Strategies Behind Biosensors for Food and Waterborne Pathogens

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Bhaskar Das, P. Balasubramanian, R. Jayabalan, N. Lekshmi, and Sabu Thomas

Abstract

Slackness in the quality control of food and water consumed by human and other animals has become a significant issue which enhances the possibilities of cross-contamination with harmful pathogenic microbes. Intake of the contaminated food and water are the causes for the over abundance of infectious diseases in both animals and humans, and this has thus emerged as a global health concern. Detection of microbial contamination in food and water has relied on conventional methods which demand intensified pre-enrichment steps followed by laborious biochemical identification techniques. Recently, most promising and advanced techniques in biological sensor development have dragged all the scientist's attention which primarily deals with rapid real-time sensing applications due to its selectivity, sensitivity and specificity. In this book chapter, the possible routes of pathogenic infections have been outlined along with its various detection mechanisms. Additionally, strategies for the biosensor development have also been elaborated based on their transducing properties.

B. Das · P. Balasubramanian
Bioenergy and Environmental Laboratory, Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, Odisha, India

R. Jayabalan
Food Microbiology and Bioprocess Laboratory, Department of Life Science, National Institute of Technology, Rourkela, Odisha, India
e-mail: jayabalanr@nitrkl.ac.in

N. Lekshmi · S. Thomas (✉)
Cholera and Biofilm Research Laboratory, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala, India
e-mail: sabu@rgcb.res.in

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8.1 Introduction

Microorganisms are present in our environment from the beginning of life and have become one of the essential parts of the nature for maintaining the eco-system. The microorganism, such as viruses and bacteria are found in every facet of the environment, due to their high adaptable nature. Among all the microorganisms, bacteria play an important beneficial role towards animals and human, but certain potentially harmful bacteria can have a profound negative impact on people due to their pathogenicity. Infringement of bacterial contamination leads to disastrous infectious diseases worldwide and can affect human health in two possible ways. Firstly, due to the lack of quality control in food processing sectors, contamination of food by bacterial pathogens (such as *Escherichia coli, Salmonella typhimurium, Campylobacter jejuni, Legionella pneumophila, Staphylococcus aureus, Streptococci*, etc.) results in numerous food borne diseases (Doyle and Buchanan 2012). It is estimated that infectious diseases cause about 40% of approximately 50 million total annual deaths worldwide (World Health Organization 2008). Secondly, inadequate access to safe and portable clean water along with poor hygiene and sanitation facilities can lead to contamination with pathogenic bacteria such as *E. coli* O157:H7*, Vibrio cholerae, Salmonella enterica, Pseudomonas aeruginosa,* etc.) (Connelly and Baeumner 2012). Waterborne pathogens are capable of causing 10–20 million deaths and non-fatal infection of more than 200 million people each year (Berry et al. 2006). These food and waterborne pathogenic bacteria are resistant to environmental conditions, and most of the human population is susceptible to these pathogens which cause high fatality rate (World Health Organization 2008). Examples of these are the incidents that took place in 1997 Hudson ground beef recall and the 1996 incident where more than 9000 fell ill, and 313 died due to *E.coli* O157:H7 contamination (Ivnitski et al. 2000).

The current general practices for controlling the outspread of microbial diseases include careful control of various kinds of pathogenic bacteria by food safety, water quality control and environmental monitoring. Conventional techniques for detection and identification of pathogenic bacteria mainly depend upon accurate microbiological and biochemical identifications (Ferreira et al. 2011). Most of these methods can be sensitive and inexpensive and give both qualitative and quantitative analysis of the tested bacteria, but still have some ambiguities due to its low efficacy for detection of pathogens in samples with a less initial load of microbes. For example, standard methods like NF EN ISO 11290-1 method for the detection of *Listeria monocytogenes* needs nearly about 7 days to produce results as they rely on the ability of these microorganisms to produce visible colonies. Compared to this technique, some newer microbiological based test like ALOA® method (AES laboratories) uses chromogenic medium in combination with *Listeria* monodisk for the detection of *L. monocytogenes* which can decrease the detection time down to 3 days. This still presents

difficulties in the quality control of semi-perishable foods (Kumar 2013). Additionally, the transformation of bacterial cells into a dormant state which are viable, but non culturable (VBNC) makes the detection of such pathogens more difficult. Biosensors are recently emerging as a rapid method of detection of microbes in food and water (Mehrotra 2016). This chapter covers a different aspect of the development of biosensors for the detection of food and waterborne pathogens. Various types of biosensors with their mechanisms are described. Additionally, a major part of the chapter is devoted to describing the pathogenicity of water and foodborne pathogens and the primary mechanistic principle for the detection of these pathogens using biosensors. Furthermore, possible drawbacks of the existing biosensor technologies with the comparison of commercial technologies are also discussed.

8.2 Importance of Food and Waterborne Pathogens

Numerous sufferings and deaths are caused by foodborne pathogens worldwide. Around five million deaths are calculated in a year under the age of 5 in Latin America, Asia and Africa due to gastroenteritis (Lanata et al. 2013). *Campylobacter* induced enteritis is a significant illness in children aged 0–4 years in Mexico and Thailand. Statistics on foodborne pathogens showed decrease in occurrences from 1996–1998 to 2005 for *Shigella, Yersinia, L. monocytogens, Campylobacter* species, *E.coli* O157:H7, and *S. typhimurium* but upsurges for *S. enteritidis, S. heidelbeg,* and *S. javiana*. Many of the foodborne pathogens are spread through reservoirs like animals and poultry. Milk, meat and egg products may act as vehicles for *E. coli* O157:H7*, C. jejuni, L. monocytogenes, S. enterica*, and *Yersinia enterocolitica* (Ferens and Hovde 2011). Novel approaches to control the pathogens at farm level help to decrease the pathogen load in processing industries. However, ready-to-eat (RTE) food products are in serious concern since RTE products do not receive any treatment before consumption (Martinović et al. 2016). Several foodborne outbreaks have happened recently as the result of consumption of minimally processed fruits and vegetables, undercooked or processed dairy products, and RTE meats (Centers for Disease Control and Prevention (CDC) 2006).

Apart from gastroenteritis, the food and water borne pathogens cause autoimmune polyneuritis, autoimmune disease (allergic encephalitis), atherosclerosis, hemolytic-uremic syndrome (Shiga like toxin from *E. coli* O157:H7), chronic rheumatoid conditions, and Guillain-Barre syndrome (*Campylobacter* infections). Based on the eating habits, some foodborne infections exist in specific countries (Martinović et al. 2016). Consumption of raw fish in Japan and meat and vegetables in Scandinavian and middle/eastern countries are the reasons for *Vibrio parahaemolyticus* and botulism cases, respectively (Brandl 2006).

Around seven decades ago, the primary pathogens transmitted through food and water were *Clostridium botulinum, Clostridium perfringens, Salmonella, and S. aureus*. During these periods, the food borne illnesses were viewed as a trouble only for a day or two rather than a danger to life (Velusamy et al. 2010). Most of the countries did not have a systematic reporting program, except UK and USA. In most of the situations, the outbreak was due to the improper handling and poor storage conditions

of food, especially poultry and meat. *Campylobacter, E. coli* O157:H7, *L. monocytogenes* and *Y. enterocolitica* were emerging when the food service establishments were educated to handle the problems with *Clostridium, Salmonella* and *Staphylococcus* (Mor-Mur and Yuste 2010). Though the new pathogens were emerging, it took several years for the health service providers to understand the seriousness of these new pathogens. Large outbreaks during 1985 and 1993 from *L. monocytogenes* and *E. coli* O157:H7, respectively, in the US, has paved way for changes in food safety policies in the US and several other countries (Kramer et al. 2006).

Through some unexplored reasons, there is always the emergence of new pathogens or re-emergence of old pathogens those are responsible for the increased occurrences of foodborne diseases (Martinović et al. 2016). Food and water borne outbreaks are not only causing human sufferings and fatalities but also distressing financial losses to food processors and producers. The reasons like (1) increased surveillance and reporting, (2) changes in the food manufacturing and agricultural practices, (3) changes in eating habits, (4) increased vulnerable populations, (5) improved detection methods, and (6) emerging pathogens with tolerance to stressed conditions are reported to be the possible reasons for the greater numbers of outbreaks in recent years (Bhunia 2008).

8.3 General Routes of Infection and Spreading Diseases

Three forms of diseases caused by foodborne pathogens are foodborne infection, intoxication, and toxicoinfection. Because of water and food are major reservoirs for foodborne illness, oral route and intestine are the primary route and site of infection, respectively. To make a successful infection, microorganism must have to pass several hurdles, and several factors have to work in a host cooperatively. The food and water borne pathogens can be transmitted even through direct contact with an infected animal or human, through soil or an arthropod vector (Conner and Schmid 2003). Following are the factors which determine the success of an infection by a foodborne pathogen (Cossart and Sansonetti 2004):

(i) Pathogens must be present in adequate numbers to initiate the infection process.
(ii) Pathogens are able to endure the changing environment of the host and must be able to multiply (presence of capsules or not).
(iii) Pathogens should find a place for their colonization through adhesion and invasion factors, and chemotaxis.
(iv) Pathogens must have some mechanisms (toxins and enzymes) to escape from the host immune system.
(v) Pathogens must damage the host tissues and cells by their component or metabolites (exotoxins, endotoxins, enzymes, etc.) that cause cell death by necrosis or apoptosis and encourage bacterial survival and multiplication.

Intact living microorganisms are necessary to initiate the foodborne infection. Upon intake with food or water, pathogens reach the intestine after passing the stomach

environment. Colonization starts in the intestine followed by crossing the intestinal barrier through the invasion process or through translocation by phagocytic M cells (Ribet and Cossart 2015). Few pathogens cause localized damage, and some may spread to the liver, spleen, lymph nodes, brain or to other extraintestinal sites. Acute food borne infections are quick and last only for short duration due to fast clearance of microorganisms by immunological mechanisms. Chronic food borne infections are long and immunological removal is not effective against pathogens. Patients recovering from a foodborne infection releases pathogens to the environment for a while. The infectious dose of the pathogens varies from 50 to 10^9 CFU (Colony Forming Units) per gram of food for live bacteria or 10^3–10^5 numbers for spores or 10–100 particles of virus or 10–100 cysts for protozoa (Kent et al. 2015). This difference in numbers depends on the infective potential of the organism, immunological nature of the host, type of food consumed, and presence and absence of the antibiotics in the host body. The human body is gifted with several mechanisms to protect itself from the invasion of the pathogens. Mucus and fluids constantly wash the epithelial cell surface of the gastrointestinal tract. Human body constantly attempts to expel the pathogens by mucus production, peristaltic movements, and by epithelial ciliary sweeping action. The presence of bile salts, proteolytic enzymes, and resident microflora also prevent colonization of the pathogens in the gastrointestinal tract (Martinović et al. 2016). After reaching the intestine, the pathogens must be able to attach themselves to the intestine and cross the intestinal barrier by the mechanisms listed in Table 8.1. Ingestion of preformed toxins (botulinum toxin, *Bacillus cereus* toxin, staphylococcal enterotoxin, and seafood toxins) results in the food borne intoxication. Actively growing pathogen releases toxins in the food which are ingested. Toxins must be ingested and absorbed in the epithelial lining of the gastrointestinal tract to cause the inflammation which evokes diarrhoea or vomiting. In case of foodborne toxiconfection, ingested bacteria along with food colonize the mucosal surface and produce exotoxins in the intestine (Iwamoto et al. 2010). Exotoxins either damage the local cells or tissues or enter the blood stream to induce the disease. Toxins of enterotoxigenic *E. coli* (heat-labile and heat-stable), *V. cholerae* (cholera toxin) and enterotoxins of *C. perfringens* are examples of exotoxins (Sibley 2004; Bhunia 2007; Ray and Bhunia 2007).

8.4 Detection of Pathogens in Food and Water

The food industry increasingly adopts several measures to improve the safety and quality of food (Scognamiglio et al. 2014). Hazard Analysis Critical Control Point (HACCP), a management tool highly promoted by various regulatory agencies in many countries is to attain a safer food supply and coordination of trading values. Rapid methods of monitoring are desirable to make the HACCP efficient (Aung and Chang 2014). Testing for the presence of specific pathogens like *C. jejuni*, *E. coli* O157:H7, *L. monocytogenes* and others is required to validate the HACCP. Hence, it is very essential to have rapid methods for the detection of the pathogens. Food and water borne pathogens are detected by several techniques (Fig. 8.1) which include culture based methods, electrical methods, methods based on ATP

Table 8.1 Characteristics of some food and water borne pathogens and toxins and their mechanism of infection (Bhunia 2007)

Pathogens	Infectious dose	Incubation period	Types of pili	Adhesion factor	Receptor	Toxins	Mode of action	Target
E. coli O157:H7	50–100 CFU	3–9 days	Type I and P pili – *E. coli*, Type IV and BFP (bundle forming pili) – EPEC	Intimin (94 kDa) – EPEC and EIEC	Translocated intimin receptor (TIR) – EPEC and EIEC	1. Hemolysin (*E. coli*)	1. Pore formation	1. Plasma membrane
						2. Heat-labile toxin (LT) (*E. coli*)	2. ADP ribosyltrans-ferase	2. G-proteins
						3. Heat-stable toxin (ST) (*E. coli)*	3.Stimulates Guanylate cyclase	3. Guanylatecyclase
L. monocytogenes	10^2–10^3 CFU	7–14 days or even longer	NA	Internalin A (88 kDa), Internalin B (65 kDa), Vip (virulence protein) (43 kDa), LAP (listeria adhesion protein) (104 kDa)	E-cadherin, cMet, gC1q-R/p32, Gp96	Listeriolysin O	Pore formation and Apoptosis	Cholesterol
Shigella spp.	10–100 CFU	12 h to 7 days, but generally in 1–3 days	NA	NA	NA	1. Shiga toxin or Shiga like toxin	1. N-glycosidase	1. 28S rRNA
						2. IpaB	2. Apoptosis	
V. cholerae/V. haemolyticus/V. vulnificus	10^4–10^{10} CFU/g	6 h to 5 days	Type IV	Toxin-coregulated pili (TCP), Mannose-fucose resistant cell-associated hemagglutinin (MSHA), mannose-sensitive hemagglutinin (MSHA)	Glycoprotein	Cholera toxin (*V. cholerae*)	ADP ribosyltransferase	G-protein

S. aureus cells	10^5–10^8 CFU/g	–	NA	Fibronectin-binding protein (FnBP)	Fibronectin	1. Alpha-toxin	1. Pore formation on	1. Plasma membrane
						2. Enterotoxins, toxin shock toxins	2. Superantigens	2. TCR and MHC II
Staphylococcal enterotoxin	1 ng/g of food	1–6 h	NA	NA	NA	NA	1. Pore formation	1. Plasma membrane
							2. Super antigens	2. Major Histocompatibility Complex (MHC)
B. cereus	10^5–10^8 CFU or spores/g	1–6 h (vomitting), 8–12 h (diarrhea)	NA	NA	NA	Heat and acid resistant nonhemolytic enterotoxin, hemolysin BL, cytoxin K, Cereulide	Pore formation	Plasma membrane
B. anthracis (inhalation anthrax)	8×10^3–10^4 spores	2–5 days	NA	NA	NA	1. Edema factor	1. Adenylatecyclase	1. ATP
						2. Lethal factor	2. Metalloprotease	2. MAPKK1/ MAPKK2
C. botulinum neurotoxin	0.9–0.15 µg (i.v. or i.m. route) and 70 µg (oral route)	12–36 h; 2 h when large quantities are ingested	NA	NA	NA	Neurotoxin	Zinc metalloprotease	Synaptobrevin, SNAP-25, Syntaxin
C. perfringens	10^7–10^9 CFU	8–12 h	NA	NA	NA	Perfringolsin O	Pore formation	Cholesterol
C. jejuni	5×10^2–10^4 CFU	1–7 days (24–48 h)	NA	CadF (37 kDa)	Fibronectin	Enterotoxin and Cytotoxin	Pore formation	Gut enterocytes

NA Not Applicable

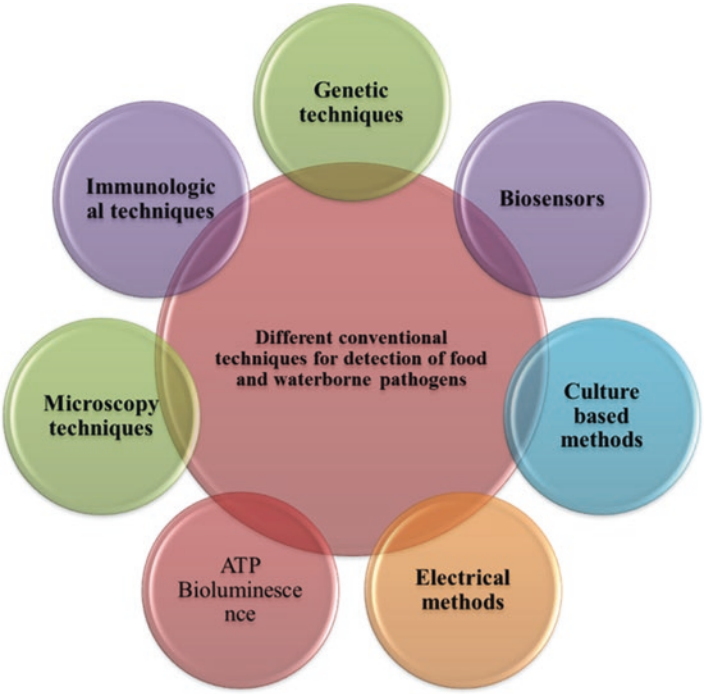

Fig. 8.1 Different detection methods of food and water borne pathogens

bioluminescence, microscopy techniques, immunological techniques, genetic techniques, and techniques using biosensor (Leonard et al. 2003). Comparison of the detection methods established for food borne pathogens is given in Table 8.2.

Culture methods detect the pathogens by growing or keeping them alive in a nutrient medium. Culture medium mainly comprises six components like amino-nitrogen compounds (peptones, other protein hydrolysates, infusions or extracts), energy sources (e.g. glucose), buffer salts (e.g. soluble phosphate salts, acetates and citrates), mineral salts and metals (phosphates, sulfates, calcium, magnesium, iron, manganese and trace metals), growth promoting factors (blood, serum, vitamins, NADH, etc.), and gelling agents (agar, gelatin, alginates, gums, etc). The basic culture medium can be made selective to allow the growth of the specific pathogens from food samples by adding selective compounds to which the pathogen of interest should be resistant (Blommel et al. 2007). Inorganic salts (sodium azide, lithium chloride, potassium tellurite added to control Gram negative bacteria; tetrathionate and sodium selenite are added to control Gram positive bacteria and coliforms), dyes (acriflavine, crystal violet, brilliant green, and malachite green), surface active agents (bile salts, cetrimide, lauryl sulphate and tergitol), and antibiotics are added to basic medium to give selective features. Apart from selective agents, components which provide differential features to the basic medium can also be added to make the basic medium a differential culture medium. Indicator dyes to indicate the pH

Table 8.2 Detection methods of food and water borne pathogens (Bhunia 2007)

Detection methods	*S. aureus*	*B. cereus*	*B. anthracis*	*C. botulinum*	*C. perfringens*	*S.enterica*
Culture methods	Baird-Parker agar; black, shiny, circular, convex, smooth with the entire margin forming a clear zone with an opaque zone (lecithinase halo) around the colonies	Determination of lecithinase activity in polymyxin-egg yolk mannitol-bromothymol agar (PEMBA)	Determination of non-hemolytic colonies on blood agar, lecithinase activity on egg yolk agar, visualization of capsules using India ink, confirmation of hydrolysis of casein, starch, and gelatin, acid production from salicin, inulin, and mannitol	Microscopic observation of cells with "tennis racket" appearance, observation of raised or flat, smooth or rough with some spreading, and irregular edged colonies with luster zone (pearly zone) when observed under oblique light on egg yolk agar	Isolation of *C. perfringens* using thioglycolate medium, tryptose-sulfite cycloserin agar containing egg yolk or brain heart infusion agar with 10% sheep blood under anaerobic incubation.	Pre-enrichment (lactose broth, tryptic soy broth, nutrient broth, skim milk or buffered peptone water), selective enrichment (Rappaport-Vassiliadis semisolid medium, selenite cysteine broth, or Muller Kauffmann tetrathionate broth) and isolation of pure cultures (Hektoen enteric agar, xylose lysine deoxycholateagar and brilliant green agar)
Cytotoxicity-based assays	Detection of mammalian cell damage by superantigens (Staphylococcal enterotoxins)	Detection of mammalian cell damage by emetic toxin	NA	NA	NA	NA
Nucleic acid-based methods	PCR based detection of enterotoxin genes (egc), exfoliative toxins A and B (etaA and etaB), methicillin- resistant (mecA) gene, and 16S rRNA	PCR based detection of toxin genes (ces, hblA, hblD, hblC, nheA, nheB, nheC, bceT, entFM, cytK, hlyII, hlyIII)	NA	Multiplex PCR for detection of *C. botulinum* serotypes A,B, E, and F	Multiplex PCR for detection of toxin genes: enterotoxin (*cpe*), alpha (*cpa*), beta (*cpb*), epsilon (*etx*), and iota (*iap*)	TaqMan based Q-PCR for *inv*A genes, NASBA method for detection of viable cells

(continued)

Table 8.2 (continued)

Detection methods	*S. aureus*	*B. cereus*	*B. anthracis*	*C. botulinum*	*C. perfringens*	*S.enterica*
Immunoassays	ELISA for superantigens	Antibody based assay for the detection of components of hemolysin and nonhemolytic enterotoxin	NA	Detection of toxins using polyclonal antibody	NA	Enzyme-linked immunosorbent assay (ELISA), surface adhesion immunofluorescent technique, dot-blot immunoassay, surface plasmon resonance (SRP) biosensor, piezoelectric biosensor, time-resolved immunofluorescence assay (TRF), fibre optic sensor
Other methods	Direct detection of whole cell or other metabolites, direct epifluorescence technique (DEFT), flow cytometry, impedimetry, ATP-bioluminescence	NA	NA	Mouse bioassay: intraperitoneal injection of toxin preparation to mice and observation for the symptoms include laboured breathing, pinching of the waist and paralysis, which develops in 1–4 days	NA	NA

Culture methods	Campylosel medium under microaerophilic conditions for *Campylobacter*	Observation of red colonies on modified cefsulodin-irgasan-novobiocin (CIN) agar with esculin (nonhydrolyzing pattern)	Observation of opaque, smooth, and round shaped with irregular edged colonies on blood agar, brain heart infusion agar, MacConkey agar	Observation of growth on selective thiosulfate citrate bile salt agar (TCBS), cellobiosepolymyxin B colistin (CPC) and mannitol-maltose agar	Usage of MacConkey, *Salmonella-Shigella*, Xylose-Lysine Deoxycholate, and Hektoen Enteric agars
	Modified charcoal cefoperazonedeoxycholate (mCCDA)for *Arcobacter* isolation				
Cytotoxicity-based assays	NA	NA	NA	NA	Plaque assay on cultured cell monolayer
Nucleic acid-based methods	PCR based detection of genes including flagellin (*fla*A), 16S rRNA, and 16S/23S intergenic spacer region for *Campylobacter*	PCR to detect yadA, virF, 16S rRNA genes and Q-PCR for *ail* gene	Real-time 5′ nuclease PCR for *pla* gene	Single or multigene-specific PCR that targets specific sequences in 16S rRNA, *tdh, trh, gyrb, toxR, ctxB, ctxAB*, and *tcpA*	Conventional and nested PCR for various species of *Shigella* which targets *ipaH, virA, ial* (invasion associated locus), LPS, and plasmid DNA
	Multiplex PCR targeting 16S and 23S rRNA genes for *Arcobacter*				

(continued)

Table 8.2 (continued)

Detection methods	*Campylobacter and Arcobacter*	*Y.enterocolitica*	*Y. pestis*	*Vibrio species (V. cholerae, V. parahaemolyticus, V. vulnificus)*	*Shigella species*
Immunoassays	NA	Indirect immunofluorescence with biopsy specimens	ELISA, direct immunofluorescence assay, and dipstick assays targeting F1 antigen or Pla protein	ELISA to detect intracellular and thermostable direct hemolysin (TDH) antigens	Wellcolex Color *Shigella* test (Latex Agglutination), EIA kits for *Shigella dysenteriae* (Shigel-Dot A), *Shigella flexneri* (Shigel-Dot B), *Shigella boydii* (Shigel-Dot C), *Shigella sonnei* (Shigel-Dot D)
Other methods	NA	Mouse bioassay for enterotoxin (Yst) determination or rabbit ileal loop assay	**NA**	NA	Rabbit ileal loop assay (RIL) to test the diarrheagenic action of toxins, newborn mouse model to study inflammation and tissue damage in the large intestine

NA Not Applicable

change in the medium (e.g. phenol red, neutral red, bromocresol purple), chromogens which can act as substrates for specific enzymes and changes the color of the medium, fluorogens which can produce fluorescence that can be detected when observed under UV illumination) (Sharma and Mutharasan 2013). Virulence factors of the pathogens can also be detected by adding blood (hemolysin), egg yolk emulsion and lecithin (phospholipase), and rabbit plasma fibrinogen (coagulase) (Jia et al. 2010). Altering the incubation conditions (pH, temperature and gaseous atmosphere) of the culture medium will also be helpful in selectively growing the targeted pathogen by suppressing the growth of others. Resuscitation media were developed to recover the injured cells by adding components which will reduce the damage to the targeted pathogen (e.g. blood, pyruvate, catalase, and cysteine to protect cells from reactive oxygen species). Several commercial kits are in the market (PetrifilmTM, CLONdiscTM, BD Biosciences, Oxoid Salmonella Rapid Test, Salmosyst®, Colilert®, ColiTrak®, and Quanti-Tray®) working based on culture methods for qualitative and quantitative analysis of food borne pathogens. Culture methods form the basis for all the detection techniques for pathogen in food. Culture based methods of detection are widely accepted due to their reliability, lower cost, ease of use, and universal acceptance (Stephens 2003; Alahi and Mukhopadhyay 2017).

An electrical method determines the response of microbial cultures to alternating current (AC) at specific frequencies. Electrolysis of growth medium and killing of microbes occurs at high currents, whereas the mediators (lipoic acid) present at the cell surface save the cells at lower current. Electrodes are required to be immersed in the growth medium or food homogenate (Luo et al. 2015). Impedance and conductance are the two parameters required to be determined either alone or in combination. Impedance is the measure of the AC equivalent of resistance to a direct current (DC) current. Capacitance and resistance are the two components of impedance at any frequency (cycles per second, Hz). Properties of the electrode, changes and conductance occurring between electrodes are related to capacitance (Singh et al. 2014). Reciprocal of the conductance is resistance. Impedance of the pure water and salt solutions is solely their conductance due to zero capacitance, whereas microbiological media have capacitance due to the presence of macromolecular content and microorganisms (Puttaswamy and Sengupta 2010). Changes in biomass results in changes in impedance but changes in conductance are the results of the effect of microbial metabolism on the charge-carrying capacity of the medium. Low molecular weight products produced through metabolism of proteins and carbohydrates are good charge carriers than the large molecular weight products. Hence, every growth medium has an impedance value based on the composition of the chemicals and inoculum (Sharma and Mutharasan 2013). Among the components of media, salts have major effect on impedance and hence the salty foods need dilution. Electrical methods are not useful in detecting pathogens in selective media with high salt levels. The assay temperature needs to be controlled due to the high-temperature coefficient of impedance which is 2% per degree (Gibson 2003; Alahi and Mukhopadhyay 2017).

ATP bioluminescence assay makes use of the availability of adenosine triphosphate (ATP) in all living cells as a energy donor. The intracellular concentration of ATP is measured to detect viable cells (Falzoni et al. 2013). Chemical energy associated with ATP is converted into light by luciferase-luciferin complex. Light detecting devices measures the emitted light and it is directly proportional to the ATP concentration. The theoretical sensitivity of this assay is high due to the availability of instruments which can detect every single photon emitted during the reaction. However, the presence of ATP in food (somatic cells) must be taken care before the assay. Somatic cells must be selectively lysed with non-ionic detergents (Triton X-100) and the released ATP of somatic cells must be enzymatically or chemically destructed. Cationic detergents are subsequently used to extract the ATP from microbes and are then measured by luciferase-luciferin. Microbial cell number is derived from the standard curves of ATP concentration or from emitted light in RLU (relative light units) to cell counting units (CFU/ml). ATP bioluminescence assay is made specific to particular pathogens by recognizing the target bacteria using antibodies or by specific bacteriophages followed by ATP assay (Griffiths and Brovko 2003; Noble and Weisberg 2005).

$$\text{Luciferin} + \text{ATP} + \text{O}_2 \rightarrow \text{Oxyluciferin} + \text{AMP} + \text{CO}_2 + \text{PPi} + \text{Light}$$

Interactions between specific antibodies and selective antigens on the pathogens are the basic mechanisms of the immunological techniques. Antibodies are targeted against the components on the outer cell wall, a protein on the flagella, or a metabolite or toxin produced by the pathogens during growth (Tlaskalová-Hogenová et al. 2011). Antibodies targeted against antigens on flagella are highly specific whereas the antibodies for somatic antigens are weak due to the sharing of antigens among a wide range of bacteria. Immunological methods are considered to be presumptive methods due to the sharing of common antigens across the microbial genus. The results from immunological methods are usually confirmed by culture based methods. Among the immunological methods ELISA (enzyme-linked immunosorbent assay) is the most widely used methods. ELFA (enzyme-linked fluorescent assays), a variant of ELISA, uses fluorescence based detection (Yeni et al. 2014). Magnetic beads coated with antibodies are used in IMS (immunomagnetic separation) to separate the target pathogen from the food components or other microbes. Different commercial kits based on immunological principles are available in the market for the detection of *Salmonella, Listeria, Campylobacter,* and *E. coli* O157:H7. Lateral flow devices are membrane based devices which rely on the immune-chromatography principle and provides rapid end-point testing. Apart from these techniques, simple agglutination tests are also widely accepted for the detection of food borne pathogens (Baylis 2003; McCarthy 2003).

Very precise detection of pathogens is possible by targeting specific sequences in DNA and RNA. Several methods are available for the amplification of specific sequences on the target pathogen. Polymerase Chain Reaction (PCR) technique relies on a thermostable polymerase, set of primers and nucleotide bases to amplify the specific sequence recognized by the primers. The amplified sequences are visualized by staining with ethidium bromide after agarose gel electrophoresis for the

presence of a band or bands of the expected size. There are several types of PCR developed by improving the sensitivity of the traditional PCR (Girones et al. 2010). Nested PCR uses two sets of primers which flank the target sequence to be amplified. Reverse transcriptase (RT-PCR) amplifies the sequences in RNA. Simultaneous detection and quantification of a nucleotide signal in Real-time PCR is made possible by continuously measuring a fluorescent reporter during the reaction. NASBA (nucleic acid sequence-based amplification) is an isothermal amplification technique which involves the simultaneous activity of avian myoblastosis virus-reverse transcriptase (AMV-RT), ribonuclease H, and T7 RNA polymerase. Target sequences in the pathogens can also be detected by hybridization technique in which the single stranded nucleotides are annealed together based on the complementarity. The techniques mentioned above do not identify several pathogens simultaneously (Singh et al. 2014). Microarray technologies facilitate the simultaneous detection of several pathogens at a time. Advances in sequencing technologies and bioinformatics have led to a tremendous increase in the use of molecular subtyping protocols for the identification of pathogens. The chemotaxonomic method of identification is based on the analysis of antigenic characteristics, whole cell protein analysis and composition of fatty acids (Fizgerland and Swaminathan 2003; Sanderson and Nicholas 2003).

Detection of pathogenic bacteria using DNA amplification method has shown promising outcomes in the field of pathogen detection where PCR is used to enhance the sensitivity of the nucleic acid based assay. Target nucleic acid segment of defined length and sequence are amplified by following three steps of PCR such as denaturation, annealing, and extension of oligonucleotide primers by using thermostable DNA polymerase (Mandal et al. 2011). PCR technique has different advantages over culture and other conventional methods due to its specificity, sensitivity and rapid accuracy. However, there are still some difficulties due to its polymerase enzyme specificity towards environmental contaminants which leads to difficulties in quantifying the generation of false positives through the detection of naked nucleic acids, non-viable micro-organisms, or contamination of samples in the laboratory (Lampel et al. 2000). From industrial application point of view, regular detection of bacterial contamination (food and water borne) using PCR technique can be expensive and complicated which requires highly skilled personnel with accuracy (Singh et al. 2014).

Among all the conventional techniques for pathogen detection, immunological detection with the use of antibodies has shown some positive result for the detection of the bacterial cells, spores and viruses (Iqbal et al. 2000). Polyclonal antibodies can be raised rapidly and cheaply as compared to monoclonal antibodies. However, the limitation of polyclonal antibodies regarding specificity encourages the development of hybridoma techniques along with recombinant antibody phage display technology (Harris et al. 2004). Since last few years, immunological methods for the detection of bacterial pathogens have become more accurate, sensitive and reproducible with many commercial immunoassays available in the market (Uematsu et al. 2006). Even though both nucleic acid-based and antibody-based detection has been able to decrease the time consumed for the assay, they are still

deficient in the ability to detect pathogens in "real-time". The requirement for a more sensitive, reliable and less time consuming and specific method of detecting a target analyte, at low cost, is the focus of many research, in particular for applications in environmental samples (Leonard et al. 2003).

The biosensor technology is one of the newly emerging techniques which offers the potential for detecting pathogens in real time. However, it still requires time-consuming pre-enrichment to detect small numbers of pathogenic bacteria in food and water. Advancement in the antibody dependent sensing techniques along with the emergence of phage displayed peptide biosensors show increased possibilities for the detection of water and foodborne bacteria (Benhar et al. 2001; Goodridge and Griffiths 2002).

8.5 Mechanism of Biosensors

A biosensor is an analytical device that consists of a bio-recognition element coupled to a signal transducer to detect an analyte of interest by converting a biological response to an electrical signal (Turner 2013). In most cases, "real-time" observation of a particular biological event (such as antigen-antibody reaction) can be done successfully by using this technology. Biosensors can enable the detection of analytes with a broad spectrum present in complex sample matrices and have shown promising outcome in areas such as food analysis, clinical diagnostics and environmental monitoring (Fitzpatrick et al. 2000). Common bio-recognition elements such as oligonucleotide probes, antibodies, enzymes, aptamers, cell-surface molecules and phages are called as bio-receptors which can recognize the target analyte molecules. Similarly, another major part of the biosensor is a transducer,which can be further classified into different aspects such as optical, electrochemical, thermometric, piezoelectric, magnetic and micromechanical or a combination of one or more than one of these techniques mentioned above (Arya et al. 2011). Biosensor devevloped to detect food and water borne pathogens must be having higher efficacy and real time validation. They should also have some basic and essential idealistic characteristics such as accuracy towards the pathogen detection with low or preferably zero probability of false positive and falst negative results (Rider et al. 2003). It has to be quick enough to produce a "real-time" response in case of perishable food analysis. The sensitivity of the biosensor has to be high to determine the pathogenic bacteria in food or water sample with a lower concentration, and it should detect false positive results efficiently. Additionally, along with higher sensitivity, the biosensor has to be highly specific for the target analyte; it should discriminate between target pathogen, toxin and other microorganisms. Along with these main features, a biosensor has to be reproducible, robust, and user-friendly (Zhao et al. 2014).

Nanotechnology has emerged as an elementary division of material science receiving global attention, owing to its wide array of applications. Nanoparticles are of great interest due to its small size, large surface to volume ratio and other novel characteristics (Dasgupta et al. 2015). Due to the wide range of applicability of this

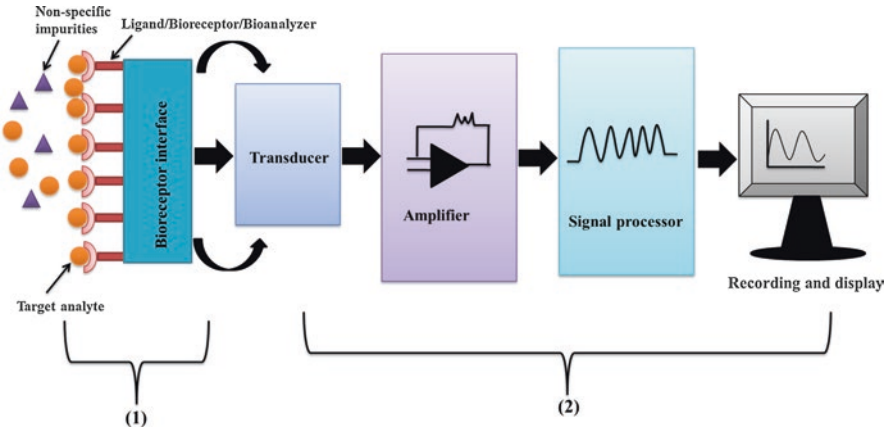

Fig. 8.2 Basic key components of a biosensor

nanoscience and technology, it has a simulated group of scientists to fabricate a nanomaterial based device for bio-analysis (combining nanomaterials with biological molecules). These bio recognition devices are capable of rapid and sensitive detection which can detect even a single cell of food and water borne pathogens within a few minutes (Arora et al. 2011). Development of nano-based materials such as nanoparticles, nanobelt, nanowire, nanofiber and nano-flakes have trans figured clinical and molecular biology by their significant use as bioanalyzer and biodetector. Yang et al. (2007) have described the application of polymeric nano-particles conjugated with biomolecules such as antibiotics, antibodies, adhesion molecules and particular DNA sequence for specific pathogen detection.

In general biosensors consist of two basic key components (Fig. 8.2) (1) Recognition element which can be categorized in to different substances such as, biological, enzymatic or cellular components (oligonucleotides, peptides, DNA sequence, and aptamers, etc.) basically termed as bio-receptors or bio-analyzers and (2) transducer (signal conversion unit). In addition, all biosensors consist of input/output interface (an electronic component which interacts with the instrument). The recognition element (bio-receptor/bioanalyzer), a ligand which binds directly or indirectly to the target molecule or component (analyte) is mainly responsible for producing a primary signal (Perumal and Hashim 2014). The transducer is the component that responds to the main signal from the recognition element and converts it into a form that can be amplified, stored, manipulated, displayed and analyzed. The signal produced by the recognition element can be generated by the direct interaction of ligand and analyte molecule which further can be analyzed directly with the help of a detector. This kind of biosensors is known as a direct (label free) biosensor (Ronkainen et al. 2010). Similarly, in some biosensors, generation of primary signal relies on the presence of any secondary molecules such as fluorescence labelled marker molecules which lead to the indirect detection of the target analyte. This type of biosensors is known as an indirect biosensor. Transducers work based on many physical principles including fluorescence, electrochemistry, optics, mass

detection, etc. For the selection of a biosensor ligand (bio-receptor), two main criteria are followed such as affinity and specificity. Antibodies are broadly used as ligands in both direct and indirect biosensors due to their specific characteristics, versatility, and strong and stable binding specific antigens. Biosensors that use antibodies as the recognition element are called as immunosensors. Commercial antibodies are readily available for many food and waterborne pathogens. Most suitable recognition elements that appear to have potential outcomes for biosensor applications includes antibody fragments (Fab) and recombinant variation of antibody fragments (Emanuel et al. 2000). Antibodies in immunosensors can be produced by genetic immunization which involves the transfer of DNA specific for the antigen to stimulate antibody production and peptides by phage display techniques (Goldman et al. 2000). In the following chapters, different strategies behind the biosensors, their mechanisms and functionality will be discussed briefly.

8.6 Different Strategies for Pathogenic Bacteria Detection

Biosensors can be classified on the basis of their basic elementary part of the sensing system such as type of bio-receptors and transducer (Fig. 8.3). Additionally, for the detection of food and waterborne pathogens, several techniques have been described that allows the direct measurement of contamination from different liquid

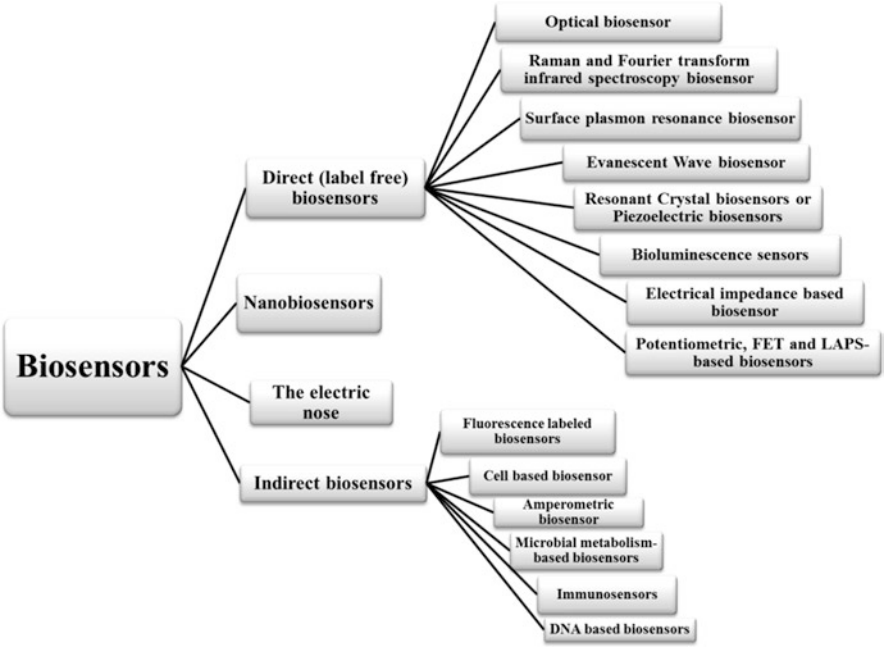

Fig. 8.3 Classification of biosensors

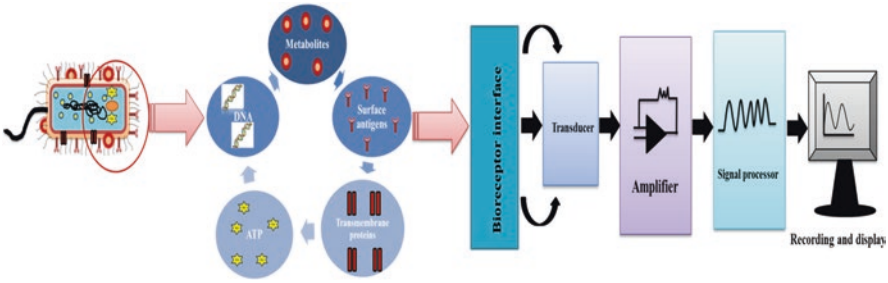

Fig. 8.4 Different strategies for detection of food and water borne pathogens

or solid interfaces (Mascini and Tombelli 2008). Several strategies have been developed to detect either the component (surface antigens, nucleic acids, and transmembrane proteins) or the metabolites produced by the pathogens (Fig. 8.4). In general, biosensors can be classified in two broad catagories (1) Direct biosensors and (2) Indirect biosensors.

8.6.1 Direct (Label Free) Biosensors

Direct (label free) biosensors are based on the direct measurement of a physical phenomenon occurring during a biochemical reaction on a transducer surface. Signal parameters such as a change in oxygen concentration, pH gradient and ion consumption, potential difference and current, resistance and optical properties can be monitored by using different electrochemical or optical transducers and amplified for the data storage and analysis. This direct technique can be further classified according to the process used for signal transduction (Perumal and Hashim 2014).

8.6.1.1 Optical Biosensors

Optical biosensors have gained popularity as the direct (label free) method for the detection of food and waterborne pathogenic bacteria amongst all of the other techniques due to their high selectivity and sensitivity. These sensors are capable of detecting tiny changes in refractive index or thickness, which occurs during the attachment of target cells or analyte component on the transducer surface. Amidst all other optical biosensors, very first commercially available sensor is fiber-optic which was marketed by Research International (Monroe, WA). The basic principle behind the detection mechanism of the fibre optic sensor relies upon the fluorescently labelled pathogen (target analyte) or toxins which when bound to the receptor molecules on the transducer surface gets excited by the laser wave at 635 nm (Tait et al. 2005). Fluorescent signals generated by continuous laser excitation were detected by the fluorescence detector in the real time system (Bhunia 2008). Various optical biosensors for the detection of pathogenic bacteria (Baeumner et al. 2003), toxins (Bae et al. 2004) and other contaminants from water and food samples have been developed so far, and out of them, the fluorescent biosensor has shown

promising outcome due to its outstanding sensitivity and specificity. In fluorescent biosensors, a fluorescent compound attached to antibodies enhances the efficacy of pathogen detection. FTIC (fluorescein isothiocyanate) is a regularly used fluorescent marker (Li et al. 2004). Other than this technique, few recently developed techniques for pathogen detection use techniques such as the Raman spectroscopy, surface plasmon resonance, laser, etc. (Yoo and Lee 2016).

8.6.1.2 Raman and Fourier Transform Infrared Spectroscopy Biosensor

Spectroscopies based on the vibrational energy such as Raman spectroscopy and Fourier transform infrared (FT-IR) are the more recurrently reported whole organism fingerprinting techniques (Kloß et al. 2013). There is always a need of culturing the microorganisms for the detection of the specific analyte from the mixture of samples to get the highest amount of biomass. Raman spectroscopy is an optical technique based on the principle of light scattering and it has been utilized by many researchers as a mean of rapid detection of bacterial pathogens from food and water samples. Schmilovitch et al. (2005) employed a disperse system of the spectrophotometer with a 785 nm diode laser to detect both Gram-positive and Gram-negative bacteria which showed clear dissimilarity between samples containing bacteria and control (without bacteria). In this approach, a pathogen of interest along with target analytes are separated from the sample with the help of capture biomolecules, which add an extra layer of specificity there by synergistically enhancing the efficacy of the sensors.

8.6.1.3 Surface Plasmon resonance Biosensor

Surface plasmon resonance (SPR) works based on the principle of optical illumination can be utilized for biomolecular analysis (Scarano et al. 2010). Additionally, plasmon represents the excited free electron present in the outer surface of the metal layer. Compatible light energy photons are the source of this resonant excitement. The amplitude of this resulting plasmon electromagnetic wave is the maximum at the interface (Anker et al. 2008). Direct label free detection of food and waterborne pathogens is also possible with this method. Application of SPR based biosensors have been described by several researchers for the detection of food and water borne pathogen such as *L. monocytogenes, C. jejuni* (Koubova et al. 2001; Taylor et al. 2006; Ray and Bhunia 2007), *Salmonella* (Oh et al. 2004), and *E. coli* O157:H7 (Subramanian et al. 2006). Additionally, many commercial SPR based biosensors have been employed by many researchers for the detection of food and waterborne pathogens in recent years. Spreeta™ biosensors was used for the detection of *E. coli* O157:H7 (Waswa et al. 2007). Biacore 3000 was utilized by Leonard et al. (2004) for the detection of *L. monocytogenes.*

In Spreeta™ biosensors, the incident light from LED reflects off a gold surface, the angle and intensity analogous to the SPR minimum is measured representing the changes in the refractive index which corresponds to the coupling of antigen-antibody complex at the sensor surface (Waswa et al. 2007). This real-time assay was conducted, and the results were obtained after 30 min. The sensitivity of the

assay was nearly 10^2–10^3 CFU/mL. Specificity of the assay was confirmed from the sensorgram as other pathogens (such as *E. coli* K12 and *Shigella*) did not show any changes during the analysis. Balasubramanian et al. (2007) also described the label free detection of *S. aureus* with the help of lytic phage as immensely precise and selective bio-recognition element and SPR based Spreeta™ sensor was chosen as a detection platform.

8.6.1.4 Evanescent Wave Biosensor

Generation of an evanescent wave can be defined as the total reflection of incident light at a particular angle. Under such conditions, an energy field is generated which penetrates a short distance past the reflecting surface. The circulation of the generated evanescent wave relies on the optical properties of the thin layer of the medium adjacent to the reflecting surface. These phenomena can be applied to the detection of pathogenic bacterial contamination in different food and water samples. Many fibre optics sensors use evanescent wave physics (Marazuela and Moreno-Bondi 2002). Evanescent based immunosensors are having a limit of detection (LOD) range between 1 and 10 ng/mL for large (>30 kDa) proteins (Nedelkov et al. 2000) depending on the molecular weight of the antigen and its affinity towards the antibody. Direct measurement of low molecular weight molecules such as mycotoxins with the molecular weight of 750 Da yield higher LODs as compared to the larger analytes. For the detection, at low range (LOD) various indirect methods such as a sandwich method or competitive assays are often used (Rasooly 2001).

8.6.1.5 Resonant Crystal Biosensors or Piezoelectric Biosensors

Resonant crystal biosensors are one of the widely used biosensors due to their simple mechanism of sensing technology and low cost. It is also known as quartz crystal microbalance (QCM) and piezoelectric biosensor (Marrazza 2014). The primary mechanism of this sensing technology relies on the changes of the acoustic resonant frequency of a quartz crystal during the attachment of a target analyte to the crystal surface. Quartz disk attached to electrodes works as a transducer in this sensor which can amplify the signal produced as an acoustic wave generated by application of an external oscillating electric potential across the device. The acoustic wave is generated through piezoelectric (PZ) effect resonating on the crystal at a particular frequency which is dependent on the mass of the analyte bound to the sensor surface. Resonant crystal biosensor allows real-time, direct and label free analysis of larger antigens which leads to the successful detection of pathogenic bacteria present in water and food samples (Law et al. 2015). Piezoelectric immunosensors were developed for *V. cholerae* (Chen et al. 2010) and *S. typhimurium* (Arora et al. 2011). Specificity of sensors are based on the receptor-analyte reaction, for example, in the immunogravimetric microbial assay (Kazemi-Darsanaki et al. 2012), for the detection of *Candida albicans* an anti-*C. albicans* antibody coated with PZ crystal was used and the sensitivity of the assay was in the range of 10^6–10^8 CFU/mL. Therewas no attachment of other species except *C. albicans* in the reaction, and no significant shifts in the frequencies were observed due to non-specific adsorption.

8.6.1.6 Bioluminescence Sensors

The emergence of bioanalytical sensing tools by using the ability of certain enzymes to produce a photon as a byproduct of their biochemical reactions have shown promising outcome for pathogen detection. This phenomenon is known as bioluminescence and can be used for the detection of physiological conditions of cells. Ulitzur and Kuhn (1987) first described the application of this bioluminescence for bacterial detection by luciferase reporter phages. In their work, they have introduced the luciferase gene in the genome of a bacteriophage, which can infect the bacterial cell. Bioluminescence sensors have been used for the detection of a wide range of bacteria (VanDorst et al. 2010). Blasco et al. (1998) have demonstrated the development of an accurate and sensitive method for the detection of *Salmonella spp.* and *E. coli.* They have used bacteriophages for the specific lysis of bacteria and the cell content released was measured by ATP bioluminescence. An increment in the sensitivity was obtained by focusing on bacterial adenylate kinase as the cell marker instead of using ATP. Emission of light was measured as proportional to the cell numbers over three orders of magnitude, and 10^3 cells were easily detectable in a 0.1 ml sample volume (Tallury et al. 2010).

8.6.1.7 Electrical Impedance Based Biosensor

Impedance is termed as the total resistance of a conductive system in AC supply and consists of two major basic part (1) capacitance which depends on the characteristics of the electrode and (2) conductance, which depends on the conductivity of the medium. In the case of impedance based biosensors, microbial metabolism results in an increase in capacitance and conductance due to the conversion of larger macromolecules and another component to its monomeric smaller form which led to increasing the charge carrying capacity (Varshney and Li 2009). This increment in the capacitance and conductance resulted in the decrease in the impedance. Therefore, the alteration of impedance, capacitance and conductance are only different ways of monitoring the test system and are all inter-related (Shimazaki et al. 2015). The relationship between impedance (Z), resistance (R), capacitance (C), and frequency (*f*) of a resistor and a capacitor series is articulated as follows (Miller et al. 2010):

$$Z^2 = R^2 + 1/\left(2pfC\right)^2$$

Bridge circuit measures this impedance phenomenon. There is always a need for a reference module to measure and exclude the nonspecific changes in the test module. Due to this requirement, one pair of electrode is used in these sensors. The reference module serves as a controller for evaporation, changes of dissolved gases, temperature and most importantly degradation of culture medium (Singh et al. 2014).

This impedance method is widely used by most of the researchers, and the Association of Official Analytical Chemists International (AOAC) accepted it as a first action method (Monaci and Visconti 2010). This approach is well established for the detection of specific food pathogens. A significant parameter for the

pathogen in food and water sample is cell viability. Viable cells can be measured by using a microscope after suspending the cells in a dye such as Trypan Blue. A new emerging biosensor for real time monitoring of the concentration of growth and physiological state of cells was proposed by Monaci and Visconti (2010). This biosensor is based on the measurement of alteration in impedance during the growth of adherent cells on integrated electrode structures (Zeng et al. 2016). The impedance of the biosensor changes according to the cell density, growth and long-term behaviour of the cells grown on the electrode at different time interval. Most impedance analysis is completed within 20–25 h. Gracias and McKillip (2004) investigated the detection of *Salmonella* using this method in 250 food samples. Food samples for the analysis were pre-enriched 14–16 h at 37 °C in peptone water.

8.6.1.8 Potentiometric, FET and LAPS-Based Biosensors

These are amongst the least common directly measurable biosensors used for pathogen detection. The working principle is based on the detection of ions present in the test solution. There is always need of one inert reference electrode and one working electrode in contact with the sample. Detection of pathogenic bacteria is possible with these biosensors by continuous monitoring of the pH changes or fluctuation in ionic concentration during the in-situ analysis conditions. Bergveld (2003) employed the use of ion-selective field effect transistor (ISFETs) for biological detection events. They have also demonstrated the fabrication of this type of biosensor using p-type silicon substrate with two n-doped regions, one acting as a source and another as a drain with a gate in between which acts as an insulator due to the presence of SiO_2 covered region, which is further over encrusted by ion selective membrane. Another new technique has been evolved for the detection of food borne pathogen by combining ISFET and potentiometry with optical detection. It is known as a light-addressable potentiometric sensor (LAPS) (Perumal and Hashim 2014; Wu et al. 2015).

8.6.2 Indirect Biosensors

8.6.2.1 Fluorescence Labelled Biosensors

Microorganisms are composed of various biological entities like proteins and polysaccharides which act as antigens in immunogenic reaction. This phenomenon permits the development of immunoassay techniques for pathogenic bacterial detection. In fluorescent immunoassay (FIA), immunoglobulins are labelled with fluorochromes which absorb short-wavelength light and then emit light at higher wavelengths which can be detected using fluorescent microscopy. Fluorescein isothiocyanate and rhodamine isothiocyanate-bovine serum albumin are most commonly used fluorochromes to tag antibodies for the detection of bacteria contaminated samples in both direct and indirect methods (Parkinson and Pejcic 2005). For detection of food borne bacteria by using FIA, food samples are pre-enriched with culture mediums because of less number of viable bacterial cells in food sample and also to reduce the interference caused by the background fluorescence producing food particulates (Perumal and Hashim 2014).

Similarly, detection of waterborne pathogenic bacteria in water samples can be done by concentrating the bacterial cells through membrane filtration. Polycarbonate filters are commonly used in this procedure (Mandal et al. 2011). Using this technique, detection of waterborne pathogenic bacteria *E.coli* O157:H7 was possible in the range of 10^5–10^9 CFU/ml within 4 h of assay time periods. Additionally, it has also been utilized for the detection of *S. typhimurium* and *Klebsiella pneumoniae.* Chowdhury et al. (1995) used a similar kind of technique for detecting *V. cholerae* O1 and O139. Bacterial cells were incubated with yeast extract in the presence of nalidixic acid which leads to the growth of substrate responsive viable bacterial cells with elongated and enlarged shape and was readily detectable using fluorescent antibody.

8.6.2.2 Cell Based Biosensor

These biosensors also serve as a dependable tool for the detection of pathogens in food and water samples. Working principle of cell based assays depends upon the electrical properties of cells to figure out the changes in the cell's vicinity (Singh et al. 2013). Due to the presence of various biological molecules at the cell surface, it functions like a capacitor where fluid acts as a resistance element. Electrical impedance detects the minute alteration in cell growth, density as well as the differences in the regular activities of the cell due to the influence of the external environment. Detection of the pathogenic bacteria in food has been done with the help of mammalian cells (Gray 2004). The detection of enzymes and cofactors from the cell system has become easier due to the massive release of metabolites (chemical compounds) into the culture medium during the assay (Fratamico and Bhunia 2005).

8.6.2.3 Amperometric Biosensor

Amperometric biosensors can detect the electrochemically active analyte which can be oxidized or reduced on the electrode. Amperometric biosensors consist of thin film construction made of gold (Au), carbon or platinum. For screen printing, the electrodes, substrates (glass, plastic or ceramic) are coated with thin films of ink in a particular pattern. Different inks can be applied to get various dimensions and shapes of the biosensor. It includes a series of basic steps, selection of the screen, selection and preparation of inks, selection of substrate and finally drying and curing stages (Arora et al. 2011). The main advantages of this technology are design flexibility, process automation, good reproducibility and a wide choice of materials. In recent years, screen printed electrochemical cells, which are cheap, affordable and can be produced in large scale, are widely used for developing the amperometric biosensors for the detection of various foods and waterborne pathogens (Lazcka et al. 2007; Velusamy et al. 2010). Disposable nature of these sensors lead to decrease in chance of contamination during electrode fouling due to the over growth of microorganism which results in loss of sensitivity and accuracy of the biosensor. Amperometric biosensor depends upon the enzyme functionality which helps to convert electrochemically inactive analyte to electrochemically active analyte through a catalytic process. Horse radish peroxidase and alkaline phosphatase have been used commonly as the functional enzymes. These biosensors are used to

develop immunosensors and genosensors for pathogen detection (Palchetti and Mascini 2008; Arora et al. 2011).

8.6.2.4 Microbial Metabolism-Based Biosensors

These types of biosensors have been developed for the detection of bacterial pathogens by their metabolic pathways. The amperometric transducers are used for tracking the biochemical reactions in the bacterial cell metabolism. In general, Clark type oxygen electrode is used for the measurement of oxygen consumed by the bacterial cells (Patel 2002; Timur et al. 2003). The detection of pathogens was carried out by the measurement of the cathodic peak current of oxygen during the proliferation period of bacterial cells (Ruan et al. 2002). One more detection mechanism using electrochemical transducer relies on the detection of specific marker enzymes after culturing the test sample in a suitable medium. By using this strategy, detection of coliforms in water and food samples have become readily possible due to the mere presence of enzymes such as β-D-glucuronide glucuronosohydrolase (GUS) and β-D-galactosidase (β-GAL). Detection of *E.coli* using conventional approaches to detect GUS enzymes or β-GAL is a much more lengthy process which relies on spectrometric detection of the bacterium. Conventionally, the bacterial cells are first treated with chromogenic substrates such as p-nitrophenyl-β-D-glucuronide (PNPG) and then monitored spectrometrically, until the release of chromophore indicators (p-nitrophenyl (PNP) and d-glucuronic acid) confirming the presence of *E. coli* in the test sample. To overcome this time-consuming protocol Mulchandani et al. (2005) developed an efficient electro-oxidative method for GUS detection using bacteria-based biosensor. They have immobilized food borne pathogenic bacteria on a carbon paste electrode that can degrade PNP and produces hydroquinone (intermediate) for oxidation at a lower potential. A rapid detection method for viable *E. coli* cells was developed by Pérez et al. (2001) using enzyme β-D-galactosidase that can convert 4-aminophenyl-β-D-galactopyranoside (4-APGal) to 4-aminophenol (A-AP) after hydrolysis**.**

8.6.2.5 Immunosensors

These types of biosensors work based on the principle of immunology where antigen-antibody interaction takes place. Specific antibodies are used for the detection of specific antigen or toxin of microorganisms. Due to the specific binding phenomenon, it has a high level of sensitivity towards the detection of particular water and food borne pathogens. Antibodies for this sake can be immobilized on the surface of the electrodes or magnetic beads that lead to differentiation of two different kinds of immunosensors (1) immunosensors based on antibody immobilized on the electrode surface and (2) immunosensors based on antibody attached on the surface of the magnetic beads (Arora et al. 2011). In this biosensors, enzyme-substrate catalysis happen in the presence of antibody and produces products such as ions, pH variation, or oxygen consumption, which further lead to the generation of electrical signal with the help of a transducer. Numerous approaches have been used in the immune-module operations of biosensors, which includes an antibody-based system for the detection of food and waterborne pathogens such as *E.coli*

O157:H7 and *Salmonella spp*. Immunomagnetic beads have also been utilized to enhance the selectivity of amperometric biosensors (Liu et al. 2001; Abbaspour et al. 2015). In this technique, *S. typhimurium* is sandwiched between antibody coated magnetic beads, and alkaline phosphatase (enzyme) labelled antibody. After that, by using a magnet, beads are localized onto the surface of a disposable graphite ink based electrode in a multiwell plate format. The presence of bacterial cells is detected by the oxidation of the electroactive enzyme product. This technique offers a LOD of 8×10^3 cells/ml in buffer sample within 80 min (Gehring et al. 1996).

8.6.2.6 DNA Based Biosensors

In recent years, newly emerged DNA based biosensorshave shown promising outcome in the field of pathogen detection. These biosensors consists of short nucleic acid sequences also known as probes with the specificity towards a particular bacterium conjugated on the surface of a transducer. The probe DNA sequence binds to the complementary DNA sequence of the target bacterium and leads to the detection of the pathogenic bacteria; this event is also known as hybridization. The level of hybridization indicates the presence of a complementary sequence in the sample which finally leads to the detection of the target pathogen (Nordin et al. 2016). Some reviewers (Kerman et al. 2003) have demonstrated the application of electrical transducers with the combination of DNA based detection. Different pathogenic bacteria can be detected easily by using disposable low-density genosensor arrays. This can be fabricated by using a screen-printed array of Au electrodes having immobilized thiol-tethered oligonucleotide and biotinylated signalling probes (Farabullini et al. 2007) for complimentary sequence detection. Analysis strategy depends on the identification of a toxin produced by the specific bacteria responsible for the production of toxin. This can be termed as one of the most crucial steps for the detection technique as the encoded gene of the target bacteria can frequently express toxin in food samples (Singh et al. 2014). Wang (2002) have successfully developed one novel detection method for the detection of *Cryptosporidium*, *E.coli*, and *Giardia* by using genosensor technology. Bacterial immobilization of specific oligonucleotides was done by using carbon pasted electrode, and chronopotentiometric techniques. Further simultaneous monitoring of hybridization outcomes were measured by real-time sensors for pathogen detection.

8.6.3 Nano-biosensors

The interplay between nanomaterials and biological system creates an emerging research field of vast importance. Unique features of nanoparticles such as small size, large surface to volume ratio and other novel characteristics make them tremendously applicable. In particular, application of nanomaterials for the development of nano-based biosensors for sensing applications has received considerable attention (McFarland and Van Duyne 2003). Integration of one dimensional (1D) nanomaterials, such as nanowires, or two dimensional (2D) nanotubes in electric devices offer substantial advantages for the detection of pathogenic bacteria and

have more advantages over conventional optical bio-detection methods (Gruner 2006). Optical-affinity biosensors based on SPR do the qualitative and quantitative measurements of biomolecular interactions between immobilized biomolecule on a metal surface and target analyte in the test solution. Application of nanotechnology in the field of optical bio-detection has emerged significantly in recent years (Kumar et al. 2015). To enhance the sensitivity of an SPR based biosensor, Au nanoparticles (NPs) have been utilized to amplify their detection level. Joung et al. (2008) employed Au NPs in a signal amplification system to enhance the sensitivity for the detection of *E. coli* 16s rRNA by using peptide nucleic-acid probes with an SPR biosensor system. DNA based biosensor for the detection of particular pathogens has become a widely used technique. Recent advancements in nanotechnology have also resulted in the development of bio-barcode assay which can provide amplification without the use of PCR and can detect many target pathogens in one sample (Li et al. 2005; Stoeva et al. 2006). This method is based on the bio-functionalization of Au NPs with a ds-DNA or ss-DNA bio-barcode, bio-receptor (such as antibody or oligonucleotide) or single component modified microparticles (MMPs) containing another bio-receptor capable of binding with the target analyte. Complexes formed by sandwiching an analyte molecule between two bio-functionalized particles are then separated using a magnet (Rowland et al. 2016). The DNA bio-barcode is released and detected by using a chip-based detection method consisting of silver (Ag) enhanced Au NPs or a fluorophore bound to the bio-barcode (Oh et al. 2006). Magnetic nanoparticles have also been utilized by many researchers for the development of a sensing technology with higher specificity and sensitivity at a lower cost. One example is the work done by Koets et al. (2009) who have employed the development of magneto-resistant sensor using supermagnetic particles as detection labels for *E. coli* and *Salmonella*. Additionally, there are many examples in the literature for novel NPs-based materials for electrochemical bio-sensing which enhances the specificity and efficacy of real-time analysis (Kumar et al. 2015). For example, screen printed carbon electrode modified with Au NPs display a 13.1-fold increase in detection of *E. coli* O157:H7 compared to the traditional screen printed carbon electrode with a working range of 10^2–10^7 CFU/mL (Lin et al. 2008).

8.6.4 The Electronic Nose

Electronic nose system has gained popularity rapidly during the last few years, the majority of its applications concentrate in the field of pathogenic bacterial detection in different food and water samples. Electronic nose system comprises of sophisticated software, data preprocessing, and statistical analysis of collected data by pattern recognition (PR) software (Loutfi et al. 2015). This technology has been used extensively in the field of sensors due to its potential for detecting target samples based on acoustic waves, conducting polymers or semiconducting materials. Several reports can be found in recent years on the use of electronic nose to detect or identify bacteria. Schiffman et al. (2001) have investigated the efficacy of the electronic nose using 15 different kinds of metal-oxide sensors to classify bacteria and fungi.

Due to the microbial metabolism, they produce some volatile organic compounds along with gases during the growth period. These metabolic products can be monitored by these sensors because they have tremendous information potential and respond to both odorous and odourless volatile compounds. Magan et al. (2001) have demonstrated the application of electronic nose biosensor using 14 conducting polymeric sensors to detect volatile profiles produced by non-inoculated skimmed milk media and media inoculated with *B. cereus* and *Candida pseudotropicalis*. Bacteria present in the test sample were detected by the automated headspace analyzer which consists of conducting polymer sensors (Osmetech Microbial Analyzer, OMA). Each of the sensors is having different sensing abilities towards various volatile organic compounds. This system was also used for screening urine specimens by sampling urine headspace and analyzing the output of the multi-detector response (Aathithan et al. 2001).

8.7 Conclusion

The upsurge of infectious diseases caused by water and foodborne pathogens are perilous global health issues. Technologies which can rapidly, sensitively, and correctly detect their presence in accordance with water and food safety regulations at clinically significant levels are essential for the upgradation of health and quality of life for millions of people. In this chapter, note on water and foodborne pathogens, infectious diseases caused by them and their specific route of infections have been discussed briefly. Additionally, numerous strategies for the development of advanced sensing techniques (biosensors) for the detection of food and waterborne pathogens have been described along with their advantages over the conventional approaches. Biosensors have great potential in detection of food and waterborne bacterial pathogens. The sensitivity of different biosensors discussed in this chapter vary relying on transducer properties and specific biological recognition elements (bio-receptors). Though several sensing strategies are developed through research, only a few approaches have shown their potential to reach the commercial market. Many of the newly developed biosensors can detect single or few analytes, but a future aspect of biosensing will be the multiple-sensing element instruments. For example, immunosensors based on the use of different antibodies are placed in an orderly arrangement in the 2-dimensional format of the chip. Using this technique, various antigens of water and foodborne pathogenic bacteria can be detected by their binding to specific antibodies at unique positions. Similarly, DNA based biosensors have also demonstrated their efficacy at low concentrations, but they require a purification process in upstream which is time-consuming.

Current and future research need to be concerned in two important cases (1) detection of pathogens in their actual environment matrixes and (2) pre-processing/pre-enrichment steps required for the analysis. Additionally, it involves miniaturisation strategies, material research and emphasis on multiplexing so that various pathogenic target analytes can be detected at once in “real-time” scale. Taken together, this technology can provide novel approaches capable of providing high

sensitivity, specificity and speed to replace the current and conventional strategies. This would hopefully, improve access to safe drinking water and safe food for consumption thereby reducing the global health issues due to water and foodborne diseases.

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