

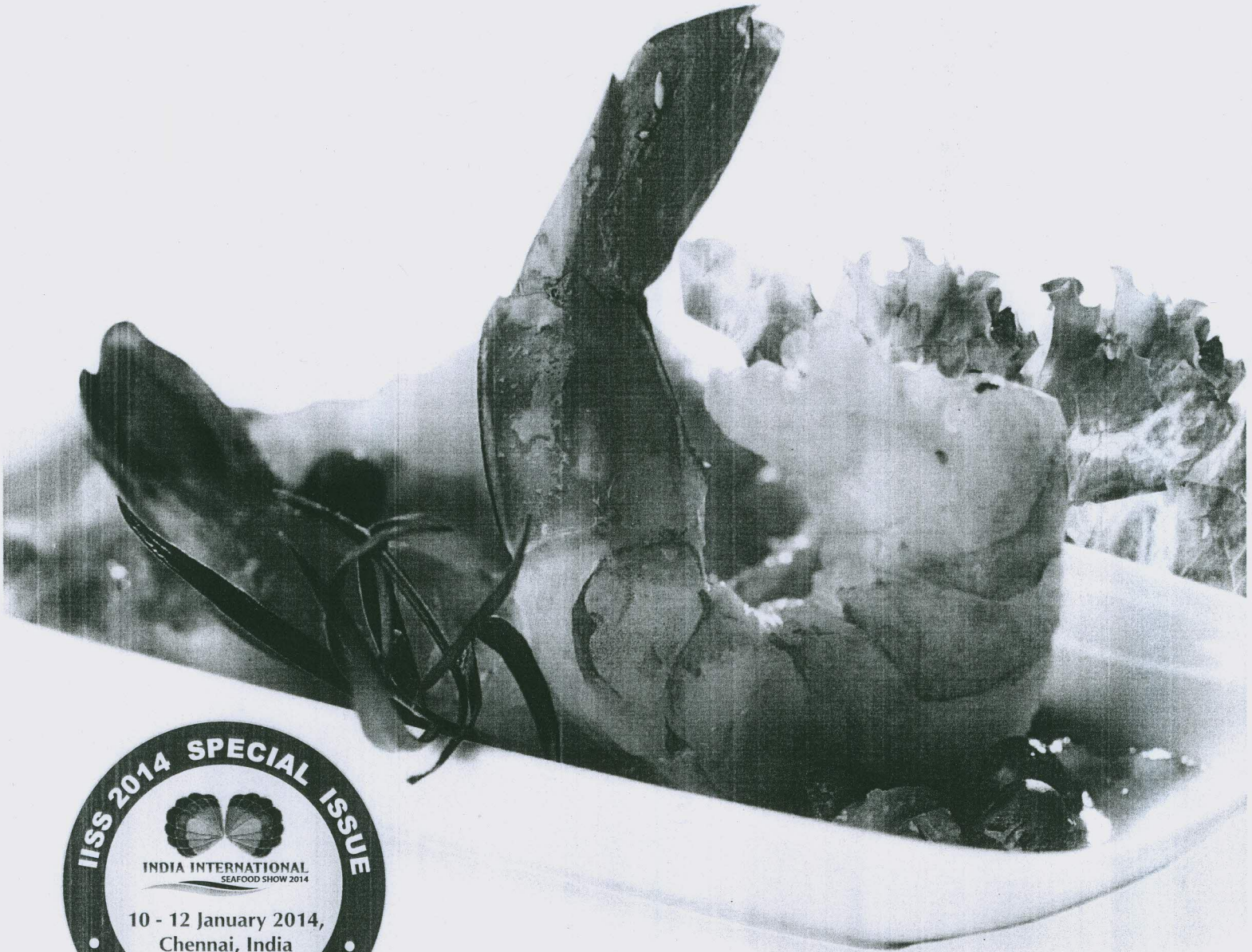
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Bacterial hazards associated with seafood and methods for their detection



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Seafood is an important part of a healthy diet providing high value protein, essential micronutrients and polyunsaturated omega-3 fatty acids. Fish production in India has increased from 41.57 lakh tonnes in 1991-92 to 86.6 lakh tonnes in 2011-12. Seafood, like any food item, has the potential to cause diseases from bacterial pathogens under certain circumstances. A number of bacterial illnesses may arise from the consumption of seafood that has either been contaminated at source or during the processing and retail chain. Such illnesses may arise from infection with the pathogenic bacteria themselves or by the ingestion of toxins formed in the foodstuff prior to consumption. Worldwide, foodborne diseases have increased continuously. Expansion of international trade, consumer demand for "lightly processed" foods, mass production of foods, and changes in eating habits were the main reasons for this. Though the global incidence of foodborne disease is difficult to estimate, it has been reported that in 2005 alone 1.8 million people died from diarrhoeal diseases and a great proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2007). Public concern regarding food safety has increased markedly over the past decade. Fish and fishery products are in the forefront of food safety and quality improvement because they are among the most internationally traded food commodities. According to

statistics published in 1999, bacteria accounted for 72% of the deaths associated with foodborne illness in USA (Mead *et al.*, 1999). The number of outbreaks with bacteria as a confirmed etiology has increased steadily since the mid-2000s.

The incidence of bacteria-related foodborne pathogens varies substantially according to geographic location. Three types of bacterial foodborne diseases are recognized: intoxications, infections, and toxicoinfections. Foodborne bacterial intoxication is caused by the ingestion of food containing preformed bacterial toxin, such as the toxins produced by *Staphylococcus aureus* and *Clostridium botulinum*, resulting from bacterial growth in the food. Foodborne infection, on the other hand, is caused by ingestion of food containing viable bacteria such as *Salmonella* or *Listeria* which then grow and establish themselves in the host, resulting in illness. Foodborne toxicoinfections result when bacteria present in food, such as *Clostridium perfringens*, are ingested and subsequently produce a toxin in the host.

Although the safety of food has dramatically improved in the last few decades, foodborne outbreaks from microbial contamination are common in many countries. According to the Centre for Disease Control (CDC), USA, the incidence of five of the seven reportable foodborne bacterial pathogens (*Campylobacter*, *Listeria*, Shiga toxin-producing *E coli* (STEC) O157, *Shigella*, and *Yersinia*) decreased from 1996 to 2010.

Seafood-associated illnesses caused by a diverse group of pathogens results in a wide variety of clinical syndromes, each with its own epidemiology. Some fish commodities are inherently more risky than others, owing to factors such as the different environments from which they come, the season during which they are harvested, and different handling and processing practices. Food safety issues associated with cultured fish and shrimp differ with region and habitat, and vary according to the method of production, management practices and environmental conditions (Reilly, 1998). The presence of pathogens causing life threatening illness in our environment, the ability of some of them to survive and/or proliferate under refrigeration and in reduced oxygen atmospheres, and, for some pathogens, the low infectious dose required for causing disease indicate the seriousness of the potential hazards with which we are faced. The food industry implements a variety of effective control measures to limit potential hazards. This generally begins on the farm with the implementation of good agricultural practices.

Prevention of seafood-borne illnesses requires an understanding not only of the etiologic agents and fish commodities associated with illness but also of the mechanisms of contamination. The pathogenic bacteria/bacterial hazards are acquired from three sources: 1. the natural aquatic environment 2. General environment- processing, retail, industry 3. Animal/human reservoir-

mainly fecal pollution of the aquatic environment. The number of pathogenic bacteria indigenous to aquatic environment and that from general environment is in general low in fish. The number of pathogenic bacteria from animal/human reservoir is different and they have different minimal infective doses. With the exception of foods consumed raw, however, the reported incidences of seafood-related disease are low. The mere presence (in low numbers) of pathogens/bacterial hazards from the aquatic and general environment is of no safety concern, not even in ready-to-eat (RTE) products (Huss *et al.* 2003). In contrast, the presence of pathogens from the animal/human reservoir is a serious safety concern for products to be eaten without (further) cooking. Growth of pathogens is likewise a serious safety concern for most RTE products.

Principal bacterial hazards associated with seafood

Seafood may be a vehicle for many bacterial pathogens. In general, the bacterial hazards associated with seafood consumption, apart from the specific case of the marine vibrios pathogenic for humans, does not differ significantly from those associated with other foods. Some types of seafood, such as bivalve molluscs eaten raw (principally oysters), other raw seafood, or lightly (fermented/smoked)/ semi-preserved (salted) seafoods, pose particular problems.

Pathogenic bacteria indigenous to aquatic and general environment

Clostridium botulinum

Clostridium botulinum is the causative agent of botulism, a potentially lethal neuromuscular disease caused by ingesting preformed botulinum neurotoxin. The organism produces seven neurotoxins that are

designated as types A through G based on their serological specificity. The types pathogenic to humans (types A, B, E and F) can conveniently be divided into two groups namely 1). the proteolytic types A, B and F, which are also heat resistant, mesophilic, and have the general environment as the natural habitat, 2). the non-proteolytic types B, E and F, which are heat sensitive, psychrotolerant, and have the aquatic environment as the natural habitat.

C. botulinum has been isolated from fresh (17-24 %) and processed (10-15%) fish and shellfish from India (Lalitha and Nirmala Thampuran, 2006). There are no reports of seafood associated botulinum outbreaks in India. Many of the botulism outbreaks in USA, Israel, New York, Cairo, UK and Finland were traced to the consumption of uncooked, under cooked, smoked, stale or fermented fish and also to seal and whale meat (Hauschild, 1993). Semi-preserved seafoods including smoked, salted and fermented fish, have also been identified as causes of botulism.

Pathogenic *Vibrio* spp.

Vibrio spp. are frequently isolated from seafood particularly, shellfish, and a number of these organisms are human pathogens (Huss, 1994). *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* are universally recognised as important human pathogens; with *V. hollisae*, *V. alginolyticus*, *V. fluvialis* and *V. mimicus* each responsible for a smaller, but substantial, number of infections (Morris, 1999). Except *V. cholerae* and *V. mimicus*, others are halophilic.

V. cholerae is the aetiological agent of cholera, the most feared epidemic diarrheal disease induced by cholera enterotoxin. Only two cholera serotypes (*V. cholerae* O1 and O139) have been shown to cause the disease.

V. cholerae O1 was isolated from fish from Cochin. A cholera epidemic caused by *V. cholerae* O139 serogroup erupted in Madras, southern India and these strains were later isolated from Calcutta and Bangladesh (Albert *et al.*, 1993; Ramamurthy *et al.*, 1993b).

V. parahaemolyticus, a halophilic organism native to marine or estuarine environment, is a well known enteropathogen and is associated with food-borne gastroenteritis due to consumption of inadequately cooked or refrigerated crustaceans and fish. The level of *V. parahaemolyticus* in Indian fishery products varies considerably. *V. parahaemolyticus* has been isolated from fresh (10-35%) and processed seafood (5%) in India. *V. parahaemolyticus* was isolated in 3.5–23.9% of patients with diarrhoea in India (Morris, 1999). In recent years, the incidence of *V. parahaemolyticus* infection has been increasing in many parts of the world, and this has been attributed to the emergence of a new clone of the O3:K6 serotype carrying only the *tdh* gene. (Matsumota *et al.*, 2000). In India, *V. parahaemolyticus* strains belonging to serovar O3:K6 and possessing the *tdh* gene but not the *trh* gene was shown to be responsible for the high incidence of *V. parahaemolyticus* infection in Calcutta during 1996. In addition to the new O3:K6 serovar, strains of serovars O4:K68 and O1:K untypable (KUT) have been associated with an increased incidence of *V. parahaemolyticus* infections worldwide.

V. vulnificus is a seafood-borne pathogen found in marine or estuarine environments, causing a rapidly fatal infection in persons with pre-existing liver disease, hemochromatosis (iron overload) or compromised immune systems. *V. vulnificus* can cause wound infections and primary septicemia. Biotype 1 of this bacterium can be

lethal to humans, biotype 2 may be an opportunistic pathogen for humans. The organism is responsible for seafood-related infections and of shellfish-associated deaths (Morris, 1999). *V. vulnificus* was recovered from fresh and processed fish/ shellfish from Mangalore and Kochi along the west coast and in seafood from Kakinada in the east coast of India.

***Aeromonas* spp.**

Aeromonas have been increasingly recognized as relevant etiological agents in gastrointestinal infections as well as extraintestinal infections such as cellulitis, wound infections, septicemia, urinary tract infections. *Aeromonas* spp. are frequently found in seafood and their products. *Aeromonas* spp. have been linked to two major group of human diseases: septicemia (mainly by strains of *A. veronii* subsp. *sobria* and *A. hydrophila*) and gastroenteritis (any mesophilic *Aeromonas* spp. but principally *A. hydrophila* and *A. veronii*) (Kirov, 2001). The other diarrhoea-associated isolates were *A. trota*, *A. veronii* biovar *A. veronii* and *A. jandaei* (Kirov, 2001). *Aeromonas* associated diarrhoea has been reported from Bombay, Calcutta, Goa, Vellore, Pondicherry and Chennai in India (Alavandi & Ananthan, 2003). *A. hydrophila* was reported in 42% samples of frozen fish and in 21% of fresh fish (21%) in India.

Plesiomonas shigelloides

Plesiomonas shigelloides has been implicated as being an enteric pathogen in sporadic cases and outbreaks of diarrhoea in various parts of the world. The most common manifestation of infection with this is watery diarrhoea. It can also cause wound infections and septicemia. The organism is mostly associated with aquatic environments and is typically

associated with seafoods (Huss *et al.*, 2003). In India, the organism was recovered from the body meat of crab (*Scylla serrata*).

Listeria monocytogenes

Listeria monocytogenes is the causative agent of human listeriosis, which can be lethal for foetuses, pregnant women, neonates and immuno-compromised persons. Severe listeriosis can cause meningitis, abortions, septicemia and a number of other maladies, some of which may lead to death. Shrimp and shellfish were implicated in listeriosis outbreaks. *L. monocytogenes* was detected in fresh and processed fish from Kochi and Mangalore, India.

Clostridium perfringens

Clostridium perfringens has been implicated as the etiological agent in many food poisoning outbreaks. The organism is widely distributed in aquatic environments, soil, intestines of animals and humans worldwide. Both raw and processed seafoods were implicated in *C. perfringens* food poisoning outbreaks in United States and Japan. This organism was detected in high percentage (68-88%) in fish from Japan and India.

Bacillus cereus

Bacillus cereus causes two types of illness – diarrhoeal and vomiting (emetic) types. Incubation period is 12 to 24 hours. Contamination of fresh and processed fish by *B. cereus* has been reported in India. *B. cereus* associated foodborne illness accounted for 2% of outbreaks with confirmed aetiology that were reported to the Centers for Disease Control and Prevention of the US FDA during 1973–1987 (Bean & Griffin, 1990). In eight *B. cereus* food poisoning outbreaks reported in USA, rice and shellfish were involved.

Pathogenic bacteria indigenous to the human/ animal reservoir

***Salmonella* spp.**

Salmonella most commonly causes acute gastroenteritis, with symptoms including diarrhea, abdominal cramps, and fever. Other clinical manifestations can include enteric fever, urinary tract infections, bacteremia, and severe focal infections. *Salmonella* is a leading cause of bacterial enteric disease in both humans and animals. Only two species of *Salmonella* are recognized at the Centre for Disease Control (CDC) in USA ; the *S. enterica* and the *S. bongori*. The former occurs in 6 sub-species. Within each of these, several serotypes exists. Thus *S. enterica* subsp. *enterica* as the largest group covers approximately 1500 serotypes. Examples of such serotypes are Enteritidis, Typhimurium or Typhi. The serotypes of the other sub-species are not named but identified by antigenic formula. The infective dose in healthy people varies according to serovars, foods involved and susceptibility of the individuals (Huss *et al.*, 2003). In India, *S. typhi* was the most predominant among *Salmonella* isolates and *S. paratyphi* B biovar Java heavily contaminated samples of marine prawns in Calcutta markets (Boonmar *et al.*, 1998). *Salmonella* was isolated from 6-17% fresh fish/ shellfish samples from Kochi and Coimbatore markets and 9-15% frozen fish samples from cold storages at Kochi and fish processing units in Mumbai (Lalitha and Nirmala Thampuran, 2006; Kumar *et al.*, 2008). *S. typhi* was the most common bacterium associated with shellfish vectored disease.

***Shigella* spp.**

Shigella is responsible for bacillary dysentery, also called Shigellosis. The infectious dose is low, approximately 10-100 cells. Among the four species

of *Shigella* known, *S. dysenteriae* causes the most severe condition of bacillary dysentery. Seafood (shrimp-cocktail, tuna salads) have been the cause of a number of outbreaks of shigellosis. *Shigella* spp. were isolated from seafood samples from Kochi, India.

Pathogenic *Escherichia coli*

Pathogenic *Escherichia coli* is a group of bacteria, including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), enteroaggressive *E. coli* (EAggEC), and enterohemorrhagic *E. coli* (EHEC/VTEC). Most infections appear to be related to contamination of water or handling of food under unhygienic conditions. EPEC causes a watery type of diarrhoea accompanied by vomiting and fever, typically in infants and young children. The EIEC produced a diarrhoeal disease similar to *Shigella* whereas ETEC causes diarrhoea resembling *V. cholerae* diarrhoea. ETEC are a major cause of diarrhoea in children in developing countries and also a cause of so-called travellers' diarrhoea in adults. ETEC strains produce two types of toxin of which one resembles the cholera toxin. Also, the DAEC and EAggEC cause various variants of diarrhoea. *E. coli* O157:H7 is the most common EHEC serotype. Shiga toxin-producing *E. coli* (STEC) O157:H7 infection, which can cause haemolytic uremic syndrome and death, is a global public health concern. In India, occurrence of *E. coli* O157:H7 has been reported in fresh seafood marketed in Mangalore and Kerala.

Yersinia enterocolitica

Yersinia enterocolitica is the causative agent of yersiniosis. Outbreaks have been associated with oysters and fish. *Y. enterocolitica* is a psychrotrophic

pathogen. In India, *Y. enterocolitica* was detected in fresh and processed seafood samples from retail markets located at Mumbai and Kerala.

***Campylobacter* spp.**

Campylobacter spp., primarily *C. jejuni* subsp. *jejuni* is one of the major causes of bacterial gastroenteritis worldwide. Campylobacteriosis is the name of the illness caused by *C. jejuni* which is also often known as campylobacter enteritis or gastroenteritis. The infective dose of *C. jejuni* appears to be small.

Staphylococcus aureus

Staphylococcus aureus is a common cause of bacterial foodborne disease worldwide. *S. aureus* food poisoning is caused by the ingestion of food that contains one or more *S. aureus* enterotoxins. The disease caused by *S. aureus* is an intoxication characterized by nausea, vomiting, abdominal cramping, watery or bloody diarrhea, and fever. The incubation period is short, typically 2-4 hours. Seven antigenically different proteins cause the disease. *S. aureus* has been isolated at levels of 2-10% in fish and bivalves but much more commonly in cooked, handled crustaceans.

Methods for detection and enumeration of bacterial hazards

Assessment of the quality and safety of foods is important in human health. Microbial testing provides the detection and quantification of pathogens and spoilage microorganisms in a variety of products. The detection and enumeration of pathogens in food are an essential part of any quality control or food safety management. The identification of pathogens and the determination of their relevance for public health, as well as their control,

is the function of risk analysis. One of the inherent difficulties in the detection of food-borne pathogenic bacteria is that they are generally present in very low numbers (< 100 c.f.u. g⁻¹) in foods in the midst of up to a million or more other indigenous microflora. Therefore, these pathogens may be lost among a background of other bacteria, and substances in the foods themselves may hinder recovery. A wide range of detection methods have been developed including cultural methods, immunological methods and molecular/nucleic acid based methods (Pina *et al.*, 2005; Velusamy *et al.*, 2010).

Traditional culture methods

Culture based standard microbiological methods are still the most widely used technique for the detection of food borne pathogens, due to their reliability and accuracy. These methods mainly rely on specific microbiological and biochemical identification. Selective liquid or solid culture media are used to grow, isolate, and enumerate the target microorganism and simultaneously prevent the growth of other microorganisms present in the food.

Enumeration of the microorganisms present in a sample is normally performed by plate count method or the most probable number (MPN) method. The plate count method is based on culturing dilutions of sample suspensions in the interior or on the surface of an agar layer in a Petri dish. The MPN method calculates the number of viable microorganisms in a sample by preparing decimal dilutions of the sample, and transferring subsamples of 3 serial dilutions to 9 or 15 tubes containing liquid culture medium, to carry out the method on 3 or 5 tubes, respectively. The tubes are incubated, and those that show growth (turbidity)

are counted. Taking into account the dilution factor, the final result is compared to a standard MPN table, which will indicate the MPN of bacteria in the product. Qualitative procedures are used when it is not necessary to know the amount of a microorganism present in a sample but only its presence or absence. Three basic protocols have been described for the detection of microorganisms: 1) direct plating on (selective) media; 2) direct (selective) enrichment and 3) pre-enrichment, followed by a selective enrichment. There has been considerable improvement in traditional methods: new equipment for homogenising samples (eg. Stomacher blender), spiral platers, (automated) colony counters and the TEMPO-method, based on the automated most probable number (MPN) techniques used in traditional microbiology for counting low numbers of micro-organisms. Biochemical and enzymatic identification kits are capable of identifying microorganisms by combining a series of biochemical tests and a database developed by the kit's manufacturer. Test kits such as

API system, Enterotube II, etc are commercially available.

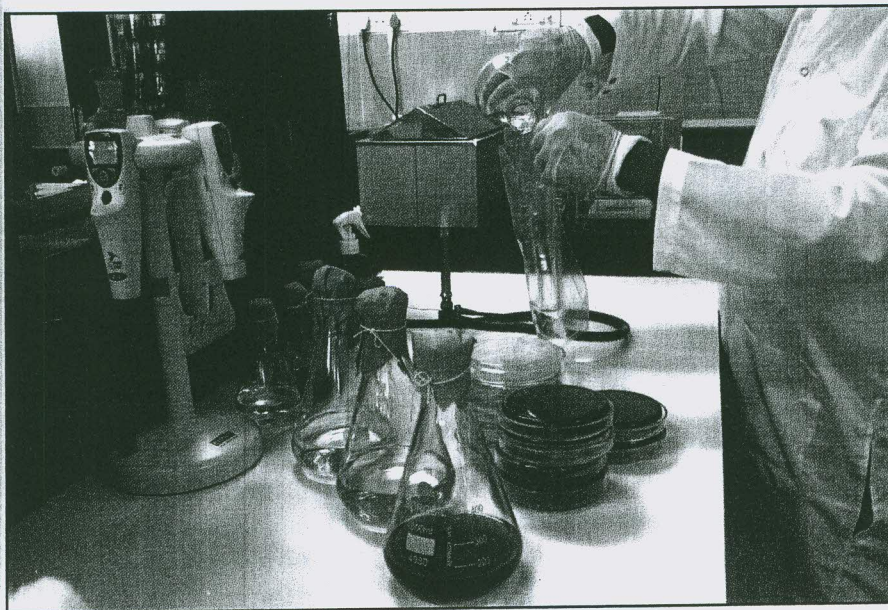
Culture methods have been used for detection of *Salmonella*, *V. parahaemolyticus*, *V. cholerae*, *L. monocytogenes*, *S. aureus*, *E. coli*, *C. jejuni*, *Y. enterocolitica* and *C. botulinum*. Cultural methods for *Salmonella* require enrichment in a non-selective pre-enrichment media, such as Buffered Peptone Water (BPW), and to increase the number of target cells as these are generally not uniformly distributed in foods, typically occur in low numbers, and may be present in a mixed microbial population. Next, primary enrichment cultures are typically inoculated into secondary selective enrichment broths, such as Selenite Cystine broth (SC), Rappaport Vasiliadis Soy broth (RVS) and incubated at elevated temperatures (37°C or 42°C for 18-24 hours) before being struck onto selective agars such as Xylose Lysine Deoxycholate agar (XLD agar), Bismuth Sulphite agar (BIS) or modified semisolid Rappaport Vasiliadis (MSRV). Culture methods for detection of *Salmonella* and *L. monocytogenes* require 7-8 days to yield results, as they rely on the ability of

pathogens to multiply in enrichment broth, produce visible colonies on selective agar which can then be characterized by performing additional biochemical and or serological tests. In addition, viable bacterial strains in the environment can enter a dormancy state where they become non-culturable (viable-but non-culturable (VBNC)) which can subsequently lead to an underestimation of pathogen numbers or a failure to isolate a pathogen from a contaminated sample. In recent years, *Vibrio cholerae*, *E. coli*, *Campylobacter jejuni*, *Salmonella* spp., *Listeria monocytogenes* and *Yersinia enterocolitica* have been reported to enter the VBNC state. Chromogenic media for the detection of *L. monocytogenes*, and *Salmonella* (eg. BBL CHROMagar (CHROMagar), chromID *Salmonella* (BioMerieux), Oxoid Brilliance *Salmonella* Agar (Oxoid) can reduce detection time down to 3 days. For detection of *Campylobacter*, 4-9 days are needed to obtain a negative result and between 14 and 16 days for confirmation of a positive result. Microbial toxins are recognized by their lethal action in mice, also known as mouse bioassay and neutralization with specific antisera (either in vitro or in vivo) for eg. *C. botulinum* toxins .

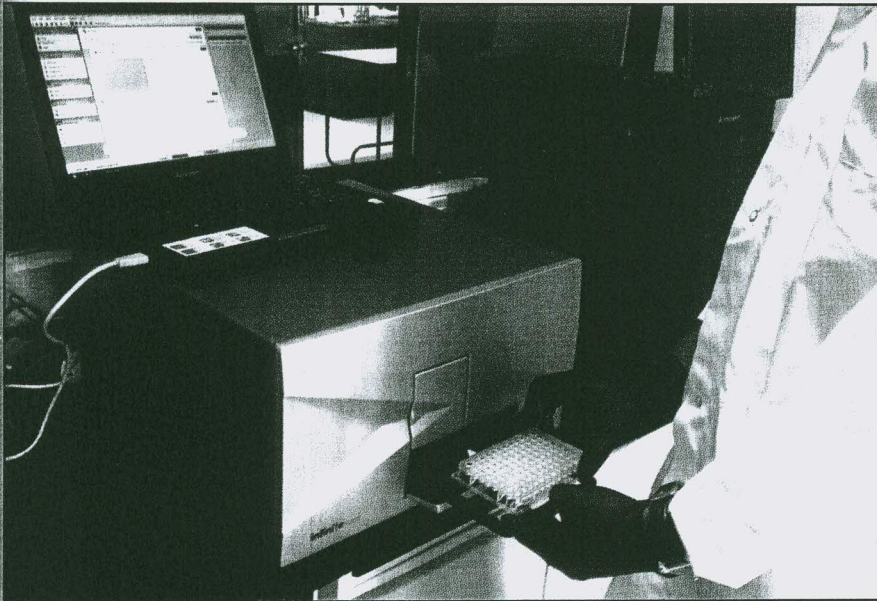
The major disadvantage of cultural methods are that they are labour-intensive and time-consuming as it takes 2-3 days for initial results, and up to 7-10 days for confirmation. This is an obvious inconvenience in food industry applications. In spite of their disadvantages, culture based methods remain the gold standard for the detection of pathogens.

Immunological methods

In recent years, many new methods have been developed for pathogen detection in the food industry. The immunology-based / antibody-based methods are perhaps



Cultural Method of isolation of pathogens



Elisa reader for detection of Salmonella

the only technology that has been successfully employed for the detection of bacterial pathogens, spores, and toxins alike. For example, immunomagnetic separation (IMS), a pre-treatment and/or pre-concentration step, can be used to capture and extract the targeted pathogen from the bacterial suspension by introducing antibody coated magnetic beads in it. IMS can then be combined with almost any detection method, e.g., optical, magnetic force microscopy, magnetoresistance (Bead Array Counter) and Hall Effect, amongst others. Other immunological detection methods include enzyme-linked immunosorbent assay (ELISA) test which is the most established technique nowadays. This assay combine the specificity of antibodies and the sensitivity of simple enzyme assays by using antibodies or antigens coupled to an easily assayed enzyme. Results can be interpreted visually (qualitatively) or using an instrumental read-out (quantitatively). Immunology-based test kits for the detection of *Salmonella*, *Listeria*, *Campylobacter*, *E. coli* O157:H7, *Pseudomonas*, *Staphylococcus aureus* are commercially available. For microbial

toxin detection, several other immunoassays have been described - reversed Passive haemagglutination (RPHA), radioimmunoassay and reversed Passive latex Agglutination assay (RPLA). Bacterial toxins (eg. *B. cereus* toxins, *C. perfringens* enterotoxins, *S. aureus* enterotoxins) can be detected by RPLA based test kits which are commercially available. Rapid methods have made a major impact on microbiological testing over the last twenty years or so. Lateral flow immunoassays have been developed for detection of pathogen and bacterial toxins which typically use a sandwich type ELISA and the majority use polyclonal antibody as a capture antibody and a monoclonal antibody as the detection antibody. Commercially available lateral flow immunoassays include DuPont™ Lateral Flow System *Salmonella*, Singlepath *Salmonella* (Merck), Lateral Flow assay Duopath® *Cereus* Enterotoxins, Lateral Flow Assay Singlepath® L'mono.

Molecular methods for detection of pathogens

Molecular diagnostics employs genetic materials (DNA and RNA) as

diagnostic targets for identifying suspected pathogens. There are many DNA-based methods developed, but only probes and nucleic acid amplification based assays have been developed commercially for detecting foodborne pathogens. Alkaline phosphatase (AP)-labeled and digoxigenin-labeled DNA probes to identify the presence of *V. parahaemolyticus* strains harboring the virulence marker *tdh* gene are commercially available.

Nucleic acid based assays, particularly PCR and real-time/quantitative PCR (qPCR), are most widely used of all molecular methods for detecting and identifying bacteria in foods. The PCR has become an important diagnostic tool in the detection of foodborne pathogens. A target DNA can be amplified 1-million-fold in less than an hour, with sensitivities in theory down to a single target pathogen. In the PCR assay, a specific amplification of a defined target-DNA is obtained by successive cycles of three steps, namely denaturation of sample DNA to obtain single strand target, annealing of short and specific primers to the target DNA and polymerisation of DNA starting from the primers by use of a thermostable DNA-polymerase. The number of amplicons are doubled in each cycle, and all but the fragments produced with the original target DNA as template will be of a fixed size, corresponding to the distance between the two primers. The amplified DNA-fragment can be visualised by agarose-gel electrophoresis.

PCR based methods are used in the detection of wide range of seafood-borne pathogens such as *Salmonella*, *L. monocytogenes*, *C. botulinum*, *B. cereus*, *Escherichia coli* O157: H7, *Y. enterocolitica*, *C. jejuni* and *S. aureus* using suitable primers. For eg. Primers were designed based on specific gene *fimA* of *Salmonella* and gene *afa* of

pathogenic *E. coli* for amplification. Several of these PCR tests (e.g., *Salmonella* BAX PCR; PCR assay amplifying *ctx* gene for *V. cholerae*) are recommended by FDA for screening foods for pathogens. Rapid and easy detection of food-borne pathogens will facilitate adoption of precautionary measures to maintain healthy food.

The different PCR methods developed for bacterial detection include (i) real-time PCR (ii) multiplex PCR and (iii) reverse transcriptase PCR (RT-PCR). PCR may also be found coupled to other techniques. Examples are “the most probable number counting method” (MPN-PCR), surface plasmon resonance and PCR-acoustic wave sensors, LightCycler real-time PCR (LC-PCR) and PCR-enzyme-linked immunosorbent assay (PCR-ELISA), the sandwich hybridization assays (SHAs) or the FISH (fluorescence in situ hybridization) detection test. Real-time PCR can provide qualitative as well as quantitative detection of pathogens. Current PCR technology allows for rapid detection of pathogens in real time, because of fluorescence monitors built into the thermocycler. Real-time PCR assays can provide information regarding pathogen cell numbers in a sample. Multiplex PCR assays provide simultaneous detection of two or more pathogens such as *V. parahaemolyticus*, *E. coli* O157:H7, *Salmonella*, *S. aureus* and *L. monocytogenes*.

PCR assays are increasingly used in food-microbiology, because they offer a sensitive and specific detection of pathogens, and they differentiate virulent bacteria from avirulent members of the same species. Genetic amplification methods have made it possible to significantly reduce assay times while maintaining a high level of

sensitivity and specificity. These methods are also able to distinguish closely related species which most antibody tests could not. PCR assays which use DNA as a target are usually not quantitative with regard to viable organisms. The issue of viability can be overcome by using RNA targets which are rapidly degraded following cell death. DNA microarray represents the latest advance in molecular technology, in which many microscopic spots of DNA oligonucleotides are arrayed series for detection of multiple pathogens in a food sample.

Biosensors have recently been introduced as analytical devices incorporating a biological material (e.g., microorganisms, nucleic acids, enzymes etc.) or a biologically derived material (e.g., recombinant antibodies, engineered proteins etc). Optical biosensors have been developed for rapid detection of contaminants, toxins or drugs and even pathogen bacteria.

The nature of food and foodborne illness has changed dramatically over the last century. The implementation of preventive food safety management programs such as the Hazard Analysis and Critical Control Points (HACCP) has greatly improved food safety, but it will not be fully effective until better methods of analysis are developed. The commitments emerging from the World Trade Organization agreements have resulted in unprecedented interest in the strengthening of food control infrastructure at country levels and Food Safety and Standards Authority of India (FSSAI) was established under the Food Safety and Standards Act, 2006. Microbial food safety is being enforced through FSSAI. In recent years, as people increasingly consume food prepared

outside the home, growing numbers are potentially exposed to the risks of poor hygiene in commercial foodservice establishments. As some diseases are controlled, others emerge as new threats. Changes in microorganisms lead to the constant evolution of new pathogens, development of antibiotic resistance in food-borne pathogens, and changes in virulence of known pathogens. In order to combat the emerging challenges, public health workers has to adapt to a changing environment with improved methods and apply novel technologies such as biosensors and microarrays for pathogen detection in foods.

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