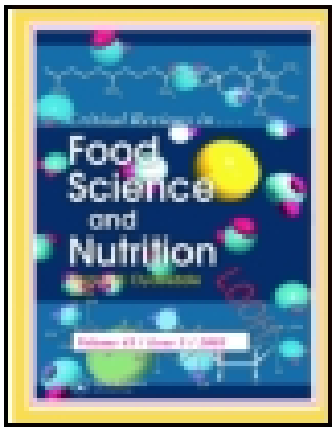

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Molecular Detection of Food Borne Pathogens: A Rapid and Accurate Answer to Food Safety

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Molecular Detection of Food Borne Pathogens: A Rapid and Accurate Answer to Food Safety

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Food safety is a global health concern. For the prevention and recognition of problems related to health and safety, detection of food borne pathogen is of utmost importance at all levels of food production chain. For several decades a lot of research has been targeted at development of rapid methodology as reducing the time needed to complete pathogen detection tests has been a primary goal for food microbiologists. With the result, food microbiology laboratories now have a wide array of detection methods and automated technologies like enzyme immunoassay, PCR, and microarrays which can cut test times considerably. Nucleic acid amplification strategies and advances in amplicon detection methodoloies have been key factors in the progress of molecular microbiology. A comprehensive literature survey has been carried out to give an overview in the field of foodborne pathogen detection. In this article, we describe the conventional methods, as well as recent developments in food pathogen detection, identification and quantification, with a major emphasis on molecular detection methods.

Keywords cell culrture based methods, immunoassays, microbes, nucleic acid based assays.

INTRODUCTION

Food borne illnesses often called food poisoning are the infections or intoxications caused due to consumption of contaminated food or food products. There are more than 250 known foodborn illnesses, most of which are caused by bacteria, followed by viruses and parasites. According to a WHO (World Health Organization) estimate, 2.2 million people worldwide die from diarrheal diseases caused by a host of bacterial, viral and parasitic organisms, which are spread by contaminated water (WHO, 2006a) and 20% of deaths among children under five in India are caused by diarrheal disease (WHO, 2006b). The Lowy Institute for International Policy (2006) estimates that a mild global outbreak of the avian flu can cost the world 1.4 million lives and close to 0.8% of GDP (US$330 billion) in lost economic output (Umali-Deininger and Sur, 2006). Foodborne diseases are widespread and becoming a growing public health concern not only for the developing countries but also for the developed nations. The estimates of centre for Disease Control (US) for the year 2011 suggest that each year roughly 1 in 6 Americans (or 48 million people) gets sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases, out of which 44% illnesses are caused by 31 known pathogens while 56% due to unspecified agents. Although the incidence of food borne diseases at global level is difficult to estimate but they pose a significant economic burden and have immense impact on international food trade and development. Recognizing the current data gap, the World Health Organization (WHO) launched the Initiative to Estimate the Global Burden of Foodborne Diseases (Hanson et al., 2012). A majority of foodborn pathogens have been identified for food borne illnesses, however, *Campylobacter* spp., *Salmonella* spp., *Staphylococus aureus, Escherichia coli* O157:H7, *Clostridium perfringens, Listeria monocytogenes,* Viruses (Norovirus, Hepatitis A), protozoa

(*Cryptosporidium*, *Cyclospora*, *Toxoplasma)* have been reported to be responsible for most of the food borne outbreaks (Alocilja and Radke, 2003; Chemburu et al., 2005; Velusamy et al., 2010). Consumption of Raw or rare meat and poultry, undercooked fish or shellfish, eggs or foods derivd from them, raw sprouts, unpasteurized juices, ciders, milk, and milk products, soft cheeses, uncooked hot dogs, luncheon meats, and deli meats items and ready to eat meats, fruits and dairy products increase the chances of getting a foodborne illness (Mandal et al., 2011).

The development of rapid means of communication such as internet and other forms of media have increased consumer awareness about food safety and public health by global transmission of information regarding the public health threats associated with food-borne diseases (Lindsay, 1997; Buzby and Unnevehr, 2003; Kafersteing, 2003; Unnevehr, 2003; Bramhmbatt, 2005; Ewen et al., 2003). Outbreaks of human mycotoxicoses have also increased understanding of the impact of mycotoxins and raised food security and public health concerns in many developing countries (Bhat and Vasanthi, 2003; Dohlman, 2003; Unnevehr, 2003). In the present day scenario, globalization of trade has increased the risks of cross-border transfer of food-borne illnesses. The food production chain has become more complex, providing greater opportunities for contamination and growth of pathogens. Many outbreaks of foodborne diseases that were once contained within a small community may now take on global dimensions.

Food safety and quality management is very important for meeting both government and private sanitary and phyto-sanitary (SPS) standards in export markets (Otsuki et al., 2001; Henson, 2003; Unnevehr, 2003; World Bank 2005). Therefore rapid detection of pathogens or microbial contaminants in food is very important for ensuring safety of consumers and for devising strategies for the control of outbreaks. Quality assurance system like HACCP (hazard analysis

and critical control point) also require food microbiology testing (Taylor, 2001; 2007; Jin et al., 2008). Presence of pathogenic microorganisms in the food chain has serious implications for the food industry as the failure to detect a pathogen may lead to a dreadful effect. Foodborne outbreaks from microbial contamination, chemicals and toxins have occurred in many countries (WHO, 2007) and have become a driving force for the development of revolutionary pathogen detction systems. Julian Turner (2012) had reported in the foodprocessing-technology.com that in the US itself, the food sector represents almost 50% of the total industrial microbiology testing market, which is more than double the size of any other industrial segment, including pharmaceuticals, wheras in Europe, the food safety testing market is predicted to top $1bn in five years suggesting thereby that protecting the public from food-borne diseases is also generating a big business.

This review discusses various methods of detection of foodborn pathogens with a major emphasis on molecular detection techniques.

CULTURE BASED TRADITIONAL TECHNIQUES

Ttraditional culture methods use selective liquid or solid culture media, to grow, isolate, and enumerate the target microorganism and simultaneously prevent the growth of other microorganisms present in the food (Jasson et al., 2010). These methods of foodborn pathogen identification involve pre-enrichment growth, selective enrichment culture and selective plating followed by biochemical identification and serological confirmation of the results. Both qualitative and quantitative culture methods are available. When only the presence or absence of a pathogen in the food sample is required to be known, the qualitative procedures are used in which presumptive colonies are grown on selective media from a known amount of food sample

(usually 25g) followed by raising of pure cultures and the identification of pathogen by various biochmical or serological test (Betts and Blackborn, 2009). In the quantitative culture methods enumeration of the microorganisms present in the food sample is performed by plate count method or most probable number method which are based on serial dilution techniques (Stannard, 1997; Betts and Blackborn, 2009; Blodgett, 2010)

Although these methods are relatively inexpensive, sensitive and are still regarded as gold standards, however the main drawback of these methods is their long analysis time and their labour intensiveness. The whole procedure takes typically between 7 and 10 days (Vunrcrzant and Pllustoesser, 1987; Biswas, 2005).

MICROSCOPIC AND OPTICAL CHARACTERISTICS BASED METHODS

Various methods based on microscopic and optical charcteristics of the appropriately stained microbial cells have been developed for assuring microbial safety of foods and food products, these include Direct Epifluorescent Filter Technique (DEFT), Flow Cytometry and Solid Phase Cytometry. DEFT is a rapid method for enumerating microbial food-borne pathogens and is used widely in the dairy industry for raw foods (Hermida et al., 2000), milk and milk products, beverages, foods etc. The technique involves capturing bacterial cells on the surface of polycarbonate membrane filters, staining with a fluorochrome such as acridine orange, and visualisation using epifluorescence microscopy (Pettipher et al.1992; Lopez-Campos et al., 2012). The DEFT count is rapid and accurate, however it is very labour intensive and is only applicable when about 10^3-10^4 CFU/g of bacteria are present in the sample.

Flow cytometry, is applied to the enumeration of viable bacteria in a sample and uses flourescent dyes for the analysis of viability, metabolic state, and antigenic markers of bacteria. This

technique quantitatively measures optical characteristics of cells when they are forced to pass individually through a beam of light (Veal et al., 2000; Lopez-Campos et al., 2012). The technique is fast, automatic, and specific subject to the availability of selectively discriminating dyes for specific types of microorganisms (Seo et al., 1998). However, the detection limit with food samples is around 10^5 –10^7 CFU/g (Betts and Blackburn, 2009).

Solid-phase cytometry (SPC) combines the principles of epifluorescence microscopy and flow cytometry (D'Haese and Nelis, 2002). Microorganisms are retained on a membrane filter, fluorescently labelled and automatically counted by laser-scanning device. Each fluorescent spot can be visually inspected with an epifluorescence microscope connected to a scanning device by a computer-driven moving stage. SPC is applicable only if the number of bacteria present is around 10^3 –10^4 CFU/g (Lopez-Campos et al., 2012).

IMMUNOLOGICAL METHODS

All immunological methods for detection of food borne pathogens are based on antigen-antibody reactions. The body produces specific antibodies in response to invading pathogen. Experimentally, these molecules are produced in laboratory animals against a specific antigenic component of the pathogen or toxin. The most important characteristic of an antibody is its ability to recognize only the target antigen in the presence of other organisms and interfering food components. In addition, the successful use of antibodies to detect pathogens depends on the stable expression of target antigens in a pathogen, which are often influenced by temperature, preservatives, acids, salts, or other chemicals found in foods.These reactions are versatile and specific but the success of an immunoassay depends on the specificity of the antibody. With the advent of hybridoma technology, it has been possible to develop monoclonal antibodies which

react only with one specific pathogen. The limit of detection for immunoassays is approximately 10^4 –10^5 CFU/g (Jasson et al. 2010; Lopez-Campos et al., 2012). Immunoassay based methods have become popular due to the development of advanced and sensitive assays as well as due to the development of mechanical devices which have automated various steps (Mandal et al., 2011). Immunoassays are available in different formats. In **Latex agglutination format** the food borne pathogen is detected using antibody coated coloured latex beads which agglutinate the target antigens (specific pathogen) and form a visible precipitate. Latex agglutination assay is simple and very fast but not very sensitive and require approximately 10^7 bacterial cells for reaction (Feng, 1997). For detection of pathotoxins antibody coated coloured beads are used to agglutinated soluble antiens or toxins in place of microbial cells and the format is called **Reverese Passive Latex Agglutination.** In the **Immunodiffusion** format diffusion of antigen is allowed through a gel impregnated with antibody and the appearance of precipitation line indicates the presence of sppecific food borne pathogen or the antigen. **Enzyme-Linked Immunosorbent Assay (ELISA)** is one of the most popular and fast methods for the detection of food borne pathogens. Double antibody sandwich assys are generally used in most of commercially available immunological kits where commercially available antibody-coated microtitre plates (primary antibody) or other solid matrices are used to capture the antigen (pathogen or pathotoxin) from target food samples, and a second antibody (secondary antibody) conjugated with an enzyme is added to form an antibody-antigen-conjugate "sandwich." Commonly used enzymes for conjugation with the secondary antibody are Alkaline phosphatase or horseradish peroxidases. An enzyme substrate is then allowed to take place to identify this whole complex, and the results can be recorded visually or with a spectrophotometer. The

detection sensitivity of ELISA is about 10^5 colony-forming units (CFU)/mL for whole bacterial cells, and a few ng/mL for toxins or protein analytes (Feng, 1997). The **Immunoprecipitation format** is also a sandwich procedure but it relies on the antibody conjugated with the latex beads or colloidal gold instead of enzyme conjugates for the detection purposes. These assays are extremely fast and simple. **Immuno-Magnetic Separation** method utilizes the antibodies coupled with the magnetic particles to capture specific bacteria from the food matrix. It is similar to the enrichment using selective media. This enriched or purified bacterial culture can then be detected by any of the any of the methods discussed above. Various kits have been developed based on Immunological methods for detection of food born pathogens which are available commercially and have been responsible for revolutionising the field of food testing (Table-1).

NUCLEIC ACID BASED METHODS

Spurred by technological developments and commercial profit motives, nucleic acid based assays have become widely available as powerful tools to assist in diagnosis and monitoring of food borne pathogens. These methods are based on the detection of the specific gene sequences (signature sequences) in the genotype of the target organism (Table-2). The sequences may be selected in such a way that they can detect a particular group, genus, species or even strain of the micro-organism. There are many DNA-based assay formats, but probes and nucleic acid amplification techniques are the most popular ones and have been developed commercially for detecting foodborne pathogens (Table -3 & 4).

Nucleic Acid Probes

Probe-based tests are widely used in the food indutry as they are easy to use. In these tests, nuclecic acid probes are immobilized to inorganic supports (**dipsticks**) so that they can be easily manipulated (e.g., washing off unhybridized DNA) without damaging or losing them. The principle behind the use of DNA probes is quite simple. It involves hybridization of the DNA sequence of an unknown microbial pathogen by a known DNA probe (lebelled DNA). The physical basis for gene probe tests stems from the structure of DNA molecules themselves. Each DNA molecule is composed of two complementary polynucleotide strands or chains held together by hydrogen bonds between specific pairs of nucleotides. The hydrogen bonds holding the chains together can usually be broken by raising the pH above 12 or the temperature above 95°C, resulting in denaturation of DNA and production of two single-stranded molecules. When the pH or temperature is lowered, the hydrogen bonds are reestablished between the complementry pairs, reforming double-stranded DNA.

The identification of target organism by DNA probe hybridization methods is based on the presence or absence of particular genes. Short single strands of DNA that are complementary to genes present in a pathogenic microbe are synthesized and labelled with radioactive or non radioactive reporter molecules. The food sample in which the presence of pathigen has to be tested is treated so that any microbial cells are lysed, releasing their DNA which is dennatured and the probe is added. Hybridization (annealing of complementary strands) then occurs between the single-stranded DNA probe and single-stranded DNA released from pathogenic microbes present in the food. If the desired targeted sequence is present then the signal is obtained due to the hybridization of the labelled probe. If the targeted sequence is absent then probe will not bind and no signal will be obtained (Feng, 1997; Glynn et al., 2006).

A gene probe consists of either an entire gene or a fragment of a gene with a known function. In addition based on the nucleotide sequence of the known gene, short pieces of single-stranded DNA can also be synthesized. In addition to DNA, probes and/or their targets can be made of RNA. A number of commercially available gene probe kits use synthetic DNA probes specific for ribosomal RNA targets. DNA: RNA and RNA: RNA hybrids are somewhat more thermally stable than DNA: DNA duplexes, but RNA molecules are quite labile at alkaline pH (Rijpens and Herman, 2002).

One of the main drawbacks of probe-based assays is that it requires a relatively large number of target cells (typically 10^4–10^5) to yield unambiguous results in a background containing large numbers of nontarget microorganisms (Rijpens and Herman, 2002). Therefore, colony hybridization assay formats are often used in which an aliquot of a homogenized food is spread-plated on an appropriate agar. After incubation, the colonial pattern is transferred to a solid support (usually a membrane or paper filter) by pressing the support onto the agar surface. The cells are then lysed in situ by a combination of high pH and temperature to denature and affix the DNA to the support. The solid support with the attached target DNA is incubated with a labelled probe. The labelled probe DNA that fails to reform the double helix is removed by washing the probe-target complexes on the support at an appropriate temperature and salt concentration. The labelled hybrid is detected by autoradiography, colorimetry or fluorescent methods depending upon whether radioactive element, colorimetric moieties, fluorescent dye or enzymes have been used to label the probe.

Target Genes for Nucleic Acid Based Assays

The first step in developing a gene based assay whether probe based or PCR based is to decide what information is needed. If a particular taxonomic group is to be identified, the probe/ primers must be directed toward a gene or region of a gene that is conserved throughout a particular species or genus, while some times one may want to know if a microorganism carrying a particular gene is present. Targetting of specific determinants of virulence are useful in assessing a risk to public health posed by bacterial contamination. The target gene for nucleic acid based assays should be present in the cell at relatively high copy number while being sufficiently heterologous at the sequence level. Popularly used target genes for gene based assaya are Genomic DNA, multicopy rRNA, genes encoding toxins or virulence factors and genes involved in cellular metabolism. In some bacterial species pathogenicity may vary considerably among isolates. Such species can represent strains ranging from highly dangerous isolates to entirely non-pathogenic strains that are biologically safe and can be used in fermentation processes or for the manufacturing of medications (Frey et al, 1998). Hence, in such cases virulence factors or their respective genes need to be targeted as they are responsible for the pathogenecity of the pathogens. (Falkow, 1988; Finlay and Falkow, 1989; Finlay and Falkow, 1997; Strauss and Falkow, 1997; Whittam et al., 1993). Although the highly conserved areas of the 16S and 23S ribosomal RNA (rRNA) genes have been used to study the relationships among distant bacterial taxa, the more variable regions of these genes are useful for differentiation of genera and species and, therefore, are used as targets for genus and sometimes species-specific PCR (Border et al., 1990; Giesendorf et al., 1992; Wan et al., 1994; Rijpens and Herman, 2002). The 16S–23S rRNA spacer region has also been successfully used for developing specific DNA probes (Barry et al., 1991; Rossau, 1991; Rossau et al., 1992; Herman et al., 1995; Gurtler and Stanisich, 1996;

Rijpens et al., 1997). Commonly used targets for the development of primers and probes for nucleic acid based detection methods are described in Table 2.

Polymerase Chain Reaction (PCR)

Developed by Kary Mullis, PCR is now a common and an extremely powerful tool that enables exponential amplification of a specific target sequence in a short time and hence greatly reduces dependence on cultural enrichment step (Mullis and Faluna, 1987). The reaction system includes a heat-stable DNA polymerase such as Taq polymerase, an enzyme isolated from the bacterium *Thermus aquaticus* that has optimum activity at 72^{o}C, a template DNA from the pathogens being detected, and two complementary oligonucleotide primers that are designed to flank the target sequence on the template DNA. The method relies on thermal cycling, consisting of a heat denaturation phase when single strands are generated from a double-stranded DNA, an annealing phase when the primers bind to the single-stranded target sequences, and an extension phase when the DNA polymerase makes a strand that is complementary to the template using a heat stable DNA polymerase in the presence of free deoxynucleoside triphosphates (dNTPs) resulting in a double replication of the starting target material. Typically, PCR consists of a series of 20-40 cycles, which amplify specific pieces of template DNA at more than a billion-fold. PCR can be extensively modified to perform a wide array of genetic manipulations (Feng, 1997; Glynn et al., 2006; Shi et al., 2010). However, PCR assays are also susceptible to interferences by food matrices, therefore a brief cultural enrichment period is required to enhance assay performance and also to distinguish viable from nonviable cells (Rossen et al., 1992). Several formats of PCR are available for detection of food pathogens.

Ligase Chain Reaction

LCR is a technique for Ligation-dependent target DNA amplification in which an enzyme, DNA ligase, is used to join two complementary oligonucleotide probes that are ligated to the target sequence. The ligated product is used as a template to join complementary oligonucleotides which, through repeated processing with enzymes, allow for the production of many copies of DNA. The amplified DNA copies can then be used to determine the presence of the target of interest at the junction of two oligonucleotide probes. In conjunction with a primary PCR amplification, LCR has become a very promising diagnostic technique. This technique utilizes a thermostable ligase and allows the discrimination of DNA sequences differing in only a single base pair (Wiedmann et al., 1994; Barany, 1991 a and b). Wiedmann et al., (1992, 1993) had developed a polymerase chain reaction (PCR)-coupled ligase chain reaction (LCR) assay for the specific detection of Listeria monocytogenes. The detection method with the chemiluminescent substrate Lumi-Phos 530 permitted detection of the LCR products in less than 3 h, so that the whole assay could be completed within 10 h.

Nucleic Acid Sequence-Based Amplification (NASBA)

NASBA is specifically designed to detect RNA. It is a transcription-dependent amplification based on promoter primers that recognize specific target sequences and enable RNA amplicon synthesis. It uses three viral enzymes viz. reverse transcriptase, RnaseH and T7 RNA polymerase, which act in concert to amplify RNA targets. A primer binds to the target RNA sequence and a cDNA strand is produced with a reverse transcriptase. RNase H then digests the template RNA and a second primer binds to cDNA, with which the reverse transcriptase is used to produce double-stranded cDNA. Last, T7 RNA polymerase is used to produce RNA transcripts via an amplification process. The main advantage of this technique is that it is

isothermal (occurs at a constant temperature), avoiding the need for expensive thermal cyclers., NASBA diagnostics have mainly been developed for the identification of viruses however in the field of food testing it has been used for the detection of *Campylobacter, E. coli, L. monocytogenes* and *Salmonella enterica* in various foods and for *Cryptosporidium parvum* in water **(**Uyttendaele, 1995 a,b and c; Min and Baeumner, 2002; Cook, 2003; Nadal et al., 2007). Both 16S rRNA and various mRNAs have been used as target molecules for detection; however the latter has the advantages in allowing specific detection of viable cells. An enrichment in nutrient medium prior to NASBA can ensure sensitivity of detection and encourage the detection of only viable target cells (Cook, 2003).

Multiplex Polymerase Chain Reaction

In food industries the key considerations for quality evaluation are the cost and limited volume of test samples. In multiplex PCR, multiple sets of primers are included in a single reaction tube (Vandenvelde et al., 1990; Bej et al., 1991), so that more than one target sequence are amplified in one reaction system. By targeting multiple genes at once, additional information can be gained from a single test run that otherwise would require several times the reagents and more time to perform. For the optimization of multiplex PCR reactions, it is very important that the annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes of different target genes should be different enough to form distinct bands when visualized by gel electrophoresis. Multiplex PCR has been successfully applied in detection of food borne pathogen detection (Elnifro et al., 2000; Rachlin et al., 2005; Lu et al., 2007; Chun et al., 2007; Teh et al., 2008; Kawasaki et al., 2009; Shi et al., 2010; Kawasaki et al., 2011).

Nested Polymerase Chain Reaction

Nested polymerase chain reaction is a modification of polymerase chain reaction. This methood is more sensitive and specific than the conventional PCR as it reduces the non-specific binding in products due to the amplification of unexpected primer binding sites. This reaction involves two sets of primers, used in two successive runs of polymerase chain reaction. The first primer set is used to amplify a target sequence, which then serves as the template for a second amplification. The second primer set lies internal of the first amplicon. Therefore secondary amplification does not occur if the primary amplification is nonspecific. A number of foodborne pathogens have been detected using this technique (Klemsdal and Elen, 2006; Kim et al., 2008; Saroj et al., 2008).

Real-Time PCR

Real-time polymerase chain reaction, also called quantitative real time polymerase chain reaction is a technique used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification. The quantity can be either an absolute number of copies or a relative amount when normalized to DNA input or additional normalizing genes. The amplified DNA is detected as the reaction progresses in real time.

Two common methods for detection of products in real-time PCR are: (1) non-specific fluorescent dyes that intercalate with any double-stranded DNA such as SYBR-green I, EtBr, and (2) sequence-specific DNA probes consisting of oligonucleotides that are labeled with a fluorescent reporter such as TaqMan, Molecular Beacons, Scorpions etc which permits detection only after hybridization of the probe with its complementary DNA target (Heid et al., 1996; Wittwer et al., 1997; Morrison et al., 1998; Vet et al., 1999; Donohoe et al., 2000; Mhlanga and

Malmberg, 2001; Saha et al., 2001; Solinas et al., 2001; Siraj et al., 2002; Abravaya et al., 2003; van der Velden et al., 2003; Tan et al., 2004). The key features of this technique are its sensitivity and speed due to which it has become a very attractive method for the detection of foodborne pathogens (Shi et al., 2010). A number of commercial kits based on real time PCR technique are already available in the market for the detection and characterization of food borne pathogens (Table-3).

Reverse-Transcription PCR

Reverse-transcription PCR is a variant of PCR in which RNA is used as the initial template instead of DNA. In RT-PCR, the RNA template is first converted into a complementary DNA (cDNA) using a reverse transcriptase and the cDNA is then used as a template for exponential amplification using PCR. The main advantage of this method is that it can detect the presence of viable pathogen in the target sample (Sharma, 2006). Reverse-transcription PCR is used not only to detect genes of foodborne viral pathogens via RNA (Gilgen et al., 1995; Arnal et al., 1999; Hewitt and Greening, 2006; Sharma 2006; Wang et al., 2008), but also to detect the specific expression of certain genes during the course of growth or infection since they are amplified at the much higher number of messenger or ribosomal RNA than the number of DNA copies present in foodborne pathogens. However, RNA is unstable, and reverse-transcription PCR is therefore more skillful at handling when quantification is required for foodborne pathogen detection (Gilgen et al., 1995; Arnal et al., 1999; Hewitt and Greening, 2006; Sharma 2006; Wang et al., 2008).

DNA Microarray

DNA microarray, commonly known as DNA chip or biochip, is a collection of microscopic DNA spots attached to a solid surface. Each DNA spot contains picomoles (10^{-12} moles) of a specific DNA sequence, known as *probes* which are used to hybridize a *target* under high-stringency conditions. Probe-target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target. Since an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in parallel. Therefore, arrays have dramatically accelerated many types of investigation. In standard microarrays, the probes are synthesized and then attached via surface engineering to a solid surface by a covalent bond to a chemical matrix. The solid surface can be glass or a silicon chip, in which case they are colloquially known as an *Affy chip* when an Affymetrix chip is used. Other microarray platforms, such as Illumina, use microscopic beads, instead of the large solid support. Alternatively, microarrays can be constructed by the direct synthesis of oligonucleotide probes on solid surfaces. DNA arrays are different from other types of microarray only in that they either measure DNA or use DNA as part of its detection system (Baldwin et al., 1999; Richmond et al., 1999; Winzeler et al., 1999; Cummings and Relman, 2000; Rhodius et al., 2002).

Using microarray technology detection of multiple target sequences can be done in a single assay which in turn enables thousands of identification assays to be conducted in parallel, where each probe represents a specific small section of a genome or a sequence common to multiple genomes (Rasooly and Herold, 2008). All array technologies share three main features: multi-target analysis, specific binding or hybridization of the target, and labeling of the target

molecules. A major challenge for microbial safety in food industry is the need to detect and characterize small numbers of microorganisms in a large sample volume, often with a background of similar organisms and interfering matrix material. Thus, for many microarray applications one of the initial steps is amplification of the specific target sequence to increase sensitivity (Rasooly and Herold, 2008).

Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is rapid, accurate, and cost-effective method for detection of target genes in the food samples (Mori and Notomi, 2009). This technology has been developed for the detection of a variety of food born pathogens as has been shown in table-4.

LAMP is a one-step amplification reaction that amplifies a target DNA sequence with high sensitivity and specificity under isothermal conditions. LAMP methodology involves three steps, an initial step, a cycling amplification step, and an elongation step. It employs a DNA polymerase with strand-displacement activity, along with two inner primers and two outer primers which recognize six separate regions within a target DNA. LAMP assay is extremely specific because the amplification reaction occurs only when all six regions within a target DNA are correctly recognized by the primers. The addition of reverse transcriptase makes it possible to amplify DNA from RNA sequences (RT-LAMP). In addition, detection is simplified by visual judgment without post-amplification electrophoresis (Hara-Kudo et al., 2005; Song et al., 2005). LAMP has been applied for the detection of many kinds of pathogens causing food-borne diseases (Lukinmaa et al, 2004). LAMP kits for detecting *Salmonella*, *Legionella*, *Listeria*, verotoxin-producing *Escherichia coli* and *Campylobacter* have been commercialized (tabe-3)

Other Methods

In addition to the above mentioned methods a number of DNA fingerprinting based techniques such as Randomly amplified polymorphic DNA, Amplified restriction-length polymorphism (AFLP), Denaturing gradient gel electrophoresis (DGGE), Pulse field gel electrophoresis (PFGE), Amplified restriction-length polymorphism (AFLP) etc. are also being routinely used for the identification of microbial pathogens by their respective DNA profiles in food industry.

CONCLUSION

Food-borne pathogens represent one of the major challenges for all stakeholders in the food chain. In order to ensure microbial safety of food, implementation of Good Hygiene Practices (GHP) and Hazard Analysis and Critical Control Points (HACCP) are used as preventive strategy in food industries. However, appropriate controls are necessary to ensure the safety of food along the entire food chain as microbial safety is a prerequisite for commercialization of foods. Exciting developments have taken place in the field of food microbiology and safety in the last two decades.

The culture-based traditional methods for microbial detection and identification are although simple, relatively inexpensive, and sensitive, but are too time-consuming for high-throughput testing. The extended time required for conventional enrichment, plating, and biochemical/serological methods to give results do not enable problems to be identified soon enough to allow the appropriate remedial action to be implemented. An ideal method must produce rapid, accurate, and reliable estimates of total viable microbial numbers. The development of molecular methods that rely on the detection of genomic elements (DNA or RNA) with or without culture could therefore be a viable alternative. Although culture-based

methods are still considered the gold standard for identification and diagnosis, molecular methods have emerged as the confirmatory method for identification in many diagnostic applications. DNA-based methods, such as polymerase chain reaction (PCR), are faster and provide more information than culture-based methods but have a limitation that they are normally limited to the analysis of a single pathogen or a small group of related pathogens. Multiplex PCR is sometimes unstable, and the possibility of generating nonspecific products has hindered its wider application in diagnostics. Microarray technology enables a significant expansion of the capability of DNA-based methods in terms of the number of DNA sequences that can be analyzed simultaneously, enabling molecular identification and characterization of multiple pathogens and many genes in a single array assay. Detection of pathogens using molecular methods requires a prior knowledge of the target sequence and the random mutation in the genomic DNA can compromise the detection. This problem can be overcome with the use of redundant probes, or primers that make extremely unlikely the simultaneous variation of all target sequences, or with the implementation of long probes and less stringent hybridization conditions. In general an ideal method must be sensitive, fast and the specific. The cornerstone of any method is its accuracy. The objective of developing a rapid assay is to reduce the time required to obtain an accurate result.

As various assay procedures are available in complex designs and formats, the onus lies on the user to exercise caution when selecting rapid methods. He need to evaluate these tests thoroughly, as some may be more suitable than others for distinct testing situations or for assaying certain types of food. Technological advancements are taking place at a great pace and next generation assays, such as biosensors and DNA chips haver already been developed which

potentially have the capability for near real-time and on-line monitoring of multiple pathogens in foods. Rapid detection kits for food spoilage and even food pathogen detection for home use also seems possible. The field of rapid methods and automation in microbiology has great potential and many exciting developments will certainly unfold in the near and far future.

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Table1 Commercially available kits based on Immunological methods for detection of food born pathogens

Name of The Kit	Assay Format	Target Organism	Firm
Campyslide	Latex agglutination	*Campylobacter*	Becton Dickinson
RIM	Latex agglutination	*E.ColiO157:H7*	REMEL
Prolex	Latex agglutination	*E.ColiO157:H7*	PRO-LAB
Ecolex O157	Latex agglutination	*E.ColiO157:H7*	Orion Diagnostica
Wellcolex O157	Latex agglutination	*E.ColiO157:H7,*	Laboratoire Wellcome
Wellcolex	Latex agglutination	*Shigella*	Laboratoire Wellcome
MicroScreen	Latex agglutination	*Listeria, Campylobacter*	Mercia
Listeria latex	Latex agglutination	*Listeria*	Microbiology International Frederick, MD
Staphyloslide	Latex agglutination	*Staphylococcus aureus*	Becton Dickinson
Aureus test	Latex agglutination	*Staphylococcus aureus*	Trisum
Bactigen	Latex agglutination	*Shigella*	Wampole Labs
1-2 test	Immunodiffusion	*Salmonella*	BioControl
EHEC-TEK	ELISA	*E.Coli* O157:H7	Organon-Teknika
RIDASCREEN	ELISA	*Staphylococcus aureus*	R-Biopharm
VIDAS	ELISA	*Staphylococcus aureus, Listeria, Listeria monocytogenes, Salmonella, E. coli O157*, Staphylococcal enterotoxin, and *Campylobacter*.	BioMerieux, Hazelwood, MO
Assurance	ELISA	*E.ColiO157:H7, Salmonella*	BioControl
HECO157	ELISA	*E.ColiO157:H7*	3M Canada
TECRA	ELISA	*E.ColiO157:H7, Listeria, Bacillus cereus* diarrhoeal toxin*, salmonella, Staphylococcus aureus*	TECRA
Premier EHEC	ELISA	Verotoxin	Meridian Diagnostics Inc., Cincinnati, Ohio
Premier O157	ELISA	E coli O157	Meridian Diagnostics Inc., Cincinnati, Ohio
Ecoli Rapitest	ELISA	*E. coli 0157*	Kalyx Biosciences, Ontario

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Transia Card *E.coli* O157	ELISA	*E.Coli O157:H7*	Raisio Diagnostics
PATH Stick	Immunoprecipitation	*Salmonella*	LUMAC
Reveal	Immunoprecipitation	*Salmonella*	Neogen
Clear view	Immunoprecipitation	Salmonella	Unipath
Cholera SMART	Immunoprecipitation	*Vibrio Cholerae*	New Horizon
Bengal SMART	Immunoprecipitation	*Vibrio Cholerae*	New Horizon
Dynal	immuno-magnetic separation	*E. Coli O157, Listeria , Cryptosporidium, Giardia,* etc	Dynal (Oslo, Norway)

Table 2 Commonly used target genes used for nucleic acid based assays

Organism	Target genes	Reference
Listeria monocytogenes	Listerolysin O (*hlyA*)	Mengaud et al., 1988
Salmonella spp	1.8 Kb HIND III	Tsen et al., 1994
Salmonella spp	Fimbrin (*fim A),* Salmonella enteritidis fimbrial protein (*sefA),* Invasin (*inv A)*	Chen et al., 2000; Seo et al., 2004; Wan et al., 2004; Naravaneni and Jamil, 2005; Iyer and Kumosani, 2010
Campylobacter jejuni	Flagellin gene (flaA and flaB), 16S rRNA, 16S/23S rRNA	Giesendorf and Quint, 1992; Oyofo et al., 1992; Rasmussen et al., 1996; O'Sullivan et al., 2000
E. coli O157:H7	H7, O157, the attaching and effacing gene (*eae*A, *ehylA),* verotoxin (*vt*1 and *vt*2 gene)	Paton and Paton, 1998
E coli	Afimbrial adhesins (*afa)*	Naravaneni and Jamil, 2005; Iyer and Kumosani, 2010
E coli O157	Shiga toxin genes *(Stx1, stx2),* the attaching and effacing gene (*eae), rfb, vt1, vt2*	Desmarchelier et al., 1998; Vuddhakul et al., 2000; Fitzmaurice et al., 2004 a and b; Iyer and Kumosani, 2010
Enterotoxigenic *E. coli(*ETEC)	heat-labile (LT) or heat-stable (ST) toxin genes	Schultsz et al., 1994
Enterohemorrhagic *E. coli* (EHEC*)* and verotoxin or Shiga toxin-producing *E. coli* (VTEC/STEC)	Verotoxin (*vt*) or *Stx*, the attaching and effacing gene (*eae),* hemolysin gene *(hlyA),* Enterohemolysin (*elyA)*	Lang et al., 1994 ; Thomas et al., 1994 ; Witham et al., 1996; Paton and Paton, 1998;
Y. enterocolitica	Enterotoxin gene *yst,* the attachment-invasion locus *ail* , and the invasin gene *inv*)	Ibrahim, et al., 1992; Kwaga et al., 1992; Nakajima et al., 1992
L. monocytogenes	Listeriolysin O-gene (*hlyA*) , invasion-associated protein gene (*iap*), hly A, hlyO, inlA, inlB, 16S/23S rRNA	Agersborg et al., 1997; Klein and Juneja, 1997; Scheu et al., 1998; Giovannacci et al., 1999; O'Connor et al., 2000; Aznar and Alarcon, 2003
Yersinia enterocolitica	Low calcium response protein *(lcrD)*	Plano et al., 1991
Salmonella typhimurium	Invasin (*invA)*	Galan et al., 1992
Shigella sonnei	Membrane associated expression of invasion	Andrews and Maurelli, 1992

	plasmid antigens *(MxiA)*	
Escherichia coli	Secreted enteropathogenic protein*(SepA)*	Jarvis et al., 1995
Erwinia amylovora	Hypersensitivity response secretion protein *(HrcV)*	Wei and Beer, 1993
Escherichia coli K-12	Flagellar hook protein(*flhA)*	Itoh et al., 1996
Staphylococcus aureus	Coagulase, Methicillin Resistance	Iyer and Kumosani, 2010

Table 3 Commercially available kits based on nucleic acid assays for detection of food born pathogens

Name of The Kit	Assay Format	Target Organism	Firm
Gene TrakTM	Gene probe	*Campylobacter*, *E. coli*, *Listeria*, *Salmonella*, *Staphylococcus aureus* and *Yersinina enterocolitica*.	GENE TRAK, Neogen, MI USA
AccuProbe	Gene probe	*Campylobacter*,	Gene probe, San Diego, CA,USA
BIND	Bacterial ice nucleation detection method	*Salmonella*.	Idetek
Probelia	PCR based	*Clostridium botulinum*, *E. coli* O157:H7, *Salmonella spp, L. monocytogenes*	Bio-Rad Hercules, CA
BAX	PCR based	*E. coli* O157:H7, *Listeria* and *Salmonella*.	Qualicon, Inc., Wilmington, DE
Dr Food kit	PCR based	*E.coli*, *Salmonella spp*, *Campylobacter spp.*, *L. monocytogenes*	Dr. Chip biotech Inc.
PCR Diagnosis-Bacteria Identification Kit	PCR based Kit for Lightcycler	*E. Coli*	Biochain
Light Cycler ® Food proof *Listeria* genus detection kit	PCR based Kit for Lightcycler	*Listeria*	Roche
Light Cycler ® Food proof Salmonella detection kit	PCR based Kit for Lightcycler	*Salmonella*	Roche
Light Cycler ® Food proof *E Coli O157* detection kit	PCR based Kit for Lightcycler	E. *coli O157*	Roche
Light Cycler ® Food proof *L. Monocytogenes* detection kit	Real time PCR based	*L. monocytogenes*	Roche/BIOTECON

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Foodproof® *Listeria* Genus Detection Kit	PCR	*Listeria*	BIOTECON Diagnostics GmbH
Foodproof ® EHEC Screening Kit	PCR Kit for the qualitative detection using real time PCR Instruments	Enterohaemorrhagic *E.coli* (EHEC) by screening for genes of Shiga toxins (*stx1* & *stx2*) and intimin (*eae*)	BIOTECON Diagnostics GmbH
Foodproof® E. coli O157 Detection Kit 5'Nuclease)	Real time (for TaqMan® instruments	*E. coli O157*	VWR Merck Millipore
Foodproof® E. coli O157 Detection Kit, Hybridization Probes	Real time (for LightCycler® instruments	*E. coli O157*	Merck Millipore
Real Art ™ *L. Monocytogenes PCR Kits*	Real time	*L. monocytogenes*	QIAGEN
Real Art ™ *Campylobacter PCR Kits*	Real time	*C. jejuni, C. lari, C. coli*	QIAGEN
Real Art ™ *Salmonella PCR Kits*	Real time	*Salmonella*	QIAGEN
Taqman® *L. Monocytogenes* Detection Kits	Real time	*L. monocytogenes*	Applied Biosystems
Taqman® *Salmonell enterica* detection Kits	Real time	*Salmonell enterica*	Applied Biosystems
Taqman® *Campylobacter jejuni* detection Kits	Real time	*Campylobacter jejuni*	Applied Biosystems
Taqman® *E.coli O157:H7* detection Kits	Real time	*E.coli O157:H7*	Applied Biosystems

SureFood® pathogen Salmonella	Real time	*Salmonella* spp	Congen
SureFood® pathogen Listeria	Real time	*L. monocytogenes*	Congen
SureFood® pathogen Campylobacter	Real time	*Campylobacter*	Congen
Artus *L.monocytogenes* PCR Kits	Real time	*L.monocytogenes*	Artus
Artus *Salmonella* PCR Kits	Real time	*Salmonella*	Artus
Artus *Campylobacter* PCR Kits	Real time	*Campylobacter*	Artus
Loopamp Verotoxin-producing Escherichia coli Detection Kit	LAMP	Verotoxin-producing *Escherichia coli*	EIKEN Chemical Co.
Loopamp Escherichia coli O157 Detection Kit	LAMP	*Escherichia coli O157*	EIKEN Chemical Co..
Loopamp Listeria monocytogenes Detection	LAMP	*Listeria monocytogenes*	EIKEN Chemical Co.
Loopamp Campylobacter Detection Kit	LAMP	*Campylobacter*	EIKEN Chemical Co.

Table 4 Nucleic acid based methods reported for detection of pathogenic microorganisms in food samples

Detection Method	Target Organism	Reference
PCR	*Shigella spp, Salmonella* and *E.coli*	Bej et al., 1991; Frankel et al., 1990; Tsai et al., 1993; Schimidt et al., 1995; Naravaneni and Jamil 2005
	Bacillus cereus	Messelhausser et al., 2007; Perry et al., 2007
	Yersinia enterocolitica	Ibrahim et. al., 1992; Nakajima et al., 1992; Perry et al., 2007
	C. jejuni	Ronner and Lindmark, 2007
	Salmonella	Bej and Jones, 1993; Malorny et al., 2003; Choi and Lee, 2004; Kim et al., 2007a; Murphy et al., 2007; Perry et al., 2007; Stark and Made, 2007; Kumar et al., 2008
	Vibrio cholerae	Bej and Jones, 1993; Shangkuan et al., 1995
	V. parahaemolyticus	Tada et al.,1992
	V. vulnificus	Brauns et al. ,1991
	L. monocytogenes	Simon et al.,1996
	Staphylococcus aureus	Wilson et al., 1991; Kim et al., 2007a; Riyaz-Ul-Hassan et al., 2008
Nested PCR	*L. monocytogenes*	Herman et al., 1995b
Multiplex PCR	Enterohemorrhagic *Escherichia coli* Strains	Gannon et al., 1997
	E. coli O157:H7, Salmonella, S. aureus, L. monocytogenes, and *V. parahaemolyticus*	Kim et al., 2007a
	L. monocytogenes, and major serotypes and epidemic clones of *L. monocytogenes*	Chen and Knabel, 2007
	E. coli O157: H7 and *L. monocytogenes*	Mukhopadhyay and Mukhopadhyay, 2007
	Bacteria from Genera Salmonellae and Shigellae	Villalobo, 2010
	E. coli O157:H7	Jeshveen et al., 2012

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	Salmonella spp., *L. monocytogenes,* and E.*coli* O157:H7	Kawasaki et al., 2009; Kawasaki et al., 2011
	Salmonella and L. *monocytogenes*	Jothikumar et al., 2003
Ribotyping	*L. monocytogenes*	Gendel et al., 2004
RFLP with PCR	*Staphylococcus* and *Listeria* spp.	Atanassova et al., 2001; Dubois et al., 2003
RT-PCR	*E. coli* O157:H7	Yaron and Matthews , 2002
LAMP	*Y. ruckeri*	Ohtsuka et al., 2005; Okamura et al., 2008,
	Salmonella spp.	Hara-Kudo et al., 2005; Wang et al., 2007
	Enterotoxigenic *E. coli*	Song et al., 2005; Yano et al., 2007.
	Campylobacter coli.32	Yamazaki et al., 2008.
PCR-LCR, nonisotopic	*L. monocytogenes*	Wiedmann et al., 1992; 1993; 1994
	Simultaneous detection of *L. monocytogenes, Salmonella, Shigella, S. aureus, Streptococcus*	Cao et al., 2011
Microarray	*pyogenes, V. cholerae, V. parahaemolyticus, V. vulnificus, Y. enterocolitica, Proteus mirabilis* and *P. vulgaris*.	
	15 *Salmonella, Shigella*, and *E. coli* strains	Loy et al., 2005
	15 serotypes of *Shigella* and *E. coli*	Li et al., 2006
	S. aureus	Sergeev et al., 2004a
	Campylobacter species (C. jejuni, C. coli, C. lari, and *C. upsaliensis*)	Volokhov et al., 2003
	Six species of the *Listeria genus: L. monocytogenes, L.ivanovii, L. innocua, L. welshimeri, L. seeligeri* and *L. grayi*	Volokhov et al., 2002
	V. vulnificus, V. cholerae, and V.parahaemolyticu	Panicker et al., 2004
	Genes for mycotoxin bisynthesis including fumonisin, aflatoxin, ochratoxin, trichothecene (type A and B), and patulin,	Schmidt-Heydt and Geisen, 2007
	Staphylococcus spp	Sergeev et al., 2004b
Multi-pathogen microarrays	18 potential biowarfare agents including *S. aureus* enterotoxin genes, *Listeria spp., Campylobacter spp.,*	Wilson et al., 2002

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	and *Clostridium perfringens*	
	E.coli O157:H7, S. enterica, L. monocytogenes and *C. jejuni*	Suo et al., 2010
	23 species of food microbial pathogens including *Salmonella spp., Shigella spp., E. coli, S. aureus, L. monocytogenes, V. parahaemolyticus, C. jejuni, V. cholerae, Clostridium botulinum,* and *C. perfringens.*	Wang et al.,2007
NASBA	*L. monocytogenes*	,Uyttendaele et al., 1995c; Blais et al., 1997; Nadal et al., 2007
	C. jejuni, C. coli, C. lari	Uyttendaele et al., 1995a; Uyttendaele et al., 1995b; Uyttendaele et al., 1996; Uyttendaele et al., 1997; Uyttendaele et al., 1999
Real Time PCR	*Bacillus cereus*	Martinez-Blanch et al., 2009
	C. jejuni	Rantsiou et al., 2010
	E. coli O157:H7	Ibekwe et al., 2006; Singh et al., 2009
	L. monocytogenes	Rodriguez-Lazaroetal., 2004; 2005; Guilbaud et al., 2005; Berrada et al., 2006; Chen and Knabel, 2007; Kim et al., 2007; Murphy et al., 2007; Oravcova et al., 2007; Perry et al., 2007; Rantsiou et al., 2008
	Y. enterocolitica	Iliev et al., 2008; Lambertz et al., 2008
	Salmonella and Salmonella Serotype Typhi	Farrell et al., 2005; Liming and Bhagwat, 2004
	O1, O139, and non-O1, non-O139 strains of V. cholerae	Huang et al. 2009; D'Urso et al., 2009
	S. aureus, L.. monocytogenes, S. typhi, Shigella spp., *E. coli* O157:H7, *V cholerae, V. parahaemolyticus, S. pyogenes*	Huang et al., 2007
	Salmonella, Shigella, E.coliO157:H7	Wang et al., 2007

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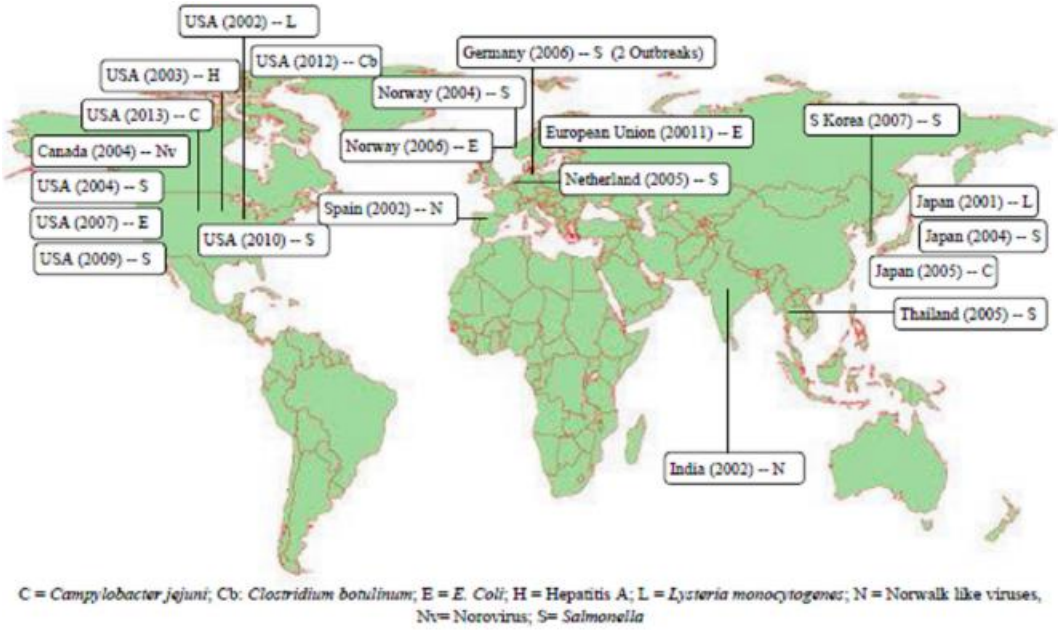

Figure 1 Major foodborne disease outbreaks (2001–2013) in the world and their causes