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Bacterial detection: From microscope to smartphone

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ABSTRACT

The ubiquitous nature of bacteria enables them to survive in a wide variety of environments. Hence, the rise of various pathogenic species that are harmful to human health raises the need for the development of accurate sensing systems. Sensing systems are necessary for diagnosis and epidemiological control of pathogenic organism, especially in the food-borne pathogen and sanitary water treatment facility's bacterial populations. Bacterial sensing for the purpose of diagnosis can function in three ways: bacterial morphological visualization, specific detection of bacterial component and whole cell detection. This paper provides an overview of the currently available bacterial detection systems that ranges from microscopic observation to state-of-the-art smartphone-based detection.

Contents

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1. Introduction

Bacteria are microorganisms that are a few micrometers in length and take the shapes of spheres, rods, or spirals. Bacteria sense and respond to temperature and pH changes, nutritional starvation, stresses to the outer and inner membranes, new food sources, toxins, and quorum sensing signals (Salis et al., 2009). One of the processes associated with bacterial infection is chemotaxis, which involves the attraction or repulsion of the bacteria from chemical stimulus (Grebe and Stock, 1998). Pathogenic bacteria are harmful species that cause bacterial infections and contagious diseases that result in many serious complications. Common pathogenic bacteria and their effects include *Escherichia coli* and *Salmonella* (food poisoning), *Helicobacter pylori* (gastritis and ulcers), *Neisseria gonorrhoeae* (sexually transmitted disease), *Neisseria meningitidis* (meningitis), *Staphylococcus aureus* (boils, cellulitis, abscesses, wound infections, toxic shock syndrome, pneumonia, and food poisoning), and *Streptococcus* spp. (pneumonia, meningitis, ear infections, and pharyngitis). Records from the Foodborne Diseases Active Surveillance Network show that between 1996 and 2005, 121,536 cases of laboratory-confirmed bacterial infections were reported in the United States, resulting in 552 deaths, of which 215 were due to *Salmonella* and 168 due to *Listeria* (Behravesh et al., 2011). Epidemic diseases caused by foodborne pathogenic bacteria are major health issues, which necessitate an immediate detection strategy (Zhang, 2013). This is because the clinical presentations for most of the pathogenic infections are not specific enough for definitive diagnosis. The main issue in diagnostic is to distinguish between the closely related sub-species and between pathogenic and non-pathogenic strains. Sensing strategies developed should be rapid and involve sensitive procedures for early screening and proper enumeration of the identified pathogenic strains. The rapid and accurate detection of pathogenic bacteria is vital for the administrations of appropriate antibiotic treatment to control the spread of the disease and to assess the drug resistance information. Detection of pathogenic agents is also a vital step for the identification of infection source anywhere from home, hospital to outdoor settings (Klompas et al., 2009; Allegranzi et al., 2011; Polin et al., 2012).

Fundamental identification of bacteria relies on the morphological features of the cells, which can be visualized via microscopic observations. Other common methods include Gram staining, culturing, and biochemical assays and sequence-based detection. In addition, probes (e.g., aptamers or antibodies) that are specific to surface/flagellin proteins of the bacterial or the bacterial whole cell, are also used to detect the presence of bacteria. These probes are often applied in biosensors for the detection of specific types of bacteria. This review provides an overview of the currently available bacterial detection strategies.

2. Microscopic examination

Microscopes are optical-based instruments that are used to visualize samples that cannot be seen by the naked eye (Fig. 1a and b). Bacteria were initially observed by the Dutch microscopist van Leeuwenhoek using a single-lens microscope (Porter, 1976). Direct observation of bacteria under microscope is the simplest and cheapest way for bacteria identification. Bacteria are identified and classified based on the various shapes, sizes and arrangements (for examples coccus, bacillus, filamentous, in pair or chain formation). Beside, based on cell wall properties, bacterial can be identified under microscopic observation due to their staining properties, such as Gram positive, negative, or acid-fast staining). The application of labeled tags (fluorophores, quantum dots etc) has also enhanced the precision of the microscopic identification of bacteria. For example, fluorophores tagged to probes specific to a given microorganism can be visualized with a fluorescence microscope. Recently, fluorescence microscope was used to visualize *Pseudomonas aeruginosa* with the aid of fluorescently labeled nucleic acid probe (Wang et al., 2011b). Steingart et al. (2006) reported that use of a fluorescence microscope that enhanced the smear microscopic visualization by 10% over the conventional Ziehl–Neelsen (ZN) staining method for *Mycobacterium tuberculosis* visualization. Another microscope, LED (light-emitting diodes) microscope, which is characterized by long lifespan and low power consumption, is beneficial in countries with limited resources. LED microscope was used to visualize *M. tuberculosis* (Trusov et al., 2009; Albert et al., 2010). Although not as sensitive as fluorescent microscopes for visualizing *M. tuberculosis*, the LED microscope provides faster reading with exceptional reproducibility and easy to be used (Bonnet et al., 2011).

Another technology that may prove to be useful for laboratories with limited resources is the portable digital microscope known as CellScope, which has the capacity of both bright-field and fluorescence microscopy (Chang et al., 2012a, 2012b). The images obtained are binarized images that have patch sizes that match the size of *M. tuberculosis* bacilli (2–4 μm in length, 0.5 μm in width). This microscope, together with the portable automated low-cost bright-field/fluorescence microscope, may slowly replace the bulky high-cost, conventional laboratory-grade optical microscopes (Schaefer et al., 2012). With the advancements of technology, microscopes with higher resolutions have been introduced, including scanning electron microscopes, transmission electron microscopes, environmental scanning electron microscopes and atomic force microscopes (Jung et al., 2010). Although microscopic examination represents a simple and cheap way for bacterial identification, it cannot be used for differentiation between different bacterial strains. However, this is possible by using sequence specific detection.

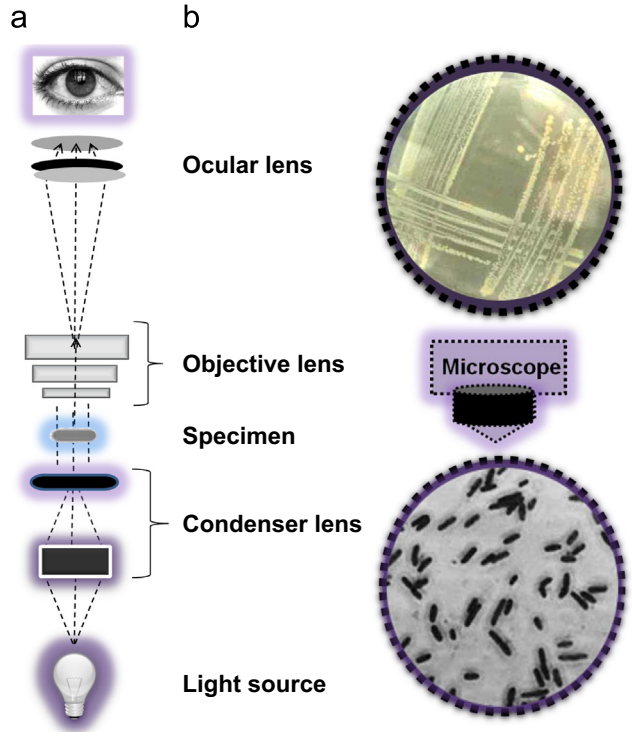

Fig. 1. Microscopic observation of bacteria. (a) Basic set-up of a light microscope and (b) Microscopic image of bacteria.