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Chapter 9

# Molecular Pathology and Infectious Diseases

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## I. INTRODUCTION

Molecular diagnostic tests can be divided into two primary groups. The first are tests designed to detect the presence of mutations in the human genome that are associated with specific diseases. These tests need to be performed only once in a specific individual's lifetime. Of course, the discovery of additional disease-linked mutations may initiate additional investigation. The second group of molecular diagnostic assays are designed to detect infectious agents (Espy et al., 2006) or the recurrence of a mutation specifically associated with a tumor the patient has had in the past (Kannim et al., 2009). Bacterial, viral, fungal, or parasitic infections can be eradicated and recur at a time in the future requiring repetition of the same molecular assay for detection. The viral load of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are used to monitor therapeutic efficacy and clinical course (Phillips et al., 2001; Bain, 2001). Minimal residual disease detection refers to the analysis of tissue/cells in a patient previously diagnosed with a malignancy to evaluate if therapy was completed successfully or if rare malignant cells are present. For example, monitoring JAK2 V617F mutation has been identified in the majority of Philadelphia chromosome-negative myeloproliferative disorders with minimal residual disease following specific treatment (Kannim et al., 2009).

In 1994, the College of American Pathologists' Patient Preparation and Specimen Handling Editorial Board initiated a two-year effort to write the final fascicle (#VII) entitled *Reference Guide for Diagnostic Molecular Pathology/ Flow Cytometry* (Kiechle et al., 1996). The book was published in 1996 with 134 entries divided into 78 human nuclear genome mutations, 6 human mitochondrial genome mutations (Kiechle et al., 1996a), and 50 infectious disease molecular detection assays (18 viruses, 18 bacteria, 12 parasites, and 2 fungi) (Table 9.1). In the past 13 years, many changes and refinements in relationship to the use of infectious disease molecular diagnostic testing in patient care have been made (Espy et al., 2006). New strategies have been devised in nucleic acid purification and amplification (Mardis, 2008). Many of these advances in molecular pathology diagnostic technologies will be described in this handbook. The following review will focus on issues that require vigilance in the face of continuous progress in our understanding of the pathogenesis and progression of infectious diseases. The goal is to improve our current approaches to the detection of infectious disease.

## II. EMERGING INFECTIOUS DISEASES

### A. How They Develop

The combination of the widespread agricultural and industrial development has resulted in the increased density of humans living in close proximity to each other. This lifestyle change from more rural existence has brought humans in closer proximity to wild animals that harbor potential future human pathogens. Wolfe et al. (2007) proposed that there are five stages through which a pathogen exclusively infecting animals (stage 1) must progress before being transformed into a pathogen exclusively infecting humans (stage 5). The authors selected 25 infectious diseases (15 common in temperate climates and 10 common in tropical climates) to evaluate.

Higher proportions of disease were transmitted by insect vectors or belonged to stage 5 pathogens in the temperate climate compared to the tropics (Wolfe et al., 2007). Animal reservoirs were more common in the tropics compared to the temperate climate (Wolfe et al., 2007; Jones et al., 2008). An exploration of the geographic locations of

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**TABLE 9.1 Bacterial and Viral Infectious Agents Described in the 1996 *Reference Guide for Diagnostic Molecular Pathology/Flow Cytometry* (Kiechle et al., 1996)**

| Bacteria | Virus |
|---|---|
| *Aeromonas spp.* and *Pleisiomonas spp.* | Adenovirus |
| Antimicrobial resistance determination | Cytomegalovirus |
| Bartonella | Enteroviruses |
| *Bordetella spp.* | Epstein Barr virus |
| *Campylobacter spp.* | Hepatitis A virus |
| *Chlamydia trachomatis* | Hepatitis B virus |
| *Clostridium difficile* | Hepatitis C virus |
| *Enterococcus spp.* | Hepatitis delta virus |
| Enterovirulent *Escherichia coli* | Hepatitis E virus |
| *Gardnerella vaginalis* | Herpes simplex virus |
| *Haemophilus spp.* | HIV 1 |
| *Legionella pneumophilia* | Human herpesvirus-6, types A and B |
| Molecular Epidemiology of Nosocomial Infections | Human papillomavirus |
| *Mycobacterium leprae* | Rabies virus |
| *Mycobacterium spp.* | Respiratory syncytial virus |
| *Mycoplasma spp.* | Rotavirus |
| *Neisseria gonorrhoeae* | Rubella virus |
| *Trepenema pallidum* | Varicella-zoster virus |

the emergence of 335 infectious diseases between 1940 and 2004 determined that there was a steady increase in disease identification with a maximum during the 1980s, which is attributable to the emergence of HIV (Jones et al., 2008). Of these events, 20.9% were caused by drug-resistant microbes primarily located in high latitudes where developed countries with readily available antibiotics are located (Jones et al., 2008).

## B. Hospital-Acquired, Antibiotic-Resistant Bacteria

Today, hospital-acquired, antibiotic resistance may occur in both gram-positive and gram-negative bacterial (Table 9.2). Methicillin-resistant *Staphylococcus aureus* (MRSA) was first described in 1968 (Zitterkopf, 2008). Bacterial culture on chromagar requires greater than 24 hours for MRSA growth. Meanwhile, the patient being tested should be in isolation, since MRSA transmission in 24 hours from person to person may be as high as 5% to 15%, depending on the distance between the two individuals. Polymerase chain reaction (PCR) with probes to identify the mecA gene, which encodes methicillin resistance, can decrease the turnaround time for detection to 2–4 hours and is more sensitive than culture. Since this screening assay is currently not reimbursed for hospitalized patients, this effort to reduce MRSA prevalence in the hospital becomes an exercise in cost avoidance. The additional cost for caring for a patient with an MRSA infection ranges from $27,000 to $35,000 per patient compared to a patient with methicillin-sensitive S. aureus (Zitterkopf, 2008). The rate of subsequent MRSA infection after identification of MRSA colonization is approximately 30% (Davis et al., 2004). The dilemma rests in the ethical question related to who should be screened. There are two viewpoints. The first approach would screen all hospitalized patients at the time of admission. The other approach uses targeted active surveillance, only screening patients at a high risk for MRSA colonization or infection. Examples of high-risk groups are listed

**TABLE 9.2 Hospital-Acquired, Antibiotic-Resistant Bacteria**

| |
|---|
| **Gram-Positive** |
| Methicillin-resistant *Staphylococcus aureus* (MRSA) |
| Vancomycin-resistant enterococci (VRE) |
| Glycopeptide-resistant enterococci (GRE) |
| **Gram-Negative** |
| Beta-lactamase-producing strains of *Escherichia coli* and *Klebsiella pneumoniae* |
| Fluoroquinolone-resistant *Pseudomona aeruginosa* |
| Ciprofloxacin-resistant *Neisseria meningitides* |
| Carbapenem-resistant *Acinetobacter spp*. |

**TABLE 9.3 Selective MRSA Screening**

| |
|---|
| **High-Risk Patients** |
| Critical-care admissions/transfers |
| Admissions from long-term-care facilities |
| Patients who have previously tested MRSA-positive |
| Dialysis patients/chronic renal failure |
| Admissions with skin and soft-tissue infections |
| Patients admitted with fractured hip from emergency department (ED) |
| **Presurgical Screening** |
| Orthopedics—Total hip and knee replacement |
| Neurosurgery—Open spinal with implant |

in Table 9.3. The goal of each of these screening programs is to eradicate MRSA from the patient to reduce the risk of subsequent MRSA infection during the hospitalization. Healthcare workers at the hospital and the patient's relatives are usually not included in these MRSA screening programs since the goal of the program is not complete eradication of MRSA in a community.

The carbapenem-hydrolyzing β-lactamase known as *K. pneumoniae carbapenemase* produces a resistant strain associated with a higher mortality in infected patients compared to patients infected with carbapenem-sensitive strains. The use of surveillance cultures can identify the population of asymptomatic colonized patients and enhance the effectiveness of a carbapenem-resistant *K. pneumoniae* infection prevention program (Calfee and Jenkins, 2008). This approach will lead to the development of a U.S. Food and Drug Administration (FDA)–approved molecular diagnostic test to detect the drug resistant bacteria for more rapid turnaround time for screening results.

## C. Bacterial Genome Sequences

On June 14, 2007, 470 genome sequences from 352 bacterial species were available online (Fournier et al., 2007) and approximately 600 projects were ongoing (Hernandez et al., 2009). This rapid progress is attributed to high-throughput sequencing technologies (Mardis, 2008) and computational assembly of sequences (Hernandez et al., 2009). These projects have provided genotype-to-phenotype revelations related to detection of resistance to antimicrobials, identification of virulence factors, and better understanding of host-pathogen interactions (Fournier et al., 2007). On a larger scale, metagenomics samples the genome sequences of a community of microbial organisms inhabiting a common environment (Hagenholtz and Tyson, 2008). One example is the Human Microbiome Project, an international effort to map human-associated microbial communities from the gut, mouth, skin, and vagina.

Sequencing of *Streptococcus suis* sequence type 7 (ST 7), which was responsible for two larger outbreaks in China (1998 in Jiangui and 2005 in Sichuan province) and sporadic infections in several provinces in China, has demonstrated the presence of the tetracycline resistant gene, tetM (Ye et al., 2008). This tetM gene is associated with the conjugative transposon Tn916. Horizontal transfer of Tn916 with the tetM gene played a central role in the evolution of the epidemic *S. suis.* ST 7 clone. Also, the three virulence genes of *S. suis*. ST 7, which include mrp, sly, and ef, were detected by PCR in all species studied (Ye et al., 2008). Horizontal gene transfer or the exchange of DNA between prokaryotes uses several mechanisms such as conjugation, transformation, and phage transduction. Horizontal gene transfer accounts for the genetic plasticity that permits the acquisition of new virulence factors, antibiotic resistance, or metabolism of toxic compounds by bacterial strains.

## D. Recent New Respiratory Viruses

Since 2000, six new respiratory viruses have been detected (Table 9.4) (Dong et al., 2008). The outbreak of severe acute respiratory syndrome (SARS) in the Guangdong province of China represents the potential international chaos that an emerging infectious disease can create (Zhao, 2007; Mazzulli et al., 2004). The first index case was located in the city of Foshan, west of Guangzhou province. He had a fever of greater than 39°C and respiratory symptoms on November 16, 2002, and was hospitalized on November 20, 2002. He recovered on January 8, 2003, but he infected his wife and

**TABLE 9.4 Six new respiratory viruses since 2000**

| Virus | Comments |
|---|---|
| H5N1 Avian influenza | New strain influenza A with limited human to human transmission (1998–2009). Detection: Sequence analysis of H and N genes; RT-PCR using specific hemagglutinin gene sequence for H5N1 . |
| Severe acute respiratory syndrome coronavirus (SARS-CoV) | Spreads by respiratory secretions and airborne transmission, RT-PCR, cloning and sequencing identified organism. |
| Human coronaviruses NL63 and HKU1 | Enveloped positive-strand RNA-viruses which cause common cold. Genetic variability and cross-reactivity must be monitored when using molecular asssays for detection. |
| Human metapneumovirus | Paramyxovirus found in winter and spring temperate climates. RT-PCR method of choice for detection. |
| Human bocavirus | Human parvovirus with high prevalence of 2 to 18%. Co-infection or true pathologic role needs more investigation. |

*Collated from (Dong et al., 2008).*

three other relatives (Zhao, 2007). SARS spread to 25 countries with 8,096 confirmed cases and 774 deaths in three months (Dong et al., 2008; Zhao, 2007). The largest outbreak of SARS outside of Asia was in Toronto, Canada, where 438 people were diagnosed and 44 people died (Mazulli et al., 2004). Symptoms included fever with temperature greater than 38°C, nonproductive cough, myalgias, headache, and malaise. The incubation period was 2 to 10 days. The disease cannot spread before the onset of symptoms or after recovery from the disease. An international effort lead to the identification of a novel coronavirus, a level 3 pathogen. The published sequence in May 2003 was the Tor 2 strain from the Toronto outbreak. Assays to detect SARS-CoV using PCR were developed for viral RNA detection. Viral load in the upper respiratory tract and feces was low during the first four days and peaks at around the 10th day of the illness (Zhao, 2007). The international economic effect of SARS has been estimated to have been $100 billion. Canceled travel and decreased hotel occupancy in China and Canada was estimated to contribute to a 1% decrease in their national economies. This outbreak demonstrates the importance of molecular techniques that were used to establish the identity of the infectious agent within seven months of the presentation of the index case. This finding was rapidly followed by RT-PCR assay for the detection of SARS-CoV in patient specimens (Espy et al., 2006). Without these techniques, the containment of this outbreak may have taken much longer.

## E. Future Directions

The increasing number of infectious organisms that threaten the health of humans is a result of multiple factors, including rapid pathogen mutation rate, threat of bioterrorism (Nelson and Wilson, 2007), need for rapid identification of the pathogen in the septic patient, transformation of nonhuman pathogen to a human pathogen (Wolfe et al., 2007), and outbreaks of emerging and novel infectious diseases (Zitterkopf, 2008; Clafee and Jenkins, 2008; Ye et al., 208; Dong et al., 2008; Zhao, 2007; Mazzulli et al., 2004; Jones et al., 2008). The general dilemma is the clinical need for the identification of the pathogen in 2-6 hours to facilitate the initiation of therapy (Zitterkopf, 2008; Calfee and Jenkins, 2008). Most culture methods for viruses, bacteria, and fungi require a day or more for results. Identification of a bacterium by molecular methods will not provide any information related to the antibiotic sensitivity of the organism. MRSA is an example of an exception to this generalization. Therefore, most bacteria will need to be cultured to complete the sensitivity studies. A microarray designed to detect the most common gram-positive and gram-negative bacterial pathogens could be used as an initial screen in the emergency department or the intensive care units. The miniaturization of the space required to extract the nucleic acid and add the reagents for PCR and the thermocycler for amplification (Holland and Kiechle, 2005) could lead to the development of a molecular diagnostic device small enough to be moved from patient to patient for point-of-care testing. This technology would be simple to use and could be employed in underdeveloped countries for rapid identification of infectious organisms (Dong et al., 2008).

# III. BIOFILMS

## A. Planktonic vs. Sessile Growth

In the microbiology laboratory, microorganisms are studied as freely suspended cells in nutrient-rich culture media referred to as *planktonic growth.* However, up to 70% of human bacterial infections are caused by microorganisms growing in sessile state or biofilm (Table 9.5) (Donlan, 2002; Lynch and Peterson, 2008).

**TABLE 9.5** Biofilm-Associated Microorganisms on Indwelling Medical Devices or in the Absence of a Foreign Body

| |
|---|
| **Associated with indwelling medical devices** |
| *Candida albicans* |
| Coagulase—negative staphylococci |
| *Enterococcus spp.* |
| *Escherichia spp.* |
| *Klebsiella pneumoniae* |
| *Pseudomonas aeruginosa* |
| *Staphylococcus aureus* |
| **Associated with no foreign body** |
| Urinary tract infections: Uropathogenic *Escherichia coli* |
| Middle ear infection: *Haemophilus influenza, Streptococcus pneumoniae, Moraxella catarrholis* |
| Chronic rhinosinusitis (cystic fibrosis): *Pseudomonas aeruginosa* |
| Native value endocarditis: *Staphylococcus lugdunensis, Enterococcus durans,* Viradens group streptococcus |

***Collated from (Donlan, 2002; Lynch et al., 2008).***

A biofilm consists of a polysaccharide matrix, DNA, and protein that form the extracellular polymeric substance. The extracellular polymeric substance may account for 50% to 90% of the total carbon of biofilms (Donlan, 2002). Biofilms may form on surgical or non-surgically placed indwelling medical devices or may be associated with chronic infections that are unrelated to the presence of foreign bodies (Table 9.5). A biofilm can be 10 to 100 times less susceptible to an antimicrobial compared to the organism grown in suspension. Several factors may play a role in this antibiotic resistance (Lynch and Robertson, 2008). First, the extrapolymeric matrix of the biofilm may trap the antibiotic. However, some antibiotics and antifungals are capable of diffusing into biofilms. Second, different genes are up-regulated and down-regulated in planktonic growth compared to biofilms. For example, *S. aureus*, when growing in a biofilm, up-regulates genes encoding enzymes involved in glycolysis and fermentation (Donlan, 2002). The metabolic shift to fermentation is attributable to the oxygen-limited biofilm environment. Biofilm growth may also up-regulate multidrug efflux pumps, which will reduce the antibiotic concentration within a bacteria. Finally, there are a subset of persister cells within a biofilm that are slow-growing and geneticaly programmed to survive exposure to antibiotics (Lynch and Robertson, 2008). They are not mutants and do not grow in the presence of antibiotics. They express a toxin, Rel E, which drives persister organism into a dormant state. High-throughput screening assays have been developed to determine the effectiveness of small molecules to disrupt biofilm development (Junker and Clardy, 2007).

**TABLE 9.6** MicroRNA found in large DNA viruses

| Virus | Number of MicroRNAs |
|---|---|
| Epstein-Barr virus | 23 |
| Kaposi sarcoma–associated herpesvirus | 12 |
| Mouse gamma-herpesvirus 68 | 9 |
| Rhesus lymphocryptovirus | 16 |
| Human cytomegalovirus | 14 |
| Herpes simplex virus Type 1 | 6 |

## B. Quorum Sensing

In biofilms, intercellular communication occurs by autoinducer-activated intracellular signaling. When the intracellular concentration of the autoinducer reaches a threshold, the bacterial population as a whole will alter gene expression (Donlan, 2002; Junker and Clardy, 2007). From 1% to 10% of a microbes genome may be regulated by this process called *quorum sensing*. The autoinducer used by several gram-negative organisms is acylated homoserine lactone (HSL). For example, quorum sensing controls the expression of virulence genes, including lasB, lasA, apr, toxA, and lasI, using two quorum-sensing systems, las and rhl. The lasI gene product directs the formation of a diffusable extracellular signal, N-(3-oxododecanoyl)-L-HSL. This autoinducer interacts with lasR to activate the virulence genes. Gene expression required for *P. aerginosae biofilm* formation can be suppressed by the presence of halogenated furanones, which are structurally similar to HSL and interfere with reception of the biofilm formation signal (Junker and Clardy, 2007). Alteration of the quorum sensing mechanism may be an effective method for treating infections that are caused by biofilms.

# IV. MICRORNAS IN LARGE DNA VIRUSES

## A. Generation of MicroRNAs

MicroRNAs (miRNAs) are 21 to 24 nucleotides long and are noncoding. They regulate gene expression by directly targeting specific mRNA molecules through mRNA degradation or inhibition of their translation. Since the first report in 2004, 100 viral miRNAs have been identified

(Grey and Nelson, 2008). The beta herpesvirus, human cytomegalovirus, generates 14 miRNAs (Table 9.6) (Rider et al., 2008). Alpha herpesvirus, herpes simplex virus type 1, and four gamma herpesviruses, Kaposi's sarcoma–associated herpesvirus, Epstein-Barr virus, Rhesus lymphocryptovirus, and mouse gamma herpesvirus 68 are also large DNA viruses that encode miRNAs.

MiRNAs are transcribed by RNA polymerase II in the nucleus as single-stranded RNA precursor called primary miRNA. The nuclear RNase III endonuclease enzyme, Drosha, excises a 65–70 nucleotide hairpin with a two-nucleotide, 3' overhang from the primary miRNA. This stem-loop structure is called pre-miRNA, which is transported into the cytoplasm by exportin 5 in a Ran-GTP-dependent manner (Rider et al., 2008). The cytoplasmic RNase III type endonuclease, Dicer, then removes the stem loop of pre-miRNA creating a 21- to 24-nucleotide double-stranded RNA with a two-nucleotide, 3' overhang on each strand. These duplexes are unwound and one strand is preferentially loaded into a protein complex called the RNA-induced silencing complex (RISC). This loaded single-stranded RNA is called the *guide strand*, which directs an endonuclease within RISC to cleave mRNAs that have perfect or near-perfect homology with the guide strand. Nucleotides in position 2 through 7 of the miRNA function as the seed region of the guide strand. Less-stringent homology between miRNA and sites within the 3' untranslated region of the target will lead to inhibition of translation rather than mRNA degradation. There are approximately 600 human miRNA sequences that regulate gene expression in such diverse areas like immune response, neural development, DNA repair, apoptosis, and oxidative stress response.

## B. Human Cytomegalovirus miRNAs

Candidate miRNA sequences were located in human cytomegalovirus (HCMV) using a computer algorithm called Stem-loop Finder (Grey and Nelson, 2008). The function and targets of these 14 viral miRNAs are largely unknown. HCMV miRNAs might down-regulate cellular mRNAs encoding antiviral factors (Cullen, 2009). Most of the other herpes viruses have their miRNA clustered in small regions of the genome, but this clustering does not occur in the HCMV genome (Rider et al., 2008). Viral protein production in large DNA viruses is temporally regulated in three phases (Cullen, 2009). The immediate early phase generates regulatory proteins; the early phase provides a more diverse array of proteins, including those needed for viral genome replication; and the late phase generates primarily structural proteins. In HCMV, most miRNAs are classified as early genes. Two miRNAs, mir-UL70 and mir UL112, are immediate early genes (Rider et al., 2008).

The role that miRNAs may play in the HCMV latent and productive replication cycles is relatively unknown. HCMV has several mechanisms available to prevent programmed cell death or apoptosis of infected cells. HCMV genome encodes a mitochondrial-localized inhibitor of apoptosis as well as an inhibitor of caspase 8-induced apoptosis. HCMV noncoding RNA, β2.7, is produced in the early viral replication phase. β2.7 holds the components of the mitochondrial respiratory chain complex I together. This stabilization ensures that the complex I will remain bound to the inner mitochondrial membrane, thus preventing apoptosis.

HCMV miRNA, mir-UL112-1, targets the mRNA that encodes major histocompatibility complex class I polypeptide-related sequence, or MICB. MICB is a cellular ligand for the activating receptor NKG2D which is expressed on natural killer (NK) cells. NK cell activation can result in the death of HCMV-infected cells. The proposed target for mir-UL112-1 is in the 3' untranslated reading frame of MICB mRNA. Cells that can produce mir-UL112-1 have less cell-surface MICB and are resistant to NK cell killing. Mutant cells without mir-UL112-1 have abundant MICB bound to NKG2D receptors and active NK cell killing (Cullen, 2009; Grey and Nelson, 2008; Rider et al., 2008). HCMB miRNA, mir-UL112-1, also down-regulates the expression of HCMV genes involved in its own replication. This restriction of viral replications helps maintain the infected cells' longevity by reducing the production of toxic viral proteins. Mir-UL112-1 targets a host mRNA that encodes on immediate early protein 72 (IE 72). IE 72 regulates the transcription of viral genes required for viral replication.

# V. LEISHMANIA

## A. Variety of Disease Presentation

Nearly two dozen distinct *Leishmania* species cause infections in multiple endemic areas in both children and adults (Murray et al., 2005). Annually, approximately 1.5 to 2 million children and adults develop symptomatic disease (cutaneous 1 to 1.5 million; visceral 0.5 million). There are approximately 70,000 deaths per year. Seventy of approximately 1000 known sandfly species transmit leishmaniasis by innoculating skin with flagellated promastigotes. The promastigotes invade macrophages or neutrophils and transform and replicate as amastigotes that infect other macrophages. The disease produced varies tremendously depending on the species, host response to the infection and region of origin (Table 9.7) (Murray et al., 2005).

Visceral leishmaniasis, kala-azar, results from widespread invasion and disabling of the mononuclear-phagocytic system by *Leishmania donovani* and rarely by *L. amazonensis* or other cutaneous pathogens, especially in immunocompromised patients. Visceral leishmaniasis is most common in southern Asia and Sudan (Table 9.7) and leads to marked organomegally, especially the spleen and liver, and is

**TABLE 9.7 Regions Where 90% of Leishmaniasis Occur**

| Cutaneous Leishmaniasis | Visceral Leishmaniasis |
|---|---|
| Afghanistan | India |
| Pakistan | Bangladesh |
| Syria | Nepal |
| Saudi Arabia | Sudan |
| Iran | Brazil |
| Brazil | |
| Peru | |

*Collated from Murray et al., 2005.*

uniformly fatal if not treated. Visceral leishmaniasis, especially in India, is due to *L. donovani donovani* and only infects humans. Spread is from human to human (Murray et al., 2005).

Mucocutaneous leishmaniasis, naso-oro-pharyngeal leishmaniasis, is a severe hyperergic and highly disfiguring disease that is endemic in parts of South America, especially Brazil. It is caused primary by *L. braziliensis braziliensis,* but sometimes by *L. braziliensis guyanensis,* and occasionally similar but not classical lesions occur in infections by organisms of the *L. mexicana* complex, especially *L. mexicana amazoniensis* (Murray et al., 2005).

Cutaneous leishmaniasis is a relatively localized and mild disease. Amastigotes circulate in active leishmaniasis and parasitemia is frequent in immunodeficiency patients with HIV associated with kala-azar. However, asymptomatic parasitemia also occurs in immunosuppressed individuals with subclinical visceral infections (Murray et al., 2005).

## B. Diagnosis

Leishmaniasis is an infectious disease that is most prevalent in underdeveloped countries that have limited access to culture techniques or molecular diagnostic methods. In these undeveloped countries, where leishmaniasis is endemic, the microscopic observation of amastigotes in clinical specimens is the preferred diagnostic method for cutaneous, mucocutaneous, and visceral leishmaniasis (Murray et al., 2005; Reithinger and Dujardin, 2007). A fingerstick blood test has been used for the rapid detection of the anti-K39 antibody using an immunochromatographic strip in the field to detect symptomatic patients with kala-azar. The sensitivity of this assay is 90% to 100% (Murray et al., 2005; Reitthinger and Dujardin, 2007).

*Leishmania spp.* is a hemoflagellates that has a giant mitochondrion called a kinetoplast located adjacent to the basal body of the flagellum, consisting of DNA maxicircles and minicircles, equaling 5% to 10% of the total DNA. The minicircles have been used as DNA targets due to their very large numbers per cell. These repeat sequences are also attractive PCR targets (Espy et al., 2006; Reithinger and Dujardin, 2007). However, kinetoplast DNA has a high degree of polymorphism and, therefore, appropriate internal controls and leishmania standard controls will need to be used to adequately monitor the quality of each assay (Espy et al., 2006; Reithinger and Dujardin, 2007). The 18S rRNA target has been used successfully in simple to complex assay designs for the identification of *Leishmania spp.* (Reithinger and Dujardin, 2007). Further work remains to be done to develop a simple, reliable, point-of-care molecular technique for use in underdeveloped countries for *Leishmania spp.* identification.

## C. *Leishmania spp.* Genome Sequences

The genome of three *Leishmania spp.* (*L. braziliensis–mucocutaneous leishmaniasis, L. major–cutaneous leishmaniasis,* and *L. infantum–visceral leishmaniasis*) and one sandfly vector (*Lutzomyia longopolpis*) have been sequenced to date. *L. major* has 8,298 coding genes and 97 pseudogenes compared to *L. infantum*, with 8,154 coding genes and 41 pseudogenes and *L. braziliensis* with 8,153 coding genes and 161 pseudogenes (Peacock et al., 2007). There is a lack of functional information related to more than 50% of the genes identified. *L. braziliensis* has features distinct from *L. major* and *L. infantum. L. braziliensis* has 35 chromosomes, while the other two have 36. *L. braziliensis* is the only one of the three that has miRNA that could function as gene-silencing agents. A gene has been identified that encodes a Dicer-like protein that converts double-stranded RNA into small, single-stranded miRNAs. Also, a gene that encodes an Argonaute protein or the "slicer," which is an endonuclease found in the RISC, which cleaves the mRNA that contain sequence homology to the single-stranded RNA called the guide strand within RISC (Peacock et al., 2007).

Efforts to determine which genes that are specifically located in one *Leishmania spp.* that direct specific host response, virulence factors, and disease severity have revealed nothing of significance at this time. However, after the functionality of the entire set of genes within the genomes are identified, this type of investigation may become more fruitful.

# VI. THE FUTURE

The exponential explosion of molecular assays for the detection of infectious agents has aided in the rapid identification and treatment of specific infectious diseases (Espy et al., 2006; Reithinger and Dujardin, 2007). The rapid detection of respiratory viruses in multiple assays is commercially available. However, the screening for Gram-negative and Gram-positive bacterial agents in a microarray or other

multiplex format is still not feasible. The use of 16S rDNA PCR has failed to detect a significant number of culture-proven newborn septic events (Jordan et al., 2006). Another target may need to be explored.

The availability of reliable, simple, portable molecular devices are needed in underdeveloped countries to detect endemic infectious diseases caused by parasites, fungi, viruses, and bacteria (Dong et al., 2008). The development of miniaturized molecular techniques using microfluidics and smaller volumes of specimen and reagents will lead the way to this solution in the future (Holland and Kiechle, 2005). We are in an era where the number of molecular assays for infectious agents needed clinically is greater than the number currently available. The years ahead will see the classic microbiology laboratory functions decrease as new molecular methods are introduced to perform the initial detection and drug sensitivity of infectious agents.

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