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Detection of Food Borne Pathogens with the Help of Traditional and Modern Molecular Techniques

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Abstract: ***Many food - borne microorganisms have become the major contamination sources in food and water and these pathogens gets involved and are affects the food safety and also causes different infections in humans which leads to the illness in human life among worldwide. As a result of consumption of foodstuff, mainly animal products which are the contaminated source with vegetative pathogens or their toxins are being involved un - naturally into the humans lifestyle nowadays. However most of these microbes have zoonotic importance and results in the significant impact on both public health and into the economic sectors. These tiny micro - organisms are being challenges in everyone's lifestyles and are the causative agents of two - thirds of human food - borne diseases worldwide with high burden in developing countries. The animals either the aquatic or farm animals are the major reservoirs of many food - borne zoonotic bacterial pathogens also the somehow dairy products, raw meats and the products which directly comes from the farms have become the major prospect in the mode of transmission of disease and infections in humans. All these food borne pathogens remove here toxins inside the humans through environment and nutrient which is provided from us and these toxins gets involve inside of the human body and started to cause infections to the humans some of the major zoonotic bacteria causes the humans - infection which are characterized mainly by gastrointestinal symptoms including nausea, vomiting, diarrhea, abdominal cramps, and other agent - specific symptoms. Some bacteria may cause severe complications. Conventional (culturing), serological, and molecular techniques are important for detection of these common zoonotic bacteria and their toxins in food. The various diagnostic and time consuming methods were performed previously which leads into the slow processing for the detection of toxins such as polymerase chain reaction technique is a traditional which was performed earlier by Kary Mullus in 1980 which was the great invention in Medical and Life Sciences field. However polymerase chain reaction is simple detection method of the bacterial toxins now - a - days and also become the slow process although in today's technologies era Medical and Life sciences have developed different techniques for quick and better result in bacterial toxins in any source and this technique is the modern technique. In this review article the main prospective is to compare between the traditional and modern technique which have become the major role in laboratories. The field for the detection of bacterial toxins includes the diagnostic devices that are integrating molecular methods, biosensors, micro - fluidics, and nano - materials and also offers the new avenues for swift, low - cost detection of pathogens with high sensitivity and specificity. These analyses and screening of food items can be performed during all phases of production. This review article presents major developments achieved in recent years in point of need diagnostics in land - based sector and sheds light on current challenges in achieving wider acceptance of portable devices in the food industry. The particular emphasis which is placed on methods for testing nucleic acids, protocols for portable nucleic acid extraction and amplification, as well as on the means for low - cost detection and read - out signal amplification.***

Keywords: Gastroenteritis, biosensors, micro - fluidic, pathogens, food

1. Introduction

Early in 460 B. C., Hippocrates reported that there is a strong connection between food consumed and human illness. The food borne pathogens (e. g. viruses, bacteria and parasites) are kind of biological agents which can cause a food borne illness events in humans. However, it can be defined as the food borne diseases as the eruption of the occurrence of two/more cases of similar illness which can be results from the ingestion of a common food. Some of different types of illness can be occurred due to food borne pathogens when the pathogens are ingested with food and establishes it or usually multiplies in the human host. The food borne pathogens can also create toxigenic criteria itself in a food product and produce a toxin which is then ingested by the human host. However, food borne illness is usually classified into following a) food borne infection and b) food borne intoxication. Since the incubation period is usually involved in microbiology and such different science field the time of ingestion of food borne pathogens until symptoms occur this is much longer than food borne intoxications. More than 200 different food borne diseases have been identified. (Bintsis, T., 2017)

However most of the pathogens have a zoonotic origin, and food products of animal origin are considered as the major vehicles of food - borne infections. The food producing animals (cattle, chickens, pigs, turkeys, etc.) are the major reservoirs for many food - borne pathogens. Animal products (meat, milk, egg, fish, etc.) all these products have high risk due to pathogen contents, natural toxins, adulterants, and other possible contaminants and the risk of food - borne diseases in humans is increasing when consumption of food of animal origin is increased in the human lifestyle. In recent research years, the food - borne pathogens have become most important public health problem worldwide and their impact on health has the significant morbidity and mortality rate, and economy is increasingly recognized sectors. According to different research reports, a huge number of people suffer from food - borne diseases each year worldwide, and around 600 million (10 people in the world) become ill due to the consumption of contaminated food and water stuff. Due to unrecognized or unreported outbreaks, statistical data of food - borne diseases are increased now - a - days all over the world. However the food - borne diseases are major health issues both in developed and developing countries, but developing countries tend to suffer from the largest share of the burden of food - borne diseases. According to the WHO, now 40%

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of the population suffer from food - borne diseases each year in developed countries, and up to 2.5 million deaths are being estimated per year in developing countries. In today's scenario, the research and health awareness has been growing on the complete human lifestyle that contributes the impact of zoonotic food - borne pathogens and being transmitted from animal - originated food. (Abebe, E et al, 2020)

The bacteria which are the causative agent of two - thirds of food - borne disease and outbreaks though around 250 different food - borne diseases, from the biological hazards the bacterial pathogens are the most serious concern cases regarding the issues of meat safety to consumers. Although, the bacterial food - borne illnesses are among the most widely spread global public health problems in recent times of research. Mostly the vertebrate animal species are the natural reservoirs for many pathogens that almost causes the human infections after transmission through food. The animal origin food supplements particularly meat (beef, mutton, and pork), dairy products (milk, cheese, yoghurt, and ice cream), and eggs are the three ways by which people gets interact to pathogenic bacteria, due to their nutritional value mainly high protein and lipid content, dairy products are a suitable the growth environment for a range of microorganisms. The contaminated raw meat is one of the main sources of food - borne disease [26]. Food of animal origin (milk, meat, and their products) can become contaminated with bacteria during food processing or slaughtering. (Abebe, E., 2020)

However the bacteria and viruses are the most common causes of food borne diseases and exist in a different shapes, sizes, types and properties. Some of the examples are *Clostridium botulinum, Clostridium perfingens, Bacillus subtilus, Bacillus cereus, Staphylococcus aureus, Norovirus, Hepatitis A virus, Escherichia coli, Campylobacter* etc. which are capable of spore formation and highly heat resistant activity. Some of the pathogenic bacteria such as *Staphylococcus aureus, Clostridium botulinum* are capable of producing heat resistant toxin. There are most of the mesophilic pathogens that are capable for the optimal growth temperature which ranges from 20 - 40 degree Celsius. However, most of the psychotrophs food borne pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica* are capable of growth under refrigerated conditions and temperatures less than 10 degree Celsius. (Bintsis, T., 2017)

The term food borne diseases or food - borne illnesses or more commonly food poisoning are used to denote gastrointestinal complications that occur following recent consumption of a particular food or drink. (Dhama et al 2014)

However food - borne outbreaks caused by toxigenic bacteria shares a common symptomatology, and in addition some of the adequate methods for bacterial toxin detection are lacking. Consequently, the proportion of "weak evidence" food borne outbreaks is particularly high in case of bacterial toxins acting as the causative agent. Furthermore, detection of bacterial toxins is exceptionally important because toxins may remain in or on the food and be ingested while the bacteria is present for the long duration of time. The methods for bacterial toxin are detected primarily and rely on immunological assays such as ELISA, lateral flow immunoassays, and agglutination tests. In some cases, toxins are evidenced by bioassays in tissue culture, or mouse neutralization testing, and for some other live animal tests, all of them time consuming with some being ethically challenging. An emerging branch of analytical methods for pathogen detections with the potential to address weaknesses of classical methods combines biosensors, microfluidics, and nanotechnologies. Furthermore, over the last decade, the field of portable sensors for food and water quality control has grown exponentially and very quickly. (Bintsis, T. (2017)

Traditional methods of identification of food - borne pathogens which causes disease in humans and are time - consuming and laborious, so there is a need for the development of innovative methods for the rapid identification of food - borne pathogens. Recent advances in molecular cloning and recombinant DNA techniques have revolutionized the detection of pathogens in foods. (Naravaneni, R., & Jamil, K. (2005)

Food borne Gram positive Bacteria

Bacillus cereus

Bacillus cereus species are the members of family Bacillaceae which are gram positive, motile rod shape bacteria. They have the ability for the formation of spores. The spores which are produced by Bacillus cereus possess appendages and pili, are more hydrophobic than outer Bacillus spores. There are different types of properties that enables the spores to adhere to various kind of surfaces and which helps to resist the removal during cleaning and sanitation. . The vegetative cells of Bacillus cereus grows at the temperature which ranges from 4 - 15 to 35 - 55 degree Celsius but prefers 30 - 40 degree Celsius, depending on the strain. Bacillus cereus produces 2 types of toxin infections which is the emetic (vomiting) and the diarrheal one both these toxins are the main cause of illness. (Granum, P. E., & Lund, T. (1997) The group of spore - forming, aerobic, facultative anaerobic, rod - shaped bacteria comprises at least eight closely related species: *B. anthracis*, *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis*, *B. cytotoxicus*, and *B. toyonensis* (Ehling et al 2019) Thus the emetic syndrome that is caused by emetic toxins which are produced by this bacteria is started grow during the growth phase in the food. The diarrheal toxins which is produced during the growth of the bacteria occurs infections in the small intestine of the human body. The food which is directly implicated in Bacillus cereus and allows the symptoms like food poisoning includes meat products, soups, vegetables, puddings, sauces, milk and milk products. The symptoms of these pathogenic bacteria include abdominal pain, nausea and diarrhea after an incubation period of 8 - 16 hours approx. The molecular detection technique for these pathogenic bacteria includes the genetic and genomic analysis which has revealed that Bacillus cereus is very identical to *Bacillus anthracis* and also includes some strains which have plasmids which resembles the toxin of Bacillus strains. The Bacillus cereus

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symptom majorly includes food poisoning methods in 2 types: -

- It causes an emetic toxin which results in vomiting and
- The second is causes an enterotoxins which involves diarrheal symptoms.

Thus the virulence factors of both the types includes that emetic toxin which causes emesis (vomiting), pathogenic infections and structure of emetic toxins which lasts for long time and have been a mystery. The toxins is mainly detected by the use of Hep - 2 cells with the procedure of isolation and determination of the structure. The enterotoxin and the properties of this pathogenic bacteria has also been lasts for long time and it involves the different enterotoxins which one of it has been characterized as three components of haemolysin HBL that is consists of 3 proteins B, L1 and L2. The HBL is suggested as the primary virulence factor in Bacillus cereus food poisoning symptoms like diarrhea. (Bintsis, T., 2017)

Staphylococcus aureus

The micro - organism which involves in commensal criteria are always present in skin, nose and mucous membranes of healthy humans and animals. (Abebe, et al, 2020) Staphylococcus aureus is an opportunistics food borne pathogen which causes the multiple infectious diseases in both humans as well as animals. (Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). Staphylococcus is comprises of many different species and subspecies. It is gram positive catalase positive, coagulase positive, oxidase positive and non - motile pathogenic bacteria. It belongs to the family Micrococcaceae. If we talk about the epidermiology of the bacteria, it has been involve interest in animals which has the majorly increment of infectious processes of disease and especially involves MRSA strain. Around 50% of healthy individuals gets infected by this bacteria in different sites of body i. e. in nasal passage, throat and skin. If we talk about animals the mastitic cow and food producing animals are very common sources of Staphylococcus aureus in raw milk and widely present in a broad host range and also includes human beings. The MRSA factors includes immune - suppression hemodialyses, peripheral malperfusion, advanced age, extended in hospital stays, residency in long term facilities, inadequacy of anti - microbial therapy, indwelling devices, insulin - requiring diabetes and decubitus ulcers. The symptoms of this pathogenic bacteria starts occuring from simple skin infection to most serious and potentially life - threatening infections such as septicemia, necrotizing factors fasciitis, infective endrocarditis, necrotizing pneumonia and toxic shock syndrome. It is usually transmitted by food sources such as livestock and poultry products and seafood and bakery products which consists of enterotoxigenic strains. *Staphylococcus aureus* is detected by molecular based and immunological based techniques. The molecular detection and identification for HRSA strains involves RT - PCR Real Time Polymerase Chain Reaction. The *Staphylococcus aureus* has the ability to produce enterotoxin which risk for the subsequent food poisoning procedure in humans. There are 9 different identified *Staphylocoocal enterotoxins* A, B, C1, C2, C3, D, E, F and G. In which type A and D are always responsible for the majority of outbreaks. (Abebe, et al, 2020)

Food - borne Virus

Viruses are the large groups of submicroscopic and very infectious a agent which are usually regarded as non - living complex molecules and contains a protein coat which is surrounded by an RNA and DNA core of genetic material, but it does not contains semi - permeable membrane. Viruses are always capable for the growth and multiplication of itself only in living cells and causes various important diseases in humans, animals and as well as in plants. Viruses are incapable of survival for long periods. There are more than 100 types of enteric viruses which are capable of causing food borne diseases in humans and animals. There are 2 most common food borne viruses which causes different kind of disease mainly in humans i. e. Hepatitis A and Norovirus. These 2 viruses are transmitted through food such as bivalve mollucus, calms, cockles, mussels and oysters. (Bintsis, T., 2017)

Hepatitis A: -

Hepatitis A viruses are environmental friendly organisms which could be transmitted by the contaminated food, water and environmental surfaces. This virus shares some major characteristics with other genera of the picornavirus family which are different from the species in genus Hepatovirus. Hepatitis A genotypes are 6 in number (I - VI) and are determined by RNA sequence analysis. Genotype I, II and III contains strains which are associated with human infections. Generally this virus is comprised of single positive stranded RNA genome of 7.5 kilobases approx and it is non - enveloped. (Bintsis, T., 2017)

The hepatitis A virus (HAV), a picornavirus, is a common cause of hepatitis worldwide. The spread of infections is generally processed through person to person or by oral intake after fecal contamination of skin or mucous membranes; less commonly, there is fecal contamination of food or water. Hepatitis A is endemic in developing countries, and most residents are exposed in childhood. (Cuthbert, J. A. (2001). HAV has a positive - stranded RNA genome of around 7, 500 nucleotides with a single open reading frame (ORF) flanked by 5′ and 3′ untranslated regions (UTRs). (Chiapponi et al 2014)

Norovirus: -

Norovirus possesses the acute viral gastroenteritis cases worldwide which are estimated about 5.4 million food borne illness. This virus is non - enveloped virus with a diameter of 30 - 35 nm and a single stranded RNA genome is about 7.5 kilobase approx. The viruses are very diverse in mature and classified into 6 geno groups and these 6 geno - groups only causes the infections in humans. The ability of this virus comprises the wide range of temperatures that freeze about 60 degree Celsius and always persists on environmental surfaces and food items which contributes to raped dissemination. However the food borne viruses can cause considerable morbidity and mortality. Hence the norovirus infections can be cured by the different precautions such as hand - washing with soap and water remains the best method for removing norovirus from fingers. In research scientists have found that norovirus can also be detect by hand sanitizers formulation which is supplemented with urea and citric acid and also proved it more effective against norovirus. (Bintsis, T., 2017)

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Gram negative Bacteria

Escherichia coli

It is gram negative, rod shaped bacteria which belongs to Enterobacteriaceae family with 5 different virulence groups that include entreo - aggregative. *Escherichia coli*, *Enterohemorrahagic Escherichia coli, Enterovasive Escherichia coli. Escherichia coli* consists of 3 micron meter in length and can be ferment as glucose and other sugar components. Thus Escherichia coli O157: H7 is best known serotypes which contains a pathotypes and can cause food borne infections in humans. The well known toxin in bacteria's are Shega toxin which is major source for food borne pathogen. Due to the over consumption of contaminated food of animals origin it causes the infections by the animal products. The Intimin gene is responsible for the bacteria's intimate adhesion to intestinal cells which causes the appearance of attachment lesions and erasure the microvilli brush border of enterocytes. Escherichia coli consists of destruction of microvilli, intimate effacing and adherence of the organisms to the enterocyte membrane. There are several different methods for the detection of Escherichia coli and its strain such as Escherichia coli O157: H7 that can be detect by culture in special media, serological and molecular assays which has been used for the detection of serotype in food, environmental and clinical samples is Sorbitol Mackonkey agar SMAC that is supplemented with cefixine and potassium tellurite both these supplement are one of the most sensitive and differential media to isolate Escherichia coli O157: H7 from the serotypes of Escherichia coli. The SMAC consists of bile salts, carbohydrate source, sorbitol and an indicator too. (Bintsis, T., 2017)

Campylobacter

Campylobacter was first reported in 1886 by Theodore Escherich and observed the bacteria as non - culturable spiral shaped bacteria. This bacteria is the great importance in human medicine and food safety. These species are the leading cause of bacterial derived food borne diarrheal disease in humans worldwide which results from contamination food of animal origin. The bacteria can also colonize in most of the warm blooded animals and poultry. *Campylobacter* is the member of the family *Campylobacteriaceae* that includes the genera Arcobacter and the species bacteriodes ureolyticus. It consists of 0.2 - 0.8 micro meter in size and is curved or special and microserophilic bacteria in size. It is gram negative bacteria with a distinctive darting motility and this darting motility is catalase and oxidase positive. However when two or more bacterial cells are grouped together, they form S or V shape of the gull wing. The *Campylobacter* incubation period varies from 3 - 5 days and in humans campylobacteriosis is characterized by watery and bloody diarrhea, abdominal pain, cramps, fever, malaise and vomiting which is specially dangerous for young ones. Camphylobacter species cause the bacterial diarrheal disease in humans and in animals too worldwide. A large and diverse number of risk factors which contribute in susceptibility of humans to camphylobacteriosis. It is transmitted through direct contact with infected animals or equipments and water also. It is also transmitted in humans through contaminated food and water. The bacteria can be diagnosed by different molecular techniques. The bacterial culture is generally used for phenotypic identification of Camphylobacter. However, many other selective broths such as Bolton broth, Camphylobacter enrichment broth and Preston broth which has been compared for the efficacy in detection of Camphylobacter species. For an alternative growth on agar and biochemical identification methods and a variety of technologies including immunoassay methods and molecular techniques PCR can be used. (Abebe, E 2020)

Molecular techniques for the detection of bacteria

There are many such advantages for the identification of *Bacillus cereus* bacteria on comparing too traditional methods with modern methods. This bacteria can be detect by several molecular techniques such as polymerase chain reaction which includes different types of polymerase chain reactions such as the DNA polymerase chain reaction, the nested polymerase chain reaction, and the real - time polymerase chain reaction. Despite the fact that the amplification stage is connected to a variety of electrophoresis techniques, including denaturing gradient gel electrophoresis, temporal temperature gradient gel electrophoresis, and pulse field gel electrophoresis (DGGE). Thus the DNA fingerprinting amplification was applied to obtain different strains of Bacillus cereus. By using specific primers which targets the gyr B gene encodes the DNA gyrase subunits B which were find out 90% in bacteria that can be isolate from hot chocolate powder and hot chocolate drinks. Ne The Bacillus cereus bacteria can also be identified using probes that are unique to each bacterium's 16S r - RNA gene using RTi PCR, which has a detection limit of 165 CFU/g of artificially contaminated pasteurised food. An inventive method has been used in current research procedures to speed up the post - polymerase processes. According to research, the time - consuming electrophoretic method for the fast visualisation system utilising gold nanoparticles, which are AUNPs, has replaced the molecular techniques for the detection of Bacillus cereus strains by polymerase chain reaction. Asymmetric PCR can also be carried out prior to the application of propidium monazide (PMA), as PCR amplification is necessary to detect viable Bacillus cereus cells. In essence, PMA is a viability photoreactive DNA binding dye that can enter the Bacillus cereus bacterial cell membrane and, upon PMA entry, binds to the DNA of the bacteria's dead cells. (DGGE). Another PCR molecular techniques low resolution gel electrophoresis. It is a laborious but although time consuming method to visualize PCR products. In this PCR technique involvement of micro which is filled with fluid it is like an artificial chip which is present and also an inexpensive PCR molecule that constitute a thermal cycler which is combined with a PCR chip which allows the amplification and real time fluorescence that can monitor and be used to detect the Bacillus cereus genomic DNA hence for an instance addition in the genomic DNA detection the addition of primers can also be conjugated with biotin and di - oxigenin that enables the lateral flow based detection of amplicons which is an alternative to the optical measurements. By using this module the negative sample exhibited a flurorescent signal neither any bond. So, from the sample to the introduction and intro detection to the result visualisation, the lab on chip PCR technique module completes all phases. When compared to the conventional

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approach for detecting bacteria, biosensors are an ideal way for detecting food - borne pathogens. Next, Bacillus cereus can also be detected from biosensors. Biosensors are small devices that have become increasingly prominent in the field of biotechnology due to their ability to deliver quick findings, simplicity, and the potential for point - of - care diagnosis. Because they allow for selective identification in many Bacillus cereus species, DNA - based biosensors have been extremely successful in the instance of Bacillus cereus. The first choice is to incubate a sample containing Bacillus cereus with magnetic or polyanitine core or shield nanoparticles that are coated with a polyconal anti - Bacillus cereus antibody. The second option involved using a magnetic field separation to separate the background matrix and collect the bacterial cells that were bound to the magnetic particles. After the matrix was separated, the final product was collected using magnetic particles that were then transferred to screen - printed carbon electrodes for voltametric detection. Bacillus cereus strains like the cereulide and emetic strains can also be found and eliminated using current molecular technology. In most cases, the Bacillus cereus eliminates enterotoxins like cereulide and emetic toxins, with emetic Bacillus cereus strains include emetic food intoxication by the synthesis of a cereulide toxin. A non - ribosomal cereulide synthetase enzyme, which is encoded by the ces gene, then produces the toxin. Thus, the harmful molecule is a cyclic depsipeptide made of three repeats of the amino acid sequence D - Oxy - Leu - Dla - L - Oxy - Val - L - Val. Cereulide is very resistant to treatment methods used in the food business and is highly stable. Although antibody - based strategies were investigated, they are most frequently utilised due to the lack of cereulide immunogen characteristics and the limited reproducibility of such tests. The toxin that is produced when cells are injured is measured by cytotoxity - based approaches. Such a variety of cells, including HEp - 2 human laryngeal carcinoma, LHO Chinese hamster ovary, and rat liver cells, were used to make cereulide. Cereulide can be quantified using substances like C - MS Liquid Chromatography Mass Spectrometry and Matrix Asserted Laser Desorption Ionization Time of Flight (MACDI - TOF), which have low cross reactivity and high specificity. Valinomycin, a drug that may be purchased, was utilised to detect the cereulide. However, the quantification of the toxin was expressed as valinomycin which was equivalent and a continuous point because of the difference between the 2 molecules in the biological and chemical assays. (Ramarao et al 2020)

Food - borne intoxications are increasingly caused by the dodecadepsipeptide cereulide, the emetic toxin produced by *Bacillus cereus*. As such intoxications pose a health risk to humans, a more detailed understanding on the chemodiversity of this toxin is mandatory for the reliable risk assessment of *B. cereus* toxins in foods. Mass spectrometric screening now shows a series of at least 18 cereulide variants, among which the previously unknown isocereulides A–G were determined for the first time by means of UPLC - TOF MS and ion - trap MS^n sequencing, ^{13}C - labeling experiments, and post - hydrolytic dipeptide and enantioselective amino acid analysis. (Marxen et al 2015)

The most reliable assay of the emetic toxin is the oral challenge in primates. The most recently research has demonstrated that the vacuole response in the HEp - 2 cells caused by the culture supernatant of B. cereus strains is correlated with emetic toxin activity. In this study, we describe the purification and structural analysis of the B. cereus toxin as a vacuolation factor on HEp - 2 cells. (Agata et al 1994)

Traditional PCR methods require amplification in a thermocycler and product separation by gel electrophoresis followed by hybridization with a probe. This is a time - consuming and laborious process. However, the products of the PCR can also be detected by using a DNA binding dye, such as SYBR Green, or through the use of fluorescent probes. The non - sequence - specific SYBR Green assay is less expensive than the fluorescent - probe - based assays utilizing Taqman probes. (Jothikumar, N., & Griffiths, M. W. (2002).

Kary Mullis created the polymerase chain reaction for the first time in 1985. Following creating a DNA suspension and boiling it at 100 degrees Celsius, E. coli DNA is extracted. The supernatant is then utilised as the DNA template for a polymerase chain reaction (PCR) after centrifugation. It takes a number of polymerase chain reaction components, including buffer, to amplify a polymerase chain reaction. Buffer gives the reaction its recognisable strength and buffering capacity. The cations in the buffer are utilised to stop the repelling force between the negatively charged backbones of dsDNA, which is what makes the annealing process possible. The basic materials utilised as primers and templates are dNTP deoxynucleotide tri phosphates. dATP, dTTP, dGTP, and dCTP are the four parts of dNTP. The primers are a 20 nucleotide long 5' - 3' oligonucleotide. Despite being complementary to the flanking strand and acting as a foundation for the DNA amplification process' extension step, which contains the target sequence and E. coli primary binding site. (Zhao et al 2014)

After the primer has been annealed, the complementary strand is formed utilising the temperature - resistant Taq polymerase enzyme by employing free dNTP. However, the invention of Real Time Polymerase Chain Reaction, which uses fluorescence to detect the presence of a specific gene in real time and does not require electrophoresis, has significantly improved the sensitivity and speed of PCR - based detection methods. Although the majority of R - PCR assays designed for the identification of O157: H7 rely on the detection of stx encoding stx - toxins, cal - intimin, and O - antigen, these R - PCR assays provide the potential to quantify absolute and relative levels of O157: H7 in complicated sample matrices. These are the several R - PCR modes, and the Taq - Man PCR is the most popular R - PCR system. The RT - PCR based test for the detection of O157: H7 viability in food and environmental samples is developed. (Zhao et al 2014)

Basically, mRNA is present in cells that are continuously growing, and unlike DNA, mRNA tends to decay quickly in dead cells. This is why RT - PCR relies on creating single - stranded complementary DNA from mRNA. The rfbE and stx 1 m - RNAs are more stable in the live cells, according to

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scientific investigations. The capacity of RT - PCR to detect stx 2 m - RNA from living O157: H7 cells is affected by the growth circumstances, with the best detection of stx 2 mRNA occurring during late log and early stationary phase of microbial development. Additionally, one of the current time - consuming approaches to find and confirm the presence of STEC in the sample is the use of biosensors. However, they are too expensive and sophisticated to be used in standard analysis. New strategies built on the usage of biosensors have so been described as a result. Biosensors combine a physicochemical detecting component with a biological identification element that is in contact. There are numerous different biosensors available for the detection of E. coli O157: H7, including fibre optic - based biosensors and resonant acoustic profiling. . (Zhao et al 2014)

Hence the modifications and automation of conventional methods in food microbiology include sample preparation, plating techniques, counting and identification test kits. The ATP bioluminescence techniques are increasingly used for measuring the efficiency of cleaning surfaces and utensils. Cell counting methods, including flow cytometry and the direct epifluorescent filter technique are suitable techniques for rapid detection of microorganisms, especially in fluids. Automated systems based on impedimetry are able to screen high numbers of samples based on total bacterial counts within 1 day. Immunoassays in a wide range of formats make rapid detection of many pathogens possible. Recently, there have been important developments in the use of nucleic acid - based assays for the detection and subtyping of foodborne pathogens. The sensitivity of these methods has been significantly increased by the use of the polymerase chain reaction and other amplification techniques. (de Boer, E., & Beumer, R. R.1999)

Molecular technique for the detection of virus

In the past, viruses were identified using an electron microscope to examine a stool solution. Even though it is somewhat labor - intensive and insensitive, this test is still considered the gold standard for virus diagnosis. Routine ELISA tests are available for the detection of non - group A rotaviruses, Sapoviruses (SaV), and the remaining NoVs, as well as for the identification of group A rotaviruses, adenoviruses, and astroviruses. Reverse transcriptase - polymerase chain reaction (RT - PCR) assays can be used to detect viral nucleic acid and make the diagnosis. The variable viral genome of NoV presents a challenge in the development of a single universal detection test. Detection of certain IgM antibodies, however, is indicative of recent infection for the hepatitis viruses. Additionally, viruses can be found in faeces and in serum by RT - PCR. As a result, there are significant discrepancies in the lowest amount required for a positive test across the different assays, ranging from a few particles (cell culture and RT - PCR) to a million particles per gramme. This has direct implications for how the results should be interpreted. A individual with a positive EM test sheds a lot of virus particles, but a positive RT - PCR test may only shed a small number of particles. As a result, there are currently no precise standards for interpreting the findings of these many tests. But because of all the complicating elements, not everyone who is ill necessarily sheds more viruses than someone who is symptom - free, and different persons appear to shed viruses at various maximum amounts. A individual with a positive EM test sheds a lot of virus particles, but a positive RT - PCR test may only shed a small number of particles. As a result, there are currently no precise standards for interpreting the findings of these many tests. To make matters more complicated, the maximal amounts of shedding appear to vary for various viruses, and those who are ill may not necessarily shed more viruses than those who are symptom - free. However, because these viruses have a very low minimum dose for infection, any sick individual can spread the illness. Even after the development of RT - PC, virus detection in food or water has proved challenging. This is due to the fact that the most significant food - borne viruses do not proliferate quickly and easily in cell culture, necessitating their direct detection in food extracts. However, due to the extremely low minimum dose required for infection by these viruses, any infected person can spread the disease. Even with the introduction of RT - PC, it has been difficult to identify viruses in food or water. This is because the most dangerous food - borne viruses do not multiply rapidly and easily in cell culture, making it necessary to directly identify them in food extracts. Therefore, the fundamental cause of the dearth of data on the unpredictability of test results from sampling is the interaction of changing viral counts and the absence of a culture system. However, a genomic study can separate the strains of all enteric viruses into subgroups. (Koopmans, M. and Duizer, E., 2004).

2. Future Prospects

Food - borne pathogens have become the common source for human illness. These can be bacteria, parasites and even viruses which can cause infections or any such kinds of deadly disease in humans as well as in animals also. In today's world biotechnology has become the important to role to detect the pathogens in food and water source. Scientists have developed different techniques which can detect their toxins in any source. Although on earlier for the detection of pathogen toxins there was the traditional technique for the detection of pathogens toxins were PCR polymerase chain reaction techniques, DNA extractions. There were different polymerase chain reaction techniques which are very helpful for the detection of bacteria, parasite and viruses. However modern biotechnology techniques have involved the biosensors and micro fluidic chip which are used to detect the food borne pathogens and their toxins which are involved in food and water source. On the comparison with modern to traditional technique traditional technique have become the good and helpful techniques for laboratories. However traditional technique are good but do not give the immediate result of detection but on comparison modern technique have developed which are biosensors and micro fluidic chip which are very important and give immediate response and result of any particular food borne pathogens. In future era these modern biotechnology techniques have become very vast and important for laboratories. So biosensors and lab on chip technique have taken the important area on laboratories rather than polymerase chain reaction and DNA extraction, but the very basic technique are also being helpful in such as different polymerase chain reaction, nucleic - acid based reaction and DNA reaction. Technologies have become very important in

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all over the field different - different technologies are developed which are very useful in mankind.

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