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CURRENT TRENDS USED IN DIAGNOSIS OF FOOD BORNE PATHOGENS

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ABSTRACT

Food borne pathogens have become a major concern worldwide as they cause several diseases such as diarrhea, stomach infection, food borne botulism, gastrointestinal infection, etc. The diagnosis of these pathogens is a global health goal and the food borne diseases lead a major crisis on health. There are many conventional methods devised for the same purpose but these methods are very time consuming and may take several days to few weeks to confirm the result. Traditional methods to detect food borne bacteria often rely on time-consuming growth in culture media, followed by isolation, biochemical identification, and sometimes serology. Hence, research is being carried out to improve the rapidness, sensitivity and selectivity for the detection of food borne pathogens. These new methods are often referred to as "rapid methods", a subjective term used loosely to describe a vast array of tests that includes miniaturized biochemical kits, antibody- and DNA-based tests, and assays that are modifications of conventional tests to speed up analysis. But these methods should be cost effective in nature and user friendly. This review paper focuses on all the conventional methods, immunological methods, PCR based methods, molecular based approaches and recent trends being used for the detection, identification and quantification of the food borne pathogens.

KEYWORDS: Food borne pathogen, food borne pathogen detection, conventional methods, immunological methods

INTRODUCTION

"Food poisoning" can be the result of either chemical poisoning or the ingestion of toxicants (intoxication). These toxicants may be found naturally or may be produced by the microorganism as a toxic metabolic product. The diseases that may be caused due to ingestion of these toxicants are botulism, salmonellosis, gastroenteritis, shigellosis, yersiniosis, etc. The most prevalent microorganisms that may lead to such infections are *Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Bacillus cereus, Escherichia coli, Vibrio parahaemolyticus*, etc. Animal by-products, such as feed supplements, may also transmit pathogens to food animals (for example, *Salmonella* and bovine spongiform encephalopathy). Seafood is another potential source of food-borne pathogens, such as *Vibrio spp., Listeria spp.* and Hepatitis A. Causes of this food borne illness may be due to consumption of food and water containing viable pathogenic microorganisms or their preformed toxins, from ingestion of pathogenic algae, parasites, and their preformed toxins through food (Frazier et al., 1988).

FOOD BORNE INFECTIONS

- **Salmonellosis**

Salmonellae spp. inhabitat in the gastrointestinal tracts of domestic and wild animals, birds, pets and insects. They cause salmonellosis in animals and birds and then remain in a carrier state (Flowers et al., 1988). The pathogens invade mucosal lining of the small intestine, proliferate into the epithelial cells, and produce a toxin, resulting in inflammatory reaction and fluid accumulation in the intestine (D'Aoust et al., 1989). Foods associated with this pathogen include beef, chicken, turkey, pork, eggs, milk and products made from them (Bean et al., 1990).

- **Listeriosis**

Listeria monocytogens can be isolated from samples such as soil, water, sewage, dead vegetation and undercooked food such as meat, milk, egg, sea foods and fish, as well as leafy vegetables and tubers. This pathogen invades body tissues and multiplies inside the body, thus releasing the toxin which may eventually lead to death (Marth et al., 1988, Racourt et al., 1994 and Lovett et al., 1989).

- **Pathogenic *Escherichia coli***

Escherichia coli typically colonize the gastrointestinal tract of human infants within a few hours after birth. The different pathogenic strains of *Escherichia coli* are EIEC, UPEC, EHEC, DAEC, EPEC and ETEC.

- Shigellosis

The pathogen carries plasmid encoded invasive traits which help the shigellae cells to invade epithelial mucosa of the small and large intestines. The pathogens are passed on directly or indirectly, from a person either suffering from the disease, or a carrier (Smith et al., 1987 and Wachsmuth et al., 1989).

- **Campylobacteriosis**

Campylobacter spp. is also an enteric organism i.e. it resides in the intestine of human beings. It has also been isolated from fecal matter, food, sewage, water and vegetables, etc. It produces a thermo labile enterotoxin that cross-reacts with cholera toxin. These strains produce an invasive factor that enables the cells to invade in epithelial cells in both the small and large intestines in humans (Stern et al., 1989 and Doyle et al., 1988).

- **Yersiniosis**

Yersinia enterocolitica inhabits naturally in intestines of food animals and birds, pets, wild animals and humans. Human carriers do not show any disease symptoms. Different types of food can be contaminated from these sources. It has been isolated from raw milk, processed dairy products, raw and improperly cooked meats, fresh vegetables and improperly chlorinated water (Doyle et al., 1986 and Scheimann et al., 1989).

- **Gastroentritis infection caused by *Vibrio spp.***

The food borne pathogenic strains can cause hemolysis because of the presence of a heat-stable hemolysin. The infection may be caused due to consumption of raw, improperly cooked or contaminated sea foods (Twedt et al., 1989).

Diagnosis of these pathogens has therefore been the most important concern regarding the food safety. Till now there have been many techniques for the diagnosis of these harmful pathogens. This paper is dedicated to some of those techniques.

CONVENTIONAL METHODS

The conventional methods for enumeration of bacteria are often very tedious as they involve a number of steps. The steps involved are pre enrichment, selective enrichment, purification and isolation of single colony, biochemical screening, morphological identification and serological confirmation. Some of these methods are pour plate method, spread plate method, streaking, MPN method, etc. Culture based methods are still the most widely used detection techniques and remain the standard method for the detection of pathogens due to their selectivity and sensitivity. The traditional 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.In the colony count method the total number of pathogens in a given sample is determined by inoculating the sample onto a growth medium by the spread-plate method or pour plate method. The other method is MPN test in which three serial dilutions are transferred into 15 tubes of appropriate liquid medium for the five-tube method. After incubation, the number of positive tubes are counted and compared to standard MPN charts to predict the approximate number of bacteria present in the given sample. MPN is based upon the use of statistical charts. These methods have been used for detection of *Listeria monocytogenes* (Artault et al., 2001, DeBoer et al., 1999 and Stephan et al., 2003), *Staphylococcus aureus*, *Salmonella spp.*, *Escherichia coli* (Aycicek et al., 2004), *Campylobacter jejuni* (Sanders et al., 2007) and *Yersinia enterocolitica* (Weagant et al., 2008).

PCR BASED DETECTION

PCRis a simple molecular technique which is specific and reproducible. PCR is a thermo cyclic reaction that is completed within 3 steps; those are denaturation, elongation and termination. During eighties and nineties in the last century PCR has become widespread method for food pathogen detection and identification of bacterial species from clinical samples (Stone et al., 1994). PCR has been applied for the detection of microorganisms from microbial cultures, tissues and directly from clinical samples. Fecal specimens are among the most complex specimens for direct PCR testing due to the presence of inherent PCR inhibitors that are often co-extracted along with bacterial DNA. Here, the enrichment step is crucial in increasing bacterial cell numbers, prior to nucleic acid extraction (thereby obtaining higher yield and good quality DNA) and primer-specific amplification, in the highly sensitive and quick detection of bacterial contaminants in food matrices. It is important to note that Standard Plate Count is done both before and after enrichment to enumerate the bacteria in

the food sample prior to PCR (McKillip et al., 2002; Nakano et al., 2004). It has been recently reported that PCR has been used for the quantification of *Listeria monocytogenes* in meat samples (Rantsiou et al., 2008), *Staphylococcus aureus* in food samples (Alarcon et al., 2006) and *Campylobacter spp*. (Bottledoorn et al., 2008) in poultry carcasses. A quadruplex qPCR assay was developed for detection and differentiation of O1, O139 and non-O1, non-O139 strains of *Vibrio cholerae* and for prediction of their toxigenic potential (Huang et al., 2009). *Listeria monocytogenes* has also been investigated in biofilms using qPCR techniques (Guilbaud et al., 2005). A real time PCR that is able to identify and differentiate between 63 different serogroups of *Vibrio cholerae* has been developed and tested for the presence of seven targets with a detection limit 1.4cfu/mL (Bielawska et al., 2012). Single stranded DNA probes and non-functionalized gold nanoparticles have been used to provide a colorimetric assay for the detection of amplified DNA of *Salmonella spp*. in food samples (Prasad et al., 2011).

IMMUNOMAGNETIC SEPERATION (IMS)

IMS uses small super-paramagnetic particles or beads coated with antibodies against surface antigens of cells, they are efficient for the isolation of certain eukaryotic cells from fluids such as blood and this principle has been used for several medical applications. Isolation of specific bacteria bound to beads by the antigen-antibody reaction has generally been accomplished by inoculating the bead samples to cultivation broths or onto solid media selective for the target bacteria. The immuno-magnetic beads have been used for detection of *Escherichia coli* (Chapman et al., 2003), *Salmonella* (Jordan et al., 2004) and *Listeria spp.* (Kaclikova et al., 2001). Antibody coated magnetic beads were added in the sample containing pathogens. The IMS technique was combined with ECL (electroluminescence) for detection of *Escherichia coli* and *Salmonella typhi* present in food samples (Yu et al., 1996).

BACTERIOPHAGE BASED DETECTION

Bacteriophage is believed to have excellent specificity and selectivity for their host. Phage also has a unique property of expression for a fusion protein bearing a foreign peptide on its surface. Hence, libraries of fusion phage might be constructed and screened to identify proteins that bind to a specific antibody (Smith et al., 1997). These phage particles can be immobilized on target of interest and the unbound particles can be washed off and tightly bound particles are eluted and used as target probes. The complete infection cycle of a virulent phage usually takes 1 –2 h, by multiplication inside the host cell, it undergoes

amplification step, which makes it possible to shorten or completely overcome lengthy pre-enrichment procedures (Brovko et al., 2012). Reporter phage-based technology has been successfully leveraged for identification of several pathogens, including *Escherichia coli* (Waddell et al 2000), *Salmonella spp*. (Kuhn et al 2012), *Staphylococcus aureus* (Pagotto et al; 1996) and *Listeria monocytogenes* (Loessner et al., 1996). *Salmonella* has been recognized by phage p22 monolayer using *Escherichia coli* and *Listeria spp.* as control bacteria (Handa et al., 2008). Gold coated SF10 glass substrates were used for bacterial capture and studied using SPR. Receptor Binding Protein (RBP) functionalized SPR substrates were used for detection of host *Campylobacter jejuni* at concentrations as low as 10^2cfu/mL.They expressed GP48 RBP as a glutathione S-transferase-Gp48 (GST-Gp48) fusion protein and immobilized onto Surface Plasmon resonance (SPR) surfaces using glutathione self-assembled monolayers (GSH SAM). Immobilization of GST-Gp48 was done onto magnetic beads which were used to capture and pre-concentrate the host pathogen from suspension (Singh et al., 2011). T4 bacteriophage and BP 14 phage have been used for the detection of *Escherichia coli*and methicillin resistant *Staphylococcus aureus* (MRSA) respectively with a detection limit of 10^3cfu/mL within a period of 20 minutes (Tawil et al., 2012).

BIOLUMINESCENT DETECTION

ATP Bioluminescence: This technique measures light emission produced due to the presence of ATP, which is involved in an enzyme substrate reaction between luciferin and luciferase (bioluminescence).This method involves the emission of light produced by reaction between luciferin and luciferase (enzyme) in the presence of ATP. The amount of light produced is proportional to the concentration of ATP which in turn is proportional to the number of microorganisms in the sample. Bioluminescence produced is used to enumerate the total microorganisms present in a sample. This method is applicable if the number of pathogens present in the sample is high i.e. more than 10^4cfu/g (Samkutty et al., 2001 and Jasson et al., 2010). Specific antibodies raised against the target organisms were immobilized on the surface of 96-well plates, and then the sample matrices containing target cells in the wells were incubated. ATP bioluminescence was measured resulting in a limit of detection of 10^4cfu/mL for both *Escherichia coli* O157:H7 and *Salmonella typhimurium* (Hunter et al., 2010).

IMMUNOLOGICAL METHODS

The basic principle involved in the immunological method is the specific binding of an antibody to an antigen. Immunoassays are appropriate for detection of food borne pathogen due to their production of specific antigens and detection of bacterial toxins. ELISA is the most commonly used method which has high specificity to the suspect antigen present in the analyte. The micro titer plate contains primary antibody specific to the target in which the food sample is added. The target can be a component of the pathogen, such as a cell or flagellar antigen or enterotoxin (Notermans et al., 1991). After incubation, the unbound antigen is washed off and the secondary antibody is added. Washing is done and the assay is developed or tag is bound to the secondary antibody. ELISA is being used to detect either whole-cell antigen targets or products (e.g., virulence determinants) of pathogens such as *Salmonella spp*., *Escherichia coli* O157:H7, *Campylobacter spp.*, *Bacillus cereus* and *Listeria monocytogenes* (Peplow et al., 1999; Chen et al., 2001; Valdivieso-Garcia et al., 2001; Daly et al., 2002; De Paula et al., 2002; Yeh et al., 2002 and Bolton et al., 2002). Even though both antibody-based and nucleic acid-based detection have greatly decreased assay time compared to traditional culture techniques, they still lack the ability to detect micro-organisms in "real-time".

DNA MICROARRAYS

Microarray analysis is hybridization based technique that allows analysis of thousands of biological sample simultaneously. This technique also proved to be useful for the screening of pathogens in the background of non-pathogens. A microarray using single nucleotide extension labeling with gyrB as the marker gene was developed targeting *Escherichia coli*, *Shigella spp.*, *Salmonella spp*., *Aeromonas spp.* and *Helicobacter spp.*, etc. (Kostic et al 2007). DNA Microarray has also been used for detecting food borne pathogens and their further analysis enables multiple pathogen characterization and molecular identification of many genes in a single array assay. Perreten and workers had hybridized DNA from two field strains that allowed the detection of 12 different antibiotic resistance genes in a *Staphylococcus haemolyticus* strain (isolated from milk of cows with mastitis). They also designed a disposable microarray for detection of up to 90 antibiotic resistance genes in gram-positive bacteria. It was tested against 36 strains and enabled the detection of multi-drug–resistant strains of *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Staphylococcus haemolyticus*, *Clostridium perfringens* and an avirulent strain of *B. anthracis* harboring the broad-host-range resistance plasmid (Perreten et al., 2005).

BIOCHEMICAL KITS

Food pathogens are important cause of a variety of human and animal diseases. Commercially available biochemical kits for the detection of toxins offer ease of use and are easy to perform. The sample volume required by these kits is very low and rapid hence may be used for clinical purposes also. Miniaturized biochemical test kits show 90-99% accuracy in comparison to conventional methods. These biochemical kits have been used for the identification of *Enterobacteriaceae spp.*, *Listeria spp.*, *Staphylococcus spp.* and *Campylobacter spp.* (Cox et al., 1987 and Dzeizak et al., 1987).

MODERN METHODS

Biosensors: Biosensor is an analytical device that integrates a biological recognition element (e.g., an enzyme, antibody, biological whole cells, aptamer, nanoparticles such as quantum dots, gold nanoparticles, silver nanoparticles) with a physical (e.g., optical, mass, or electrochemical) transducer, whereby the interaction between the target and the bio-recognition molecules is translated into a measurable electrical signal (Thevenot et al., 2001). Biosensor is "a self-contained analytical device that incorporates a biologically active material in intimate contact with an appropriate transduction element for the purpose of detecting (reversibly and selectively) the concentration or activity of chemical species in any type of sample" (Arnold et al., 1988). Biosensors are devices that detect biological or chemical complexes in the form of the transducer. The biological elements include antibodies, enzymes, cofactors, microorganisms, etc. These elements are used for the fabrication of biosensors.

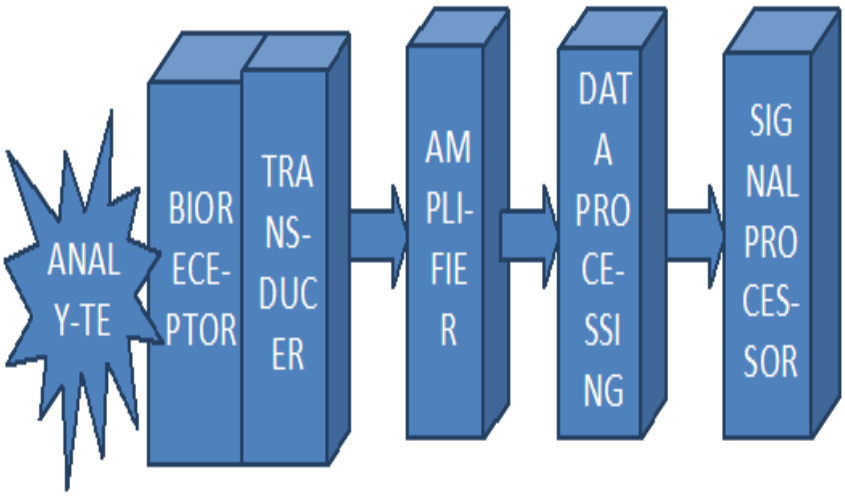

Figure 1: illustrates a schematic diagram of a biosensor

Biosensors are classified in four basic groups, depending on the method of signal transduction: optical, mass, electrochemical and thermal sensors. Optical biosensors work on the principle of light absorption, fluorescence, luminescence, reflection, Raman scattering and refractive index for conventional and analytical techniques. These biosensors provide rapid, highly sensitive, real-time and high-frequency monitoring without any time-consuming sample concentration or sample pre-treatment steps. Electrochemical sensors operate by reacting with the analyte and producing an electrical signal proportional to the analyte concentration. It consists of a sensing electrode (or working electrode) and a counter electrode separated by a thin layer of electrolyte.

Classification of biosensors

1. **On the basis of types of transducers**

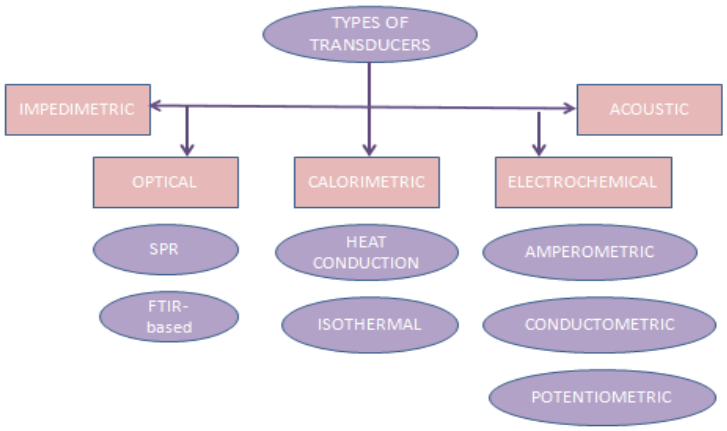

Figure 2: Types of transducers

2. **On the basis of types of bioreceptors**

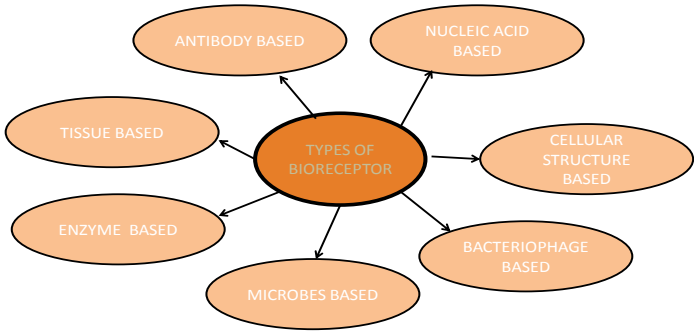

Figure 3: Types of bioreceptors

Nano biosensors have silica nanoparticles doped with fluorescent dye molecules and antibodies which react with antigens on the bacteria surface. Each of these nanoparticles contains thousands of dye molecules and nanoparticles are suitable to attach themselves to each bacterium. Fluorescent signal arising from the dye due to the reaction between antibodies and antigens can be amplified and measured. These sensors have been used for the detection of *Escherichia coli* (Otles et al 2010). Gold nanorod probes have been used for the simultaneous detection of *Escherichia coli* and *Salmonella typhi* with a detection limit 10^2 cfu/mL in less than 30 minutes based on Localized Surface Plasmon Resonance (LSPR). The amino-terminated gold nanorods were functionalized with antibodies to construct gold nanorodbioprobes (Chungang et al., 2008).

S.No.	Bioreceptor type	Transducer type	Pathogen detected	References	Year
1.	Nucleic acid	Amperometric	*Escherichia coli*	Li K. et al., 2011	2011
2.	Nucleic acid	SPR	*Salmonella spp.*	Zhang D et al., 2012	2012
4.	Bacteriophage	Amperometric	*Escherichia coli*	Singh et al., 2013	2013
5.	Filamentous E2 phage	Magneto elastic sensors	*Escherichia coli*	Li S. et al., 2010	2010
6.	T4 phage	SPR	*Escherichia coli*	Arya et al., 2011	2011
7.	T4 phage	SPR	*Escherichia coli*	Tawil et al., 2012	2012
8.	Phage	SPR	*Campylobater jejuni*	Singh et al., 2011	2011
9.	Antibody	FTIR Nanobiosensor	*Escherichia coli*	Mura et al., 2012	2012
10.	Antibody	Electrochemical sensor	*Bacillus cereus, Escherichia coli*	Emma B. Setterington et al., 2012	2012
11.	DNA aptamer	SWCNT based potentiometric sensor	*Escherichia coli*	Zelada et al., 2010	2010
12.	Aptamer	Impedimetric	*Salmonella typhi*	Labib et al., 2012	2012
13.	Antibody	LSPR	*Escherichia coli, Salmonella typhi*	Chungang Wang et al., 2008	2008
14.	DNA	Nanomaterials coated electrochemical sensor	*Listeria monocytogenes*	Kashish et al., 2015	2015
15.	DNA probes	Nanomaterials	*Staphylococcus, Pseudomonas, Kleibsella, Streptococcus, Lactobacillus, Enterococcus,Escherichia, Lactobacillus, Haemophilus, Proteus, Acinitobacter, Citrobacter, Stenotrophomonas*	Chung et al., 2013	2013

In DNA biosensor the bioreceptors used for pathogen detection is nucleic acids. Here the target nucleic acids are identified by matching with the complementary base pairs. Every

organism has unique genetic makeup i.e. the DNA sequences of every organism is different from the other. DNA biosensors are constructed by the immobilization of the oligonucleotide sequence (probe) onto a transducer that is able to convert a biological event into a measurable signal. Single stranded DNA biosensor is designed for the detection of the *Bacillus cereus* group species (Vijayalakshmi et al., 2009).

Electrochemical sensors operate by reacting with the analyte and producing an electrical signal proportional to the analyte. An electrochemical sensor consists of reference electrode, sensing electrode and counter electrode. Due to miniaturization of biosensors, the sample and reagent volume has been reduced. Cyclic voltammetry is a common electrochemical measurement technique in which a cyclic electric potential is applied between the electrodes and the resulting current flow is measured.

Carbon and gold electrodes have been used in the recent years for the detection of *E. coli*. DNA based electrochemical sensor has been used for the diagnosis of *Klebseilla pneumonia, Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa* and *Pseudomonas mirabilis,* etc. An ion-channel based biosensor was developed for rapid detection of *Campylobacter spp*. The sensing element composed of a stainless-steel working electrode covered by artificial bilayer lipidmembrane (BLM*)*. Antibodies to bacteria embedded into the BLM are used as channel forming proteins. The biosensor showed a very good sensitivity and selectivity to *Campylobacter spp.* (Ivnitski et al., 2000). Lysis of *Escherichia coli, Pseudomonas mirabilis, Klebseilla pneumonia* and *Pseudomonas aeruginosa* with Triton X-100 and lysozyme or Triton X-100 and lysozyme followed by NaOH resulted in successful electrochemical detection of 16S rRNA (Joseph et al., 2007).

Aptasensors are those biosensors which have aptamer molecules as their biorecognition element. Aptamers are small oligonucleotide molecules of RNA and DNA selected by Systematic Evolution of Ligands by Exponential enrichment (SELEX), binds selectively to its target through folding into a complex three-dimensional structure that bind with a high affinity and specificity to their target molecule. RNA aptasensor has been used for the detection of *S. typhi,* SWCNT potentiometric aptasensor is been reported to diagnose pathogenic strain of *Salmonella typhi*at a very low concentration of 0.2cfu/ml to 10^6cfu/ml. This potentiometric aptasensor showed a high specificity towards *S.typhi* as it showed no response for *Escherichia coli* and *Lactobacillus caesei* (Zelada et al., 2009). Impedimetric aptasensor has been used for the diagnosis of *Salmonella typhimurium*, highly specific DNA

aptamer were selected against this bacteria via SELEX technique. This sensor was so specific that it could distinguish *Salmonellatyphi* from other *Salmonellaspp.* (Labib et al., 2012). SELEX procedure was used for probing *Staphylococcus aureus*, in this a panel of eleven ssDNA were used out of which five were found to be highly specific to *Staphylococcus aureus* (Cao et al., 2009).

Optical biosensors have been designed in the area of food borne pathogen detection for detecting *Listeria monocytogenes* by the help of fiber optic sensor with a detection limit of 10^3-10^4 cfu/mL, taking 2.5 hrs (Bhunia et al., 2007). Gold nanoparticles have been used to develop a nanobiosensor for the detection of *E. coli O157:H7* in feed stock, where antibodies have been used as the biorecognition element. Diffferent dilutions of *E. coli* were taken and the shift in the SPR peak was monitored with respect to control. Also the color of the solution changed from red to blue for all dilutions except for the control (free bacteria). The color changed from red to blue in test samples due to binding between *Escherichia coliO157:H7* and specific site of its antibodies which conjugate with AuNPs (Ali et al., 2014). As mentioned in figure 2, optical biosensors can be classified into two types: SPR and FT-IR. Jue Wang and his colleagues had developed a new version of SPR biosensor for rapid and simultaneous detection of mixed bacterial infections caused from pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Clostridium tetani* and *Clostridium perfringens*. They enhanced the sensitivity of the instrument by attaching specific DNA probes to the biosensor (Wang et al., 2011). FT-IR spectroscopic method has been used for detecting *Escherichiacoli* O157:H7 strains with a detection limit of 10^5cfu/g. The analysis was done by separating the live and dead cells by filtration and IMS techniques followed by chemometric techniques such as OPUS, TQ Analyst and WinDAS softwares (Davis et al., 2010). FT-IR platform was linked to specific antibody to mesoporous titania thin for detection of *Escherichiacoli* O157: H7. The minimum detection limit was 10^2cfu/mL in less than 30 minutes (Mura et al., 2012). Carbon nanotube biosensors were covalently attached to Salmonella monoclonal antibody and immobilized onto a glassy carbon electrode to detect the presence of pathogens (Jain et al., 2012).

Grating-coupled surface plasmon resonance imaging (GCSPRI) has been used for detection of *Pseudomonas aeruginosa exotoxin A (ntPE), Bacillus globigii, Mycoplasma hyopneumoniae, Listeria monocytogenes, Escherichia coli* and M13 bacteriophage simultaneously. It can be used as disposable biosensor chips and produced in mass at low

cost. This instrument measures binding over 1000 regions of interest (ROIs) as it employs a microarray of specific antibodies immobilized on the sensor chip (Marusov et al., 2012). Gold nanoparticles have been used to make a strip test for the detection of *Vibrio cholerae O139* using monoclonal antibodies. The limit of detection was 10^4 cfu/mL within 20 minutes (Pengsuk et al., 2013).

CONCLUSION

Conventional pathogen detection methods are sensitive but they have delayed response time. Hence, the analytical techniques like optical, electrochemical, impedimetric, potentiometric, etc. biosensors can be used for the rapid detection of pathogens. Although these methods have some disadvantages as well, considering sensitivity and cost. Optical techniques possibly provide better sensitivity relative to electrochemical detection, but they are expensive. In terms of simplicity, miniaturization and cost, methods that use nanoparticles as bioreceptors can be developed.

FUTURE PROSPECTS

To devise a biosensor, one should focus on the transducer to be used. The parameters one should keep in mind while designing it are sensitivity, cost, specificity, detection limit and performance. Optical techniques are much more sensitive in comparison to other methods, but their cost and complexity makes them unappealing. However, electrochemical techniques are user friendly but quite insensitive and their performance is poor at times. Research needs to be carried out to increase the detection limit (between 10 and 100 cfu/ml) and performance. Cost is a major hurdle that needs to be taken care of, so that commercialization of biosensors can come into picture.

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