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Introductory Chapter: Why Do We Need Rapid Detection of Pathogens?

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1. Importance of pathogens

A pathogen is defined as an infectious biological agent, which can be a virus, bacterium, fungus, or other microorganism being the first link in the chain of infections and diseases. We are all exposed to pathogens in our everyday life, but normally they cause no harm as the body's immune system eliminates them. In order to survive and multiply, pathogens must be able to colonize the host, replicate, and spread to a new host.

Pathogens can be divided into human, animal, and environmental pathogens [1, 2]. The two major subdivisions of environmental pathogens are foodborne and waterborne pathogens [3]. The key difference between environmental pathogens and human along with animal pathogens is their ability to survive and thrive outside the host [3].

Environmental pathogens are defined as microorganisms that normally spend a substantial part of their lifecycle outside hosts. They are born in the water, soil, air, food, and other elements of our surroundings, and influence individual organism [3]. Foodborne diseases are caused by the consumption of food or water contaminated with pathogens or their toxins. The common foodborne pathogens, which are responsible for most of the foodborne disease outbreaks, are *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella enterica*, *Bacillus cereus*, *Vibrio* spp., *Campylobacter jejuni*, and *Clostridium perfringens* [2].

The increasing demand for street food and for minimally processed ready-to-eat products has increased concerns about food safety [2]. We should carefully control the production processes in the food and agricultural sectors to assure high standards for food quality and safety. Most waterborne pathogens (*Salmonella typhimurium*, *Vibrio cholerae*, *Legionella*, *Escherichia coli* O157:H7, and *Campylobacter jejuni*) do not grow in water, and are introduced into drinking-water supplies with human or animal feces. These pathogens can initiate infections in the

gastrointestinal tract following ingestion [1]. It has been calculated that diseases caused by waterborne pathogens have an annual economic cost around 1 billion dollars in the US and nearly 12 billion USD worldwide [1]. Based on this threat, infections caused by contaminated water have a considerable impact and testing of the safety of drinking-water should be improved.

Treating infections with broad-spectrum antibiotics in cases where timely treatment is unavoidable, but the causative agent has not yet properly identified is a common practice. This can cause major damage to the normal microbiota of host organism and pose a global threat of spreading drug-resistant bacteria [4]. Decades of research into antibiotic development has produced highly effective and safe antibiotics, giving excellent tools for prevention and focused fight with bacterial infections [4]. However, release of each new drug has been inevitably followed by a rapid propagation of resistant pathogens. This issue has become a serious threat, causing annually at least 23,000 deaths in the United States [4] and about 25,000 deaths in the European Union [5].

In US, it is suggested that around 80% of the nation's annual antimicrobial consumption is used in food animals for medical procedures, disease prevention, and growth promotion [6]. So, the misuse of antibiotics due to insufficient identification of infection-causing pathogens in veterinary has even a bigger impact on the spread of drug-resistant bacteria.

The availability of modern detection methods plays a key role in the speed and quality of monitoring, surveillance, and quantitative microbial risk assessment, and has a major influence on implementing the best practices to prevent threats [1].

2. Current methods for pathogen detection

How to detect small numbers of pathogens in large numbers of harmless microflora in a large and complex sample matrix? How to make sure that the strains recovered are indeed pathogenic?

The gold standards for pathogen detection are culture-based methods [7, 8]. The culture-based methods or count methods of culturing and colony – detecting of microorganisms – are based on the integration of the sample into a nutrient medium in which the microorganisms can multiply, thus providing visual confirmation of their growth [9]. Although these methods are simple, easily adaptable, and generally inexpensive, they are laborious, limited by low sensitivity (false negative results), and require relatively long time to perform as they depend on the ability of the microorganisms to grow in different culture media [9]. It commonly takes 2–3 days to get initial results, and up to 1 week to get final information about the specific pathogen causing the infection or disease with culture method [9].

In recent decades, many new methods have emerged for the rapid diagnostics of bacterial infections. Microbiological analysis are based on the detection of microorganisms by visual, immunological, or genetic means, either before (enumerative methods) or after enrichment of samples [9].

The most widespread methods for pathogen detection are polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). PCR method is very specific and can be used to identify microorganisms that cannot be readily cultured. However, the PCR method which requires amplification, isolation, and quantification of DNA is a complex technique to use and requires costly instruments and trained personnel [10]. In comparison with PCR analysis, ELISA is less complicated and less expensive, but real-time detection is not possible due to the need of incubation of samples for 2–3 h [10]. Therefore, neither PCR nor ELISA techniques meet the criteria of carrying out on-site rapid analysis of pathogens, therefore alternative methods are in urgent need. The main advantages of rapid detection techniques are the possibility of earlier interference and faster focused action to potential problems, but also improved throughput of analysis.

Novel technologies for the detection of pathogens are of critical importance, and extensive research and development activities are going on with the aim to reduce assay time and reduce the amount of manual labor by automating methods whenever possible [8, 9]. The sensitivity of assessment is another major parameter in cases when potential risk of infections is caused by low number or a single pathogen.

Modern biotechnologies are important in many fields: agriculture, medicine, environmental monitoring, and in food industry as they are improving the ability to detect pathogens quickly and effectively. Nevertheless, the development of new methods has many challenges. These methods should be capable of concentrating pathogens and removing matrix-associated inhibitors, should be simple, rapid, and inexpensive; they should be able to eliminate or reduce the need for culture enrichments and minimize the chance for false-positive results [8].

In recent years, there has been a constant growth in the field of pathogen biosensing due to modern developments of novel electronic devices. Biosensor-based technologies commonly rely on the specific recognition of antigen epitopes of pathogen targets by a recognition agent-like antibodies or aptamers. These immunosensing technologies offer prospective features like real-time, on-site, simultaneous multiplex detection of different pathogenic agents integrating the selectivity of biomolecules and the processing power of modern nanoelectronics [11]. One must also remember that even having established a rapid and reliable method for the detection of pathogens, we should remember that detection technology is not the only aspect to consider and we still have to follow strict sampling procedures to avoid contamination. Otherwise, the results can be meaningless or even worse—misleading.

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