

Review article**Innovations of Biotechnology to diagnose and Cure Various Human Diseases**Yumna Zaheer¹, Arooj Fatima¹, Hafiz Saad Bin Mustafa^{2*}, Ejaz-ul-Hasan² and Tariq Mahmood²¹ Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture Faisalabad, Pakistan.² Oilseeds Research Institute, AARI, Faisalabad, Pakistan.*Corresponding Author's email: saadpbg@gmail.com

Abstract: Modern world is on the edge of threat of pandemic infectious diseases which are likely to emerge after a period of several events occur inside human system and subsequently spread rapidly within human throughout the world. Today, although despite of extraordinary advances in the development of curative measures; increased global interrelationship yet laxity of sewage systems enhance the chances of diseases. The frequency of cancer is on the rise in Pakistan; about 300,000 individuals were affected by cancer every year. Non-availability of good quality drinking water leads to the consumption of heavy metals that may contain lead, copper, Nickel, Molybdenum and Chromium responsible for major persistent diseases such as cholera, cancer, diarrhoea, hair loss, typhoid and anemia that affect not only the health but also has adverse outcomes on the economic stability of societies. Modern Biotechnology is an emerging booming field, involving approaches by which human beings modify living organisms or use them as tools to combat against various human diseases. The advent of Nanotechnology and its advantages in the biotechnology has transmogrified human healthcare. Recombinant DNA technology has improved the celerity, specificity and susceptibility of various diagnostic assays. It is also worth notable that the occurrence of cancer is on the peak in Pakistan but the government authorities have not been providing adequate facilities for sedative care. In this scenario, Biotechnology plays a major role by developing anticancer drugs such as cannabinoids and nanoparticle encapsulation materials such as curcumin extracted from cannabis sativa and Rhizome of turmeric respectively and by introducing RNAi pathway to eradicate cancer from living world effectively.

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Introduction

Biotechnology is an advanced approach that involves the manipulation of living organisms and biological systems to make or to modify products and services for the welfare of mankind. Since the history of biotechnology is ancient, developments in the field of genomics, cell biology, genetics and tissue engineering have distinguished a variety of astonishing applications, given the field a new momentum. Its enhancement through the development of nanotechnology and information technology has strengthened the research (Awais, *et al.*, 2010). International competition is driving among biotechnology companies to introduce new items and techniques. The increasing awareness of biotechnology as a social and economic driver has prompted governments in developed countries to financially support their local biotechnology companies to promote research, development and marketing of objectives and commodities (Anon., 2002a). Increase in population explosion with their incurable diseases and undetermined environmental issues paves the way for biotechnology companies to develop quality products and assistance (Anon.,

2002b). In the coming years, recombinant DNA technology and molecular medicine is expected to be widely used in human health care. Advancements in surgical tools such as angioplasty, hybrid imaging technique and laser surgery will not only enhance the chances of survival but also cost effective and highly non-invasive for the patient. Advancements in tissue culture techniques enable artificial production of tissues for repair and renewal purposes (Anton *et al.*, 2001). In vitro production of large amount of biochemically derived extra cellular proteins of medical importance have provided a huge prospective for pharmaceutical industries. Some recombinant DNA products being used in human therapy are: Insulin for diabetic patients, Human growth hormone (GH), Erythropoietin (EPO) against anemia. Interferons, Granulocyte-macrophage colony-stimulating factor used for stimulation after a bone marrow transplant. Several interleukins, Tissue plasminogen activator (TPA) for diffusing blood clots. Adenosine deaminase (ADA) for curing severe combined immunodeficiency (SCID). Angiostatin and endostatin used as anti-cancer drugs. Hepatitis B surface antigen (HBsAg) provided as a vaccine

against hepatitis B virus (Hayward, *et al.*, 1991 and Hilson, *et al.*, 1987). The motive of the present study is to explore and introduce the latest pathway of biotechnology in human health sciences in order to establish new dimensions for the medicament that are more efficient, effective, more accurate, no side effects and cost effective. Biotechnology has played an important role in the improvement of plants, animals and human health (Ali *et al.*, 2014; Kiani *et al.*, 2013; Arshad *et al.*, 2015; Butt *et al.*, 2015; Javed *et al.*, 2015; Shan *et al.*, 2015; Hussain 2015).

Diagnosis of viral diseases

Viruses are capsules involving genetic material inside. These tiny entities are much smaller than bacteria. Polyvalent vaccines are needed to combat against these viruses particularly HIV/AIDS. Organism cause severe illnesses in humans such as smallpox, common cold and hemorrhagic fever.

Virus – host relations for system analysis

Scientists have beautifully summarized the properties of biological systems that make them attractive for systems examination having auspicious properties, exuberance and modularization (Aderem, 2005). Virus host relationship for global investigations is generally interesting due to their peculiar traits. A life threatening situation prevailing in macaques in 1918 where people are infected by influenza virus, auspicious properties involves the creation of abnormal innate immune responses (Kobasa, *et al.*, 2007). Exuberance of HIV through undetermined and rapid low-accuracy throughput is widely recognized as determinant for continuous inflammation and resistance. Uetz's work shows the networks of lethal human diseases such as varicella zoster virus and KSHV that depicts the phenomena of modularization (Uetz, *et al.*, 2006). These examples depicts that virus-host interactions provides a bright opportunity for research on wide scale.

A. Detection of nucleic acids

i. Nucleic acid extraction:

First step in the molecular diagnostic procedure is extraction of pure nucleic acid that acts as a template in the reaction mixture. The assay may yield false results if the final material has not been decontaminated. To prove the absence of PCR inhibitors the assay is important for the quality control of the nucleic acid extraction. Internal controls can be either a house-keeping gene, an endogenous gene, a constant basal cell-expressed gene, such as gluceraldehyde-3-phosphatase (GPDH3) or the β actin, or an exogenous nucleic acid that is not present naturally in the preparation but added in the extraction step (Belák and Thorén, 2001). The development and availability of the robotic extraction platforms not only minimizes the risk of contamination, but also enables processing of large numbers of samples under

constant reaction conditions and minimal operator manipulation. Ultimately, these platforms have contributed to the development of efficient and durable diagnostic assays, shortening the processing time required per sample from hours to minutes (Belák, *et al.*, 2009; Tariq *et al.*, 2014; Dar *et al.*, 2014).

ii. Polymerase chain reaction (PCR) and real-time PCR

PCR works on the same process of DNA replication and results in the artificial production of desired DNA sequences in large quantities from a mixture of diverse sequences (Saiki, *et al.*, 1988). Multicenter studies shown the continuous detection of positive results along with false positive that is having negative samples, indicating the existence of impurities (Schweiger, *et al.*, 1997). A template, independent of the target DNA, known to produce a PCR product with specific primers can be used as a control for the PCR inhibitors, thus signifies false-negative results (Belák, *et al.*, 2009). The generation of signal in a real-time PCR assay has been limited, until recently, such as intercalating dyes (SYBR Green and Eva Green), hydrolysis probes, hybridization probes and dye-labelled oligonucleotide ligation (Belák, *et al.*, 2009; Nazir *et al.*, 2014; Siddiqui *et al.*, 2015). A modification of this method employ matrix-assisted laser desorption-Ionization (MALDI), which identifies the molecular weights of the PCR products and compares them with known databases (Lipkin, 2010). Additional improvement in the sequencing technology has been achieved by converting from photometric to chemical detection of the PCR reaction in real time (Anon., 2012; Zameer *et al.*, 2015). Fourth generation sequencing platforms, such as Nano pore sequencing technologies, long read extension methods and procedures based on direct video recording of nucleic bases, have already been developed as a proof of principle (Anon., 2012).

iii. Isothermal amplification

Isothermal amplification technologies offer the benefits of omitting thermocycling, enabling DNA amplification at constant temperature. These techniques involve single primer isothermal amplification (SPIA), nucleic acid sequence-based amplification (NASBA), signal-mediated amplification of RNA technology (SMART), loop-mediated isothermal amplification (LAMP), isothermal multiple displacement amplification (IMDA), Helicase-dependent amplification (HDA), strand displacement amplification (SDA) and strand invasion-based amplification (SIBA), transcription-mediated amplification (TMA) (Gill & Ghaemi, 2008). As a result of the Amplification process loop is generated at the end of each complementary strand which is extended frequently. Emergence of turbidity

in the reaction mixture or the generation of a fluorescent signal using fluorescent dyes are the indications of an amplification process (Gill and Ghaemi, 2008).

iv. **Diagnosis by RFLP and related DNA-based approaches**

RFLP procedure involves the isolation of the targeted pathogen, extraction of DNA or RNA, and lastly the nucleic acid digestion with one or a set of restriction enzymes. It is considered that different restriction enzymes are at the beginning, so the process of analyzing numerous molecular fingerprints digesting with various restriction enzymes can be tackled (Loza.Rubio, *et al.*, 1999). PCR product will not be generated if the site is mutated that was previously compatible with the primer, on gel as a result different pattern is produced by amplified segments of the DNA. Conversion of dominant RAPD marker to a co-dominant SCAR marker means that a single PCR product is sufficient to identify a specific site in the genome (Lewin, *et al.*, 2002).

v. **Genome sequencing**

The technique involves the detection of DNA derived from infectious agents and identified in order to ameliorate and transmogryfy. At present this the conclusive distinguishing procedure. Since 1977, the Sanger method (Sanger, *et al.*, 1977) has been the dominant strategy and gold standard for DNA sequencing. In addition to the analysis and comparison of different sequence motifs, the technology also offers the feasibility of predicting the tendency of pathogens to mutate into more pathogenic strains, tracing forward the spread of an outbreak or infection e4). Mass tag-PCR has been successfully used for simultaneous detection and differentiation of syndromic diseases, such as respiratory diseases, diarrhea's, encephalitis /meningitides and hemorrhagic fevers (Lipkin, 2010). These new technologies have made it possible to identify an unknown pathogen (emerging pathogens) or to identify a variant that is present in small quantities within a mixture (Kunin, *et al.*, 2008). High-throughput sequencing is a challenge for bioinformatics solutions needed to analyze the vast quantities of data generated, in order to answer specific biological queries including possible amplifications of high numbers of unexpected pathogens and their interactions with the host cell genome (Kunin, *et al.*, 2008; Javed *et al.*, 2015).

vi. **DNA microarray technology**

Ability of Genome sequencing to immobilize thousands of DNA segments on a surface leads to the discovery of DNA microarray technology (Cassone, *et al.*, 2006). A genome of an entire microorganism can be easily characterized on a single disposition, making it conceivable to accomplish genome-wide research

(Ye, *et al.*, 2006; Akondi and Lakshmi, 2013). The oligonucleotide-based array and the PCR product-based array are the two types of DNA microarrays that are being used (Panicker, *et al.*, 2004). A DNA microarray involves the arrangement of fabrication, preparation of the probe, hybridization of probe and data analysis (Call, *et al.*, 2001). They have the ability to give detailed investigation and compare the expression levels of enormous number of genes quantitatively but can also be used in the detection of selected DNA fragments qualitatively (Call, *et al.*, 2003a; Call, *et al.*, 2003b; Perreten, *et al.*, 2005). Microarrays have the potential to discriminate and categorize a variety of viral pathogens causing gastroenteritis including rotavirus and astrovirus (Chizhikov, *et al.*, 2002; Honma, *et al.*, 2007; Jaaskelainen and Maunula, 2006; Lovmar, *et al.*, 2003).

B. **Detection of proteins**

New molecular diagnostic techniques provide unprecedented detection and distinguishing methodologies which are essential for the detection and recognition of infectious agents and their impact on the antibody production (Belak, *et al.*, 2009). In addition to eukaryotic systems production of various recombinant proteins produced in prokaryotes have been used in diagnostic assays (Balamurugan, *et al.*, 2004).

B. **Detection of antigens**

i. **Antigen-capture enzyme-linked immunosorbent assay (Ag ELISA)**

It is an advanced technique that facilitates the detection of antigen from infectious agent present within animal before or during clinical diagnosis. A squeezed testing pattern is generally followed by Ag ELISA using capture and antibody detection. Combination of anti-species is utilized if the detection of antibody is not labeled (Trueblood, *et al.*, 1991). Capture antibody detects the target antibody from other surpassing protein in specimen suspensions and enhances the chances of detection by ensuring that the suspensions are semi-concentrated. Strong binding to the infectious agent, identification of highly conserved epitope particular for the target organism and the potential to bind with the ELISA plate without losing the susceptibility for reaction are some of the desired characteristics polyclonal antibody. Recognition by the capture polyclonal antibody, a second polyclonal antibody that is determining an epitope is also attach with the ELISA plate is generally used in an indicator system. Whereas, identification of polyclonal antibody is difficult having inclusive horizontal reactivity and to enhance the probability of reaction against all antigenic modifications polyclonal antisera is preferred (Molina, *et al.*, 1993).

iii. **Fluorescence antibody test:**

Fluorescent antibody test (FAT) is used for detection of pathogens in animal tissues, using specific antibodies against the target antigen. The antibodies are labelled with fluorescent dye. Once the sample is prepared according to the aforementioned laboratory procedure, labeled antibodies are added in the sample, sample is incubated under defined conditions, washed to remove the unbound antibodies and examined under fluorescent microscope. Visible fluorescence indicates at the binding sites of the specific antibodies. The method is commonly used for detection of the rabies virus in the brains of dead animals and classical swine fever virus in tissues of suspected pigs. As it is based on direct binding of the labelled antibody to the antigen present in the specimen, it is commonly called direct immunofluorescence (Libeau, *et al.*, 1994)

iv. Immuno-histochemistry

It involves the site detection of antigens in fixed tissues. This diagnostic technique offers a number of advantages over other diagnostic techniques that involves the sample is consented conveniently, probability regarding safe handling of human pathogens, determination of unfeasible organisms, primitive studies of the preserved samples (Haines & Clark, 1991). Diagnosis of abnormal prion protein in brain tissue is also detected by immune-histochemistry to assure scrapie and other transmissible diseases such as spongiform encephalo-pathies and has been ratified to be more effective than the histo-pathological examination criterion (Thorgeirsdottir, *et al.*, 2002).

Bii. Detection of antibody

i. Agglutination: Agglutination is a method using the characteristic of specific antibodies to bind many pathogenic organisms into single cluster thereby forming large complexes, which are easily precipitated. The precipitation can be macroscopically or microscopically perceptible. Different technical applications using this principle are widely used for specific antibody detection against various diseases. The method can be helpful for detection of both antibodies, when known antigen is used and when well defined sera are used (Mulder and Brans, 1952).

ii. Haema-glutination inhibition test:

The haema-glutination inhibition method depends on the quality of some pathogens (mainly viruses) to nonspecifically agglutinate erythrocytes. In the existence of specific antibodies, this action of the virus can be 'blocked' and it will not be able to agglutinate the erythrocytes. The assay for qualitative and quantitative detection is used both for antigens and antibodies. It has been used broadly used for the identification of serotype specific antibodies against avian influenza, peste des petits ruminants (PPR) and others. It can also be used for the diagnosis of antigen (detecting presence of an avian influenza virus from

the allantoic fluid after tested isolation) (Mulder & Brans, 1952).

iii. Competitive ELISA (cELISA):

It is an immunoassay used to quantify antibody or antigen by using a competitive approach. This method offers significant advantages over other techniques of ELISA that is does not involve the testing for species-specific enzyme-labeled conjugates samples which are extracted from many species under test. Many antigens are difficult to purge. When an indirect assay is used, due to non-specific binding it can generate large background values. The principle is based on the variance between the detecting antibody and serum that is to be tested. Detection of the specific antibody binding is detected by using an appropriate anti-species conjugate. Expected colour reduction is acquired due to the presence of antibodies attached with the antigen in the analyzed serum, which is why prevented the detection of specific binding of antibody (Mignon, *et al.*, 1991).

iv. Immuno-blotting

This technique is performed in the diagnostic laboratories to diagnose infectious organisms based on the specificity of antigen to identify a particular serological response. False-positive and negative results obtained from other diagnostic tests can be corrected by immune-blotting (Molina, *et al.*, 1993). For an instance detection of an antigen has been used in millions of specimens of brain stem cells in Europe for the identification of prion protein (Schaller, *et al.*, 1999). The method of immunoblotting involves the following steps: (i) Sample preparation (ii) Using gel electrophoresis for the resolution of antigen (iii) Separated polypeptides are then transferred to a membrane made of nitro-cellulose or poly-vinylidene difluoride (iv) Blocking On membrane nonspecific binding positions are blocked (v) Incubate along with detecting antibody (vi) Identification of attached antibody (SCHALLER, *et al.*, 1999).

C. Proteomics

The application of proteomics in the diagnosis of infectious diseases is emerging and proved to have a considerable importance. Studies revealed that the proteomic analysis of serum samples expressing seven serum proteins is changed considerably in patients suffering from chronic hepatitis B virus. Likewise, diagnosis of Creutzfeldt-Jakob disease is assisted by proteomics that indicates seven proteins in cerebrospinal fluid which are distinguished between patients with altered or infrequent Creutzfeldt-Jakob disease (Choe, *et al.*, 2002).

D. Production of antigens by recombinant DNA technology

Genome sequences of numerous pathogens have already been determined. A gene responsible for an antigen or immunogen can easily be cloned with PCR

technologies and characterized by nucleotide sequence (Anon., 2012). In short, the amplified DNA can be cloned in a bacterial plasmid vector and expressed as a recombinant antigen. The cloned gene can be designed in a way that the recombinant protein is fused at its N or C-terminal with a detection tag that can be used for its purification by affinity chromatography. There are many tags but the most popular are polyhisidine (6-8 residues), glutathione S-transferase (GST) or the Flag peptide Flag composed of 8 amino acid residues. Then determination of the antigenicity of the gene products. Research shows that the antigen gene in silico, obtained from the data of genome sequences, escalate the progression of diagnostic kits and vaccine through systematic screening (Tortorella, *et al.*, 2000).

Treatment of Human viral Diseases:

RNA interference is an extremely potent and skilled tool to reduce expression of targeted genes. Under special control system and providing selection of best delivery tool, the technology of RNAi will proved to be successful either performed in cell culture labs or providing within biological system of humans to cure against viral infections (Mollaie, *et al.*, 2013).

RNAi a way to cure HIV infection:

Human immunodeficiency virus (HIV) was the first infectious virus shown to be hindered by RNA interference because of the well perceived life cycle and gene expression pattern of HIV (Lee, *et al.*, 2002). Successive studies shows that a variety of other viruses are also inhibited by RNAi Mechanism including hepatitis C virus (HCV), poliovirus, hepatitis B virus (HBV) and many others (Leonard, *et al.*, 2005). A recent strategy takes asset by targeting

the mutants directly of escape mutants in vital genes (Brake and Berkhout, 2005). Introduction of siRNAs or shRNAs to HIV infected cells is the most challenging impediment. The target cells are generally consist of monocytes, macrophages and T lymphocytes. An organized delivery of siRNAs to T lymphocytes is not practicable since the insusceptibility of the vector itself inhibit to conduct multiple injections which would be otherwise necessary for prevention. It is not problematic to use viral vectors to transfer anti-HIV that codes for shRNA genes (John, 2006) Isolation of T cells from patients is an advanced approach followed by transduction, development of the transduced cells and transfusion. In an ongoing research, T lymphocytes are transduced with a lentiviral vector that codes for anti-HIV antisense RNA from an HIV-infected patient (Dropulic, 2001). The expanded transduced cells are afterwards transfused into patients. Vectors nurturing genes that codes for siRNAs would also follow this type of therapeutic approach. To transduce isolated stem cells with vectors nurturing the therapeutic genes is a different prospect. This RNAi approach has the potential to transduce all the hematopoietic stem cells that are competent of being infected by the virus. Prior to transfusion Hematopoietic stem cells are congregated from the patients and transduced outside the body of the patient. Retroviral vectors were transduced into hematopoietic stem cells have revealed the effectiveness of this approach in two clinical trials (Amado, *et al.*, 2004). Figure 1 shows the contrasting features betwixt siRNA and miRNA pathways.

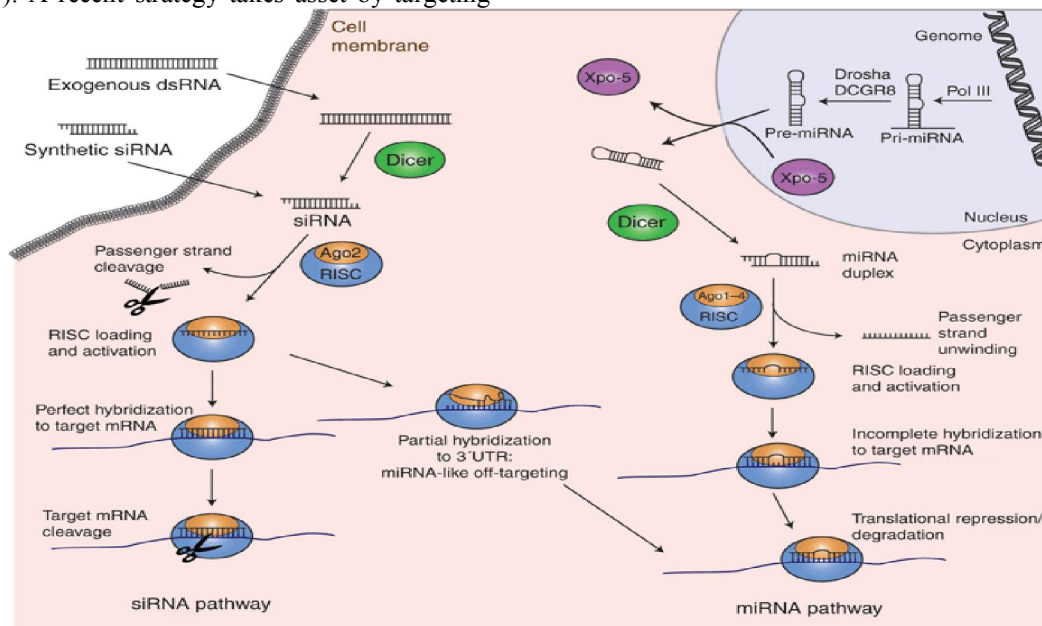


Figure 1. The contrasting Features betwixt siRNA and miRNA Pathways

In this scenario as RNAi is emerging as a formidable approach than other antisense or ribozyme approaches, expansion of this technology for clinical trials of humans against HIV treatment is predicted to occur in the near future (Rossi, 2006 and Kanasty, *et al.*, 2012).

Diagnosis for cancer:

Aging is fundamental factor of abnormal and uncontrolled cell division and spreading of cells. It can spread to any part of the body and can metastasise to distant sites. With the passage of time many types of cancers are diagnosed in humans depending on the chaotic events (Renan, 1993). The most prevalent types of cancer are lung, stomach, colon, brain and breast cancer. Through Pathological analysis of many organ sites disclose aberrations that indicate the process through which cells develop successively from normal to a series of precancerous states into intrusive cancers (Foulds, 1954). These types of examinations have revealed clearly that the genomes of tumor cells are inevitably transmuted at multiple sites having blockages through lesions and changes in normal number of chromosomes (Kinzler and

Vogelstein, 1996). Process of transforming cultured cells involve several steps: Before they acquire tumorigenic competence mice cells require at least two established genetic changes, while transformation is difficult in human trials (Hahn, *et al.*, 1999).

Quantum Dots-Based Diagnosis:

Nanotechnology brings revolution in the diagnosis and treatment of cancer (Peng and Yan, 2010). Creation of quantum dots (QDs) is one of the most remarkable developments in label technology. Quantum dots belong to a heterogeneous class of engineered nanoparticles having distinguishing chemical and optical properties that make them suitable for various potential implementations varies from the field of medicine to energy (Genger, *et al.*, 2008). Because of their peculiar photo-physical properties, QDs can exceed the constraints of conventional dyes and are promising fluorophores for fluorescence imaging within biological systems (Figure 2). Quantum dots are used to detect specimens of ovarian cancer cells of various types using wavelength of extreme emission and with high specificity and sensitivity (Wang, *et al.*, 2004).

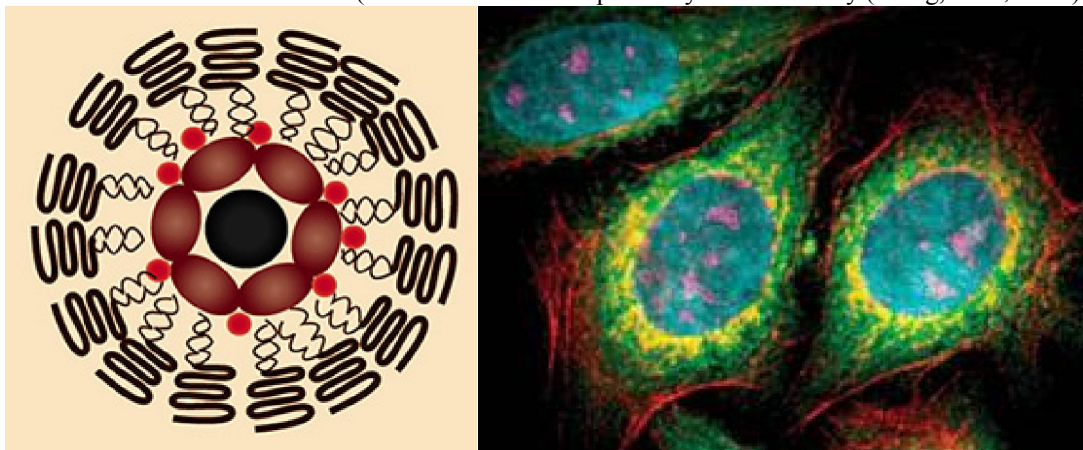


Figure 2. Researchers develop cargo ships that contain fluorescent "quantum dot" nanoparticles to target and destroy tumors.

Quantum dots latched on to cancer cells

For successful bio imaging of ovarian cancer cells a research revealed that biocompatible quantum dots plated with natural protein silk fibroin are tagged as a fluorescent label (Nathwani, *et al.*, 2009). Multicolor Quantum dots were used as bio conjugates for quantitative profiling and simultaneous detection of multiple clinically significant tumor biomarkers, in breast cancer cells has been reported (Yezhelyev, *et al.*, 2007). For a person having prostate cancer, early diagnosis is depended on the prostate-specific antigen and prostate cancer detection has been transformed by the progression of prostate-specific antigen based screening, as an encouraging step forging ahead in the Prostate-specific antigen epoch where prostate cancer

was recognized at an earlier stage than ever before (Freedland, *et al.*, 2008, Noguchi, *et al.*, 2000). Likewise, use of single-chain antibody fragments conjugated with quantum dots shown to have several advantages which were endorsed by detecting prostate cancer (Wang, *et al.*, 2008).

miRNA in Cancer Diagnosis

The miRNA seems to be specific for a normal tissue or malignancy. Investigators have revealed that the miRNA mode for poorly differentiated human neoplasms are more tumor-specific than the corresponding mRNA expression patterns (Cao *et al.*, 2011). Expression of miRNAs in human solid tumors has confirmed a number of miRNAs as potential targets of anticancer therapy (Castaneda *et al.*, 2011).

A group of microRNAs located on chromosome 13 has been found to be modulated by an important transcription factor that is c-Myc, overexpressed in many human cancerous cells. Expression analysis of microRNA reveals that it would be worthwhile to study micro RNA in the diagnosis of cancer (Chaulk, *et al.*, 2011; Zhao, *et al.*, 2013).

Cancer Therapy

Polymeric nanoparticle-encapsulated curcumin:

Advanced research exploiting nano-curcumin is affirmed to have beneficial impact from the effects of curcumin against cancer and other diseases (Savita Bisht, *et al.*, 2007). A recent study shows the effectiveness of an approved anti-cancer agent that is Abraxane™, which is an albumin nanoparticle and combination of paclitaxel, in the field of drug delivery (Gradishar, 2006). In order to develop balanced and organized carriers for hydrophobic anti-cancer drugs, scientists flourished cross-linked polymeric nanoparticles and demonstrated that these non-viable polymeric formulations are non-toxic and have the potential to act as carrier for hydrophobic compounds, provided artificially or within biological systems (Savita Bisht, *et al.*, 2007). It has been proved that Curcumin was not to be toxic for normal cells of the body, involving parenchymal cells of liver, epithelial cells of kidney, fibroblasts and white blood cells against cancer cell lines at the dose provided for curative effectiveness (Choudhuri, *et al.*, 2005). Limited clinical trials have been performed using curcumin through oral administration, even with highly exposed gastrointestinal mucosa, shown to have minimal adverse effects on humans (Cruz-Correa, *et al.*, 2006).

miRNA as a pathway to treat Cancer

Recent research predicts the association of miRNAs with cancer development and its growth in human body, and now these small regulatory RNAs could serve as targets of anticancer gene therapies (Avci, *et al.*, 2013). Antisense molecules can inhibit the activity of oncogenic miRNAs, and their efficacy have been tested by reducing the miRNA activity on reporter genes having miRNA-binding sites (Krutzfeldt, *et al.*, 2005). Antagomirs represent a new class of oligonucleotides designed to antagonize specific miRNAs. Tumor suppressors that can function as miRNAs can also be lost in cancer, as well as overexpressed. Manipulation of this miRNA expression strategy is not only employed to treat various type of cancers but are also currently being used in several other clinical trials to treatment of several other diseases (Castaneda, *et al.*, 2011; Ye, *et al.*, 2013; Zhu, *et al.*, 2013).

Dramatic Effects of Cannabis:

Brain cancer is one of the most malignant types of cancer to treat with efficacy and the chances of survival for the patients who are being suffered from

this fatal disease is often very low. Cannabis has been used as a medicinal plant for centuries and now it is revealed as a potential treatment against cancer. But our aim is to throw light on the fact that cannabis have dramatic effects on brain cancer. A new research found that cannabis helps to reduce the growth of brain cancer cells. Recently it is revealed that two active chemical components, tetrahydrocannabinol (THC) and cannabidiol (CBD), extracted from the cannabis plant shown to have remarkable effects in reducing the size of brain tumors. In a present research regarding human clinical trials, scientists are trying to focus on the possibility of combining cannabinoids with irradiations. It has been published in the journal of Molecular Cancer Therapeutics that the most beneficial results along with the drastic reduction in the size of brain cancer cells have been obtained when treated with both irradiations and cannabinoids. The ongoing research on cannabis provides one of the best remedy ever to save the lives of humans in the future effectively against brain cancer but at present this is the most life threatening disease worldwide (Katherine, *et al.*, 2014).

Bacterial Diseases



Product containing cannabinoids actively used against brain cancer treatment

Bacteria are present everywhere in the universe. There are many diseases which are caused by Bacteria and are a major threat for our life. Some common bacterial diseases are given below:

- a. Anthrax- Caused by *Bacillus anthracis*.
- b. Borreliosis- Caused by *Borrelia spp*,
- c. Lyme disease- Caused by *B. burgdorferi*,
- d. Brucellosis- Caused by *Brucella abortus*,
- e. Salmonellosis- Caused by *Salmonella spp*
- f. Tetanus- Caused by *Clostridium tetani*.
- g. Leprosy- Caused by *Mycobacterium leprae*
- h. Plague- Caused by *Yersinia pestis*,

Diagnosis and cure of bacterial diseases

Biotechnology has been using in medical field to cure different diseases with the help of products obtained from living organisms. Antibiotics were first used 2500 years ago when mouldy curds were made from soybeans to cure infection.

Vaccination

Scientists are using different techniques to make vaccines according to the infection of disease causing microbes and according to the reaction of the body against that microbe. Additionally, the physical characteristics of microbes also matter.

Live, attenuated vaccines-

Vaccines that contain attenuated (weakened) disease causing microbe. It do not cause disease but boost up the immune system of a body to make it remember. These vaccines are very strong and give a very strong reaction to immune system for lifetime. It is used against viral diseases, like measles, mumps and chickenpox. A person who does not has a strong immune system, it is not safe for him to use live attenuated vaccine for example for those patients who are receiving chemotherapy. These vaccines should be kept in refrigerator to stay potent. There is also a threat that the weakened microbe may mutate back to its virulent form and become a cause of fatal disease.

Inactivated vaccines

In these, the microbe can't change back to its original virulent form because it is killed with heat and radiations etc. These vaccines are freeze and dried and then can be stored easily. Examples of inactivated vaccines are mainly against cholera, bubonic plague and hepatitis A. (Biotech & med. Res.).

Subunit vaccines

These vaccines do not include complete disease-causing microbe, antigens are used that the immune system the most. Antigens are used as 'markers' on the surface of a microbe, and this part is recognized by the immune system's T-cells, and then to T-cells get bind to that microbe. Scientists make antigens with the use of recombinant DNA technology. In this case the vaccine is called a recombinant subunit vaccine, for example the vaccine against the hepatitis B virus.

Toxoid vaccines

These are vaccines that are used against those bacteria that secrete toxins, for example diphtheria and tetanus. The vaccine causes the immune system to produce antibodies against the toxin, which attach to the toxin and block its action.

Conjugate vaccines

Some harmful bacteria have an outer coating of sugar molecules named polysaccharides. This coating covers the antigens (markers) on the surface of the bacteria so that the immature immune system of a child can't identify it. Scientists link antigens or toxoids from a microbe which an immature immune system can recognize to the polysaccharides, thus

making a conjugate vaccine. For example, the vaccine against Hemophilic influenza type B (Hib).

DNA vaccines

Several types of these vaccines are already being tested in humans. A DNA vaccine uses the genes of the microbe that code for the antigens of that microbe. When genes enters in the body, they are adopted by body cells, and then command the body cells to make antigens. The antigens then activate the immune response. DNA vaccines can be stored and produced easily. Vaccines against herpes and influenza have been tested.

Recombinant vector vaccines

Recombinant vector vaccines are just like DNA vaccines, but they use an attenuated microbe as a vector to support the DNA of the disease-causing microbe in the body of organism. The vector virus 'infects' body cells, thereby supplying the DNA to the body cells. Scientists are making efforts of doing work on viral-based and bacterium-based recombinant vector vaccines such as against HIV, rabies and measles.

Phages as therapeutic agents

Phages have several advantages over traditional antibiotics as therapeutic agents (Pirisi, 2000; Sulakvelidze *et al.*, 2001; Matsuzaki *et al.*, 2005). Phages are very much common in environment and frequently utilized in foods (Bergh *et al.*, 1989). Human beings are exposed to phages since birth and therefore they established the normal microflora of the human body. They are commonly found in human gastrointestinal tract, skin and mouth, where they are present in saliva and in the plaques of teeth (Bachrach *et al.*, 2003). Phages are have been shown to be unintentional contents of some vaccines and sera commercially available (Merril *et al.*, 1972; Geier *et al.*, 1975; Milch, *et al.*, 1975). Therefore, without interrupting the natural microflora phage therapy is used to disrupt their particular bacterial host (Lorch, 1999; Sulakvelidze *et al.*, 2001; Duckworth, *et al.*, 2002). Phage therapy approach seems to be very approachable and benign recognized by clinical studies. Scientists appraised the potential of natural phages against Escherichia, Salmonella, Enterobacter and Brucella and are antibiotic-resistant (Matsuzaki *et al.*, 2005; Kumari *et al.*, 2010). Modified phage have been evaluated increasingly because of restrains of phage therapy with the use of lytic phages. The safety concerns relating impulsively propagating live microorganisms and the non-uniformity of phage therapy give outcome in the cure of infections which are caused by bacteria specifically induced scientists to explore more phages which are controllable (Skurnik *et al.*, 2007). New modified phages have been seen that show an excellent success to overcome challenges to primitive phage therapy (Moradpour, *et*

al., 2011). Techniques involving antibiotic therapy to combat against bacterial infections, gram negative bacteria release endotoxins that can cause fever or in adverse situations trigger life-threatening shocks (Theil, *et al.*, 2004). To overcome the endotoxin release, recombinant phage developed from *P. aeruginosa* through genetic modifications proved to be efficient and powerful anti-infection agent (Hagen, *et al.*, 2003; Hagens *et al.* 2004). Phage therapy holds the benefit of disrupting the membrane connected with endotoxins (Parisien, *et al.*, 2008). Due to biosafety issues related with the preparation of therapeutic phage, each phage that is used therapeutically has gone through exhaustive characterizations (Payne & Jensen, 2000; Carlton, *et al.*, 2005; Hanlon, 2007; Mattey *et al.*, 2008; Ilayas *et al.*, 2014).

Clinical application of bacteriophages

Phages when injected in six patients targeted staphylococcus near the base of cutaneous boils (furuncles and carbuncles), resulted in improvement in 48 hours and reduction in pain, swelling and fever. Merabishvili and workers (2009) used phage cocktail, consisting of exclusively lytic bacteriophages for the treatment of *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections in burn wound patients in the Burn Centre of the Queen Astrid Military Hospital in Brussels, Belgium. The first controlled clinical trial of a therapeutic bacteriophage preparation (Biophage-PA) showed efficacy and safety in chronic otitis because of drug resistant *P. aeruginosa* in UCL Ear Institute and Royal National Throat, Nose and Ear Hospital, London, UK (Wright *et al.*, 2009). Several clinical trials on phage therapy in humans were reported with the majority coming from researchers in Eastern Europe and the former Soviet Union (Abdul-Hassan, *et al.*, 1990; Sulakvelidze, *et al.*, 2001). One of the most extensive studies evaluating the application of therapeutic phages for prophylaxis of infectious diseases was conducted in Tbilisi, Georgia, during 1963 and 1964 and involved phages against bacterial dysentery (Babalova, *et al.*, 1968). The most detailed English language reports on phage therapy in humans were by Slopek and co-workers who published a number of papers on the effectiveness of phages against infections caused by several bacterial pathogens, including multidrug-resistant mutants (Slopek, *et al.*, 1983, 1984, 1985; Kucharewicz-Krukowska, *et al.*, 1987; Weber-Dabrowska, *et al.*, 1987). Phages have been reported to be effective in treating various bacterial diseases such as cerebrospinal meningitis in a newborn (Stroj, *et al.*, 1999), skin infections caused by *Pseudomonas*, *Staphylococcus*, *Klebsiella*, *Proteus*, *E. coli* (Cislo, *et al.*, 1987), recurrent subphrenic and subhepatic abscesses (Kwarcinski, *et al.*, 1987), *Staphylococcal* lung infections ((Ioseliani, *et al.*, 1980; Kaczowski,

et al., 1990), *Pseudomonas aeruginosa* infections in cystic fibrosis patients (Shabalova, *et al.*, 1995), eye infections (Proskurov, 1970), neonatal sepsis (Pavlenishvili, *et al.*, 1993), urinary tract infections (Perepanova, *et al.*, 1995), and cancer (Weber-Dabrowska, *et al.*, 2001). Abdul-Hassan, *et al.* (1990) reported on treatment of 30 cases of burn-wound linked antibiotic-resistant *Pseudomonas aeruginosa* sepsis. Bandages soaked with 1010 phages/ml were applied three times daily. Half of the cases were found to be improved. Markoishvili, *et al.*, (2002) reported the use of PhagoBioDerm, the phage impregnated polymer, to treat infected venous stasis skin ulcers. To patients that had failed to respond to other treatment approaches, PhagoBioDerm was applied to ulcers both alone and, where appropriate, in combination with other treatment strategies. Complete healing of ulcers was observed in 70% of the patients. Mushtaq, *et al.*, (2005) reported that a bacteriophage encoded enzyme, endosialidase E (endo E) selectively degrades the linear homopolymeric α -2, 8-linked N acetylneuraminic acid capsule associated with the capacity of *E. coli* K1 strain to cause severe infection in the newborn infant. In one of the study, PhagoBioDerm (a wound-healing preparation consisting of a biodegradable polymer impregnated with ciprofloxacin and bacteriophages) was used in three Georgian lumberjacks from the village of Lia who were exposed to a strontium-90 source from two Soviet-era radiothermal generators they found near their village. In addition to systemic effects, two of them developed severe local radiation injuries which subsequently became infected with *Staphylococcus aureus*. Approximately 1 month after hospitalization, treatment with phage bioderm was initiated. Purulent drainage stopped within 2–7 days. Clinical improvement was associated with rapid (7 days) elimination of the *S. aureus* resistant to many antibiotics (including ciprofloxacin), but susceptible to the bacteriophages contained in the PhagoBioDerm preparation (Jikia, *et al.*, 2005). Leszczynski and co-workers (2006) described the use of oral phage therapy for targeting Methicillin Resistant *Staphylococcus aureus* (MRSA) in a nurse who was a carrier. She had MRSA colonized in her gastrointestinal tract and also had a urinary tract infection. The result of phage therapy was complete elimination of culturable MRSA (Leszczynski, *et al.*, 2006).

Mass Spectrometry

Mass spectrometry has become a significant and analytical tool in biology in the past two decades. In principle, mass spectrometry exhibits high-throughput, sensitive and specific analysis for many applications in microbiology, including clinical diagnostics and environmental research. Recently, different mass

spectrometry methods for the classification and identification of bacteria and other microorganisms, as well as new software analysis tools, have been developed (Sacha, *et al.*, 2010).

Therapeutic antibodies

Antibodies are highly definite, naturally evolved molecules that recognize and eliminate pathogenic and disease antigens. The past 30 years of antibody research have hinted at the promise of new versatile therapeutic agents to cure cancer, autoimmune diseases and infection. Technology development and the testing of new generations of antibody reagents have changed our view of how they can be used for prophylactic and therapeutic purposes. The therapeutic antibodies of today are genetically engineered molecules that are designed to ensure high specificity and functionality. Some antibodies are loaded with toxic modules, whereas others are designed to function naturally, depending on the therapeutic application (Brekke, *et al.*, 2003; Hussain *et al.*, 2015).

Causes of Human Urinary Tract Infection

Urinary tract infections (UTIs) are the most common bacterial infections and are mainly caused by Uro-pathogenic *Escherichia coli* (UPEC). UTIs are typically considered extracellular infections, it has been recently demonstrated that UPEC bind to, invade, and replicate within the murine bladder urothelium to form intracellular bacterial communities (IBCs). These IBCs dissociate and bacteria flux out of bladder facet cells, some with filamentous morphology, and ultimately establish quiescent intracellular reservoirs that can seed recurrent infection. This IBC pathogenic cycle has not been investigated in humans. The presence of exfoliated IBCs and filamentous bacteria in the urines of women with chronic cystitis suggests that the IBC pathogenic pathway characterized in the murine model can occur in humans. The findings support the occurrence of an intracellular bacterial niche in some women with cystitis that can have important implications for UTI recurrence and treatments from women with acute UTI (David, *et al.* 2007).

Detection of Sexually Transmitted Infections

1) Detection of CT and GC Infections

A major achievement in screening for CT and GC genital tract infections came with the recognition that by using highly sensitive NAATs, the organisms could be detected in urine from infected women (Lee *et al.*, 1995). After this discovery, many investigators explored the use of vaginal swabs for detection of CT and GC by ligase chain reaction and transcription-mediated amplification (Hook, *et al.*, 1997 and Sary *et al.*, 1998). These studies demonstrated that vaginal swabs are as sensitive as cervical swabs for the detection of CT and GC and more sensitive than urine

specimens. Moreover, a multicenter study underscored the susceptibility of urine specimens to technical errors resulting from relatively demanding processing requirements, particularly the centrifugation step, which was unnecessary for vaginal swab specimens (Schachter *et al.*, 2003). However, for detection of GC in women, not all NAATs show acceptable performance with specimens other than endocervical swabs. Neither urine nor SOVs showed adequate sensitivity for detection of GC with the Roche Amplicor PCR assay (Knox *et al.*, 2002). It has been clearly showed that the vaginal swab is an acceptable and accurate specimen type, and use of swab specimens is more sensitive than use of first-catch urine specimens for detection of CT and GC using transcription-mediated amplification.

Quorum-sensing signals

Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. The bacterium *Pseudomonas aeruginosa* permanently colonizes cystic fibrosis lungs despite aggressive antibiotic treatment (Singh, *et al.*, 2000). This suggests that *P. aeruginosa* might exist as biofilms structured communities of bacteria encased in a self-produced polymeric matrix in the cystic fibrosis lung. (Singh, *et al.*, 2000) Consistent with this hypothesis, microscopy of cystic fibrosis sputum exhibits that *P. aeruginosa* are in biofilm-like structures. *P. aeruginosa* uses extracellular quorum-sensing signals (extracellular chemical signals that cue cell-density-dependent gene expression) to coordinate biofilm formation. Here we found that cystic fibrosis sputum produces the two principal *P. aeruginosa* quorum-sensing signals; however, the relative abundance of these signals was opposite to that of the standard *P. aeruginosa* strain PAO1 in laboratory broth culture. When *P. aeruginosa* sputum isolates were grown in broth, some showed quorum-sensing signal ratios like those of the laboratory strain. When we grew these isolates and PAO1 in a laboratory biofilm model, the signal ratios were like those in cystic fibrosis sputum. Our data support the hypothesis that *P. aeruginosa* are in a biofilm in cystic fibrosis sputum. Moreover, quorum-sensing signal profiling of specific *P. aeruginosa* strains may serve as a biomarker in screens to identify agents that interfere with biofilm development (Singh, *et al.*, 2000).

Nuclear magnetic resonance spectroscopy

Pneumonia, an infection of the lower respiratory tract, is caused by any of a number of different microbial organisms including bacteria, viruses, fungi, and parasites. Community-acquired pneumonia (CAP) causes a major number of deaths worldwide, and is the sixth leading cause of death in the United States. However, the pathogen(s) responsible for CAP can be difficult to identify, often leading to delaying in

appropriate antimicrobial therapies. In the present study, we use nuclear magnetic resonance spectroscopy to quantitatively measure the profile of metabolites excreted in the urine of patients with pneumonia caused by *Streptococcus pneumoniae* and other microbes. We found that the urinary metabolomic profile for pneumococcal pneumonia was significantly different from the profiles for viral and other bacterial forms of pneumonia. These data demonstrate that urinary metabolomic profiles may be useful for the effective diagnosis of CAP (Slupsky, *et al.*, 2009).

Molecular diagnostics of infectious diseases

Nucleic acid amplification technology has opened new avenues of microbial detection and characterization, such that growth is no longer required for microbial identification (Daud, *et al.*, 2014). In this respect, molecular methods have surpassed traditional methods of detection for many fastidious organisms. The polymerase chain reaction (PCR) and other recently developed amplification techniques have simplified and accelerated the in vitro process of nucleic acid amplification. The amplified products, known as amplicons, may be characterized by various methods, including nucleic acid probe hybridization, analysis of fragments after restriction endonuclease digestion, or direct sequence analysis. Rapid techniques of nucleic acid amplification and characterization have significantly broadened the microbiologists' diagnostic arsenal. However, most phenotypic variables commonly observed in the microbiology laboratory are not sensitive enough for strain differentiation. When methods for microbial genome analysis became available, a new frontier in microbial identification and characterization was cleared (Wagar, *et al.*, 1996)

Traditional Microbial Typing

Biotyping

Traditional microbial identification methods rely on phenotypes, such as morphologic features, growth variables, and biochemical utilization of organic substrates. The biological profile of an organism is termed a biogram. The determination of relatedness of different organisms on the basis of their biograms is termed biotyping. Investigators must determine which profile variables have the greatest differentiating capabilities for a given organism Kilian M, Sorensen I, Frederiksen W. Biochemical characteristics of 130 recent isolates from *Haemophilus influenzae* meningitis. For example, gram stain characteristics, indole positivity, and the ability to grow on MacConkey medium do not aid in the differentiation of nonentero-hemorrhagic *Escherichia coli* from *E. coli* O157:H7. However, sorbitol fermentation has proven to be an extremely useful characteristic of the

biochemical profile used to differentiate these strains (Clin, *et al.*, 1979).

PCR-based diagnostics

Molecular diagnostics are revolutionizing the clinical practice of infectious disease. Their effects will be significant in acute-care settings where timely and accurate diagnostic tools are critical for patient treatment decisions and outcomes. PCR is the most well-developed molecular technique up to now, and has a wide range of already fulfilled, and potential, clinical applications, including specific or broad-spectrum pathogen detection, evaluation of emerging novel infections, surveillance, early detection of biothreat agents, and antimicrobial resistance profiling. PCR-based methods may also be cost effective relative to traditional testing procedures. Further advancement of technology is needed to improve automation, optimize detection sensitivity and specificity, and expand the capacity to detect multiple targets simultaneously (multiplexing). This review provides an up-to-date look at the general principles, diagnostic value, and limitations of the most current PCR-based platforms as they evolve from bench to bedside (Yang, *et al.*, 2004; Burdi *et al.*, 2014; Khan *et al.*, 2015; Afzal *et al.*, 2015; Qureshi *et al.*, 2014ab).

Fungal diseases

Fungi are found in every part of universe. There are about 1.5 million various species of fungi in the world but only about 300 cause diseases in human beings. Fungi found in soil, plants and in human's skin inside and above the human skin. Many fungi are not dangerous but some types cause severe health threats (Garcia-solache, *et al.*, 2010 and Hawksworth, 2001). Most common fungal diseases are:

- a. **Aspergillosis** - caused by the fungus *Aspergillus* and mostly occurs in people with lung diseases or weakened immune systems.
- b. **Coccidiomycosis (Valley fever)** - Caused by *Coccidioides*, a fungus found in the soil of dry, low rainfall areas.
- c. **C. neoformans infection** - Caused by *Cryptococcus neoformans*, a fungus that lives in soil throughout the world.
- d. Cryptococcal meningitis is major problem in resource-limited countries with a high burden of HIV/AIDS.
- e. **C. gattii infection** - Caused by *Cryptococcus gattii*, a fungus which lives in soil in tropical and subtropical regions of the world.
- f. **Fungal eye infections** - Fungal eye infections are mostly caused by an eye injury. Different types of fungi can cause fungal eye infections.
- g. **Pneumocystis pneumonia (PCP)** - An illness caused by the fungus *Pneumocystis jirovecii*.

PCP is one of the most frequent and severe opportunistic infections in humans with weakened immune systems, particularly people with HIV/AIDS.

h. **Ring worm** - Ringworm is a common skin infection that is caused by a fungus. It's called "ringworm" because it can cause a circular rash (shaped like a ring) that is mostly red and itchy.

i. **Sporotrichosis** - Caused by the fungus *Sporothrix schenckii*. The fungus lives in the whole world in soil, plants, and decaying vegetation.

Diagnosis of Fungal Diseases

1. Conventional-PCR

Conventional PCR has emerged as a major tool for the diagnosis and study of phytopathogenic fungi and has contributed to the alleviation of some of the problems related with the detection, control and containment of plant pathogens (Henson, *et al.*, 1993; Martin *et al.*, 2000). PCR is a sensitive technology that offers several advantages over the traditional methods of diagnosis: micro-organisms do not need to be cultured the technique possesses the potential to detect a single target molecule in a complex mixture, and it is versatile.

Depending on the design of the primers, both narrow and broad selectivities are possible, thus enabling the detection of a single pathogen at the species or strain level. However, despite these advantages, the adoption of PCR for routine detection of plant pathogens has been slow due to technical limitations related to the postamplification procedures, which are necessary to detect amplicons. In fact, although PCR techniques can considerably lessen the use of time needed for diagnosis compared to conventional culturing methods, they require additional work when Southern blot or sequencing are required to identify the PCR products. Moreover, conventional PCR is unreliable for quantitative analysis (Ginzinger *et al.*, 2002). Four main chemistries are used to detect and study phytopathogenic and antagonistic fungi. These chemistries can be grouped into amplicon sequence non-specific and sequence specific techniques (Mackay *et al.*, 2002; Ahmad *et al.*, 2014; Rasheed *et al.*, 2014; Altaf *et al.*, 2015).

2. Detection of Fungal Infection in Human Blood

A novel panfungal PCR assay which detects the small-subunit rRNA gene sequence of two major fungal organism groups was used to test whole-blood specimens obtained from a series of blood or bone marrow transplant recipients. The 580-bp PCR product was identified after amplification by panfungal primers and hybridization to a 245-bp digoxigenin-labeled probe. The lower limit of detection of the assay was approximately four organisms per milliliter of blood. Multiple whole-

blood specimens from five patients without fungal infection or colonization had negative PCR results. Specimens from 11 infected patients gave positive PCR results. Blood from three patients with pulmonary aspergillosis had positive PCR results: one patient's blood specimen obtained in the week prior to the diagnosis of infection by a positive bronchoalveolar lavage fluid culture result was positive by PCR, and blood specimens obtained from two patients 1 to 2 days after lung biopsy and which were sterile by culture were positive by PCR. The blood of four patients with candidemia, three patients with mixed fungal infections, and one patient with fusariosis also had positive PCR signals. The panfungal PCR assay can detect multiple fungal genera and may be used as an additional thing to conventional methods for the detection of fungal infection or for describing the natural history of fungal infection. Further studies are needed to define the sensitivity and specificity of this assay for the diagnosis of fungal infection prior to the existence of other clinical or laboratory indications of invasive fungal infection (Jo-Anne, *et al.*, 1997).

3. Discovery of a sexual cycle in *Aspergillus fumigatus*

Aspergillus fumigatus is a saprotrophic fungus whose spores are ubiquitous in the atmosphere. It is an opportunistic human pathogen in immunocompromised individuals, causing potentially lethal infections and is linked with severe asthma and sinusitis. The species is only known to reproduce by asexual means, but there has been accumulating evidence for recombination and gene flow from population genetic studies, genome analysis, the presence of mating-type genes and expression of sex-related genes in the fungus. *A. fumigatus* possesses a fully functional sexual reproductive cycle that leads to the production of cleistothecia and ascospores, and the teleomorph *Neosartorya fumigata* is described. The species has a heterothallic breeding system; isolates of complementary mating types are required for sex to occur. We demonstrate increased genotypic variation resulting from recombination between mating type and DNA fingerprint markers in ascospore progeny from an Irish environmental subpopulation. The capability of *A. fumigatus* to engage in sexual reproduction is highly significant in understanding the biology and evolution of the species. The presence of a sexual cycle provides an invaluable tool for classical genetic analyses and will be helpful for research into the genetic basis of pathogenicity and fungicide resistance in *A. fumigatus*, with the aim of improving methods for the control of aspergillosis. These results also yield insights into the potential for sexual reproduction in other supposedly 'asexual' fungi (Céline, *et al.*, 2009).

4. Identification of Disease gene through nonsense-mediated mRNA decay inhibition

Premature termination codons (PTCs) have been shown to initiate degradation of mutant transcripts through the nonsense-mediated messenger RNA (mRNA) decay (NMD) pathway. Gene identification by NMD inhibition (GINI) used to, to identify genes harboring nonsense codons that underlie human diseases. The NMD pathway is pharmacologically inhibited in cultured patient cells, resulting in stabilization of nonsense transcripts. To distinguish stabilized nonsense transcripts from background transcripts upregulated by drug treatment, drug-induced expression changes are measured in control and disease cell lines with complementary DNA (cDNA) microarrays. Transcripts are ranked by a nonsense enrichment index (NEI), which is associated with expression changes for a given transcript in NMD-inhibited control and patient cell lines. The most promising candidates can be selected using information such as map location or biological function; however, an important advantage of the GINI strategy is that a priori information is not essential for disease gene identification. GINI was tested on colon cancer and Sandhoff disease cell lines, which contained previously characterized nonsense mutations in the MutL homolog 1 (MLH1) and hexosaminidase B (HEXB) genes, respectively. A list of genes was produced in which the MLH1 and HEXB genes were among the top 1% of candidates, thus validating the strategy (Noensie, *et al.*, 2001).

5. Comparative genomic analysis

Leishmaniasis is an infectious disease that is prevalent in Europe, Africa, Asia and the Americas, killing thousands and debilitating millions of people each year. With 2 million new cases reported annually and 350 million people at risk, infection by the insect-transmitted *Leishmania* parasite represents an important global health problem for which there is no vaccine and few effective drugs. At least 20 *Leishmania* species infect humans, and the spectrum of diseases that they cause can be categorized broadly into three types: (i) visceral leishmaniasis, the most serious form in which parasites leave the inoculation site and proliferate in liver, spleen and bone marrow, resulting in host immunosuppression and ultimately death in the absence of treatment; (ii) cutaneous leishmaniasis, in which parasites remain at the site of infection and cause localized long-term ulceration; and (iii) mucocutaneous leishmaniasis, a chronic destruction of mucosal tissue that develops from the cutaneous disease in less than 5% of affected individuals (Marsden, *et al.*, 1986).

a) Retrotransposons and RNAi

In addition to selection pressure acting against chromosomal rearrangements, *Leishmania* may lack

some of the machinery that generates diversity in other eukaryotes. Lack of transposable elements would favor chromosome stability and is seen in the genomes of *L. major* and *L. infantum*. In other kinetoplastid parasites, namely *T. brucei* and *T. cruzi*, several classes of transposable elements are present (the non-long terminal repeat (LTR) retrotransposons, *ingi/L1Tc* and *SLACS/CZAR* and the LTR retrotransposon *VIPER*), but the *L. major* genome has only remnants of *ingi/L1Tc*-related elements (DIREs), suggesting their loss during evolution of the *Leishmania* lineage (Bringaud, *et al.*, 2006). Similarly, *L. infantum* and *L. braziliensis* also contain the *ingi/L1Tc* DIREs.

The telomeres of *L. braziliensis* contain a family of 20–30 previously unknown DNA transposable elements, each including putative reverse transcriptase, phage integrase (site-specific recombinase) and DNA and/or RNA polymerase domains, which we have called 'telomere-associated transposable elements (TATEs)'. The TATEs and their bordering regions are highly conserved and are inserted only in the telomeric hexamer repeats at the same relative position (GGGup arrowTTA). As observed for most mobile elements, a duplicated motif (TT), present on either side of the transposable element, seems to correspond to a target site duplication. Unlike non-LTR retrotransposons, the TATEs do not contain an APE-like endonuclease domain but they do contain a putative integrase-like domain (site-specific recombinase), related to the transposase domains of other transposable elements, that may contribute to the observed telomeric site specificity. The telomeres seem to contain clusters of tandemly arranged TATEs, including short elements probably derived from full-length elements by internal deletions. It has not been possible to determine the precise organization of the TATEs owing to their repetitive nature. In many eukaryotes, the effects of retrotransposable elements can be regulated through a RNA silencing mechanism such as RNAi. Despite its demonstration and utility in *T. brucei* (Ngo, *et al.*, 1998), RNAi has not been detected in other kinetoplastid species including *L. major* and *T. cruzi* (Robinson, *et al.*, 2003).

Comparison revealed genes in *L. braziliensis* that may be involved in the RNAi pathway. A hallmark of this pathway in other eukaryotes is Dicer activity, which converts double-stranded RNA (dsRNA) into small interfering RNA (siRNA). A divergent gene (Tb927.8.2370) encoding a Dicer-like protein (TbDcl1) has been described in *T. brucei*. The TbDcl1 protein bears the two RNase III-like domains typical of Dicer and is required for generating siRNA-sized molecules, and its downregulation results in a less efficient RNAi response. An ortholog of TbDcl1 has

not been found in *T. cruzi* or *L. major*, trypanosomatids that lack a functional RNAi pathway. *L. braziliensis*, however, contains a similar gene (L) that is endowed with two conserved RNase III domains. Dicer activity could also be carried out by a combination of independent proteins carrying the relevant dsRNA-binding domain, DEAD/H box RNA helicase and RNase III domains. The RNase genes implicated in this complex are missing in *L. major* and *L. infantum*, but present in the *L. braziliensis* genome at regions of otherwise conserved synteny between the *Leishmania* species (Shi, *et al.*, 2006).

Argonaute, an endonuclease involved in the dsRNA-triggered cleavage of mRNA, is another crucial component of the RNAi machinery and, unlike *L. major*, *L. braziliensis* contains an ortholog of the functional argonaute gene (TbAGO1) present in *T. brucei*. A second gene containing an argonaute PIWI domain (TbPW11), which was originally identified in *T. brucei* and has orthologs in both *Leishmania* and *T. cruzi*, has been shown not to be involved in the RNAi pathway (Durand-Dubief, *et al.*, 2003). Argonaute, an endonuclease involved in the dsRNA-triggered cleavage of mRNA, is another crucial component of the RNAi machinery and, unlike *L. major*, *L. braziliensis* contains an ortholog of the functional argonaute gene (TbAGO1) present in *T. brucei*. A second gene containing an argonaute PIWI domain (TbPW11), which was originally identified in *T. brucei* and has orthologs in both *Leishmania* and *T. cruzi*, has been shown not to be involved in the RNAi pathway. TbAGO1 can be replaced by the human gene encoding Argonaute, suggesting that TbAGO1 encodes the endonuclease activity required for mRNA target degradation in the trypanosome RNAi pathway. The *L. braziliensis* gene contains the typical argonaute domains PAZ and PIWI, the latter of which contains key amino acids essential for TbAGO1 activity. In addition, the *L. braziliensis* AGO1 gene encodes an amino-terminal RGG domain, which is present in TbAGO1 and shown to be essential for association with polyribosomes (Shi, *et al.*, 2004). Examination of the syntenic regions on chromosome 11 in *L. major* and *L. infantum* revealed remnants of AGO1, suggesting that the RNAi machinery has been lost from the *Leishmania* subgenus to which they both belong. In the alternative subgenus *L. viannia* (which includes *L. braziliensis*), RNA viruses have been characterized, however, suggesting that this lineage could have retained RNAi as an antiviral defense mechanism. The RNAi machinery also have a role in regulating the functions of transposable elements (Stuart, *et al.*, 1992).

Genes differentially distributed between species

Only one gene locus has been directly implicated in *Leishmania* disease tropism. In *Leishmania*

donovani, the causative agent of visceral leishmaniasis, A2 gene products are required for parasite survival in visceral organs; by contrast, *L. major* contains only A2 pseudogenes. Three genomes in parallel (using ACT software25) for species-specific genes that might contribute to differences in disease presentation, immune response and pathogenicity. Despite the broad differences in disease phenotype, we found that few genes are specific to individual *Leishmania* species (Zhang, *et al.*, 2003). Clear instances were found where tandem duplication, followed by diversification, accounts for species-specific differences; for example, copies of a hydrolase gene in *L. infantum* (LinJ31.3030) and an adenine phosphoribo-syltransferase gene in *L. braziliensis* (LbrM26_V2.0120) seem to have arisen and diverged from an adjacent gene. Larger tandem gene arrays are a characteristic feature of all kinetoplastid parasite genomes, facilitating increased protein expression in the absence of gene regulation by transcription initiation. Although correctly assembling highly repetitive regions is technically difficult from randomly sequenced DNA, the depth of assembled reads provides an indication of the number of repeat units present in specific regions. The largest family of surface-expressed protein genes in *Leishmania*, the amastins, are specifically expressed by intracellular parasites in the host. In *L. major*, the largest amastin array (comprising 21 out of 54 amastin genes) is interspersed with repeat units of the unrelated tuzin genes that encode proteins of unknown function. Although similar in organization, the amastin-tuzin array seems to be reduced in size by at least half in *L. braziliensis* (on the basis of the depth of coverage of reads across this repeat region). By contrast, the surface-expressed GP63 zinc metalloproteinases, which function in host cell binding and parasite protection from complement-mediated lysis, are encoded by a repeated gene cluster which seems to be enlarged fourfold in *L. braziliensis* as compared with *L. major* or *L. infantum* (Yao, *et al.*, 2003).

Gene evolution

In addition to the small number of species-specific and differentially distributed genes, other genetic factors are likely to show differences between the species. We therefore searched for genes with signatures of positive selection as an indicator that they may be involved in host-pathogen interactions. Those genes with the highest ratios of non-synonymous to synonymous mutations (dN/dS) were, for the most part, involved in undefined biological processes. Approx8% of genes seem to be evolving at different rates between the three *Leishmania* species and are involved in a spectrum of core processes (including transport, biopolymer metabolism, cellular

metabolism, lipid metabolism and RNA metabolism), which influence parasite survival in the host and disease outcome (Christopher, *et al.*, 2007).

Conclusion

Biotechnology does not only involve the manipulation of DNA (or the hereditary material of an organism) by means of genetic engineering. Rather, the technology encompasses the use of living organisms (plants, animals, bacteria or viruses) or biological processes to make useful products. We utilize the biological process of fermentation that takes place in yeast to make bread, wine and beer. The earliest farmers have been using the principles of biotechnology to improve their crops or livestock by selecting plants or animals with desirable traits and using them for propagation (plants) or breeding (animals). Biotechnology aims to target the causes of diseases and not the symptoms. And that's why biotechnology offers one of the strongest hopes for patients to cure diseases. Biotechnology offers patients a variety of new solutions such as:

- a. Unique, targeted and personalized therapeutic and diagnostic solutions for particular diseases or illness.
- b. An unlimited amount of potentially safer products
- c. Superior therapeutic and diagnostic approaches
- d. Higher clinical effectiveness because of the biological basis of the disease being known

Biotechnology medicines and therapies use proteins, enzymes, antibodies and other substances naturally produced in the human body to treat diseases, including genetic disorders. Biotechnology also uses other living organisms, plant and animal cells, bacteria, viruses and yeasts – in the large-scale production of medicines for human use.

Applications of biotechnology include diagnostic tests that use biotechnology materials to detect the presence or risk of disease such as cancer, genetic diseases or pollution of a cell or material. There are three primary areas in healthcare in which biotechnology is currently being used: medicines (also including advanced therapies such as cell and gene therapy), vaccines and diagnostics. So, biotechnology is one of several tools that can be efficiently used to diagnose and cure the human diseases. It also improves human health by innovating new ways.

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