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Advancements in Microbial Pathogenesis and Molecular Diagnostics

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Abstract: Infectious diseases remain a major global health challenge, intensified by antimicrobial resistance, climate change, and the emergence of new pathogens. Despite significant advances in molecular biology, a knowledge gap persists regarding the early detection, host-pathogen interaction mechanisms, and effective control of infectious agents. This article provides an extensive review of recent progress in microbial pathogenesis and molecular diagnostics, including PCR, next-generation sequencing, microarray analysis, and bioinformatics tools. The analysis highlights how modern molecular techniques enhance pathogen identification, track antimicrobial resistance, and contribute to vaccine and therapeutic developments. Findings demonstrate that integrating molecular diagnostics into clinical practice significantly improves the accuracy and speed of diagnosing infectious diseases,

leading to better patient outcomes and public health preparedness. However, challenges remain in data interpretation, regulatory approval, and ensuring access in low-resource settings. The study underscores the urgent need for interdisciplinary research and innovation to address these limitations and combat the evolving threat of infectious diseases.

Keywords: microbial pathogenesis, molecular diagnostics, antimicrobial resistance, infectious diseases, PCR, next-generation sequencing, bioinformatics

1. Introduction to Microbial Pathogenesis

Despite considerable progress in vaccines, antibiotics and global health initiatives, the world continues to be confronted by infectious disease, underscoring a need for their survey, prevention, containment, and treatment [1]. Pathogens, whether of bacterial, viral, parasitical, or fungal nature, not only threaten our health but represent a significant burden to the health care system. The burden associated with infectious diseases disproportionately affects low- and middle-income countries. A key threat to global public health safety, along with transforming infectious agents and natural outbreaks, is the unintentional or deliberate misuse of biological agents. The World Health Organization has published biannual reviews of significant events for the past five years where infectious pathogens either emerged or re-emerged and which had serious public health repercussions.

Pathogen antimicrobial resistance (AMR) has been an increasing concern and has been rising steadily. By 2050, deaths associated with AMR are projected to be 10 million. The ongoing climate change is exacerbating the problem by leading to the expansion of territory of species, such as persistors and vectors of malaria and heat-sensitive Vibrio cholera that lead to the emergence and re-emergence of pathogens [2]. Therefore, strategies to prevent, contain, and better understand the burden of infectious diseases are essential. Over the years, there has been vast technological development in the fields of next generation sequencing, genomics, transcriptomics, metagenomics, proteomics, and metabolomics. These techniques together have delineated in much more detail the genetics of pathogenicity and mechanisms of host pathogen interactions. It has also provided much information about the defensive part of the interaction including immune response, intrinsic and adaptive immunomodulation. However, despite these efforts, major gaps still exist unnecessarily prolonging our knowledge in that field. One of the biggest gaps in our undervalue of successful host invasion by pathogens is what the physiological changes during colonization and infection are. For decades, this field was somewhat in the shadow simply because either direct observation of physiological changes in vivo in infected host or the isolation of sufficient numbers of infecting pathogens for accurate in vivo experiments was not possible.

2. Historical Perspectives on Microbial Diseases

Microorganisms, including pathogenic bacteria, viruses and fungi, have had devastating effects on

people since the beginning of recorded time. Physicians have struggled to diagnose and treat these diseases since the time of Hippocrates. The concept of avoiding sickness was the primary tool available, physicians recognizing that disease came from "bad air," "miasmas" or "ill humours" and telling civic leaders to avoid mass gatherings and fens or malarial areas. On a more practical level, they would also suggest fortunetellers or ominous birds calling bad news, all of which would frighten good sense into people and made them realize that human intellect was limited and powerful forces could inflict disease. Gradually, and with the Renaissance in Europe, the intellectual elite moved away from belief in supernatural phenomena and dependent on reason and evidence. The establishment of the Royal Society in 1660 was prompted by the desire to accumulate and disseminate knowledge based on experiment and best evidence. William Harvey's discovery of blood circulation in 1628, lauded by the Royal Society, epitomized real research instead of pondering the ancients. Antony van Leeuwenhoek's microscopic observations of "animalcules" were made 200 years before knowledge of bacteria and protozoa. In 1796, Jenner's method of making patients immune to smallpox by injecting cowpox (vaccination) paved the way for rapid advances in infectious diseases research within the new framework of Western science [2].

3. Current Trends in Microbial Pathogenesis Research

Despite tremendous progress in global health initiatives over the last century, the world continues to be confronted by infectious disease, underscoring the ever urgent need for their surveillance, prevention, containment, and treatment. For many individuals in industrialized countries, infectious disease is no longer the cause of death. However, emerging, and re-emerging global pathogens, increased travel, urbanization, climate change, global population pressures, profound demographic changes, and a burgeoning cohort of the immune-compromised have conspired to maintain this group as a formidable, indeed growing, world presence. These agents, while disparate, share a common theme: they seek, and obtain, the ability to colonize (commensals), infect (transient enterics), invade tissues and organs, and then adapt—establishing themselves as dynamic human pathogens. Such agents represent a significant overlap with those pathogens termed biowarfare, or bio-defense, threat agents, hence their research relevance.

Recent historical perspectives have highlighted the continual tasks that are presented globally. Emerging agents still concern, and have relevance today: Ebola, Marburg, HIV/AIDS, SARS, H5N1, XDR-TB, Chikungunya, and most recently MERS-CoV. Despite a significant global public health response, antimicrobial-resistant (AMR) pathogens have continued to emerge [1]. Recent trends in AMR are particularly troubling, with a steady rise in resistant HIV, Mtb, S. pneumonia, Enterococci, Salmonellae, Shigellae, Acinetobacter, and increased resistance to second- and third-line drugs. It is projected that by 2050 there will be 10 million associated AMR deaths, with a potential global cost of 100 trillion USD. Despite a significant research response since the landmark papers of the 1960s to 1980s, we are still far from understanding the associated mechanisms. Climate change has also unquestionably led to the re-emergence of pathogens, most notably the range expansion of Vibrios, where global warming has been identified as a key factor. Thus, despite a wave of notably productive research over the last few decades that has paved the way to vaccine formulation, a more detailed understanding is vital for the rational design of Mtb adjuvant therapy. At a time when a growing proportion of the global population is immune compromised through malnutrition, HIV, or transplantation, the need for these surveys has never been more acute. Finally, the genome era marches on apace, and pathogens and disease states are now increasingly subject to high-throughput investigations. Translating these often disparate findings to a better understanding of disease causation and pathogenicity remain a substantial challenge, particularly in the case of biotic disease states and complex interactomes.

4. Molecular Mechanisms of Pathogenicity

Despite tremendous progress in global health initiatives, the world continues to be confronted by infectious disease, underscoring the continued need for infectious disease surveillance, prevention,

containment and treatment [1]. Humans coexist with a vast array of pathogens and an even larger array of non-pathogenic microbes which protect hosts from pathogenic colonization. Humanity and its pathogens share a long and complex history. Pathogens not only represent a significant burden to our health but, consequently, a significant burden to our health care and economies. Pathogenic antimicrobial resistance (AMR) has been rising steadily over the past decade, with associated deaths projected to be 10 million by the year 2050. In addition to AMR and vast genetic diversity, climate change and globalization have led to the emergence and re-emergence of pathogens, further complicating the burden of infectious diseases. Together, these considerations underscore the pressing need for developing strategies to reduce these burdens. In this context, it is essential to better understand the molecular mechanisms of host colonization, infection and disease.

In the post-genomic era, a plethora of bioinformatics tools have been developed and utilized for understanding gene regulations, intracellular signaling pathways and protein-protein interactions of known genes and gene products involved in host-pathogen interactions [3]. There have been vast technological developments over the past decade in next-generation sequencing and proteomics which have successfully dissected the genetics of pathogenicity and the fundamental mechanisms of host-pathogen interactions at the molecular level. However, one of the biggest gaps in the understanding of host invasion by pathogens is the physiological changes during colonization and infection, especially during the initialization of this process. In the course of evolution, hosts and microbes have developed mechanisms to survive and out-compete each other. Pathogens have the upper hand because they are well-equipped with a vast diversity of proteins to evade host defenses. Moreover, host immunity is a double-edged sword. While it clears pathogens from the host system, in some cases, it can also be detrimental to the host.

4.1. Virulence Factors

What is a virulence factor [4]? That question should be easier to address in light of the avalanche of literature on this topic. Yet, defining a 'virulence factor' is more difficult than one might think. By just limiting the 'virulence factors' to proteins that are exported/secreted by a pathogen and shared by men, greater agreement in the literature might be achieved. However, it is difficult to justify excluding the products of chromosomal genes and/or the products of genes that might be exported by a different mechanism. That being the case, one is left with considering virtually every protein that a pathogen might encode and the multitude of ways in which it could persist for long durations in harsh environmental conditions.

One of the earliest known virulence factors was actually a polysaccharide that could resist innate host defenses and give bacteria some modicum of invulnerability. Even this definition is fraught with difficulties because the pathogen must first enter the host to be lethal. And in order for that to occur, the host defenses must be avoided or thwarted. In order for the pathogen to avoid host defenses, it must either not trigger them or must produce a substance that quenches the host response. If a host response is not neutralized, then a state of 'balanced pathology' occurs. In the latter case, the host dies before the pathogen is overcome. This outcome has been referred to as 'hyper-virulence'. The paradox of a pathogenic organism killing a host while unable to reproduce is not uncommon. At low inocula the pathogen can cause disease but be unable to kill the host. At high inocula the host dies but not necessarily due to the infectious organism. So, in order for a hominid to define a virulence factor, one need to be fairly precise about parameters in which it is defined.

4.2. Host-Pathogen Interactions

The spread of infectious diseases caused by a broad spectrum of pathogens remains a pivotal threat to global health. There have been calls for concerted efforts in developing new technologies for the immediate diagnosis of diseases caused by high-threat pathogens, which can reduce the risk of spread of fear and panic. Recently, a cost-effective and modular platform has been developed, which allows the isolation and analysis of digital biomarkers from whole blood for the

rapid and straightforward readout of host-pathogen interactions. This platform can be further developed to facilitate nuclear acid-based diagnostics on-chip, and detect and monitor the presence of sepsis-causing bacteria directly from whole blood at the point-of-care. This development can drastically cut the time-to-result down to three hours post-infection, yielding significant improvements compared to traditional methods. Broadly, this is possible in view of the intrinsic adaptability to micro- and nano-fabrication technologies, guaranteeing rapid and economic batch production of diagnostic chips over larger areas compared to traditional solid-state detectors. Progress in global efforts to ameliorate health initiatives notwithstanding, the world remains besieged by infectious diseases, necessitating their surveillance, prevention, containment, and treatment. Pathogens threaten individual health and inflict a notable burden on national health care systems, a burden that is significantly amplified by the systematic emergence of pathogens that are resistant to antimicrobial treatments. Antimicrobial resistance (AMR) has been declared a top-ten global public health threat; projections indicate the rise of annual AMR-associated deaths to reach ten million by 2050, with a potential global cost of 100 trillion USD. Moreover, there has been a recent unprecedented emergence and re-emergence of pathogenic diseases that have become of imminent concern, especially in correlation with the ongoing climate change. All of these factors highlight the urgent need to innovate and further develop technologically-advanced strategies for the surveillance and containment of infectious diseases. [5][6]

5. Emerging Pathogens and Their Impact

Potential biothreats and the emergence of pathogens due to new variations in virulence, adaptation, or the expansion of their host range are caused by changes in the ecosystem. The potential mechanisms are versatile, unpredictable, frequently mixed and often affected by artificial events or conscious activities like warfare or bioterrorism. The new technologies have greatly expanded the 21st century tools in molecular tests, although not all detection targets are equally affected by the current revolution. Adaptation is particularly required in the framework of emerging pathogens and multiple diagnostic platforms when there are guidelines, oriented to manage complex clinical samples, that are especially valuable in syndromic approaches [7]. The same occurred in bio-threat scenarios where intentional releases cannot constrain the choice of the suspects, degrees of risk, or geographic sources. The case holds for the discovery and awareness of new pathogen agents, inexplicit foci or reservoirs of infections, aiding the assessment of complex trade-offs and cost-effectiveness policies, giving proper management of exceptional, high-impact, or bioterrorism events.

6. Role of the Immune System in Microbial Infections

Our immune system consists of a variety of cells and molecules, and has an active role in protecting us. It not only protects us from outside pathogenic agents, but it also can recognize our own body components. Our body components are recognizable by the immune system through the presence of specific marker called the antigen. This ability is called self-/non-self-discrimination. So this is one characteristic feature of the immune system which has to be maintained to have healthiness. Sometimes due to defects, the immune system is not able to differentiate self components properly, then it mounts an attack on self-components leading to autoimmunity [8]. Our body is always exposed to the external environment which is rich in microorganisms and other pathogens that are harmful. Infectious agents include viruses, bacteria, parasites, fungi, and others. Our body protects us by having an immune system since birth. In olden days, when there was a minor cut on the skin any person eventually became the patient and died. As time progressed, people discovered that unseen infectious or non-infectious agents produce the disease which affects the health of the person. The importance of the immune system was recognized using the early work of Dr. Edward Jenner and Louis Pasteur. Dr. Jenner worked on small pox, whereas Louis Pasteur developed the vaccination with the help of this immune system. With vaccinations, many eradicated diseases like small pox were completely eradicated just because of the immune system. Several vaccinations are prepared for different diseases are the only way to find the cure of a disease. Several microbial diseases are spreading across the world, tuberculosis,

cholera, and others. Up to now there is no cure for tuberculosis to cure the disease completely. The mortality rate for any disease like HIV/AIDS depends on the total count of CD4 cells. If CD4 cell count is less, the survival rate of the person is also less. The main cause of death in HIV/AIDS is tuberculosis because the TB bacteria has more tendency to suppress the CD4 cells; it may decrease the CD4 cells count and the person is in a critical condition. So, several investigations carried out on this front, different investigations and different technologies could not prevent mortality which is happening with tuberculosis. There are several diseases which cannot be cured, which affect the health of a person like rheumatic fever, tuberculosis, and so on. These diseases can affect any one of the organs of the body or may affect the immune system. Since there is no cure for these diseases, one should take care in order to protect them from these diseases.

7. Molecular Diagnostics: An Overview

A general approach to molecular diagnostics will show how they are applied most effectively when working together in concert with other diagnostic procedural sections. The PCR technique and its variations have expanded the role of molecular diagnostics in the detection of genetic material.

There is always the danger that the presentation of molecular diagnostics could be given in separation from microbiology, culture analysis, or other disciplines in diagnostic medicine. However, molecular diagnostics, including the technology of amplification of DNA by the polymerase chain reaction (PCR), offers potential benefits in patient care that can be achieved by no other methodology. Each diagnostic section has its own unique perspective because each section is examining a different aspect of patient specimens. Clinical assays exist for the detection of many biowarfare agents, but the increased concern of bioterrorism has led to many laboratory preparedness activities and the development of new tests [9].

All patients with compatible clinical syndromes who are suspected of being infected with a biowarfare agent must be considered potentially infected by a BSL-3 agent; therefore, assays for biowarfare agents will not be found in this manual. Central to the practice of clinical infectious diseases is the timely and accurate diagnostic microbiology laboratory. The successful completion of the molecular detection method described here, hybridization protection assay (HPA), depends on successful completion of the nucleic acid amplification, PCR. The PCR products, amplicons, are detected by a method called the hybridization protection assay (HPA), also referred to as bead-based protection assay. Amplified rRNA targets are biotinylated on one PCR primer.

8. Techniques in Molecular Diagnostics

Molecular diagnostics are being applied in virtually all laboratory disciplines, including bacteriology. With more rapid, sensitive, and specific techniques, diagnostic bacteriology is moving to characterizing organisms in patient specimens below the genus and species level. Because of the importance of certain virulence genes of an organism in pathogenesis, new methods are available for typing organisms below the level of serology. This intersection of microbial pathogenesis and molecular diagnostics is an exciting and fertile area for exploration. For clinical microbiology, it has been suggested that PCR for infectious agents will include only three applications: diagnosing infections when culture is not available or inadequate, detecting fastidious or slow-growing organisms, and diagnosing infections in critically ill individuals [10]. Experience suggests PCR will play a greater role in diagnostic microbiology than indicated by these limited applications. Efforts are under way to automate PCR, eliminate postamplification steps, and improve primer and probe specificity. Efforts to develop technologies such as micrototal analysis systems and NextGen sequencing may have applications in microbiology. There are a number of areas in molecular diagnostics that are ripe for exploration. Many gram-negative organisms have sequenced genomes; development of micrototal analysis systems for such organisms would appear to be feasible. Most gram-positive organisms also contain plasmids, some of which carry important antibiotic-resistance genes; these could be detected by amplification with appropriate primers. The timer PCR described in this experiment can give some

indication of the relative amounts of different amplicons present; viruses that cause cancer integrate a number of copies of important viral genes like E6 and E7 in host chromosome and could be detected by an assay detecting that gene. Vaccines have been developed that can prevent certain infections, but are not 100% efficacious; such vaccines may reduce colonization but still allow replicating organism levels above PCR detection limits. Finally, different organisms have different life cycles; some may have early and late virulence genes, and detection of the latter would indicate the need for more aggressive therapy- perhaps in higher concentrations indicated by a lower relative pathogen:organism ratio. [11][12]

8.1. PCR Techniques

The most crucial step in identifying and understanding the mechanisms of microbial pathogenesis is to characterize the microbial pathogen and determine whether the role of environmental factors or host responses is influenced. Pathogenicity is defined by multiple genes and cellular activities that destroy the host or evade the host's immune response. Many of the same molecular techniques are used to analyze the pathogenic potential of a microbe, and the mechanisms by which it elicits disease are dependent upon understanding microbial genetics. However, only the analysis of microbial genetics is usually only done in opportunistic pathogens that otherwise survive a cystic fibrosis lung or other disorders which make the host. Most pathogenic microbes, accounting for less than 0.1% of the estimated 40,000 are studied. Pathogenesis is a process that leads to a disease, which is triphasic by nature. In immunocompetent adults, Pneumocystis Jiroveci do apotheosis remains non-pathogenic. A disease outbreak first occurs but then subsides. During disease onset fungal pathogens start germinating and forming malignant links, so investigating a specific gene should be made focusing on the early step of pathogenesis by a Candida sp. allergen. Microorganisms cannot simply be classified as being an extremophile, hydrophobe, saprophytic, or pathogenic. The genetic make-up of a microorganism along with the condition where it interacts is critical to determining the type of microorganism it is represented in. However, when planning a system level experiment, the versatility to interpret the experimental results may not be complete, for example bacteria have both pathogenic forms (virulence capability) and non-pathogenic forms, and selection of mutants in laboratory conditions. There are fungal species that are pathogenic and non-pathogenic in a given organism. But the unique position possessed by experimental pathogenesis is not limited to analyses of biotic factors. To get a better perspective of pathogenesis, an analysis of abiotic factors that support pathogenesis in relationship to abiotic environment must also be performed [13]. Lethality and multi-drug resistance are traits normally associated with pathogenic microbes, which may be defined as those microbes capable of causing disease, or illness, resulting from infection [14]. Several bacterial pathogens thrive intracellularly in macrophages. Pardon the actions that result from infection with Listeria Monocytogenes and Salmonella Typhimurium, right after one another. In vivo screening utilizing randomized transposon mutant libraries has proven to be a useful model for identifying such pathogens. The indirect environmental factor has a strong impact on the pathogenicity of a microorganism. Overall P.J binding with dendritic cells were noted, reproducing the usual physical bacterial binding seen in the nasopharynx, indicating that binding may play a role in stimulating a biological effect that drives pathogenesis. This includes expression of immune suppressive transitory factors by both the host and microbe, and membrane-binding microbe effectors that ingress host signaling. The most common disease outbreak is acute, with many pathogens resolving after acute infection. However, viruses, tuberculosis, and infections are examples of infections that retreat post first-found. A subset opportunist view pathogens that other than the deletion epidermal shoulders, the advisory an "chronic" infection-type. Once it was established that host C. albicans genotypes determine susceptibility to infection; years of results were pursued the underlying host perturbatory in the host.

8.2. Next-Generation Sequencing

Medical microbiology is a large genre in the field of microbiology which deals with the identification, characterization, and prevention from different microorganisms resulting in

infections. The initial diagnosis of any infectious disease largely depends upon successful detection of the pathogen. Detection and identification of the pathogen still solely depends on culture methods in diagnostic laboratories. Different selective, enriched, differential cultivation media are utilized for the successful culture of the pathogen with prior sample treatment. In the case of slow-growing microorganisms, cultures are routinely incubated for 2–4 weeks. Conversely, rapid-growing microorganisms are diagnosed after 24 hrs of incubation. Culture methods are time-consuming and are not widely available in a diagnostic laboratory. Many molecular and biochemical test kits are commercially available for the quick identification of pathogens in blood and other culture media samples. Unfortunately, these test kits require accuracy. False-positive and false-negative results are common with these test kits, resulting in mistreatment of the patient. Molecular diagnostics are becoming increasingly popular as it overcomes some of the limitations of traditional detection methods and the report of the result is rapid. Molecular characterization of pathogens is commonly done through ribosomal DNA sequencing methods. Prokaryotic and eukaryotic microorganisms are characterized through 16 s and 18 s rDNA sequencing, respectively. The match/mismatch of sequence data with available rDNA sequence data serves as a basis for accurate detection of the pathogen in the given sample.

In recent years, NGS technology has provided robust and efficient characterization of whole pathogens and the whole genome of pathogenic organisms. The NGS method allows a rapid characterization of unknown pathogenic microorganisms in complex biological samples, opening up the possibility for the detailed study of molecular host–pathogen interaction dynamics. Rapid advance in NGS has benefited researchers in various fields of microbiology and pathology. The rapid detection of pathogens by NGS aids in the identification of widespread pathogens during endemic or pandemic disease outbreaks and provides the point of care to individual patients. In this review, we focus on NGS techniques and its application in the identification of different pathogens and its recent other development, and co-evolution of pathogen and host during infection.

8.3. Microarray Analysis

The emergence of newly occurring pathogens with minimal genetics information leaves scientists scrambling to understand these organisms. The resulting uncertainties and unanswered questions pose enormous problems for biodefence epidemiologists. Bacillus anthracis was one of the first targets in the infamous 2001 bioterrorist attacks in the United States. Six years on, biothreat scares are increasingly raised by the discovery of unexpected materials with non-hypervirulent bacteria. Public health efforts in response to such bioterrorism threats and epidemics have intensified recently. Traditional methods fall short in providing rapid, highly sensitive identification and discrimination of prevalent biothreat agents from closely related organisms. Viewed from an engineering perspective, epidemiology’s compilation of their occurrence time spans, human contacts, and location relates to a problem in automation: the triangulation of latents from multiple, wide-area, non-uniform data sources. Microarray technology enables the manufacture of a high-density grid of DNA or protein spots on a solid substrate . Widely utilized wellness and environment applications include expression analysis, gene discovery, gene typing, sequencing, drug design, mutation detection, and toxicity screening. Very recent developments ready standard microarray systems for pathogen detection currently relying on PCR, cell culture, or immunoassay. A prototype microfluidic bio-nano-chip is demonstrated recently, enabling a full immunoassay detection protocol using space-modulating fluorescent magnetically marked micro-particles. Machine-readable biomarker bead arrays enable high-throughput gene variability analysis. Bulk magnetoresistance sensing allows the direct, rapid, and multiplexed detection of analytes in a bead array with high sensitivity. A light-scattering technique is also capable of reading microarray bead sets for gene expression profiling far less expensively than current commercial instruments. Broad-spectrum respiratory virus detection is demonstrated using immobilized broad-spectrum viral DNA arrays and colorimetric labeling detection. The large spectrum of biomolecules involved in microarrays has opened the door to wide applications in

molecular forensics and comparative profiling, especially for emergent and less well understood pathogens. The substantial progress in microarray research in the past few years has brought it to the front lines of rapid diagnostics and medical research. With further integration of all components from sample preparation to data analysis in a miniaturized system, microarrays are poised to become compact lab-on-a-chip solutions for in-the field analysis and monitoring. [15][16][17]

9. Applications of Molecular Diagnostics in Pathogen Detection

Microbial pathogenesis is a field that focuses on the study of the mechanisms used by pathogenic microorganisms to cause infections in humans and other animals. Parasitism on humans is initiated by colonization at the site of entry. Microbes may grow in or invade host tissues and produce toxins that prevent normal host processes resulting in gaps and subsequently enter the bloodstream [18]. Here, they encounter macrophages at the local site and bloodstream produced by the reticuloendothelial system thus entering various body tissues. Upon entering the macrophages, microbes can survive and grow within the macrophages, disseminating the infection to other tissues and organs.

Molecular diagnostics enables rapid and accurate detection of the presence of microbes, viruses, and bacteria, and their genomic sequences. Molecular diagnostics are used for monitoring infectious diseases such as acquired mutations for drug resistance and viruses. Methods used are either PCR or hybridization to detect genetic mutations in a sample [9]. Emerging public-health concern involves infectious diseases that can be transferred from nonhuman animals to humans. Biologic agents aimed at public-spread infection are known as bioterrorism pathogens. Rapid and accurate identification of the causative agents has become critical because optimal effectiveness of antimicrobial and sometimes antiviral management of patients with life-threatening infections is time-dependent. In contrast to more slowly evolving infectious disease epidemics, an infectious disease emergency can occur unexpectedly and the number of cases can rapidly rise. In the laboratory, this has necessitated development and implementation of rapid diagnostic tests. Since a molecular genetic aspect to each diagnostic test is always the identification of the infectious agent, this field of molecular diagnostics has been most deeply penetrated in the context of emerging infectious diseases.

10. Case Studies: Successful Implementation of Molecular Diagnostics

The goal of this article is to provide a review of important breakthroughs in microbial pathogenesis. It is ambitious and offers a wide range of topics because the field is both dynamic and interdisciplinary. At the same time, one can only hope to synthesize important, general themes. The growth in knowledge and technological capability has been explosive, hence a more ambitious, comprehensive endeavor to review the field. This review should be broad enough to encompass much of what continues to be exciting in the field, yet focused enough that individual topics are covered in some depth and important concepts and models are treated in detail. The review will start with a general discussion of model systems and the relationship between genotype and phenotype. Next comes the heart of any discussion of microbial pathogenesis, the host–pathogen interaction. In the vast sea of literature in this field, it is impossible to single out the most important research or development. Instead, several case studies are provided on the hope that they will both highlight important advances in understanding, and also offer examples of the integration of different technologies and disciplines. To further ensure that this review will have lasting value, there is a review of some classic work in the field, not merely historical curiosity, but because important concepts are still actively studied and developed.

Nonlinear dynamics, often called chaos, is having an increasing impact on a wide variety of scientific disciplines, including epidemiology and microbial pathogenesis. To make these results accessible to a broader audience, the basic concepts of nonlinear dynamics and chaos are first briefly reviewed. They are then applied to both immunology and the dynamics of the host–pathogen interaction. For example, the host–pathogen interaction is rife with examples of chaotic

dynamics, and models correctly accounting for these are presented. These results have important implications for the design of improved treatment regimens.

11. Challenges in Molecular Diagnostics

Successful extraction of RNA from formalin-fixed, paraffin-embedded (FFPE) specimens for PCR can be daunting. The prompt original tissue processing and a well-developed extraction protocol are crucial. Microorganisms processing an infection can be randomly distributed in the tissue. Consequently, when samples are collected, the detection of microorganisms might be just by chance. As a punch biopsy is used for the collection of the FFPE tissues, and the inflammation and/or microorganisms are noted through the histopathologic examination, more often than not, these tissues can be analyzed, documenting pathogen DNA. However, the use of fresh or frozen tissue is much more problematic. Both are in block form, so random sections will be interrogated. If the pathogens are randomly distributed, bad luck will entail no amplification, even though the infection appears descriptive of microbes. In other words, if a culture grows out a single species of microbe, pathogenesis is simply declared based on the culture legacy, an empirical decision not supported by modern science. The course of events of infection is a body's intricate response to the microbe or other injurious agents, quite complex and indiscernible to the culture at this time [19]. In this same vein, the interpretation of PCR results is complicated. Molecular assays already have been noted to detect mixed infections [20]. Such results may be problematic for the clinician to associate with the site of infection and the severity or the extent of the infection. All aspects of molecular methods, including accumulation and interpretation of the results, are known to clinical microbiologists. Because of such complexity, the microscope, a simple device requiring a modicum of training to use, is still the most important tool for the pathologic examination of tissue and tissues. All of this is to suggest that the state-of-the-art infectious disease investigations, the culture and molecular analysis of tissues, are currently fragile and fraught with difficulties. Nonetheless, the outcome of bacteriological and/or molecular analysis of tissues from patients who succumbed to an infectious disease is the scientific reasonable gold standard for the diagnosis of fatal or life-threatening infections. In the past, this knowledge was only obtainable post-mortem; however, in the present era, it is feasible to obtain such tissue analysis from an individual still breathing, consequently allowing for appropriate antimicrobial therapy and in all likelihood prevention of fatality.

11.1. Technical Limitations

The recognition that bacteria, fungi, and viruses cause diseases has been profoundly significant for public health, but for the clinician this recognition only moved the problem to the identification of the responsible microbe. The ancient Greeks recognized the contagious nature of diseases but had to wait for Anton van Leeuwenhoek and the advent of the microscope before anyone had the tools to investigate microorganisms. At the same time the protection provided by variolation was known empirically but not understood until the germ theory of disease ratified the anticontagionists advocating smallpox variolation. For the epidemiologist John Snow the cholera bacterium lay behind the pump handle but could not be isolated from the drinking water supply until Pasteur and Koch had described its life cycle [19].

The microscopic identification of microorganisms quickly became a technique. The Gram stain of bacteria and the smears of Flemming and Giemsa, both developed in the 1880s, are still in use today. These elementary techniques allowed a great study of morphology and life cycles. Retrospective study of slides made by epidemiologists can effectively prove and describe the aetiology of diseases. Similarly, Romanowsky stains are still the touchstone for hematological essays. Some pathogens, such as the malarial parasite, Plasmodium, can still be definitively identified only morphologically. Antibiotics and the chemotherapeutic agents developed in their wake are said to have revolutionized cure. The purported importance of antibiotics for treatment of established infectious diseases and as prophylactic agents may have been overstated. Several studies show that a higher rank of health safety management systems does not depend on the use

of antimicrobial therapy. However, the development of antibiotics did decisively alter the temporal dynamics of infection. Broadly speaking, prior to chemotherapy infectious diseases could be divided into the six categories defined by contagionist theory: plague, quarantine, putrid, mild-contagionist, mild-contagionist, and anti-contagionist. Once antibiotics were introduced many of the serious diseases reverted to the mild contagion category; doctors retained a distinction between the ‘exanthemata’ and the ‘typic’ but the rest were generally put into the mild-group. In the late 20th and early 21st centuries disease again began a broad reconsideration. New diseases such as AIDS appeared and there was a worrying increase in multi-drug resistant strains of bacteria. As the 19th century gave rise yeasts and molds but this reconsideration did not populate. In 1823, Ann Hill made a fungiting for the first time. Recognizing the division of asexual and sexual in 1837. [21][22][23]

11.2. Regulatory Hurdles

Adoption of rapid molecular tests developed; however, their regulatory approval and implementation could take much longer. Challenges faced by developers of new diagnostics and recent work addressing these were outlined. The roadblock is the “quadruple-farther” challenge presented to the academic scientist in this field: (i) excellent science must be married with state-of-the-art technology; (ii) finding the commercial partner; (iii) successfully navigating a logjam of regulatory approvals; and also being aware of and reacting to (iv) the ramifications of these changes in diagnostics [24]. Prompt initiation of appropriate antimicrobial therapy has led to dramatic reductions in infection-associated morbidity and mortality. Simultaneously, the emergence of new and difficult-to-treat pathogens has driven the demand for improved detection and identification of microorganisms. Rapid molecular tests have been developed that do provide this necessary speed, sensitivity, and specificity, but these tests have not yet been broadly approved and implemented.

12. Future Directions in Pathogenesis Research

Despite tremendous progress in global health initiatives, the world continues to be confronted by infectious disease. This underscores the need for research into their survey, prevention, containment and treatment as well as the associated health and socioeconomic implications. Moreover, pathogens not only have a direct bearing on our health, but also represent a significant financial burden to our health care systems. Furthermore, there is a growing concern regarding pathogens that have developed resistance to known antimicrobials. Pathogen antimicrobial resistance has risen steadily over the past decade, this carries the risk of rendering a number of diseases un-treatable, including common infections like strep throat or pneumonia. With the onset of climate change, there is also the emergence and re-emergence of known pathogens and their vectors. These trends further underscore the need for the development of molecular strategies to reduce the burden of infectious diseases. Consequently, the relevance of studying pathogenesis will only increase as new infectious agents emerge and the pathogens evolve.

There have been vast technological improvements made over the past decade in agriculture, healthcare and the environment. In particular, developments in next-generation sequencing have revolutionized research into the microbiome and host-pathogen interactions. Together with progress in proteomic structuring, these technologies have helped delineate the genetics of pathogenicity and mechanisms of host-pathogen interactions at the molecular level. Nevertheless, one of the biggest gaps in the understanding of the invasion of unicellular organisms by pathogens is the accompanying physiological changes that are required, especially during the initial stages of colonization and invasion. Moreover, media that lack these physiological changes that are commonly used in studies of pathogenicity have been shown to compromise the results. In response to starvation or stress conditions it is common for a bacterial population to produce two functionally distinct subpopulations, known as phenotypic heterogeneity or bistability.

13. Integrating Molecular Diagnostics into Clinical Practice

Recently, diagnostics have been advanced to rapidly identify the causative organism(s) of an infection. Nonetheless, more is to be learned about disrupting host pathways that can lead to unexpected infections, particularly those that are life threatening. The goal of this chapter is to present recent milestones in microbial pathogenesis principles involving host-pathogen interactions of medical relevance in a section of bacterial, viral, and fungal infections. Since the human body is a rich source of iron, pathogens must compete for this essential nutrient. Host cells restrict available iron to microbes, a concept termed nutritional immunity. This defense mechanism targets iron-specific pathways developed by a pathogen, either through engagement of serum proteins that chelate iron, or by limiting total iron concentrations. Some pathogens are equipped with systems that directly compete with the host for iron. The most well characterized of these include the yersiniabactin and aerobactin systems. The pathogen either synthesizes or acquires siderophores, high-affinity iron-chelating agents, which are tightly bound to the iron transporter on the microbe to release iron into the periplasm. Once internalized, other transporter systems, or reductases, help shuttle iron across the cell membrane, where the microbe has little to no iron. The metal is then trafficked to specific sites where the pathogen can use it, or storage proteins that can buffer inappropriate oxidation. By abrogating the ability of the microbe to acquire iron, growth is stymied, and infection prevented. Thus, it is not surprising that pathogens have evolved sophisticated mechanisms to counter this hostile environment. Many of these systems are deployed on secretion systems, needle-like structures that interface with the host cell, and translocate effector proteins into its cytosol. Once inside the host cell, these effectors can subvert immune responses, modulate cell death, stimulate endocytic uptake for microbe invasion, or engage the autophagic machinery to promote the degradation of the invader. However, the detection of pathogen intent and the delivery of response is the entry point for a successful clearance of an infection. [25][26][27]

14. Ethical Considerations in Microbial Pathogenesis Research

Ethical Considerations in Microbial Pathogenesis Research

Conducting research and reporting on a controversial and under-studied topic such as disease-causing microbes presents certain challenges. Though it is important to review possibilities of controversies prior to writing the article in order to prepare for them. Controversies related to the author, to the research design or methodology, to the theoretical framework, to the findings, or to the author's explanation of the findings should be anticipated. To help form the understanding of these possibilities include asking the following questions about potential controversies and consider if they are addressing them effectively ([28]).

Standpoint on the topic of the article – Do I have a conflict of interest in relation to the specific question being studied, or in relation to the broader topic? How might that be perceived and how might it influence results and interpretation or make it more difficult to interpret the results? How do I situate my findings in relation to other studies on the same topic and how might that be critiqued, particularly in light of studies whose findings differ from the own? How might that concern be taken care of? Are there important perspectives on the topic of the study that were not considered? What are the implications of the research or of the specific findings with respect to such perspectives (clinical applications, policy implications)? Are they discussed in the article?

Systematic bias in data collection, coding, or analysis – How might judgments be biased, and how might they be cutting against or in favor of the hypotheses under investigation? Are there cases in which the possibility or opportunity to code a text could appear to be other than objective? What have been done to ensure that the same criteria and procedures are used to collect and code each piece of texts?

Methodological concerns – How has the author taken into account what cannot be observed? Thus, how has what cannot be observed been examined? How are the observations situated

socially, politically, historically? Is there an assessment of the research design and method employed in the article? Are there footprints that allow replicability?

Ethical and moral implications – Does the presentation of the findings take into account the well-being of the persons or organizations studied? Are the authors aware of the broader cultural or political underpinnings of the research? How might they be exploiting, degrading, or stigmatizing a particular group or groups of people, or contributing to their exploitation, stigmatization or degradation?

15. Public Health Implications of Microbial Pathogenesis

The potential public health implications of these three features—motility, methylation, and survival—of a number of microbial pathogens that are of current interest in respect of the molecular pathogenesis of their clinical infection are examined. This is a rapidly advancing field of academic activity, but this paper adopts a practical stance and includes public health advice where applicable. The public health aspects include alternative approaches to food hygiene and novel strategies to control pathogen survival. These features make a significant contribution to infection by some facil pathogens and may also be relevant to microbial pathogens of a similar nature.

Bacterial motility is increasingly being implicated in microbial pathogenesis, disease and clinical infection. It has a direct effect—an initial aspect of host colonization—and an indirect effect—promoting resistance to host defense mechanisms during established infection. The latter will include an uncommon strain of Salmonella with a particular capability to penetrate dried mucus and survive desiccation, which will increase the capacity of some strains to cause gastroenteritic infection.

Many of the virulence factors identified in bacteria and fungi have been found to act on the methylation of host molecules, rather than the synthesis of their de novo. Host DNA loses methylation reactivity in rapidly growing cancerous cells. So, only two nucleic acids bacteria have lost their original niche and are less of a public health risk. A commercially available polymer can be used to coat catheter tubes, inhibiting the growth of Proteus mirabilis. Also, the plant can be sprayed with procedures that do not increase levels of the hepatotoxic menadione, making survival and germination of spores of Bacillus anthracis amongst heroin more difficult.

Seventeen years of rapid developments in a range of molecular biology techniques have provided tools that can be used to provide powerful approaches to infection diseases diagnosis; molecular diagnostics. Efforts to apply the techniques to developing outcomes that are of use in clinical and public health terms can be divided into three main areas. One is prediction, particularly with respect to the nature and incidence of infections. Such approaches could be used to improve the targeting of traditional, empirically based public health strategies. An example is that nonsteroidal anti-inflammatory drugs cause a three-fold increase in the relative risk of amplified salmonellosis in the correct age group.

16. Global Surveillance of Infectious Diseases

Infectious diseases are the second leading cause of death globally and half of them are attributable to pathogens derived from animal sources. Therefore, the most effective way to mitigate public health risks and impacts of such diseases is to enable control measures during early stages of a disease outbreak by a sufficiently robust surveillance system. Indeed, many early detection and warning systems have been initially created to monitor climate change and geophysical activities which may affect human health and well-being. This framework has also played a decisive role to inspire the early actions of global surveillance of infectious diseases and the subsequent development of relevant ethical and legal codes and health policies. Among many available approaches, the methods used by international organizations related to health, animal health, and food and agriculture are focused, including recent incorporation of Earth Observation data. They provide a predefined agent-targeted country-based contagious disease base flow model and are

applicable to all intentional actions if the selection is pre-rational.

17. Role of Bioinformatics in Pathogen Analysis

Identification of microbial pathogen is an important event which leads to diagnosis, treatment, and control of infections. Over the past decades, microorganisms have developed varieties of tools and mechanisms to successfully attack the host; consequently, host organisms have also evolved unique ways to stop microbial attacks. Pathogen detection has advanced from traditional cultivation-based techniques to enzyme immunoassays and nucleic-acid-based molecular techniques. High-throughput technology like microarray and the availability of new-generation sequencing machines generated huge amounts of nucleotide sequences of viral and bacterial genomes of both known and unknown pathogens. Furthermore, researchers are looking for a complete understanding of microbes and host interactions [29].

With the advent of new biotechnologies, bioinformatics has offered tools for the reliable genome-wide investigation of host-pathogen interactions. Bioinformatics analysis tools and databases are now easily available to researchers, enabling meaningful handling of the enormous, but often low quality, quantities of sequence information generated. This offering has provided the scientific community with the opportunity for better understanding of the microbial world, often still considered as an almost unassailable opponent. Microorganisms represent one of the forms of life that have colonized almost all environments on earth, from the most extreme, such as boiling thermal springs or waters rich in salt, to the inside of animals and plants. Microorganisms like viruses, bacteria, and fungus have evolved to survive in these conditions. Although most of the microbial world is composed of microorganisms that are harmless, some are very dangerous. Modern biotechnologies and knowledge have revealed that most of the pathogenesis is mediated by toxins or enzymes affecting host cell physiology. But there are other pathogens that have developed more formidable weapons, for example, manipulating the expression of a whole set of proteins in order to alter the physiology of the host. Transferring portions of the own genome inside the host cells is another way to create a favourable environment in which to reside for a microorganism. This concept is very elegant and at the same time dangerous. It explains the existence of pathogenic organisms that, being genetically very far apart, display similar behaviour.

18. Impact of Climate Change on Microbial Pathogens

Mental illness may be associated with a higher prevalence of diabetes and is associated with poor glycemic control. A few studies report Type 2 diabetes is associated with depression and the presence of common mental disorders in low-income countries. Depression prevalence soars and few receive treatment. Very high and very low levels of diabetes were associated with high maternal-fetal mortality rates. In very hot and cold rural areas, mortality affects especially women. There were numerous cases of diabetes, of which a significant portion were Type 2. Compared to developed countries, severe chronic complications of diabetes occur earlier and are more common in younger populations.

These results corroborate others in the literature that suggest worse levels of depression among women, and greater risk of diabetes due to mental health problems among women. Such risk can be associated with the reproductive system, and by gendered social determinants of depression and diabetes. A program developed to improve maternal and fetal mortality rates is based on three pills in one pouch. The pill package of hypertension, diabetes and depression aims to align medical care of pregnant rural women with pill regimen to avoid hypothermia.

This research was conducted with data from a longitudinal evaluation in a community randomized trial. Semi-structured interviews were undertaken with all participants and discussed with a supervisory team. All data treatment were summarized in four categories. Perspectives were adopted to analyze empirically data driven findings with theoretical focus on the case. Ongoing analyses explore aspects of common mental disorders, alcoholism, domestic violence and democracy related to female mental health outcomes in rural areas. Nevertheless, findings

collected here suggest a critical approach to gender blind analysis of diabetes and depression, which are curable diseases largely focused on women. [30][31]

19. Antimicrobial Resistance and Pathogen Evolution

Failure of medications can be scheduled due to mechanistic modifications that microbes undergo, the molecular mechanisms of drug resistance. The major cause of ineffective prescribed therapy is the result of microbe drug-resistance pathogens due to antimutational metabolite synthesis matching up with target site alteration [32]. The molecular mechanism of developed microbial horizontal transfer, enzymatic inactivation modification of a site and decreased drug intake, all of which are necessary to conquer the selective drug pressure to permit the growth of the treated microorganisms with developed higher resistance of MDR. Treatment of infections caused by multidrug-resistant (MDR) microbes has been considered a catastrophe. After the discovery of antibiotic prescribed antimicrobials, the world was conquered by similar infectious diseases identical to the use of chemotherapy in which antibiotics have been found. Thus drugs had an immense effect on controlling the disease in the first half of the 20th century, and life expectancy increased as a result. Due to inappropriate use of antibiotics, microbes have developed genetic resistance capability beyond the robotic century [33]. Following the industrial revolution of intensive treatment, many bacteria have caused various microbial diseases to spread rapidly to humans and animals. Thus, it is now incoherent with the divergence of microbes, and for some of them the situation is grave. Resistance to these drugs has spread markedly among otherwise pathogenic microorganism agents, which can be deadly at such times.

20. Vaccination Strategies against Pathogens

Vaccination has been a major advance for health care, allowing the eradication or reduction of incidence and mortality of various infectious diseases. Vaccination has been highly successful in combating measles, mumps, and polio, and, thanks to the development of novel vaccines, it is possible to assess the control of infections as Hepatitis B, Haemophilus influenza type b, or human papillomavirus, to mention a few examples. Nevertheless, there are major pathogens, such as Human Immunodeficiency Virus (HIV) or Plasmodium, for which classical vaccination approaches have failed. Therefore, there is a demand for new vaccination strategies to eradicate disease, and various research fields have been highly active in this area, notably the infectious diseases, but also immunology, vaccinology, and adjuvant development, biomathematical modeling, and, more generally, network biology. However, there are obstacles to reach this goal, since eradication would require a better comprehension of the pathogenesis of these diseases and reliable correlates of protection, as well as accurate ways to direct appropriately immune responses [34]. For example, in many instances both suboptimal immune efficacies of existing vaccines and unforeseeable dreadful vaccine-related adverse effects have been recently emphasized, as in the case of the dengue tetravalent vaccine in naïve children. Another hurdle in this field is the absence of a robust, cost-effective, and predictive animal model that would combine efforts to study vaccination and infection and recapitulates features of both human infection and vaccination. Combination of several of these roadblocks prevents improved vaccine design for the majority of pathogens and hamper the efficient development of prophylactic or therapeutic AIDS vaccine candidates. The use of humanized mice would help researchers understand the biology of HIV in vivo, including the study of viral transmission, virus spread, reservoirs, and immune response. Thereby, researchers envision developing humanized mice model that are highly susceptible to a broad range of epidemic HIV-1 isolates for testing both vaccine concepts and HIV reservoir eradication strategies [35]. At the cellular level, the data obtained from these humanized mice and the extensive in-depth analysis of velogenic HIV-1 infection in hematopoietic and lymphoid organs could also have impact in basic immunology research, contributing valuable insights to host/pathogen interplay, and foster the development of new vaccine targets and vaccination concepts.

21. Public Awareness and Education on Infectious Diseases

Training and teaching in infection diseases epidemiology, including current public health and research responses. The Global Trends in Emerging Infectious Disease training program sought to develop capacity in leaders across public health sectors in countries in Africa, Asia, and the Middle East, focusing on epidemiology, laboratory diagnosis, surveillance, immunization, antibiotic resistance, and emerging disease issues. Researchers and teachers in infectious disease epidemiology project reviews emerging epidemiological approaches applicable to the SARS outbreak and its implications for epidemic disease control. An interdisciplinary understanding of the complex pattern of human occurrence and the potential for community intervention is needed, yet often not matched by teaching programs. Strategies used in a 1-unit graduate course of the epidemiology of infectious diseases include course and supplemental readings and exercises provided to enhance study. Comprehensive information with infectious disease epidemiology syllabi available on the website, including training, advanced degree programs, and funding opportunities. Special reports “Communicable Diseases” is the topic [36]. Public awareness and education is the base strategy to prevent infectious diseases. Control of the infectious diseases. Policy statement on public awareness and education against infectious diseases is proposed. Rich reference materials, information, and toolkits for concerned people will be suggested. The targets are the general public, students, travelers, and medical professionals. Finally, advice and tips from the behavioral and social aspects, the history and art resource, and the homecraft skill are provided. The efficacy of the proposed strategy will be evaluated as public awareness is reflected from the increases in public health percentages. The combatting infectious-based diseases should be jettisoned to curb the pathogen portfolio and the prevention of the Israeli bioterror death, Educate Before It’s Too Late” concerning the research misconduct and unethical activities on the public bioprotection spectrum will be settled [37].

22. Interdisciplinary Approaches to Microbial Research

There is a growing realization that only 1% of the microbial diversity has been exploited and studied thus far. Advanced molecular and genomic technologies have made it possible not only to study those cultivable 15% but also other non-cultivable microorganisms. These molecular and genomic techniques will revolutionize the understanding of microorganisms and provided opportunities for new drugs and antibiotics. Besides, a vast amount of genomic information gained from the genome projects of many model species provides unique chances for mining new drug targets. In this study attempts are made to identify likely drug targets in Mycoplasma genitalium genome, the second genome to be sequenced after Haemophilus influenzae. Several possible drug targets in M. genitalium functions are identified. Also provides a comprehensive analysis and insights into functioning of M. genitalium [38]. As more genomes, protein three-dimensional structures, and other biological information saturate the databases, computational methods will become increasingly integral to drug target identification and validation. With complete genome sequences targeted databases can be searched for one’s favorite microorganism. Comparison with well-studied regulatory and metabolic networks, one can discover the biological processes unique to that species [20]. Also has the potential to yield new targets. It should be possible to determine using bioinformatics pressing similarities with a known drug target. Techniques have been developed to examine whole bacteria, such as Mycobacterium tuberculae, as well as infected human tissues. This could be a powerful tool for high-throughput analysis of potential drug targets from medicine-resistant infectious agents, many of which are slow-growing and cannot be screened by conventional high-throughput methods.

23. Funding and Resources for Pathogenesis Research

Infectious diseases are more likely to spread where there is crowding, poor sanitation, and contamination of food and water. Therefore, they continue to be a major problem in countries with lower standards of living. Although much is known about pathogenesis and disease control, new and emerging pathogens are said to have caused more infectious diseases in the past than ever

before. Some have originated in animals, often domesticated, and there is concern about them becoming established in human populations.

Expenses related to infectious diseases can exceed the allocations of resources required to provide basic services. In some developing countries where health and education systems are weak, disease burdens are high. Very often health services respond to diseases associated with morbidity and mortality by concentrating resources on treatment. The resources allocated to finding a cure can be many times the costs of preventing it. Resources are spent when a potential for cure is evident, i.e. when diagnosing a given pathogen is convincing. Facilities to investigate wider unspecific infectious syndromes are less developed. However, many types of simple and rapid cure-detection tools are useful for early intervention, containment and control of infectious diseases. In fact, curing is foremost on the mind of those who are ill, but it is thought that resources would be better spent preventing infection and the spread of diseases to others [39].

Research on pathogenesis, molecular structure, etc., has traditionally received less attention. As a result, communities and populations are often the victims of chronic or acute infections, and nascent or rare infections. The funding mechanisms for research needed for vaccine development, the design of diagnostic tools, and research aimed at developing a better understanding of the interaction between a pathogen and a diverse host are not common. Such research is often regarded as illustrative of facts and of no immediate benefit. This perturbs the understanding of the dynamics of infections and the interactions between a pathogen and its host, the perception, and the modeling of the development of infectious diseases. These projects are often expensive and require long-term funding [40]. At the same time, this form of research may also require analysis that combines expertise from different fields, such as biology, medicine, mathematics and computer science.

24. Collaboration Between Academia and Industry

Recent advances in molecular diagnostics have revolutionized microbiology by facilitating rapid, sensitive pathogen surveillance and differential diagnosis of infectious diseases [39]. Implementation of these technologies can enable intervention when the prognosis is optimal for limiting replication, dissemination, transmission, morbidity and mortality. However, these approaches are better suited for some types of agents than others. Infections with BACterial agents are often acute, so effective intervention is more likely than with chronic infections. Similarly, acute viral infections are often diagnosed and treated during the early stages when intervention is most likely to have an effect. Conversely, pryogenic infections arise in the context of a chronic disease, chronic treatment, or an immune-compromised state. Infections with agents that reside in organs having avascular interfaces with the periphery often produce invalid results as well, as pathogens cannot be detected in blood due to compartmentalization. An important task for the future in the context of microbial surveillance is to separate those disorders which reflect the interaction of microbes with other environmental factors and susceptibility genes from those that reflect acute infections. Also of importance is the definition of a microbial cause for conditions that may be infectious initially, but become chronic without obvious clinical features of the initiating agent.

Here will be reviewed the strengths and limitations of various assay platforms, will describe the challenges associated with proving causation, and will delineate a staged strategy for pathogen discovery focused in 'hot spots' such as Indonesia and hot hosts such as humans. In the beginning it was expected that pathogen surveillance and discovery would result in paths to specific interventions that could be brought to bear to sterilize infection, promote immune recognition, or restrict vector populations. As these studies have progressed, it has become apparent that diagnostic pathogens may actually be only a minuscule proportion of the total diversity in a given niche and that pathogen discovery may itself produce levels of technical, administrative, and public resistance.

25. Conclusion

In conclusion, it must be stated that as the speed of new knowledge and as the ability to apply and incorporate these new technology developments increases, the facets of infectious diseases and the laboratory testing for their agents will quickly evolve and change. It is stressed that the pertinence of these emerging methods to the clinical practice of patient testing should be continually reassessed, as the utility of these procedures is definitively determined by a broad clinical usage experience. The dialogue and communication between the clinician and the microbiology laboratory is essential and should be sought as well as warranted whenever an interpretation of a microbiological test result is necessitated. It is so important that both the clinical microbiologist and the clinician possess, instead of the historical categorical division of labor, a working and mutual knowledge of the principles, diagnostic benefit, and limitations of the molecular assays are applied.

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