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A REVIEW ON FOOD POISONING AND RAPID METHODS FOR DETECTION OF BACTERIAL TOXINS

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Abstract:
Foodborne disease is one of the most common health problems in world-wide. Foodborne pathogens can be found in various foods and causes various illness in humans. Foodborne diseases lead to morbidity and mortality in the few population and they have emerged as a growing public health and economic problem in many countries during the last 2 decades. In India there are no systematic studies to understand the types of foods involved and the etiological agent causing the disease. Food poisoning covers a wide range of food-borne diseases caused by an equally wide range of microorganisms. Most cases of food poisoning are infections caused by bacteria such as Salmonella, Staphylococcus aureus, Clostridium species, Listeria monocytosis, Yersinia parahaemolyticus, Campylobacter, Shigella, Toxigenic E. coli, Bacillus cereus, Vibrio species, etc., or by some viruses and parasites. Less common but equally important from a food safety point of view is food poisoning as a result of intoxication. Intoxication results from ingesting pre-formed bacterial toxins in food. Live bacterial cells need not be present for food illness to develop. There is also a third type, intermediate food poisoning, caused by toxins produced during bacterial growth in the gut. Traditional methods can be time-consuming and difficult to perform. Rapid ELISA and RPLA-based methods are faster and simpler to use. Lateral flow immunoassay shows potential for detecting botulinum toxins. Techniques such as Polymerase chain reaction greatly affect the detecting of food-associated pathogenic bacteria and their toxin. This review paper highlights types of PCR techniques used to detect various pathogens and their toxins.

Keywords: Food poisoning, Toxin Detection, Various techniques.

INTRODUCTION:

Foodborne disease is a major public health concern among worldwide, it is mainly due to the high incidence of foodborne diseases over the past 20 years. However, it is very difficult to evaluate the global incidence of foodborne diseases as few cases are under-reported especially in developing countries, but the increased incidence of foodborne diseases was reported in many parts of the world. [1,5] The WHO estimates that there are more than 1000 million cases of acute diarrhea annually in developing countries, with 3-4 million deaths[6]. Factors leading to food poisoning, and enteric pathogens have developed a variety of strategies to overcome the host defense mechanism, understanding the virulence factors employed by these organisms is important in the diagnosis and treatment of clinical diseases. Numerous organisms must adhere to the gastrointestinal mucosa as an initial step in the pathogenic process, thus the organism that can plays a major role in the normal bowel flora and colonize the mucosa has an important advantage in causing diseases. The production of one or more exotoxins is important in the pathogenesis of numerous enteric organisms such toxins include enterotoxins, which cause watery diarrhea by acting directly on secretory mechanisms in the intestinal mucosa, cytotoxins which destroy mucosal cells and associated inflammatory diarrhea, neurotoxins which can act directly on the central and peripheral nervous system[7]. There are also two other groups of toxins, those that alter the cytoskeleton and those with neurotoxic activity. However, some toxins may present activity corresponding to more than one of the groups described in [Table 1].

Pathogenic bacteria possess an array of virulence factors that allow them to colonize, invade, and replicate within an immune-competent host. Many bacteria produce toxins, enzymes, and pigments, toxins and enzymes play an important role in pathogenicity. Bacterial toxins are virulence factors that manipulate host cell functions and take over the control of vital processes of living organisms to favor microbial infection. The mechanism by which pathogens interfere with host cellular processes often involves toxins secreted across their outer membrane through different secretion systems or directly injected into the host cell through the bacterial type 3 secretion system [T3SS] or T4SS secretion apparatus[8,9].

Table-1: Different types of Toxins produced by Bacteria.

TOXIN TYPE	DEFINITION

Enterotoxin	It produces a net secretion in ligated intestinal Segments without historical evidence of intestinal Lesions or damage to non-erythrocyte cells in vitro test. It stimulates the increase in the Sport circuit current [ISC] and the potential difference [PD] in the using chamber without Evidence of intestinal damage. This result involves the secretions of electrogenic anions. Additionally, a toxin can impair electrically neutral NaCl absorption, which also results in a net Secretion of ions.
Cytoskeleton-altering toxin	It alters the cellular form and has been frequently shown to be caused by the F- actin rearrangements. The toxin can cause limited cell damage but is not Lethal, and it may or may not be associated with the Evidence of net secretion in vivo or in vitro disease Models in intestinal epithelial cells.
Cytotoxins	It causes cell or tissue damage, usually ending with Cell death. The toxin may or may not be associated With net secretion in vivo or in vitro disease model In intestinal epithelial cells.
Neurotoxins	Involve the release of one or more Neurotransmitters from the enteric nervous System. It alters the activity of the smooth muscle in the intestine.

BACTERIAL TOXINS:

Bacterial toxins are mainly divided into three types based on their mode of action. Type 1 toxin, type 2 toxins, type 3 toxins.

Type I toxins: This type of toxins will disrupt the host cells without the need to entry into the host cell. Few intracellular targets of type 1 toxins have been identified, possibly due to the difficult nature of analyzing proteins that are poisonous to their bacterial hosts. These also include super antigens [Sag's] produced by *S. aureus* and *S. pyogenes*.

Type II toxins: There are two toxins namely hemolysin and phospholipases which plays a role in destroying the host cell membranes to invade and interrupt host defense processes within the cells. Damages host cells release danger-associated molecular patterns [DAMPs] that bind to pattern recognition receptors [PRRs] causing the release of inflammatory cytokines.

Type III toxins: They are also known as A/B toxins, mainly due to their binary structure, main principle is to disrupt host cell defenses. B component of these toxins binds to the host cell surface, while the A component possesses the enzymatic activity to damage the cells[8].

DETECTION METHODS:

❖ **Biological Assays:**

Bioassay and related tests remain the method of choice for some bacterial toxins [e.g. Botulinum toxins], many bioassay formats have been described, including the whole animal tests, [e.g. Monkey and kitten emesis tests, mouse lethality test, and guinea pigs skin tests], and cell culture system[10]. The detection limit is quite low and the technique is highly selective compared to other detection methods. The advantage of the test is it requires expert personnel to complete the test. Another disadvantage is that the testing kits are expensive for some sampling tests.

❖ **Immunological Assays:**

The immunological detection technique is used for the detection of pathogens[12]. The antigen-antibody binding is utilized widely in the immunological detection of pathogens from Gram-Negative bacteria which includes Enzyme Immunoassay [EIA], Enzyme-linked immunofluorescent assay [ELFA], Enzyme-linked immunosorbent assay [ELISA], and other immunological methods, are frequently used in detection. They require less time to prepare the assay than a culturing technique[14,15].

❖ **Polymerase Chain Reaction:**

The general PCR technique has been used in many applications for pathogen detection in food and has been reviewed previously. The specificity of a typical PCR regime is a result of DNA sequence-specific oligonucleotide primers that initiate repetitive rounds of in vitro replication from a target gene fragment through denaturation, primer annealing, and new strand synthesis[16,17].

❖ **Real-Time PCR:**

The basis PCR cycling series can be divided into three phases, Exponential, Linear, and Plateau. The exponential phase of amplification is the most accurate stage for quantification of products,

rather than the plateau phase when reaction conditions are suboptimal and the relative amplicon band intensities of a set of templates that we are varying concentrations before PCR are now essentially equivalent. PCR assay that measure's reaction progress during each amplification, rather than after reaching a plateau, represents an attractive means of obtaining real-time quantitative data for rapid and sensitive detection by Uniplex, Multiplex, Nested, PCR, or fingerprinting-based variations on the common theme of PCR to detect DNA or RNA[18,19]. Such methods, termed real-time PCR, are also referred to as rapid-cycle, kinetic, or homogenous PCR, and can require less than 40 minutes for amplification and data analysis[20,21].

❖ **RNA Assay: - Monitoring Virulence Gene Expression in Food Pathogens:**

Although DNA is most generally selected as a target molecule when designing a PCR-based detection assay for food-borne pathogens, differentiation of living from dead bacteria is not possible because DNA is quite persistent in dead cells[22]. Moreover, traditional traditional-culture-based approaches for enumeration of sub-lethally injured, viable but non-culture [VBNC], or both types of bacteria are not accurate because the selective media employed prevents many such bacteria from growing to visible, countable colonies. Pathogenic bacteria can become sub-lethally injured or stressed during specific stages of food processing or storage, and enter a VBNC state, but still pose a threat to the consumer[23]. Commercially available dyes that interact with DNA from dead cells and prevent traditional PCR-based detection have been augmented by confocal laser scanning microscopy, although such methods are labor-intensive for multiple samples[24]. Therefore, in order to detect and monitor pathogenic food-borne bacteria accurately, as well as to assess virulence gene expression, RNA-Based methods must generally be used.

When selecting RNA as a determinant of a cell's physiological state, one must bear in mind that ribosomal RNA [rRNA] is not an appropriate target because bacterial ribosomes are stable for at least 48 hours after cell death[24,25]. Ultimately, only m RNA is ideal to use as an indicator of either the metabolic status of bacteria or to ascertain the presence of VBNC pathogens[25]. In practice m RNA amplification by RT-PCR has been used to monitor cell viability in bacteria of reverence to the food industry[26].

❖ **Nucleic Acid Sequence-based amplification:**

Alternatively, a more rapid means of RNA analysis has been applied in studies of virulence gene expression in bacteria and viruses for Clinical Microbiology and lends itself particularly well to viable cell determination. First described by Compton et al, nucleic acid-based amplification [NASBA] is an isothermal cyclic series of reactions that use RNA as a template combined with an enzyme cocktail[32].

❖ **Microarrays:**

However, traditional methods in molecular biology generally work on a "one gene in one experiment" basis, which means the throughput is very limited and the whole picture of gene function is hard to obtain. In the past several years, the technology of DNA microarrays has attracted tremendous interest among molecular biologists and offers much in the way of high-throughput analysis of virulence gene expression in food-associated pathogenic bacteria or simultaneous detection of multiple pathogens[27]. DNA microarray technology has been a most powerful technique in areas of clinical and environmental microbiology since its inception and will likely demonstrate great potential in the food industry as a sensitive means of detecting gene expression in the battery of target pathogens[28,29,30].

❖ **Prevention of bacterial food poisoning:**

Storage of foods will help to prevent from food poisoning. There are lot of preservation method to store the food and beverages. Frozen storage of foods will also help in prevention of contamination. Many forms of bacterial food poisoning can be prevented, even if the food has been contaminated, by adequately cooking the food, and either eating it directly and quickly, or freezing it effectively. Prevention of food poisoning has to be done by the health authorities by strengthening the disease surveillance system in all the eatable products. The health authorities should strengthen considerably the foodborne disease surveillance system and follow it with efficient education and extension activity or various aspects of food safety.

CONCLUSIONS:

It is hard to estimate that foodborne illness occurs due to different microbial hazards. Detection of pathogens and toxins at an early stage is crucial to avoid food poisoning and other problems. Bioassays and related methods are still the test of choice for detecting many toxins. These assays require purification of the toxin before testing. Techniques such as PCR acceptance of various Nucleic Acid-based methods for rapid and sensitive detection of food-associated pathogenic bacteria have in some cases replaced traditional methods for bacterial enumeration in food. These approaches afford the ability to amplify DNA or RNA, as well as detect and confirm target sequence identity. Evidence of this systemic review suggests that rapid diagnostic assays such as PCR are much simpler and cheap, and therefore have to adapt widely in food microbiology.

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