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Review

Molecular methods for pathogen and microbial community detection and characterization: Current and potential application in diagnostic microbiology

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ABSTRACT

Clinical microbiology laboratories worldwide have historically relied on phenotypic methods (i.e., culture and biochemical tests) for detection, identification and characterization of virulence traits (e.g., antibiotic resistance genes, toxins) of human pathogens. However, limitations to implementation of molecular methods for human infectious diseases testing are being rapidly overcome allowing for the clinical evaluation and implementation of diverse technologies with expanding diagnostic capabilities. The advantages and limitation of molecular techniques including real-time polymerase chain reaction, partial or whole genome sequencing, molecular typing, microarrays, broad-range PCR and multiplexing will be discussed. Finally, terminal restriction fragment length polymorphism (T-RFLP) and deep sequencing are introduced as technologies at the clinical interface with the potential to dramatically enhance our ability to diagnose infectious diseases and better define the epidemiology and microbial ecology of a wide range of complex infections.

Contents

1. Introduction

Defining the etiological agent(s) of infection facilitates more effective patient management by tailoring specific targeted antibiotic therapy. Clinical microbiology laboratories worldwide have historically relied on phenotypic methods (i.e., culture and biochemical

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tests) for detection, identification and characterization of virulence traits (e.g., antibiotic resistance genes, toxins) of human pathogens. However, limitations to implementation of molecular methods for human infectious diseases testing are being rapidly overcome allowing for the clinical evaluation and implementation of diverse technologies with expanding diagnostic capabilities. Molecular methods have been increasingly exploited to diagnose classic and fastidious pathogens, since the advent of the polymerase chain reaction (PCR) (Mullis and Faloona, 1987). Some infectious agents identified and largely characterized in the past two decades using molecular methods include; Hepatitis C virus (Choo et al., 1989), *Tropheryma whipplei* (Relman et al., 1992), *Mycobacterium genavense* (Bottger et al., 1992), *Bartonella* (previously *Rochalimaea*) *henselae* (Matar et al., 1993), Hantavirus (Sin Nombre virus) (Chapman and Khabbaz, 1994), *Chlamydia pneumoniae* TWAR agent (Kuo et al., 1995), Herpesvirus 8 (Huang et al., 1995), the SARS Coronavirus (Drosten et al., 2003; Ksiazek et al., 2003), Metapneumovirus (Mackay et al., 2003), and Influenza A H1N1 (Renesto et al., 2000; Dawood et al., 2009).

Commercial suppliers have been steadily expanding the number of molecular diagnostic assays for infectious diseases testing (http://www.fda.org). Despite these continuing advances, a microbial etiology remains to be defined in a number of cases of human disease that have epidemiological, clinical and/or histological features of infection. Worldwide populations are increasingly being put at risk for novel contagions, particularly zoonotic agents or bacterial pathogens carrying acquired antimicrobial resistance genes (Church, 2004). Broad application of highly sensitive molecular technologies to common infections is also revealing that these diseases are not mono-microbial as previously thought, but rather, due to a disorder in complex microbial communities.

The human microbiome comprises 100 trillion microbial cells, outnumbering the somatic cells in the body by at least an order of magnitude (Savage, 1977). It was suggested early on that in order to properly appreciate human biology the 30,000 genes encoded in the human genome must be placed in the context of the millions of genes encoded by our microbial counterparts that inhabit the gastrointestinal tract, the genitourinary tract, the oral cavity, the nasopharynx, the respiratory tract, and the skin (Davies, 2001). The human microbiome project was designed to sequence 1000 novel bacterial reference genomes and the entire collective of microbial genomes from 250 healthy individuals (Turnbaugh et al., 2007; Peterson et al., 2009). A vast number of human commensal microbial species have yet to be identified and characterized. The composition of the human microbiota is unique and varies significantly between body sites. A recent study showed that 3.3 million non-redundant genes (150 fold greater than the human genome) were assembled from 1000 to 1150 distinct microbial species from 124 human gut samples (Qin et al., 2010). The diversity of the human microbiome is also affected by environmental factors, diet, and the host immune system (Ley et al., 2006). The essential role that commensal micro-organisms play in human health is becoming increasingly recognized for its functional involvement in normal development, nutrition, immune regulation and colonization resistance (Brook, 1999; Hooper, 2004; Mazmanian et al., 2005; Li et al., 2008; Clarke et al., 2010). There is also evidence accruing that the functional capacity of the human microbiome may be altered in patients with obesity and other chronic conditions (Qin et al., 2010).

The molecular methods for diagnostic microbiology are diverse. The advantages and limitation of techniques including real-time polymerase chain reaction, partial or whole genome sequencing, molecular typing, microarrays, broad range PCR and multiplexing will be discussed. Finally, terminal restriction fragment length polymorphism (T-RFLP) and deep sequencing are introduced as technologies at the clinical interface with the potential to dramatically enhance our ability to diagnose infectious diseases and better define the epidemiology and microbial ecology of a wide range of complex infections.

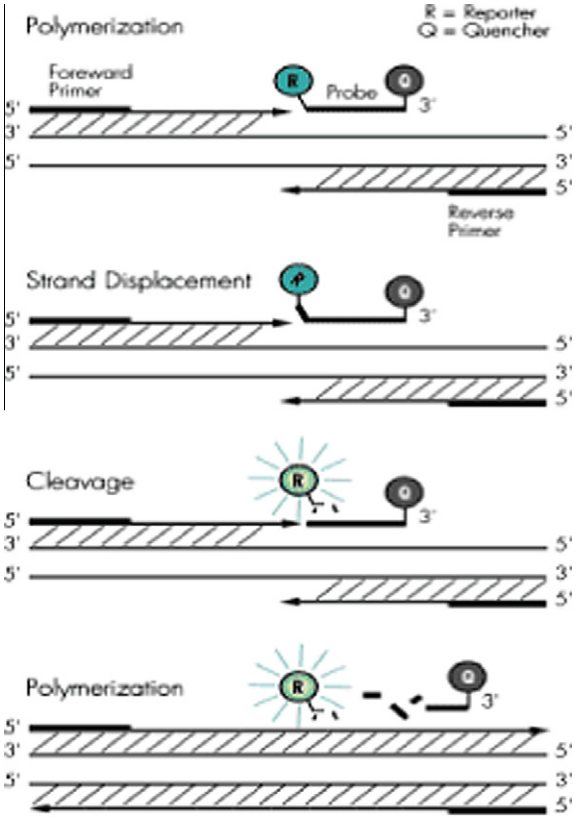

Fig. 1. Principles of real-time PCR Probes**. **Illustration from Heid et al. (1996).

2. Pathogen detection, surveillance and discrimination

Diagnosis of infectious diseases by using PCR involves target amplification methods and often nucleic acid sequence analysis of the amplicon. Unlike phenotypic characteristics that may be variable, molecular amplification methods take advantage of the use of stable genotypic characteristics. Another major diagnostic advantage of PCR is that the detection of nucleic acid does not require the labor intensive cultivation of microbial biomass. The bacterial genome provides variable regions for species identification and virulence factors or toxins loci can be useful for defining the pathogenicity of an organism. In addition to standard PCR, complete genome sequences have greatly facilitated the rapid development of other molecular identification methods for pathogen detection including nucleic acid hybridization (probes) (Procop, 2007), ligase chain reaction (Drancourt et al., 2000), strand-displacement amplification (SDA) (Walker et al., 1992a,b), transcription-based amplification (TAS, TMA, NASBA and 3SR) (Kwoh et al., 1989; Compton, 1991; Fahy et al., 1991) and loop-mediated isothermal amplification (LAMP) (Notomi et al., 2000). The enormous amount of nucleic acid sequence data from human pathogens has enhanced the diagnostic capabilities of sequenced-based identification and genotyping methods of both common and uncommon micro-organisms (Fournier and Raoult, 2011).

2.1. Real-time PCR

Despite the development of alternative amplification technologies, PCR remains the most widely used method in both research and diagnostic laboratories for pathogen detection (Lisby, 1998;