INTRODUCTION
1.1. Introduction

Food contamination demotes the presence of harmful chemicals and microorganisms which can cause consumer illness. All food we consume should be free from contamination and spoilage at all stages of its journey from its starting place until it reaches the consumers. However, food contamination is a severe public health crisis, causing foodborne diseases that affect many people every year. Hence, awareness of probable sources of food contamination is an important part of good nutrition and good health. Non microbiological contamination refers to the unintentional or accidental addition of by-products and other physical and chemicals agents (Gabriel, 2008; Dougherty, 2006). Microbiological contamination refers to intended or accidental addition of, infectious material like bacteria, yeast, mould, fungi, virus, prions, protozoa or their toxins (Gabriel, 2008). Food contamination can be microbial or environmental, with the latter being more uncommon. Environmental contaminants that can contaminate the food chain include pesticides, heavy metals, and other chemical agents (Dougherty, 2006). Bacteria are a major source of microbial contamination of food. Microorganisms (commonly called microbes or bugs) are ubiquitous found all around us, in the air, on the ground, in fact everywhere we look. They are believed to be the first life forms to colonise our planet, so starting the evolutionary journey to where they are today. They are still the bedrock of the food chain, providing sustenance for a host of other creatures, whilst adapting to take advantage of every available source of energy, no matter how seemingly inhospitable the environment is. All they need is moisture to give them a kick start. In 1999 it was estimated that food-borne pathogens were responsible for 76 million illnesses annually, resulting in 5,000 deaths (Mead et al., 1999). Reports recognized Salmonella, Listeria and Toxoplasma as the major causative agents, responsible for 1,500 of the reported deaths. Additionally to the health risk associated with contaminated foods, the food producer often faces devastating economic impact. In 2007, 21.7×10^6 lb of ground beef owing to contamination with E. coli O157:H7 resulted in the Topps Meat Company going out of business after 67 years of operation (Neuman, 2011). Certainly the consolidation of food producers means that the
larger companies have a larger accountability toward protecting the food supply. Infectious diseases whose occurrence have increased in the past 20 years, threatens to increase in the near future. 31 pathogens are known to cause food-borne illness (CDC 2011). Many of these food borne pathogens are tracked by public health systems that cause diseases and outbreaks.

Food-borne pathogens are a major risk to food safety. Most food-borne illness is caused by contamination by microbial pathogens that have entered the food chain at some point from farm to fork. Food-borne illness can also be caused by toxins (microbial or chemical) that have infected food. The bulk of cases of food-borne diseases are mild and self-limiting, although unpleasing. But, infrequently, more serious illness or even death may result. Around one-third of individuals in developed countries are affected by food-borne illness each year. In addition to disease caused by direct contamination by food-borne pathogens, food-borne illness can also be caused by microbial toxins that contaminate food (Udakis, 2010). Few bacterial species contaminating food include *Salmonella*, *Bacillus cereus*, *Escherichia coli*, *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium perfringens*, *Listeria monocytogenes*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus sp.* etc.

*Bacillus cereus:* has been distinguished as a cause of food poisoning since 1955. There are only a few outbreaks per year reported by CDC. Between 1972 and 1986, 52 outbreaks of food-borne illness related to *B. cereus* were reported to the CDC (in 2003, there were two), but this represents only 2% of the total cases which have occurred during these periods. Since, it is not a reportable disease, it usually goes undiagnosed. *B. cereus* causes two types of food-borne illnesses. Emetic type, which is characterized by nausea, vomiting and abdominal cramps within 1 to 6 hours of infection. It resembles *Staphylococcus aureus* (staph) food poisoning symptoms. The diarrheal type is manifested primarily by abdominal cramps and diarrhea has an incubation period of 8 to 16 hours. It may be a small volume or profuse and watery diarrhea. This resembles food poisoning caused by *Clostridium perfringens*. In both types, the illness usually lasts less than 24 hours after
onset. In a few cases symptoms may last longer. The short-emetic form is caused by a preformed, heat-stable emetic toxin, ETE. The long-diarrheal form of illness is mediated by the heat-labile diarrheagenic enterotoxin Nhe and/or hemolytic enterotoxin HBL, which causes intestinal fluid secretion, probably by a number of mechanisms, including pore formation and activation of adenylate cyclase enzymes (Bacillus Food Poisoning. Cambridge City Council,(www.cambridge.gov.uk/sites/www.cambridge.gov.uk/files/docs/Bacillus%20food%20poisoning.pdf).

Among these bacteria, Bacillus cereus group occupies an important position. This group consists of B. thuringiensis, B. mycoides, B. pseudomycoides, B. anthracis and B. cereus. (Ankolekar et al., 2009) B. cereus is ubiquitous in nature, isolated from soil and growing plants, it is also well acclimatized for growth in the intestinal tract of insects and mammals (Stenfors Arnesen et al., 2008). Among different species of the B. cereus group, B. cereus causes two types of food poisoning the diarrheal type and emetic type. In recent years, there have been an increasing number of well-documented reports substantiating the role of B. cereus as a food poisoning organism (Wong, 1988). 48% of B. cereus isolated from seafood, produced both the hemolysin BL (HBL) and nonhemolytic (NHE) enterotoxins, while 94% and 50% produced NHE or HBL toxins, respectively. At least one of the three genes of the NHE complex was detected in 99% of the isolates and all three genes were observed in 69% of the isolates. Only one B. cereus isolate showed the presence of the cereulide synthetase gene, ces (Rahmati and Labbe, 2008). The enterotoxin genes hblA, hblC, hblD, nheA, nheB and nheC were found in full-fat milk product isolates of B. cereus with frequencies of 37.0%, 66.3%, 71.7%, 71.7%, 62.0% and 71.7% respectively (Zhou et al., 2008). Prevalence and toxigenic profiles of Bacillus cereus isolated from dried red peppers, rice, and Sunsik in Korea have been reported by Kim et al., (2009). At the Danish retail market, among 48,901 samples of ready-to-eat food products, 0.5% had B. cereus-like bacteria above $10^4$ cfu/g (Rosenquist et al., 2005).

The development of improved hygiene, vaccines and antimicrobial agents during the twentieth century is one of the greatest health achievements as
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there was dramatic reduction in the incidence of infectious diseases. Food, the most important energy source, might be easily contaminated by pathogens if not handled hygienically (Mead et al., 1999). To date, numerous studies have examined the detection and diagnosis of food-borne pathogens with the goal to prevail over problems associated with traditional microbiological detection techniques such as being time- and labour-intensive, long culture time, dependency on enrichment and selective culture, and difficulty of quantitative analysis.

Traditionally B. cereus contamination is identified by plating technique on specific media. The growth of the isolate being able to be identified by 48h. B. cereus enterotoxin production can be detected using the B. cereus reversed passive latex agglutination (RPLA) test kit (Unipath-Oxoid, Columbia, Md.), which identifies the L2 component of the tripartite toxin HBL. With the Bacillus diarrheal enterotoxin visual immunoassay (Tecra Bioenterprises, Pty, Ltd., Roseville, Australia), the NheA antigen from the Nhe complex could be detected.

PCR was used for the detection of B. cereus toxin genes encoding cytotoxin K (cytK), haemolysin BL (hblA, hblC, hblD), non-hemolytic enterotoxin (nheA, nheB, nheC) and EM1 specific of emetic toxin using specific primers with the 23S and 16S rRNA internal transcribed sequence (ITS) as an internal control (Yang et al., 2005). Other Bioassays of Enterotoxins and Emetic Toxin such as Vascular Permeability Reaction (VPR) (Kramer et al., 1982), Ligated Rabbit Ileal Loop Test (Kramer et al., 1982), Rhesus monkey feeding test (Kramer et al., 1982), Mouse-lethality Assay (Thompson et al., 1984), Cytotoxicity Assay (Thompson et al., 1984), Aggregate-Hemagglutination technique (Gorina et al., 1975). However, these assays detect only the toxin produced in the food samples.

High throughput screening of an increasingly diverse array of both fresh and processed foods requires that pre- and postharvest food safety practices be dynamic, sensitive, specific, as well as versatile and cost-effective for large sample numbers. No single assay or method will address all of these criteria
optimally, particularly culture-based techniques. A new generation of innovative methods and technologies for pathogen enumeration and detection in contaminated foods is essential to obtain on-field, detection that are rapid, sensitive, and specific for the target pathogen (Smith et al., 2000; Feng, 2001; Rijpens and Herman, 2002). The reliability, cost, and novelty of some of these methods may still limit their adoption and scale-up practicality in a food processing scenario, obligating many laboratories to rely more on traditional microbiological methods for quality control/quality assurance of foods (Jaykus, 2003). These methods are essentially designed around the recovery and (or) enumeration of viable bacteria in the food matrix. Familiarity and acceptance among food processors with methods such as the standard plate count or selective and differential media for bacterial isolation and detection as well as commercially available biochemical profiling systems for identification of specific food isolates continue to serve as the basis in many food microbiology laboratories that lack the necessary resources to utilize some of the emerging molecular-based technologies. Novel means of detecting and enumerating bacteria of interest are continually being reported, and though the majority entail molecular biological approaches, many still fall into the category of conventional techniques.

In order for any conventional or molecular-based detection format to be a feasible tool in the food industry for hygiene monitoring or quality control, it must demonstrate reproducible sensitivity (ability to detect target cells or molecules at very low levels), marked specificity (ability to exhibit positive results in a high background of non-target molecules or cells), speed in obtaining results, low cost per assay, acceptability, ease of use by the scientific community, food microbiology laboratory staff for standardizing protocols and data interpretation (Fung, 2002; Udakis, 2003). Since no single approach satisfies all or even most of these criteria, the user has been forced to prioritize the features of each available format against the needs of the facility and implement the most practical method(s) accordingly.
1.2. Bibliography


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