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Molecular Diagnostic Methods and Their Application to Patient Care: Clinical Microbiologist's Perspective

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Abstract In the era of Human Immunodeficiency virus, emerging and re-emerging of various microbial infections, the status of a clinical microbiology laboratory performance is different in the developed and developing nations. On the one side application of rapid, accurate and advanced molecular laboratory techniques have contributed to better diagnosis, patient care and management in developed nations, the developing and economically weak countries are still plagued by ill equipped and undertrained laboratory personnel unable to afford the cost associated with acquiring infrastructure and man power. Clinical microbiology laboratories in the developing nations should be revolutionized so as to improve patient care and to contribute effectively in the control of infectious disease epidemics and pandemics that are of public health importance.

Keywords: *clinical microbiology, molecular methods, point of care, rapid and accurate laboratory testing*

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1. Introduction

In view of the current situation where the public at large are at risk of contracting various established, emerging and re-emerging infectious diseases, it becomes imperative that accurate and cost effective diagnostic techniques would prove to be effective in prompt laboratory diagnosis and specific treatment. Traditional diagnostic microbiology laboratories rely on conventional culture and serological methods for the confirmation of various bacterial, fungal, parasitic and viral infections. In the low socioeconomic and the developing third world countries access to reliable diagnostic laboratory testing methods is limited due to their inability to acquire well qualified personnel, standard and sophisticated equipments. It has been noted that population in the developing nations suffer from huge morbidity and mortality related to absence of or inaccurate laboratory diagnostic testing systems [1]. Human Immunodeficiency Virus (HIV), since its discovery three decades ago has been a challenge for the medical fraternity during which world has also seen the emergence and re-emergence of various microbial infections. HIV infection confirmation and later disease management involves the use of advanced and molecular methods, which are not freely available in developing nations that carry most of the burden of HIV disease [2]. Emergence of respiratory viral infections including the influenza virus, which has to be immediately diagnosed to stop the spread of infection at the acute stages of the disease requires well established laboratory facilities that are not available at many tertiary care centres in economically weak nations. It has been observed that lack of point of care accurate laboratory diagnostic methods results in mismanagement of the patient resulting in severe morbidity and mortality.

2. Clinical Microbiology Laboratory: Status in Developing Nations

The status of in-house laboratory diagnostic testing is still in the early stages in economically backward countries where many health care centres are not adequately equipped and the samples are outsourced for diagnosis to a distant place, which in turn leads to delays resulting in impaired patient care. Advanced laboratory facilities remain elusive for the rapid diagnosis of various infectious diseases; that could be helpful in initiating appropriate antimicrobial agents and thereby better patient care [3]. It has been noted that with more than 25 million people living with HIV, the burden of infectious diseases is huge in sub-Saharan African region where 12 million people die each year and most of the deaths are attributed to infectious diseases [4].

Developing countries that carry huge of the burden of HIV, Tuberculosis, Malaria and other infectious agents are not properly equipped to diagnose, manage and monitor progression and control their spread [5]. Laboratory diagnosis of common parasitic infections involves microscopic, immunologic and molecular techniques. Microscopic methods are currently the most common and cost-effective means for diagnosing parasitic infection / infestation in developing nations which is plagued by the non-availability of well trained lab personnel. Cultural,

Immunologic and molecular methods of diagnosis have proven to be very expensive and have not been used commonly in the developing nations [6,7].

3. Significance of Rapid and Accurate Laboratory Testing

Rapid testing in a clinical microbiology laboratory though is not mandatory and most of the diseases are diagnosed by using traditional and conventional phenotypic methods (cultural, biochemical and antimicrobial susceptibility testing), utility of such rapid and molecular methods gain significance during an emergency including an outbreak situation. Specific diagnosis of an infectious disease is essential in some cases including the paediatrics age patients' care in which a decision on how to manage has to be taken immediately to reduce the morbidity and mortality [8]. Rapid molecular methods aid in quantitative detection of antigen, antibody and the nucleic acid as is essential for various infectious agents including the HIV, Hepatitis B virus and the Hepatitis C virus. Clinical microbiology laboratories may perform better with the availability of facilities, instrumentation and the skilled man power to perform detection of toxin genes, resistance genes and various others microbial virulence determinants using various rapid and molecular diagnostic techniques [9]. Studies have demonstrated the importance of advanced blood culture techniques for identification and antimicrobial susceptibility testing along with molecular methods like target amplification by different PCR's, gene sequencing, pyrosequencing, reverse hybridization, mass spectrometry and microarray analysis which aid in specific diagnosis and deciding on the specific treatment options and management of the infectious disease [10,11]. In the era of pandemics where infectious agents rapidly spread through countries, clinical microbiologists, infectious disease specialist's should concentrate on improving staff competence, acquiring resources, accrediting laboratories and adding automation for rapid diagnosis and minimise the impact of epidemics on public health.

Previous studies have highlighted the role of molecular diagnostic methods in diagnosis, management and monitoring of infectious diseases. Studies have also stressed on the need for quality, accuracy and reliability of laboratory findings which are used by clinicians to manage the patients [12]. Studies have also confirmed that there is a need to have standard protocols, proficiency testing programmes and reference material for various molecular diagnostic testing methods to avoid variability in test results among different laboratories [13]. With increase in the incidences of nosocomial infections, which are difficult to control, diagnostic laboratories will surely benefit from the advances and availability of molecular testing facilities, which reduces the turnaround time, increases sensitivity and accuracy. Utility of novel molecular methods in the diagnosis and management of viral infections including HIV, HBV, HCV, Dengue virus, Ckikungunya, influenza virus, other respiratory viruses (SARS) and many others have revolutionized clinical microbiology laboratories mostly in the developed nations [14,15]. Existing literature not only emphasizes the need to have rapid and accurate molecular diagnostic testing methods but also stresses on the importance of maintenance, regular validation and up gradation of the technology especially in case of pathogens that regularly show genetic variations like the Influenza virus [16]. In the era of bioinformatics and gene sequencing, real-time genomic typing holds promise in specific identification of the causative pathogen, its resistance profile and acts as a guide to better patient care. A recent study has applied minimum core genome sequence (MCG) typing of bacterium *Streptococcus suis*, a zoonotic human pathogen and identified single-nucleotide polymorphisms which helps in the recognition of serotypes that cause serious human infections and those of significance to public health [17]. Another recent research has elaborated on the importance of using rapid, accurate and cost-effective whole genome sequencing (WGS) in identifying and management of infectious diseases that are of public health importance. This study has applied Multi-locus sequence typing (MLST) against clinical isolates of *Campylobacter* to examine long term trends in disease transmission patterns and outbreaks [18].

4. Advanced Clinical Microbiology Laboratory Testing

From the time since the first discovery and applicability of Enzyme immunoassays (ELISA's), for the detection of various infectious agents way back in 1980's, clinical microbiology laboratory has slowly moved in to a new era, where traditional methods are supported by the newer nucleic acid methodologies which help clinicians in the diagnosis and management of patients suffering from various microbial infections. Previous studies have impressed on the need for professional standards for clinical / medical microbiologists who assist in diagnosis, therapy and management of infectious diseases [19]. Studies have demonstrated the use of nano-materials and in-vitro nano-diagnostic testing for the detection of medically important pathogens which can be helpful in accurate diagnosis, prevention of spread and reducing the health care costs [20].

Mass spectroscopy has recently been the new approach to definitively identify various microorganisms to species level. MALDI-TOF MS (Matrix Associated Laser Desorption / Ionization-Time of Flight Mass spectroscopy) is an analytical method used for the detection of proteins (fatty acids, metabolites of microorganisms and oligo / polysaccharides) and DNA molecules. Its application in clinical microbiology laboratory is as an alternative to traditional identification systems and Enzyme Linked Immunosorbent Assays (ELISA's) and commercially used as Bio Merieux Vitek MS system. Mycobacterial speciation, identification of fastidious microorganisms, microbial typing for epidemiological purposes, virulence studies and antimicrobial susceptibility or resistant determinants are some of the applications of MALDI-TOF MS in a diagnostic microbiology laboratory [21]. As the conventional methods delay the culture results, such technology along with Nucleic acid sequence based amplification (NASBA) can be very useful in the diagnosis and management of invasive fungal infections [22].

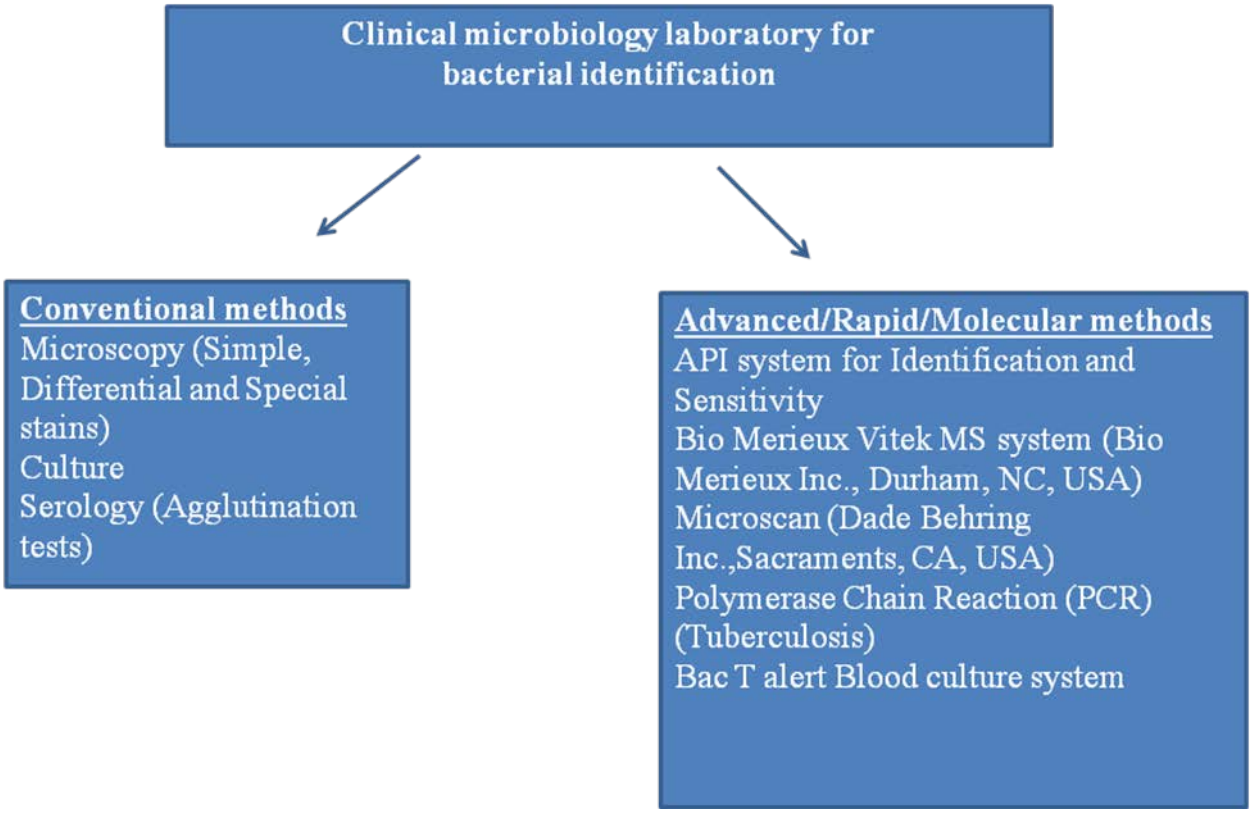

Figure 1. Clinical microbiology laboratory for bacterial identification

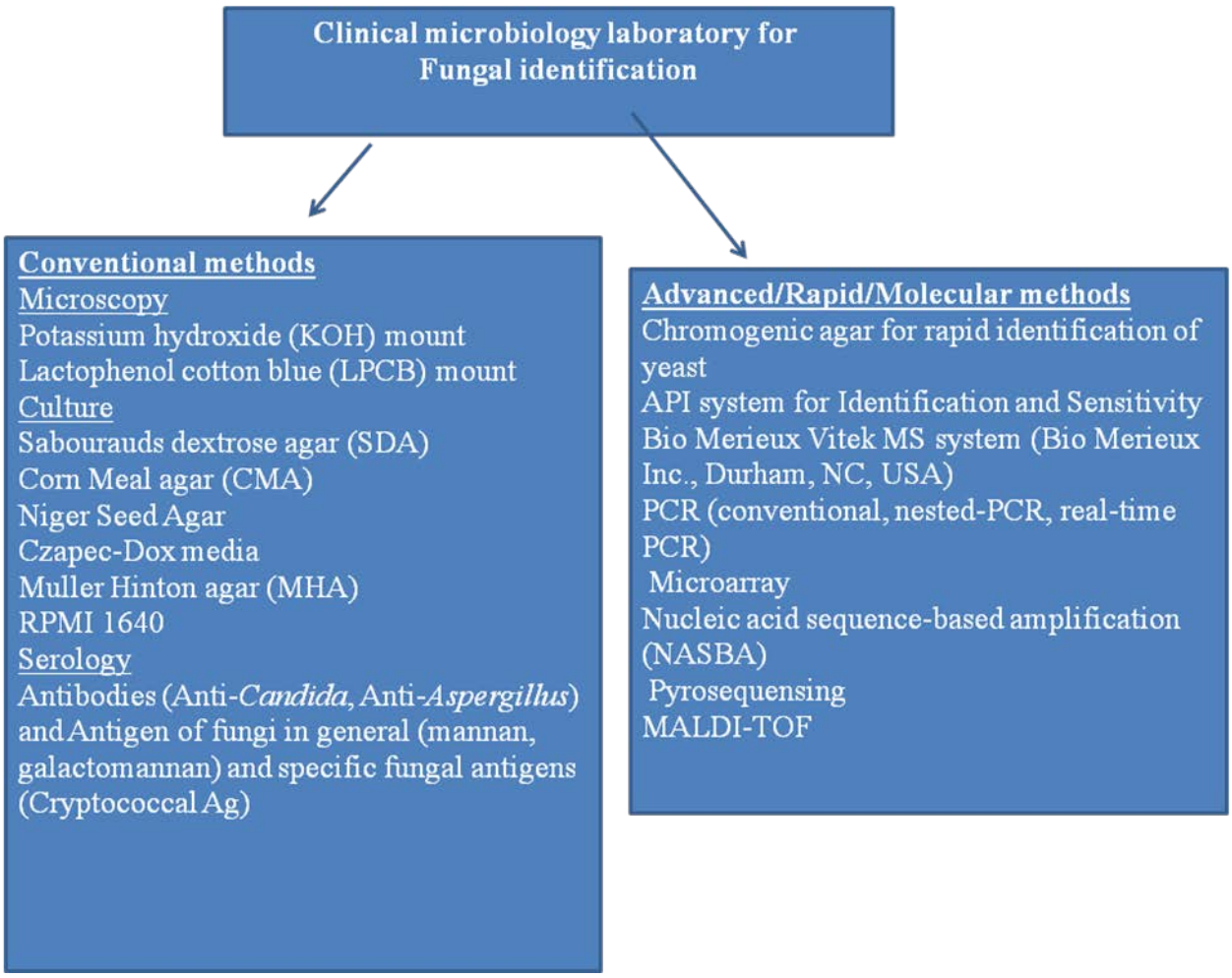

Figure 2. Clinical microbiology laboratory for fungal identification

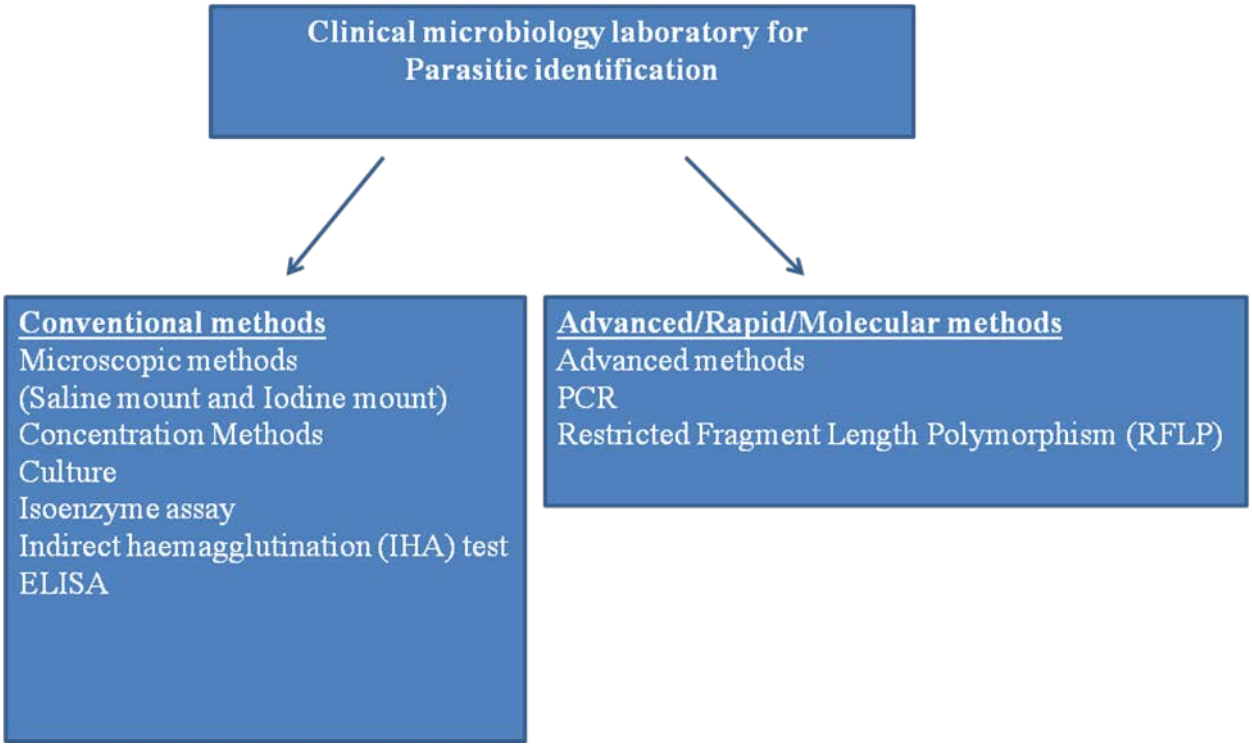

Figure 3. Clinical microbiology laboratory for parasitic identification

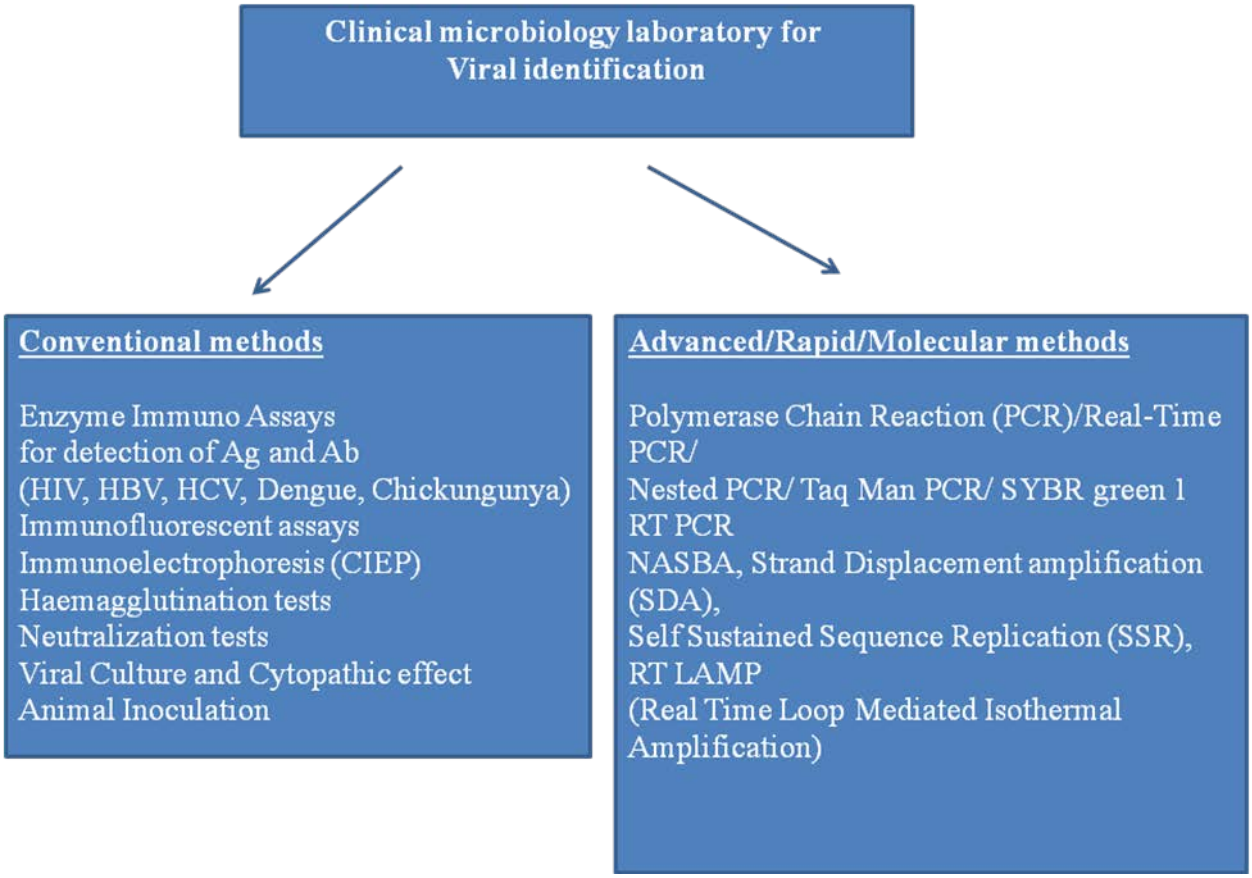

Figure 4. Clinical microbiology laboratory for viral identification

5. Point-of-Care Testing

Point of care (POC) clinical microbiology lab facility was established to study the role of such set up in improving the patient care with regards to diagnosis, therapy and patient management. This study emphasized that though real time management of patients presenting with infectious disease emergency (bacteraemia / fungaemia) has improved with POC, it has cautioned on the aspects relative to the costs when compared to conventional lab [23]. In another study from Germany that was conducted retrospectively including the cases reported

during the influenza virus pandemic (A/H1N1 / 2009) compared the results of POC with other tests (Direct fluorescent antibody, PCR) and have found that POC testing is not as sensitive and specific as PCR [24]. Role of a clinical microbiology laboratory in the management of patients located in Intensive care units (ICU's) has been highlighted by an Indian study that had elaborated on the need for readiness on the part of laboratory and clinical microbiologists intervention in the management of various acute infectious conditions (septicaemia, meningitis, pneumonia), infections by highly resistant bacteria (Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococci* (VRE), other Multi Drug Restistant microbes), Invasive fungal infections (IFI's) and health care associated infections (HAI) [26,27,28]. It has been noted that nucleic acid tests (NAT) can be beneficial in providing high-quality laboratory results with reduced turnaround times and in turn provide better patient care at reasonable cost [29]. Infectious disease diagnostics may revolutionize clinical microbiology laboratory in the future with the addition of rapid and accurate diagnostic test to detect various microbial infections. This is not without possible drawbacks that include cost constraints, false negative and false positive results.

6. Laboratory Data Management and Surveillance

Studies have impressed on the need to have electronic laboratory reporting (ELR), that is facilitated by physician reporting the identified case in association of clinical microbiologists in confirming the infectious disease and come out with a communicable disease report (CDR), which facilitates readiness during outbreaks and will be useful in studying the demographic and epidemiological aspects of an infectious disease [30]. Another important aspect in the diagnostic microbiology laboratory is the lab-based surveillance, which basically means systematic recording of data which includes clinical, laboratory parameters from clinical / public health laboratories. Infectious disease surveillance includes elucidation of data on various food / water borne pathogens, sexually transmitted infections including blood-borne diseases, vector-borne bacterial, fungal and viral infections, upper and lower respiratory tract diseases, infections of the urinary tract, infections transmitted from mother to child and many others. Surveillance of infectious diseases will be helpful in understanding the demographic details, impact on public health and be useful in the initiation of effective prevention and control measures. Networking of national and international clinical and laboratory data will prove to be effective in understanding the susceptibility / resistance profile of prevailing microorganisms, and help in coming out with prescription based strategies to reduce the further emergence of resistance. Laboratory surveillance would prove effective in public health perspective in alerting for future outbreaks or in control of epidemics / pandemics [31].

7. Conclusion and Future Implications

Conventional microbiology laboratories fail to diagnose infections caused by microbes that are not observed by routine microscopic methods and those fail to grow in routine culture media (Numerous viruses, spirochaetes (*Treponema*, *Chlamydia*, *Mycoplasma*). Therefore use of rapid, automated, advanced and molecular methods would discover new pathogens, help researchers to develop new interventions (vaccines and drugs) and assist in better patient management [32,33]. From the available literature it is evident that clinical microbiology laboratories in the developed nations have technically evolved and the application of molecular methods has enhanced accuracy of laboratory identification and improved patient care and management. Molecular diagnostic testing is still confined to only few centres (reference laboratories) in most of the developing nations including India, where even the surveillance mechanisms are inadequate owing to the cost associated with acquiring such advanced technology. Further it should be noted that in future clinical microbiology laboratories show enhanced performance with use of molecular diagnostic technology, bioinformatics, and genome informatics. Emergence of peptide and nano- diagnostic methods will usher in erudite laboratory performances and improve patient care and management.

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