

REVIEW ARTICLE

Applications of Metagenomics in the Investigation and Analysis of Microbial Diversity from Un-Cultured Soil of Hospitals

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Abstract

Hospitals play a vital role in health and well-being of human beings. However, the biomedical waste generated by hospitals pose a serious threat to general health due to the high potential of disease transmission through pathogenic bacterial strains from hospitals' dumpsites via any mode of contact. Therefore, methods for the analysis of microbial diversity in the environmental samples surrounding the hospitals' dumpsites are required for better understanding of Hospitals Acquired Infections' (HAI) transmission. Metagenomics provides solution to this problem by providing methods that allow the isolation of microbial DNA from environment that cannot be cultured and hence has never been studied before. The utilization of metagenomic approaches in investigation of bacterial diversity in hospitals' natural environment could provide with the results that can contribute toward understanding the involvement of hospitals in transmission of HAIs and to the development of strategies and protocols for proper disposal of biomedical wastes generated by hospitals accordingly.

Key Words: Metagenomics, Hospitals, Dumping System, Hospitals Acquired Infections (HAIs), Biomedical waste management.

Introduction

Bacterial infections are the second leading cause of death worldwide [10,30]. They have major contribution in the diseases that have infected human and animal lives throughout the history.

Bacteria are ubiquitous and are present everywhere on earth. They are also responsible for many mild to severe infectious diseases causing the deaths of millions of the human and animal lives in the past. Several epidemics of bacterial infections have been documented including the epidemics of smallpox, leprosy, diphtheria, tuberculosis; lower respiratory tract infections and meningococcal infections in ancient Greece and Egypt [33]. Many severe epidemics of plague in ancient times have destroyed several civilizations. History is enriched with epidemics of many infectious diseases that have devastated the human population globally. It was not until the middle of the 19th century when bacteria were found as the cause of these infectious diseases that had been infecting the human population since the ancient times [27]. The identification of microorganisms as causative agents aided towards the better understanding of epidemiology of these infectious diseases which further assisted in formulation of preventive strategies and treatments [8]. The advances in the disciplines of microbiology and immunology offered more detailed contribution toward the prevention and treatment of these diseases. But despite these strategies microbial infections continued on infecting human populations and caused the epidemics of different microbial infections in different years of 19th and 20th century.

What is Metagenomics?

It is estimated that approximately 99% of the microorganisms present in the natural environment cannot be cultured and hence cannot be studied within the laboratory conditions [2, 29]. Metagenomics provides an excellent way to study those novel microorganisms within the natural environment. Metagenomics is a method of examination of DNAs present in the natural environments through direct DNA isolation.

The term metagenomics first used by Handelsman in 1998, refers to the study of environmental DNA [12]. It was referred as study of collective genomes of the members of microbial communities from natural environment. Metagenomics involves cloning and analysis of those extracted environmental genomes without the use of culturing methods. Therefore, it offers the opportunity to explore the diverse microbial communities from the earth's natural environment of soil, which includes even those microbes which cannot be cultured and hence might not be studied before [26] (Figure 1).

Metagenomics emerged as the result of efforts in analysis of microbial diversity in natural environments including soil, water and even gastrointestinal tracts of organisms [29].

Metagenomic approaches are used for the isolation and analysis of novel DNA species from the natural environment. Nowadays, metagenomics can roughly be divided into two approaches; functional metagenomics and sequence-based metagenomics.

1. Function-Based Metagenomics

In function-based metagenomics or also called functional metagenomics, DNA is extracted from a selected environment in the form of the pool of genomes of all organisms present in that community. These extracted genomes are then fragmented and cloned into an organism that can be cultured in order to create metagenomic libraries. These libraries are then further subjected to DNA sequence analysis or functional analysis based on functions expressed by the surrogate host [26].

Functional metagenomics is also a best approach for identification and analysis of genes of unknown functions or source. Studies have demonstrated the efficacy of PCR in detection of any specific gene but it has its limitation as it can only probe the gene with known sequence. While the functional metagenomics obviate the need of gene sequence and rely on expression vector for identification of gene through their expressed function and hence proved to be more efficient than PCR [16]. Using this approach many novel antibiotic resistant genes can be identified from the environmental sources such as oral, soil and gut microbiota. Functional metagenomics has contributed in discovery of many novel gastrointestinal associated antibiotic resistant genes including *tet* (37) and beta-lactamase genes [17,18] (Figure 1).

2. Sequence-Based Metagenomics

In contrast to function-based metagenomics, sequence-based metagenomics approach specifically relies on sequence analysis to provide the basis for the prediction of the function associated with newly discovered metagenomic sequence [26, 28]. In case of sequence-based metagenomics, studies often focus on a specific gene of interest, also called “Anchor” for the identification of metagenomic clones. Most commonly these anchor genes are ribosomal RNA gene that can be used for the identification of bacterial species. For example, studies have been conducted in which metagenomic library was screened for 16S rRNA gene, which was then sequenced for further diversity analysis of the community [26]. In prokaryotes 16S ribosomal RNA gene has been commonly used for the investigation of biodiversity as this gene is universally present in all

prokaryotes and thus allows the identification of bacterial species present in variety of environmental samples. There are examples of many studies in which researchers have used approach of 16S rRNA gene identification and sequencing for the analysis of microbial diversity. Once the 16S rRNA gene sequences are obtained from the metagenomes extracted from environment, they can be further compared with reference databases such as GenBank and RDP (Ribosomal Database Project) which includes more than 800,000 16S rRNA sequences [24].

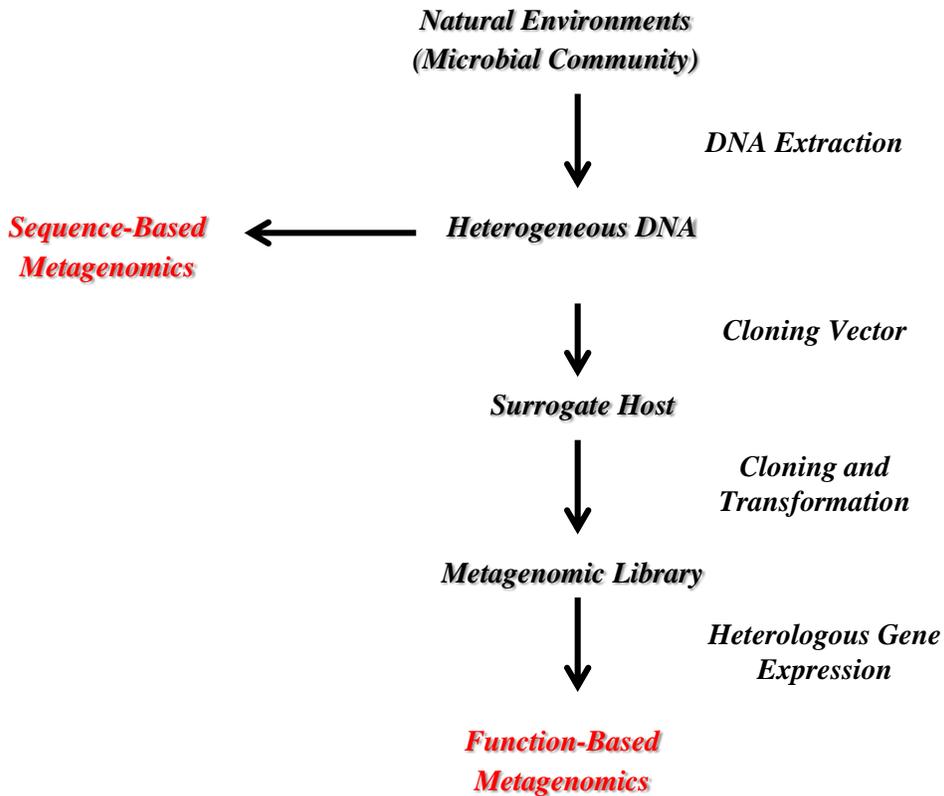


Figure 1: Metagenomics, Study of the collective genomes of the microbes from natural environment. DNA is extracted directly from the natural environment and then cloned into a surrogate host. Metagenomes are then studied either by sequencing (Sequence-Based Metagenomics) or screening for expression of activities of interest (Function-Based metagenomics).

These applications include identification of novel genes such as many antibiotic resistant genes, development of novel enzymes and isolation of many medical products from microbes such as antibiotics [19].

Metagenomics has fulfilled the demand of novel enzymes and biocatalysts through production of many important enzymes such as lipases, cellulase, amylase, xylanase and proteases on industrial level [19].

Metagenomics also has medical applications in discovery of new antibiotics. Antibiotics Turbomycin A and B have been isolated by Doreen *et al* in 2002 through metagenomic library construction of soil microbial DNA. There are examples of over 30 other natural compounds derived from marine microbes through metagenomics which are used in medical treatments. Example includes didemnin B (Aplidine™) and thiocoraline being in clinical or preclinical studies for the treatment of different types of cancers [19].

Industrial revolution has aided to the contamination of ecosystem through accumulation of many anthropogenic compounds and aromatic hydrocarbons by various ways such as petroleum spillage and the incomplete combustion of fossil fuels. Microorganisms can assist in biodegradation of these aromatic compounds as they are involved in many biogeochemical cycles and act as key drivers in degradation of many aromatic rings such as benzene, toluene, and xylene. Metagenomics also has potential in detection and elucidation of new genes and pathways for biodegradation of aromatic compounds. Studies have been reported in which functions-based metagenomic approaches has been employed in this regard. For example, in 2009 bacterial species have been reported by Marcos and coworkers in cold marine ecosystems which are involved in degradation of polycyclic aromatic hydrocarbon (PAH). In this study 14 distinct groups of genes have been identified which have shown significant relatedness with dioxygenases from Gram-positive bacteria. This indicates the potential of marine environment in presence of many unidentified dioxygenases genes [19].

Although metagenomics is the field of microbiology which is quite young and unique but it has contributed to discoveries of many novel microbial species and made significant contributions to microbiology and biotechnology word [26].

Hospitals Environment and Microbial Diversity

The natural environments of hospitals are getting colonized by a vast variety of microbial species. Hospitals are specifically enriched with vast variety of pathogenic bacteria [4]. These bacterial species from the hospitals cause many hospital-acquired infections (HAIs) also known as “nosocomial infections”. Nosocomial infections caused by the pathogenic bacteria from the hospitals are the leading cause of morbidity and mortality globally and their rate is increasing rapidly worldwide [13]. According to estimation nosocomial infections are the fifth leading cause of death in hospitals [23]. According to an estimation around 1.7 million hospital acquired infections and 99000 deaths occur annually in the United States alone. These infections are reported to be transmitted either directly through contact with healthcare personnel or indirectly through contaminated environmental surfaces. Studies provides evidence for involvement of hospitals contaminated environments in transmission of many common healthcare-associated pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Acinetobacter*, *norovirus*, and *Clostridium difficile* [25].

Healthcare workers (HCWs) are also at the risk of acquiring hospitals related infections through occupational exposure to body fluids or blood. A well-documented example of such infections is tuberculosis (TB), severe acute respiratory syndrome (SARS), H5/N1 avian influenza and H1/N1 swine influenza [20].

Similar to the definition of any other natural ecosystem, natural ecosystem of hospitals also comprised of non-living elements such as soil, air, sunlight and water and living species including plants, microorganism, insects and animals [15]. Natural ecosystems of hospitals and health care institutions contain billions of different pathogenic microorganisms interacting with humans, with each other and with their environment [16].

Understanding of microbial diversity in hospital environments is very important for improvement of human health care system [24]. As the microbial diversity of the hospitals natural environment plays very important role in dissemination of hospital acquired infections therefore the insight of hospitals microbial diversity is required in order to understand their interaction with human and health care system. This understanding can further assist in eradication of possible modes of transmission of hospital acquired infections and thus can help in elevating the health care standards in hospitals. Thus, the monitoring of the hospital environment for the objective of analysis of

microbial diversity present in it is an essential element in the control of nosocomial infections associated with the hospitals [9].

In any particular environment the composition of diversity of microbiome is regulated by two types of ecological processes; the dispersal resources and selection of certain microbial species through environmental conditions. Similarly, in hospitals, environment microbial diversity is regulated through dispersal resources or ways through which already present bacteria are dispersed from one place to another, already present microorganisms on surfaces and in the air and also through the interaction with human bodies, while the selection of specific microbial species by the environment occur through the changes in air temperature and humidity level. These environmental changes can cause the feasibility for certain species of microbes and hence their abundance [7].

Recently the advancement in high-throughput molecular techniques and availability of culture independent methods has made the study of microbial diversity feasible from different locations of hospitals [7]. The most common approach used for the study of microbial diversity from hospitals environment involves PCR amplification and ultimately the sequencing of gene encoding small subunit ribosomal RNA (16S rRNA) directly from environmental samples. 16S rRNA is ~1500 base pair gene that codes for a catalytic RNA which is part of the 30S ribosomal subunit. The gene is present in all prokaryotic cells and has desirable properties that make it most suitable marker [31]. This approach is more effective than the culture-based approach of exploring microbial diversity. Studies conducted by researchers have concluded that use of 16S rRNA amplicon sequencing approach has yielded the phylogenetic spectrum from hospital environments highly similar to that of human pathogens which implies that hospital environments might be the potential reservoirs in transmission of those pathogens [7].

There are examples of many studies that have been conducted on evaluation of efficiency of 16S rRNA gene sequencing in clinical microbiology. For example, in 2003, the suitability of 16S rRNA sequencing technology has been evaluated by Bosshard *et al* in identification of clinical strains of aerobic gram-positive rods [6,31].

Biomedical Waste (BMW) of Hospitals

Biomedical waste (BMW) or medical waste, which is the contaminated infectious material generated by hospitals or health care facilities includes syringes, body fluids, blood by products, laboratory samples, bacterial cultures or pharmaceutical wastes. Proper handling and disposal of BMW is very important to avoid transmission of infectious bacteria.

Hospitals dumping system and wastewater methods do play a major role in polluting the natural environment with vast diversity of pathogenic and non-pathogenic bacteria and contributing in development of novel antibiotic resistant strains in the natural environment as well. This kind of waste material does not only pose great threat to the personnel handling them but also to the natural environment and ultimately to public health. As these biomedical wastes, if transmitted through any mode can become detrimental for human health, therefore their adequate disposal is very crucial. A general practice for the disposal of hospitals' waste is incineration. Although incineration is considered as an indiscernible way to destroy most of deleterious pathogens, however, this practice is even more harmful for the environment because of the fact that it emits many biological, chemical and radioactive toxic air pollutants in the environment like dioxins which has been linked to cancer [22]. Another concern regarding the disposal of waste material generate by hospitals, is the dumping this material within or near the territory of hospitals. This improper disposal, without taking any careful measures has caused serious threats to environment and public health [16]. It has promoted the spread of infections through vectors such as flies, mosquitoes, insects or stray animal feeding on these wastes [16]. These vectors when come in contact with general population impose health risks. Therefore, improper handling and disposal of biomedical wastes can spread infections to waste management personnel (Sweepers, rag pickers) from sharp instruments such as needles or blades if came in contact [22].

Waste Management System in Pakistani Hospitals

In Pakistan the dumping and disposal system of is neglected in the hospitals and is still an under developed discipline. According to a study, hospitals in Pakistan produce about 250,000 tons of waste annually [14]. Despite this large scale of waste production there is no awareness regarding the medical waste management in Pakistani hospitals including the segregation, collection, storage, transportation and disposal [1]. Pakistan has its Waste Management Rules 2005, proposed by the Environment Protection Act (1997) for the management of hospital waste but unfortunately

hospitals in Pakistan do not comply with Healthcare waste management (HCWM) practices and hence expose patients and hospital staff to serious bacterial infections [3, 14]. Approximately every hospital in Pakistan does not have their own disposing system for medical waste and hence depend on private firms for collections and disposal of medical wastes. According to a survey by Environment Protection Agency (EPA) except Shifa International Hospital (SIH) and Kahuta Research Laboratories (KRL) every hospital in Islamabad does not have their own disposing system and rely on National Cleaner Production Centre (NCPC) for waste disposal. In remote areas of Pakistan hospitals do not even take into consideration the need of proper waste disposal system and throw or burn the wastes near or into the hospital territory. Another dark side of this kind dumping of medical waste is reuse of used syringes that resold in the market [14]. This malpractice of hospital disposal system imposes a serious threat to environment and human life. Introduction of hospitals' wastes contaminated with antibiotic resistant bacteria to the natural environment is assisting in emergence of novel ARGs within bacteria present in soil and water.

Soil as a Source of Microbial Diversity

Soil is the source of many microorganisms; 1g of soil contains as much microorganisms as number of humans on the earth [32]. Soil is enriched with bacterial diversity, containing both cultureable and un-cultureable bacteria. The diversity of microorganisms present in particular type of soil is determined through complex interaction of many environmental factors such as soil PH, temperature or moisture level. The microorganisms present in soil are either indigenous to environment or they can be directly or indirectly introduced into it. The indirect ways of introducing certain species in the soil include dumping of wastes or sewage or contaminated water. The soil microbiome diversity is furthermore regulated through competition between bacteria, archaea, viruses or fungi. Certain molecular mechanisms such as Horizontal gene transfers further contribute in emergence of new variety of pathogens [5].

In the previous studies researchers have studied the food and water samples from the hospitals and health care institutions with the aim of exploring them for related infections. There is however many soil related infections as well which are also needed to be considered [5].

Discussion

Metagenomics is a newly emerged discipline for the analysis of environmental microbial genomes. This approach involves the direct extraction of microbial DNA from environmental samples and their further analysis through cloning into surrogate hosts or *in silico* analysis through sequencing and computational bioinformatics tools. The advancements in the sequencing and high throughput technologies and bioinformatics tools is revolutionizing the metagenomics in investigation and analysis of microbial communities from natural environments [24].

The researchers have employed the approach of isolating the prokaryotic 16S rRNA genes from the environmental samples to study their microbial diversity as these genes have been commonly found in all prokaryotes and their sequence is highly conserved in many bacterial species. This approach makes possible the exploration of microbial diversity in particular environment through isolation of 16S rRNA gene from extracted DNA and then comparing these gene sequences to already present sequences from the databases such as GenBank [24].

The pathogenic bacteria present in the hospitals are associated with many serious hospital-acquired infections now days. Microbial diversity of the hospitals' natural environment plays very crucial role in dissemination of hospital acquired infections and hence the insight of hospitals' microbial diversity needed to be studied in order to understand their interaction with human and health care system. Thus, the monitoring of the hospital environment for the objective of analysis of microbial diversity present in it is an essential element in the control of nosocomial infections associated with the hospitals [9].

Before the introduction of metagenomics scientists can only rely on culturing methods to study the bacterial species in environments but now metagenomics has made possible the study of those bacteria species that cannot be cultured in the laboratory through direct DNA isolation from environment. Metagenomic approaches can help in examination of new resistant strains of bacteria that may have evolved through the methods of horizontal gene transfer (HGT) or mutation.

Use of metagenomic approaches in investigation of microbial diversity in hospitals' natural environments can contribute toward building the better health standards of the hospital. The details of bacterial species present in soil and water samples can help in understanding the microbial flora present in natural environment of hospitals. Potential pathogenic and drug resistant bacteria can

also be identified through researches conducted by using metagenomic approaches. The results obtained from such researches can aid in providing motivations to hospitals' waste management authorities to regulate the biomedical waste management strategies and protocols in order to elevate health standards in hospitals and eliminate the risks of hospitals acquired infections (HAIs) transmission which are considered to be the fifth leading cause of death in hospitals [23].

Future Implications

Hospitals' dumping system and wastewater systems may be playing a major role in polluting the natural environment with vast diversity of pathogenic bacteria and also contributing in development of novel antibiotic resistant strains in the natural environment.

By considering the potential involvement of hospitals' dumping system in transmission of pathogenic bacteria and ultimately the hospitals acquired infections there are certain actions that needed to be taken. These include the improvements in Healthcare waste management (HCWM) practices in hospitals. These practices in the hospitals needs to take possibility of presence of pathogenic bacteria in biomedical wastes into consideration and should design the protocols of waste handling, transport, storage and disposal according to that. Not only this, but they should also train their waste handling staff according to these protocols for their safety. A proper place for dumping of hospitals wastes and a safe system of disposal is also required in order to eradicate the possibility of transfer of these pathogenic bacteria from dumpsites to population through animals and insects. In Pakistan particularly every hospital should be regularly checked by the government for its compliance with Waste Management Rules 2005 (Environment Protection Act 1997), of the Government of Pakistan. There should be a proper regulated hospitals waste disposal system based on legislated waste management plan.

Studies utilizing metagenomics in investigation of microbial diversity present in natural environments should be conducted on large scale. Studies have previously been conducted on the food and water samples from the hospitals and health care institutions with the aim of exploring them for related infections that may have been transmitting to humans but there are however many soil related infections as well which are also needed to be considered [5]. More studies can be conducted on different indoor or outdoor samples from the hospitals such as soil, air and drinking or sewage water in order to find their role in development and transmission of pathogenic bacteria.

Conclusion

Hospitals biomedical waste disposal system has very crucial role in transmission of hospital acquired infections (HAIs) through transfer of pathogenic bacteria from contaminated hospital wastes to natural environment such as soil and water. The present study reviews the use of metagenomics in direct DNA isolation from diverse environmental samples and further analysis of these genomic data in understanding of microbial diversity present in these environments through utilization of 16S rRNA gene sequencing methods. The study also reviews the hospitals natural environments, the microbial diversity present in them and their role in transmission of hospitals acquired infections to humans. The biomedical waste disposal system of hospitals and their role in HAIs transmission has also been tackled in this study.

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