicately balanced thanks to the ubiquity of bacteria. Even though only a tiny fraction of all bacteria are pathogens, the alarming rise of bacteria resistant to antibiotics is having far-reaching and deadly effects. A double-stranded circular molecule of DNA contains the genetic information of prokaryotic organisms like bacteria. In the right environments, they can quickly grow and spread, posing a serious threat of infection (Table 1). The cell wall features that determine whether bacteria are Gram-positive or Gram-negative also determine how susceptible they are to antibiotics⁶.

Viral infections

Viruses are tiny pathogens that require a host cell to proliferate, infecting microbes, plants, and animals, and causing diseases like influenza, HIV/AIDS, hepatitis, and COVID-19. Bacteriophages, viruses that infect bacteria, help regulate bacterial populations. Viruses impact ecosystems, human health, scientific research, and technology. Understanding viruses has led to vaccines and antiviral drugs, enhancing disease prevention and treatment. Viruses are also used in gene therapy to transfer genetic material and correct faulty genes. Viral mutations contribute to genetic diversity and host evolution, with studies providing insights into virus adaptation, drug resistance, and immune evasion (Table 2).

Fungal infections

Fungi are eukaryotic microorganisms, including molds, mushrooms, and yeasts. They are crucial in food and drink production (bread, beer, cheese), decomposition, and nutrient recycling. Fungi play vital roles in industry, research, medicine, agriculture, and ecosystems. As

decomposers, they support nutrient cycling and soil health. Some fungi cause plant diseases, impacting agriculture. They produce antibiotics like penicillin and other medicinal compounds. Fungal conservation is important due to habitat loss and environmental changes. Fungi are model organisms in genetic and molecular research, aiding understanding of symbiotic interactions, adaptation, and evolution. Fungi cause mycoses in humans, ranging from skin infections to severe systemic diseases, especially in immunocompromised individuals. They also cause various infections in animals and plants, affecting agriculture and ecosystem health. Fungal infections in animals can be cutaneous (skin, hair, nails), such as ringworm caused by dermatophytes like *Trichophyton*. In plants, fungi infect roots, stems, leaves, and fruits, causing diseases that threaten food security and crop productivity (Table 3). Effective management strategies are essential to mitigate these impacts⁷.

Protozoal infections

Eukaryotic single-celled protozoa are found in soil and water, playing a crucial role in microbial food chains as a food source for other microbes. They consume bacteria and algae, making them vital components of microbial food webs and nitrogen cycling. Soil protozoa influence soil fertility, organic matter decomposition, and nutrient cycling in terrestrial ecosystems. Protozoa, like *Giardia lamblia* (causing giardiasis) and *Plasmodium* (causing malaria), are pathogens causing human diseases. Protozoal diseases are a global health concern, especially in regions with inadequate sanitation and healthcare infrastructure. Diseases like malaria cause substantial morbidity and mortality, particularly affecting vulnerable populations like children and pregnant women. Certain protozoal infections, if untreated, can lead to chronic health issues and complications, impacting the quality of life. These organisms have complex life cycles, multiple transmission routes, and various clinical manifestations. Malaria, Amoebiasis, Giardiasis, Toxoplasmosis, Trichomoniasis, Leishmaniasis, Chagas Disease, African Trypanosomiasis, Babesiosis are some examples of protozoal infection. Animals can have candidiasis from fungus *Candida albicans*, which manifests as white patches on throat, inside cheeks, or tongue and is often accompanied by pain and trouble swallowing⁸.

Microbial techniques

In microbiology, microbial techniques encompass a wide range of approaches to studying, manipulating, and analysing microbes.

Microbial Techniques for Diagnosing Bacterial Infections
 The microbial techniques for diagnosis of bacterial infections are mentioned in Figure 2.

Clinical Culture

Clinical culture is essential for diagnosing bacterial infections. Specimens (e.g., blood, urine, sputum, wound swabs) are collected aseptically and inoculated onto culture

media to grow and identify bacteria. Proper collection and transport of samples are crucial for maintaining bacterial viability and ensuring accurate diagnosis. The sample is streaked onto specific culture media (e.g., blood agar, MacConkey agar) to promote bacterial growth. Plates are incubated under optimal conditions, and technicians regularly check for bacterial colonies or color changes.

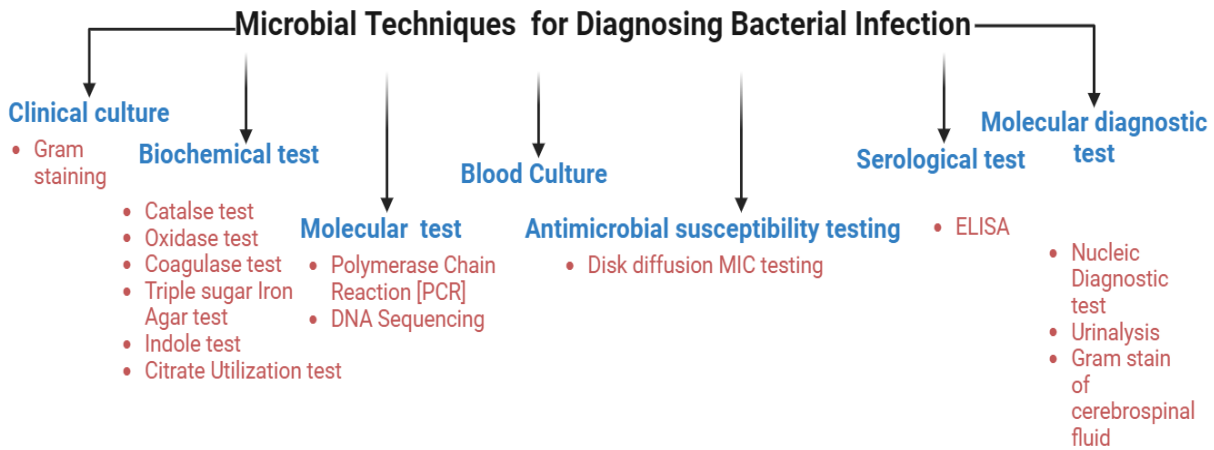


Figure 2: Microbial Techniques

Microbial Techniques for Diagnosing Viral Infections

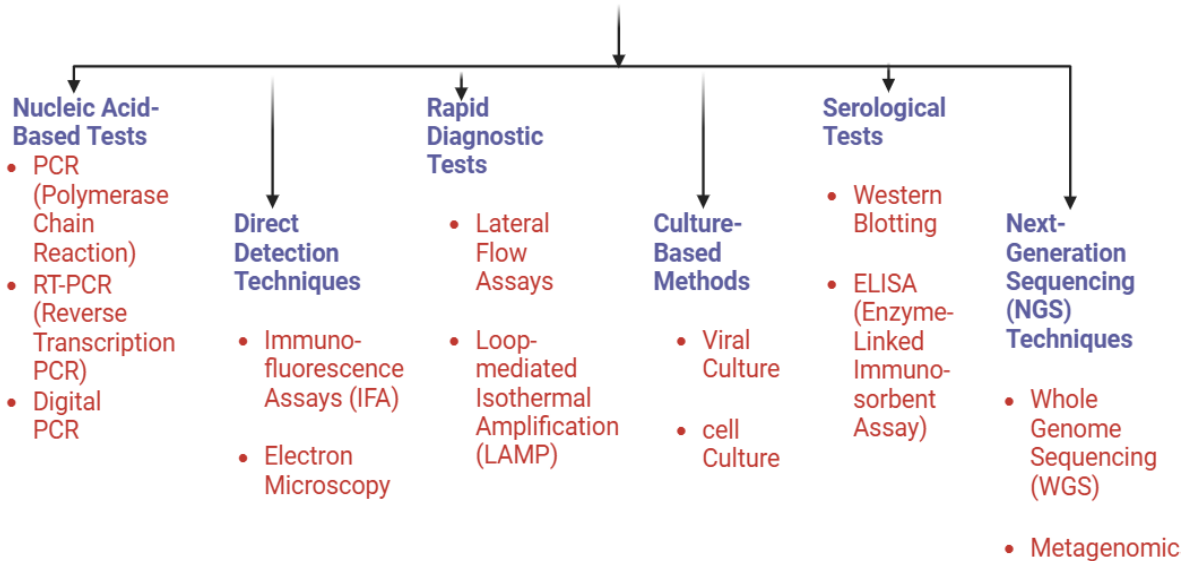


Figure 3: Microbial Techniques for Diagnosing Viral Infections

Microbial Techniques for Diagnosing Fungal Infection

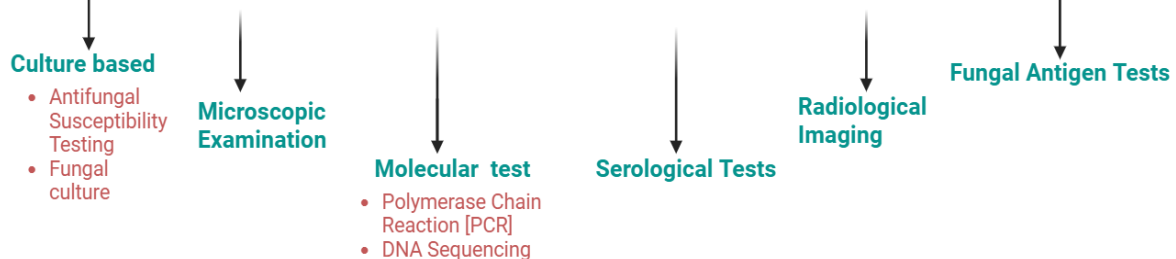


Figure 4: Microbial techniques for diagnosis of fungal infections

Single colonies are isolated and sub-cultured for further testing. Bacterial species are identified using microscopy, biochemical tests, and automated systems. Antibiotic susceptibility testing determines the most effective treatment options.

Gram Staining

Gram staining classifies bacteria as Gram-positive or Gram-negative based on cell wall characteristics. The process involves applying specific stains to bacterial cells, providing initial information about their morphology. Bacteria are spread on a slide, air-dried, and fixed with heat or chemicals (e.g., methanol) to prevent washing away during staining. As an initial step in Gramme staining, crystal violet is flooded onto a slide to turn all of the cells purple. Afterwards, iodine is introduced as a mordant, which causes the crystal violet to form a complex. A crucial step in avoiding erroneous results is the decolorisation of Gram-negative bacteria using ethanol or acetone. When Gram-negative bacteria are counterstained with safranin or basic fuchsin, they take on a pink or crimson hue. Microscopically, bacteria with a thick peptidoglycan layer (gram-positive) look purple, while those with a thinner peptidoglycan layer and outer lipid membrane (gram-negative) look pink or red. Gram staining is essential for quickly identifying bacterial cell wall characteristics.

Biochemical Tests

Biochemical tests involve assessing bacterial metabolic activities to identify species-specific traits. These tests confirm the identity of the isolated bacteria, facilitating more precise treatment plans. Biochemical tests are essential in identifying and characterizing bacteria based on their metabolic activities. Biochemical tests help differentiate between bacterial species by assessing their metabolic capabilities, enzymatic activities, and utilization of specific nutrients. Common Biochemical Tests are as follows,

Catalase Test

The catalase enzyme decomposes H_2O_2 into its component oxygen and water. Bacteria that generate catalase will cause bubbles to occur. Positive result—bubbles. Commonly used to differentiate between *Staphylococcus* (catalase-positive) and *Streptococcus* (catalase-negative).

Oxidase Test

Identifies existence of cytochrome c oxidase, an enzyme involved in electron transport chain. Positive result—color change after application of oxidase reagent. Helpful in differentiating between various genera, like *Pseudomonas* (oxidase-positive) and *Enterobacteriaceae* (oxidase-negative).

Coagulase Test

Identifies microorganisms that can manufacture the clotting enzyme coagulase. A clot has formed, which is a positive outcome. A tool for differentiating coagulase-positive *E. coli* and coagulase-negative Bacteria and germs.

Triple Sugar Iron (TSI) Agar Test

Evaluates sugar fermentation, gas production, and hydrogen sulfide production. Based on color changes and gas production in the TSI agar, aiding in differentiating between *Enterobacteriaceae* species.

Indole Test

Measures the production of indole from tryptophan by bacterial enzymes. Positive result—formation of a red color afterward addition of Kovac's reagent. Differentiates between bacteria like *Escherichia coli* (indole-positive) and *Enterobacter aerogenes* (indole-negative).

Citrate Utilization Test

Determines capability of bacteria to consume citrate as a carbon source. Positive result—color conversion of medium from green to blue. Used in differentiating *Enterobacteriaceae*.

Molecular Methods

These molecular methods offer high compassion and specificity, allowing for rapid and precise identification of bacteria, as well as finding of virulence factors or resistance genes.

Polymerase Chain Reaction (PCR)

PCR is a sensitive molecular technique which amplifying and identifying fungal DNA or RNA sequences present in patient samples for detection of fungal infections. This method allows targeted treatment and accurate, specific and rapid diagnosis of fungal pathogen. For the analysis DNA or RNA is extracted from patient specimens such as blood, tissue biopsies, respiratory secretions, cerebrospinal fluid, or other bodily fluids containing the suspected fungal pathogen and PCR amplifies specific regions of fungal DNA or RNA using primers designed to target conserved sequences unique to the fungal pathogen of interest. The multiple cycles of denaturation, annealing, and extension are carries out by PCR machine. The DNA strands split apart during denaturation, and the primers anneal to the complementary sequences. The targeted fungal sequences are synthesized into new DNA strands by DNA polymerase, which expands the primers. The patient sample has the specific fungal pathogen, as indicated by the presence of amplified fungal DNA/RNA.

DNA Sequencing

DNA sequencing is another powerful molecular technique which analyzing the genetic material of fungi present in patients sample for the detecting and identifying fungal infections. This technique involves determining the precise sequence of nucleotides in fungal DNA, provided that precise information about the fungal species and aiding in correct diagnosis and management decisions. Fungal DNA is examined by sequencing systems, like Sanger sequencing or next-generation sequencing (NGS), to recognise species, invention mutations, and understand genetic diversity. Using databases and bioinformatics tools, the acquired sequences are inspected in order to match them by earlier available sequences and conclude which specific fungus species are existing in the patient sample.

Blood Cultures

Blood cultures perceive bacteria in the bloodstream by culturing blood samples. This method is crucial for diagnosing systemic infections and guiding treatment. Blood is drawn aseptically from a vein into specialized bottles with culture media to support bacterial growth.[100]Collected blood is added to multiple culture bottles with different media, including aerobic and anaerobic, to support various bacterial growth needs. Bottles are incubated at 37°C and monitored for growth.

Positive bottles are subcultured onto agar plates for bacterial isolation and identification. Automated systems continuously monitor for growth, signaling positive results, which are then further analyzed using biochemical tests or molecular techniques.

Antimicrobial Susceptibility Testing (AST)

Disk Diffusion Method or MIC Testing

Antibiotic Susceptibility Testing (AST) evaluates bacterial isolates' response to antibiotics, guiding effective therapy selection. The MIC test finds the smallest concentration of antibiotic that stops bacteria from growing. This technique aids in treatment decisions, assesses susceptibility, and monitors resistance patterns by preparing a range of antibiotic concentrations. The dilution series is prepared according to guidelines and clinical breakpoints. A standardized amount of bacteria is added to wells or tubes with varying antibiotic concentrations, which are then incubated. The concentration at which no discernible bacterial growth occurs is known as MIC. Automated systems can provide precise MIC values. MIC testing is essential for determining bacterial susceptibility, guiding effective therapy, and addressing antibiotic resistance⁹.

Serological Tests

ELISA

ELISA detects bacterial antigens or antibodies in patient samples. These tests aid in diagnosing bacterial infections and assessing the patient's immune response. Bacterial antigens or antibodies are immobilized onto a microplate well. Unbound sites on the plate are blocked to prevent non-specific binding. The bacterial sample (e.g., serum, culture supernatant) is added to the wells and allowed to interact with the immobilized antibodies or antigens. The plate is washed to remove unbound substances. A labeled secondary antibody or enzyme substrate is added to detect the antigen-antibody binding. Enzymes catalyze a color change or produce a fluorescent signal. Optical density or fluorescence is measured using a spectrophotometer or a specialized ELISA reader.

Molecular Diagnostic Tests

NAATs

NAATs detect bacterial DNA or RNA in patient samples. (NAATs are advanced molecular techniques used for the detection, identification, and characterization of bacterial DNA or RNA. NAATs target specific DNA or RNA sequences unique to bacterial pathogens, enabling rapid and accurate identification of infections. Samples such as blood, urine, sputum, or tissue are collected for analysis from which bacterial DNA or RNA are extracted and purified. Utilizes specific primers or probes to amplify bacterial DNA or RNA sequences of interest. Identifies and quantifies the amplified nucleic acids using fluorescent probes or other detection methods. NAATs have revolutionized bacterial determination by offering rapid, sensitive, and specific detection of bacterial nucleic acids. Their applications in clinical diagnostics, surveillance, and research continue to advance our understanding of bacterial infections and guide effective treatment strategies, contributing significantly to modern healthcare and infectious disease management.

Urinalysis

Urinalysis is essential for diagnosing urinary tract infections (UTIs). It includes testing physical, chemical, and microscopic properties of urine to detect abnormalities, including bacterial presence, which indicates UTIs or other systemic issues. Initial urine analysis includes visual examination for color, clarity, and odor; cloudy urine may indicate bacteria. Dipstick tests detect nitrites (bacterial presence) and leukocyte esterase (white blood cells). Urine sediment microscopy examines centrifuged samples for bacteria, white blood cells, and red blood cells, aiding in bacterial identification and quantification. Bacterial count (CFU/mL) helps determine clinical significance. A high bacterial count often indicates infection, but distinguishing between contamination and true infection is crucial. This is achieved by evaluating patient's symptoms, inflammatory markers, and the type and quantity of bacteria present.

Gram Stain of Cerebrospinal Fluid or Other Body Fluids

Gram staining of cerebrospinal fluid (CSF) and other sterile body fluids is crucial for diagnosing bacterial infections like meningitis. This method rapidly evaluates bacterial existence and morphology by staining the fluid samples. Staining by crystal violet, iodine, alcohol or acetone, and safranin follows sample collection by lumbar puncture or sterile methods. The sample is then heat-fixed and spread on a slide. To aid in the early diagnosis and treatment, the stained smear is inspected below a microscope to decide bacterial features.

Microbial Techniques for Diagnosing Viral Infection

The microbial systems for viral infection identification are given in Figure 3.

Nucleic Acid-Based Tests

PCR (Polymerase Chain Reaction)

In the field of viral diagnostics, it is a fundamental method. Even in low viral loads, it can be detected because it amplifies certain parts of the viral genome. With the use of real-time PCR (qPCR), viral load can be measured. Strengthening and detection of viral genetic material (DNA or RNA) in clinical samples can be attained through the use of PCR, a cutting-edge molecular diagnostic method for the identification of viral infections. When it originates to recognising several viral infections, making management options, and assisting in disease surveillance and management, this knowledge is currently vital. By binding to preserved sequences in the viral genome, modified primers in PCR intensify particular areas of viral nucleic acids. A DNA polymerase enzyme arbitrates the amplification progression by cycling the DNA molecule over denaturation, annealing, and extension. PCR confirms specificity for confident viral strains or species by targeting different areas in the viral genome. The sensitivity, specificity, and speed of detection through potential by PCR have caused a sea variation in viral diagnostics.

RT-PCR (Reverse Transcription PCR)

In edict to amplify RNA viruses, RT-PCR transforms viral RNA into cDNA. By perceiving and amplifying viral RNA in clinical samples, RT-PCR is one of the best potent molecular diagnostic techniques for viral infections. Since RNA viruses essential first undergo reverse transcription to transform their RNA into cDNA previously PCR amplification, this method is predominantly crucial for

these viruses. With its exclusive combination of high compassion, specificity, and speedy detection capabilities, RT-PCR has completely transformed viral diagnostics. It is now crucial in recognizing a wide variety of viral infections in all categories of clinical contexts. After cDNA synthesis is complete, primers intended to bind to preserved viral genome sequences are used in PCR to amplify specific portions of the viral cDNA. Subsequent amplification, several detection methods are used, such as fluorescent probes, real-time PCR, or gel electrophoresis, to visualize or quantify the produced cDNA. This is comparable to traditional PCR.

Digital PCR

The accuracy of quantifying viral DNA/RNA concentrations in a sample using this technique whereby samples are partitioned into thousands of separate reactions is remarkable. One such technology is the digital polymerase chain reaction (dPCR), which can detect viral infections with a remarkable level of sensitivity and accuracy. The digital dPCR method is an invaluable technique in virology used for infection diagnosis, viral load monitoring, and understanding viral dynamics because it provides unprecedented sensitivity and accuracy for quantification of viral nucleic acids. The capability to identify low titers of viruses and quantifying viral nucleic acids has significant implications in clinical diagnostics, treatment monitoring, and in virology research¹⁰.

Serological Tests

ELISA

Understands antibodies made in reaction to viruses or viral antigens. In viral infection analysis, immune response observing, and vaccine development, ELISA tests are beneficial due to their adaptableness, high sensitivity, specificity, and ease of use. The basis of ELISAs is the perception of specific binding among antibodies or viral antigens and their gratis molecules. It can recognise viruses by perceiving their antigens or by detecting antibodies that the host immune structure has made in reaction to viruses. The reaction among a substrate and enzymes involved to antibodies sources a discernible shift in color or fluorescence. Virus antigen or antibody concentration and strength of color or fluorescence are directly connected.

Western Blotting

For the persistence of perceiving and distinguishing viral proteins and antibodies in clinical samples, virologists regularly use the robust and precise laboratory method known as Western blotting. Using this method, the host immune system's antibodies or viral antigens can be located and identified. To better comprehend viral pathogenesis, assess immune responses, and diagnose viral infections, Western blotting is a crucial tool in virological research.

Culture-Based Methods

Viral Culture

In viral culture, viral isolation, proliferation, and characterisation are all greatly supported. It comprises isolating certain strains of viruses and studying their traits by growing them in host cells in a controlled setting. The virus particles are then cultured in laboratory containers with the appropriate host cells after clinical samples such as blood or respiratory secretions have been processed.

Injecting host cells derived from patient samples allows viruses to grow and replicate. Cellular changes that manifest as cytopathological consequences (CPEs) are a hallmark of viral infections. We may do further techniques, such as molecular assays or immunofluorescence, to confirm the presence of certain viral antigens or nucleic acids.

Cell Culture

The use of cell culture in the diagnosis of viral infections is a crucial technique in the study of virology. This method relies on the controlled cultivation of host cells to analyze, isolate, and detect viruses in clinical samples. The choice of cell lines is determined by viral compatibility. After coming into contact with clinical materials such as blood or respiratory secretions, these cell cultures enable viruses to replicate. Cultures show evidence of viral infection in the form of cytopathic effects (CPEs), viral plaques, and changes in cell structure. Additional techniques for determining the existence of viral antigens or nucleic acids include neuroimaging, polymerase chain reaction, and electron microscopy¹¹.

Next-Generation Sequencing (NGS) Techniques

Whole Genome Sequencing (WGS)

By determining the viral genome, this powerful and comprehensive molecular method may be used to diagnose viral infections. This technique allows for the sequencing of a whole viral genome, which provides a wealth of information on the virus's structure, genetic variations, evolution, and potential implications for public health, treatment, and diagnosis. A virus's whole genome, including its coding and non-coding regions, may be read and mapped using whole genome sequencing (WGS). The massive amounts of sequence data generated by WGS are the result of the simultaneous sequencing of millions of DNA fragments. For this aim, high-throughput sequencing tools like next-generation sequencing are often used. Aligning and evaluating the generated sequences will be the next phase, which will involve the application of sophisticated bioinformatics tools and procedures. Because of this, we will be able to fully rebuild the viral genome and identify any modifications or mutations.

Metagenomics

Through the study of microbial genomes as a whole, metagenomics has emerged as a cutting-edge tool for the detection of viral infections in clinical samples. Emerging as a state-of-the-art instrument in virology, it is a robust and all-encompassing approach. This method improves our understanding of all viruses, including newly discovered ones, viral diversity, and viral ecology within complex microbial ecosystems. The field of metagenomics allows for the direct sequencing of clinical samples containing a diverse array of microorganisms, including viruses. Using bioinformatics tools, viral genomes are distinguished from other forms of microbial genetic material, and the genetic sequences are constructed, annotated, and categorized from the sequencing data. Metagenomics may identify and characterize novel and known viral sequences in a sample by using de novo assembly methods or by comparing sequence data with databases of known viral genomes. Genomic research has the potential to reveal many things

about the functional abilities of viral populations, such as genes associated with virulence or medication resistance. Metagenomics has revolutionized viral diagnostics by providing a holistic view of viral populations inside complex samples. Because of its ability to detect both known and unknown viruses, examine viral diversity, and monitor viral dynamics, it is a powerful instrument in the field of virology for the diagnosis of infections, the research of viral ecology, and the direction of public health programs¹².

Direct Detection Techniques

Immunofluorescence Assays (IFA)

It employs fluorescently-labeled antibodies to identify viral antigens in cells or tissues, providing a visual confirmation of viral presence. In vitro antigen detection (IVAD) is a typical method for diagnosing viral infections. It is a very sensitive and sophisticated method in the field of virology. Utilizing the fact that antibodies bind exclusively to viral proteins, this method enables the visualization and identification of viruses inside cells or tissues. To identify viral antigens in patient samples, interactional fluorescence amplification (IFA) employs antibodies that have been fluorescently tagged. Antibodies recognize and bind to specific viral proteins and structures upon interaction with infected cells and tissues. Clinical specimens such as swabs, tissues, or bodily fluids are collected and processed for the purpose of analyzing cellular structures and antigens. Virus antigens are targeted by primary antibodies that have fluorescent compounds like FITC or rhodamine linked to them. Antibodies are able to emit light of a certain wavelength when stimulated. To make samples bind to viral antigens, they are first treated with labeled antibodies. Following this process, the targeted antigen-antibody complexes are isolated from any unbound antibodies. A visual signal may be produced that shows the distribution and location of viral antigens inside cells or tissues by causing the attached fluorescent antibodies to emit fluorescence when exposed to specified light wavelengths. The samples are examined under a fluorescence microscope, which reveals fluorescently tagged viral antigens, to verify the existence of the virus.

Electron Microscopy

Delivers viral images in high quality. However, it is seldom used due to its complexity. One potent imaging tool in virology is electron microscopy (EM), which allows for direct visualization and ultrastructural characterization of viruses. This method allows for the observation of the size, shape, and complex structural features of viruses, which aids in viral identification and provides valuable data for research and diagnosis. Specialized preparation techniques are used to preserve viral particles and generate thin sections suitable for electron microscopy when clinical materials (such as tissues, bodily fluids, or pure viral suspensions) are believed to include viruses. A beam of electrons is used to bombard the prepared samples, which are then placed in a vacuum. The viral particles' ultrastructure can be seen in pictures created by electron interactions with the sample, which generate signals. Skilled individuals examine the electron micrographs in

order to detect and categorize virus particles according to their distinct shape and other structural characteristics¹³.

Rapid Diagnostic Tests

Lateral Flow Assays

Rapid and easy-to-use diagnostic methods for detecting viral antigens or antibodies in clinical samples are lateral flow assays (LFAs) or lateral flow tests (LFTs). These diagnostics, which look like pregnancy tests, are perfect for use at the point of care in areas with limited resources or during epidemics because they give fast findings without the need for specialized equipment. Sample application pads, conjugate pads, a test line, and a control line are components of a nitrocellulose membrane strip that is used in LFAs. A specimen (such as saliva or blood) is placed onto the sample pad. As the sample moves across the strip, it encounters immobilized antibodies or antigens at the test line, creating a visible line. This line is used to identify viral antigens or antibodies. Including a control line ensures that the test is valid. When lines appear in the control zone alone, it means the results are negative; when lines appear in both the test and control zones within the given period, it means the results are positive.

Loop-mediated Isothermal Amplification (LAMP)

Rapid and stable at room temperature, LAMP is a nucleic acid amplification method that is ideal for use in point-of-care diagnostics. Using strand-displacing DNA polymerase and customized primers, it constantly amplifies specific DNA or RNA sequences at a temperature of 60-65°C. Viral nucleic acids can be identified by detecting the enormous amounts of DNA with characteristic stem-loop structures produced by LAMP using turbidity, colorimetric dyes, or fluorescence reporters.

Microbial Techniques for Diagnosing Fungi Infections

Diagnosing fungal infections often involves a combination of clinical assessment, microscopic examination, and laboratory-based techniques to identify and confirm the presence of fungi¹⁴ (Figure 4).

Microscopic Examination

Direct Microscopy

Direct microscopy diagnoses fungal infections by examining clinical specimens under a microscope. This rapid, simple method involves preparing and staining samples to visualize fungal structures, aiding in fungal identification. Microscopic examination of clinical specimens (e.g., skin scrapings, sputum) uses stains like KOH or calcofluor white to visualize fungal elements under a microscope. This rapid, cost-effective method is essential for initial diagnosis of fungal infections.

Histopathology identifies fungal infections by examining biopsied tissue samples under a microscope. Tissue sections are processed, stained (e.g., H&E, periodic acid-Schiff), and analyzed to visualize fungal elements, determine the infection's extent, and characterize fungal species. This technique provides crucial information on fungal invasion, inflammatory response, and tissue damage, aiding in diagnosis and patient management.

Culture-Based Techniques

Fungal Culture

Fungal culture is a key diagnostic technique for identifying fungal infections by cultivating and isolating fungi from

Table: 4 Application of microbial techniques for diagnosis of microbial infections

Bacterial	Viral	Fungi
Clinical Culture	Nucleic Acid-Based Tests PCR (Polymerase Chain Reaction) RT-PCR (Reverse Transcription PCR)	Microscopic examination
Gram staining	Serological Tests ELISA (Enzyme-Linked Immunosorbent Assay) Western Blotting	Culture-Based Techniques: Antifungal susceptibility testing Fungal culture
Biochemical test		
Oxidase test		
Catalase Test	Culture-Based Methods	Molecular Techniques:
TSI Agar Test	Viral Culture	Polymer chain reaction
Indole Test	Cell culture	DNA sequencing
Coagulase Test		
Citrate Utilization Test		

clinical specimens. Specimens (e.g., skin scrapings, nail clippings, respiratory secretions) are placed on specialized media (e.g., Sabouraud dextrose agar) and incubated at specific temperatures (25-30°C for molds, 35-37°C for yeasts). Colonies are examined for morphology, color, and texture to aid preliminary identification, with further tests (microscopy, biochemical assays, molecular methods) confirming fungal species and assessing antifungal susceptibility.

Antifungal Susceptibility Testing (AST)

AST assesses fungal isolates' sensitivity to various antifungal drugs. Methods like broth microdilution or disc diffusion are used. Fungal isolates, cultured from patient samples, are standardized to a specific concentration. They are then applied to agar plates with different antifungal drug concentrations. After incubation, the growth patterns are examined to determine drug efficacy and fungal susceptibility. This process is essential for selecting effective antifungal treatments¹⁵.

Molecular Techniques

Polymerase Chain Reaction (PCR)

PCR amplifies and identifies fungal DNA or RNA in patient samples, detecting fungal infections. This method enables targeted, rapid, and specific fungal pathogen diagnosis. DNA or RNA is extracted from patient specimens (e.g., blood, tissue, respiratory secretions) and PCR amplifies specific fungal DNA or RNA regions using targeted primers. The PCR machine cycles through denaturation, annealing, and extension, where DNA strands separate, and primers bind to complementary sequences. DNA polymerase synthesizes new DNA strands from the targeted fungal sequences, expanding from the primers. The presence of amplified fungal DNA/RNA indicates the patient sample contains the specific fungal pathogen.

DNA Sequencing

DNA sequencing is another powerful molecular technique which analyzing the genetic material of fungi present in patients sample for the detecting and identifying fungal infections. Discovering the precise nucleotide sequence in fungal DNA is the goal of this approach, which aids in correct diagnosis and treatment choices by giving specific information about the fungus species. Sanger sequencing and next-generation sequencing (NGS) are two sequencing methods that are used to study fungal DNA. These methods

help us comprehend genetic diversity, identify species, and find mutations. Using databases and bioinformatics tools, the acquired sequences are compared with previously published sequences in order to identify the particular fungus species present in the patient sample.

Serological Tests (Antibody Detection)

Fungal infections may be more accurately diagnosed with this method, which involves detecting antibodies in patient serum or other bodily fluids. As a defense mechanism, the immune system manufactures antibodies that specifically target antigens produced by fungi after an infection. Immunodiffusion and ELISA may detect specific antibodies against fungal antigens, indicating exposure or ongoing infection. Although serological tests provide evidence of exposure or disease, it is crucial to interpret the findings with caution due to their limits, specificity, and timeliness. Together with other diagnostic methods, antibody detection enhances our knowledge of fungal infections and enables more accurate patient management.

Imaging Techniques (Radiological Imaging)

Fungal infections may be detected and evaluated using radiological imaging by observing the structural alterations in the organs or tissues that have been invaded by the fungus. When it comes to detecting and monitoring fungal infections, imaging modalities including X-rays, MRI, CT scans, and even nuclear medicine methods may be helpful. MRI, CT scans, or X-rays may help diagnose and track the progression of fungal infections in deeper tissues or organs. Radiological imaging is a valuable diagnostic technique for diagnosing and evaluating fungal infections. It may reveal the exact location, size, and characteristics of the fungal invasion in various organs and tissues..

Antigen Detection (Fungal Antigen Tests)

Fungal antigens or its components in fungal infected person can be diagnosed through fungal antigen tests. These tests detect Fungal elements can be detected directly through these tests which contributing in the identification of fungal species and guiding appropriate treatment strategies. Certain assays identify fungal antigens (such Aspergillus galactomannan and Cryptococcus antigen) in physiological fluids (including blood and cerebrospinal fluid), which can help with the diagnosis of invasive mold infections. Fungal antigen assays, which provide quick and non-invasive ways

to identify particular fungal components, are important tools in diagnosis of fungal infections^{16,17} (Table 4).

Role of microbial techniques in environmental sustainability

Microorganisms are very tiny organisms of the environment that may include bacteria, virus, fungi, protozoa and protists etc are widely distributed in the environment and due to neglectable size that are responsible for various diseases in humans, animals as well as plants. The infections that are caused by these microorganisms are having mild to severe effect on human health, animals and agriculture¹⁸. For the diagnosis and prevention of these infections there are various microbial techniques are developed. Microbial techniques for bacteria, virus and fungi are developed for the diagnosis of the microbial infection in environment. When it comes to quantifying and evaluating bacteria, microbial approaches are crucial for environmental sustainability¹⁹. The use of microbial approaches has greatly improved pollution monitoring and management, which is essential for environmental sustainability. PCR identifies microbial contaminants and measures biodiversity, while microscopic analysis counts and characterizes microorganisms involved in bioremediation. Immunological assays detect specific toxins and pathogens, providing valuable information on contamination and environmental shifts. Biosensors offer real-time data on pollutants and microbial activity for timely responses. Lateral flow assays enable quick, on-site testing for contaminants, and microfluidic devices support efficient, high-throughput environmental analysis. Collectively, these techniques enhance our ability to effectively monitor and manage environmental pollution²⁰.

CONCLUSION

Microorganism like bacteria, viruses, fungi, algae, protozoa and protists are widely spread throughout the environment which may directly or indirectly impact the environmental sustainability by affecting the humans, animals and plants. These microorganisms are responsible for various microbial infections like UTIs, gastrointestinal infections, Tuberculosis (TB), gonorrhoea, cholera, Lyme disease Candidiasis, Aspergillosis, Cryptococcosis, Dermatophyte infections etc in humans Feline Immunodeficiency Virus (FIV), rabies virus, Canine Parvovirus (CPV), Mannheimia haemolytica and Bordetella bronchiseptica etc in animals and Late blight of potatoes and tomatoes, Fusarium wilt, Corn smut, Gray mold, Alternaria leaf spot, potato tuber necrotic ringspot disease, stunted growth, and deformities in fruit, Tristeza disease and cauliflower mosaic disease etc in plants. Presence of these contrasting microorganisms in the environment is recognized by different techniques like Clinical Culture, Gram staining, Biochemical test, Nucleic Acid-Based Tests, PCR, RT-PCR, Serological Tests, ELISA, Western Blotting, Microscopic examination, Antifungal susceptibility testing, Molecular Techniques, DNA sequencing etc. So for the better environmental sustainability it is required to focus towards research on quantitative and qualitative analysis of these microorganisms.

REFERENCES

- Ahmed R, Umar AU, Shukla CP. Microbial indicators for monitoring pollution and bioremediation. In *Microbial Inoculants 2025* Jan 1 (pp. 413-432). Academic Press.
- Dhillon SK, Chung TH, Dhar BR. Bioremediation meets biosensing: leveraging microbial electrochemical cell-based biosensors. *Reviews in Environmental Science and Bio/Technology*. 2025 Jun 5:1-41.
- Iqbal S, Begum F, Nguchu BA, Claver UP, Shaw P. The invisible architects: microbial communities and their transformative role in soil health and global climate changes. *Environmental Microbiome*. 2025 Mar 25;20(1):36.
- Liang X, Yang X, Sha N, Wang J, Qiu G, Chang M. Application of eDNA Metabarcoding Technology to Monitor the Health of Aquatic Ecosystems. *Water*. 2025 Apr 8;17(8):1109.
- Dhara A, Dutta R. A review on sources and distribution of polycyclic aromatic hydrocarbons (PAHs) in wetland ecosystem: focusing on plant-biomonitoring and phytoremediation. *Environmental Science and Pollution Research*. 2025 Mar 18:1-23.
- El-Sheekh MM, El-Kassas HY, Ali SS. Microalgae-based bioremediation of refractory pollutants: an approach towards environmental sustainability. *Microbial Cell Factories*. 2025 Jan 14;24(1):19.
- Hait M, Patel D, Izah SC. Molecular Techniques and Technologies in Biomonitoring for Environmental Sustainability. In *Biomonitoring of Pollutants in the Global South 2024* Jun 6 (pp. 605-637). Singapore: Springer Nature Singapore.
- Babafemi OP, Ajani TF, Binuyo MO, Ajagbe AO, Owonibi SK, Ogwu MC. Biomonitoring for sustainable development. In *Biomonitoring of Pollutants in the Global South 2024* Jun 6 (pp. 191-239). Singapore: Springer Nature Singapore.
- Ogidi OI, Ajoko IT, Tawariwei AM. Sustainable Application of Genetic Ecotoxicological Techniques in Biomonitoring for Environmental Sustainability. In *Biomonitoring of Pollutants in the Global South 2024* Jun 6 (pp. 667-692). Singapore: Springer Nature Singapore.
- Shelke YP, Badge AK, Bankar NJ, Badge A. Applications of artificial intelligence in microbial diagnosis. *Cureus*. 2023 Nov 24;15(11).
- Hait M, Sahu P, Biswas S, Izah SC. Sustainable Application of Artificial Intelligence in Biomonitoring for Environmental Sustainability: Challenges and Prospects. *Biomonitoring of Pollutants in the Global South*. 2024 Jun 6:747-78.
- Kumar V, Chhetri A, Dey JK, Debnath A. Microbial Indicators for Monitoring Pollution and Bioremediation. *Microbes Based Approaches for the Management of Hazardous Contaminants*. 2024 Aug 21:390-6.
- Adetunji CO, Ukhurebor KE. Recent trends in utilization of biotechnological tools for environmental sustainability. *Microbial Rejuvenation of Polluted Environment: Volume 3*. 2021:239-63.

14. Huang CW, Lin C, Nguyen MK, Hussain A, Bui XT, Ngo HH. A review of biosensor for environmental monitoring: principle, application, and corresponding achievement of sustainable development goals. *Bioengineered*. 2023 Dec 31;14(1):58-80.
15. Gavrilescu M, Demnerová K, Aamand J, Agathos S, Fava F. Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *New biotechnology*. 2015 Jan 25;32(1):147-56.
16. Gavrilescu M. Environmental biotechnology: achievements, opportunities and challenges. *Dynamic biochemistry, process biotechnology and molecular biology*. 2010;4(1):1-36.
17. Ahmad M, Pataczek L, Hilger TH, Zahir ZA, Hussain A, Rasche F, Schafleitner R, Solberg SØ. Perspectives of microbial inoculation for sustainable development and environmental management. *Frontiers in microbiology*. 2018 Dec 5;9:2992.
18. Crowther TW, Rappuoli R, Corinaldesi C, Danovaro R, Donohue TJ, Huisman J, Stein LY, Timmis JK, Timmis K, Anderson MZ, Bakken LR. Scientists' call to action: Microbes, planetary health, and the Sustainable Development Goals. *Cell*. 2024 Sep 19;187(19):5195-216.
19. De Micco V, Amitrano C, Mastroleo F, Aronne G, Battistelli A, Carnero-Diaz E, De Pascale S, Detrell G, Dussap CG, Ganigué R, Jakobsen ØM. Plant and microbial science and technology as cornerstones to Bioregenerative Life Support Systems in space. *npj Microgravity*. 2023 Aug 24;9(1):69.
20. Eisenstein BI. New molecular techniques for microbial epidemiology and the diagnosis of infectious diseases. *Journal of Infectious Diseases*. 1990 Apr 1;161(4):595-602.