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Review article

A Review on Analytical Methods for the Detection of Foodborne Pathogens

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ABSTRACT:

In both developed and emerging nations, food safety has become a topic of immense importance as a matter of safeguarding public health. There have been multiple cases of acute ailments due to the consumption of food contaminated with pathogens. The infections and illnesses caused by food borne pathogens have emerged as a significant cause leading to several health hazards to humans. From the cultivation stage to the ingestion of food, there are various stages during which the pathogens can infect the food crops or food products by developing multiple colonies of the pathogen which would prove fatal for human consumption. The vital microorganisms or pathogens involved in food degradation are bacteria, mycotoxins, viruses, and other parasites which could lead to dreadful infections and worse conditions for human health. Therefore, it has become necessary to detect pathogens promptly to assure the safety of food products. To control food safety, it has become essential to use detection methods that are accurate, sensitive, and rapid. Various analytical techniques such as chromatographic methods, spectroscopic methods, and various detector-sensitive methods have been elaborated as well and their contribution in the identification of pathogens has been mentioned in brief. Analytical methods play a critical role in the detection of food borne pathogens, offering a combination of sensitivity, specificity, and practicality for routine testing in food laboratories. Continued innovation and improvements in these methods are essential for ensuring food safety and safeguarding public health. The review emphasizes the capability of analytical techniques for detecting a wide range of microorganisms and rapidly analyzing their contamination in foodstuffs.

Keywords: Chromatography, Food analysis, Mycotoxins, Pathogens, Spectroscopy

1. INTRODUCTION

Foodborne diseases are defined as any illness of toxic or infectious nature caused by the consumption of food or water contaminated with pathogenic bacteria, viruses, or harmful parasites [1]. Any pathogenic bacteria in food have the potential to cause excessive harm and serious health issues in both animals and human subjects. Food borne illnesses are frequently caused by pathogens such as bacteria, viruses, fungi, yeast, and parasites as shown in Figure No. 1 [2].

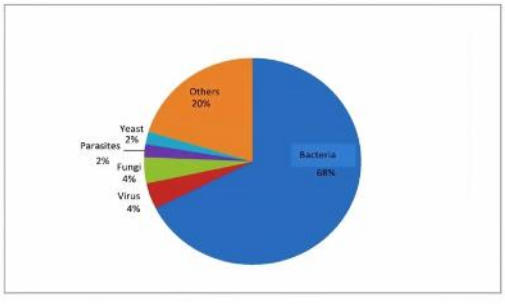

Fig 1: Pathogens causing Food borne Diseases

The presence of few Colony-Forming Units (CFUs) of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, *Listeria monocytogenes*, and species of Clostridium, Bacillus, Vibrio, Shigella, and Pseudomonas can result in illness [3].

The quality of food is not only affected by bacterial pathogens but also by several fungal pathogens called mycotoxins. The word "mycotoxins," means "fungi poison," is derived from the Greek words "mikes" and "toxin." Since the discovery of aflatoxins in the1960s, all mycotoxins have been linked to serious illnesses, including actions that are mutagenic, carcinogenic teratogenic, and immunotoxic [4]. Mycotoxins have become a major concern worldwide due to their significant effects on the health of humans and animals and hampering productivity rates [5-7].

Due to the presence of prevalent microorganisms in food, water, and the environment, it has become the need of the hour to ensure the safety and quality of food and other edible products. The World Health Organization (WHO) estimates that these pathogens are responsible for 23 million food borne illnesses and 5,000 deaths in Europe every year [8]. The other agents that comprise 20% of the total causes of food borne infections are chemicals such as pesticides, fungicides, insecticides, and rodenticides which become part of the food at the time of cultivation and harvesting only [9]. A huge portion of the Indian population is also affected by food borne illnesses by ingesting infected foods. In India,

there are reportedly 120,000 food-related deaths annually and an estimated 100 million cases of food borne illness [10]. Food borne diseases caused by microbial contamination are a major problem for public health worldwide. The proliferation of food-borne pathogens has raised the possibility of food contamination. In humans, these microbes induce gastroenteritis, which results in food poisoning, nausea, and diarrhea.

The identification of harmful microorganisms quickly, accurately, and in real-time has been the focus of extensive research in recent years. The detection of food borne pathogens in food products can be done using a variety of common analytical standard techniques including UV Visible spectroscopy, High-Performance Liquid Chromatography (HPLC) (generally for mycotoxins), Mass Spectroscopic(MS) Methods, Surface Enhanced Raman Spectroscopy (SERS), Lateral Flow Chromatographic Assay Methods, Near Infrared (NIR) Spectroscopy, Fourier Transform Infrared(FT-IR) Spectroscopy and many more.

2. ANALYTICAL TECHNIQUES INVOLVED IN QUALITY ASSESSMENT OF FOOD PRODUCTS

Ultra-Violet (UV)-Visible spectrophotometry

The identification of food contaminants can be accomplished using the widely used absorption spectroscopy technique known as ultraviolet-visible spectroscopy, or UV-visible spectroscopy. It works on the principle of Lambert-Beer law which states that " When light is incident on a homogenous medium, a part of it is reflected, a part of it is absorbed and the remaining is transmitted."It is used to identify organic food pollutants [15]. Low amounts of cyanide (CN-) which is a poisonous ion can be fatal to humans [16]. Due to its occurrence in fruit pits, bitter almonds, and plants, it is a toxin of concern for food security [17]. Cyanide ions can greatly hinder metabolic functions and interfere with the enzyme activities. To overcome this issue, UV-visible spectroscopy was used.

Infrared (IR) Spectroscopy

Since the early detection of food borne pathogens is crucial for protecting human health, early food safety monitoring is a significant concern now a day. Traditional microbiological methods are accurate and precise but they still take time to get results. Therefore, the use of infrared spectroscopy combined with a chemometrics approach yields early results. The identification and measurement of organic molecules using infrared spectroscopy is commonly done in analytical chemistry and has proven to be effective in the food industry [18]. This technique works by identifying the functional group in the structure of the molecule. The IR region is divided into three types based on the wavenumber, that is, Near IR, Mid IR, and Far IR.

Different bacterial strains of harmful bacteria display distinctive Mid-Infrared (MIR) spectra, primarily due to differences in the components of their membranes [19]. In the case of contaminated food, the infrared spectral range differs if the bacterial strain is specific. It works on the general principle of a fiber evanescent wave spectroscopy (FEWS) method. The IRFEWS coupled technique for spectrum analysis is precisely used for the detection of bacterial strains in food.

Despite having the same biochemical components in all pathogenic organisms such as proteins, polysaccharides, phospholipids, and nucleic acids, pathogens differ in the ir number and distribution which results in a unique FT-IR spectrum for each pathogen. Each spectrum has a wavelength range of 800-3800cm^{-1} but the most helpful FT-IR features for identifying bacteria come at wavenumbers of 1000–3000 cm^{-1}. This technique helped in the early identification of pathogens in food matrices which serves as a major advantage in the health field [20].

Mass Spectrometry

This technique is used to detect the molecules by identifying their mass-to-charge ratio and giving the respective spectra for each molecule. Mass spectrometry for pathogen detection is based on the Matrix-assisted laser desorption ionization, time-of-flight (MALDI-TOF) technique. This technique is useful in the identification, analysis, and characterization of several target bacteria [21]. MALDI-TOF MS has lately become an effective method for the identification of clinical isolates because of its quick turnaround times, minimal sample volume requirements, and affordable reagent costs. All types of pathogens found in food can be detected by this technique in short turnaround times [22].

A wide variety of biomolecules including nucleic acids, peptides, proteins, carbohydrates, and other molecules can be detected by this method.

Thus, MALDI-TOF allows early and direct identification of pathogens as compared to conventional techniques. It involves the detection of L. monocytogenes from broth culture using whole-cell microbial proteomics and MALDI-TOF techniques. Although the method used is known as whole-cell mass spectrometry, it involves rupturing of the intact microbe after being exposed to the matrix which typically contains acetonitrile and trifluoroacetic acid (TFA). The whole-cell method is most likely to identify ribosomal proteins, cell structure proteins, cold shock proteins, storage proteins, and nucleic acid binding proteins [23]. MALDI-TOF uses food enrichment broth directly for the identification of L. monocytogenes without growing the bacterium on solid media.

The standard strain of L. monocytogenes was foundresponsibleforcontaminatingvariousfoodssuchasfat-freeprocessed milk, camembert cheese, and cantaloupe. Experimentation regarding other pathogenic strains such as S. aureus and E. coli was also performed to analyze the

quality of food being contaminated by these strains. For all experiments, the bacteria were grown on brain heart infusion (BHI) agar undergoing incubation for 24 hours at 37 °C. Non-selective BHI broth was used as enrichment for foods contaminated with Listeria [23].

Raman Spectroscopy

To analyze the quality of food, several approaches can be used such as microbiological methods, sensory analysis, biochemical methods, and physicochemical methods. Raman spectroscopy can identify different kinds of chemicals and organic molecules as well as their physical structures by the formation of bonds [24].

When a strong monochromatic light source, particularly a laser beam, illuminates a sample, photons are scattered, and it is discovered that the majority of the scattered light has the same wavelength as the laser light as a result of this application. Asaresul to facollision between the sample and incident photons, there is a change in the wavelength of photons which is known as Raman Scattering. There are two possibilities after Raman Scattering, either the photons gain energy and get displaced to a longer wavelength or if the photons lose energy, they will move towards a shorter wavelength. The technique detects the microorganisms in foods, chemicals in food, food additives, food adulterants, and contaminants. The detection of viruses and bacteria can be done using a variety of analytical techniques.

Dehydrated Enterococcus faecal is subjected to FT-Raman spectra. In the 2700–3000cm^{-1}range which belonged to CH3, CH2, and CH) functional groups, specific C–H stretching bands were seen and the C–H bond formation band was evidentat1450 cm^{-1}[25]. *Bacillus sphaericus* (B. sphaericus), *Rhodotorula mucilaginosa* (R. mucilaginosa), *Pseudomonas uorescens* (P. uorescens), *Micrococcus luteus* (M. luteus) and *Bacillu ssubtilis*(B.subtilis) colony samples were examined in various food samples using FT-Raman. . At a wavelength of 785 nm incident light, it was discovered that thespectra of M. luteus, B. subtilis, and P. uorescens had entirely distinct spectra from one another [26].

Studies on the analysis of food borne viruses using Raman spectroscopy are quite rare. The commonly reported foodborne virus is Hepatitis A. Raman Spectroscopy utilizes the acyl groups of the active enzyme for identification. There have been numerous researches for the detection of food borne viruses by this technique but none of them have turned out to be fruitful yet.

To find minute levels of pesticide residues, different fruits and vegetables were analyzed using micro-Raman and FT-Raman spectroscopy [27]. Raman spectra of a few herbicidesnamely atrazine, prometryn, and simetryn were detected in the solid phase as well as in polar and non-polar solvents. The experimental and theoretical data were compared, and the results were analyzed.

Another study highlights the presence of Deoxynivalenol in members of the genus Fusarium such as wheat and barley28. The presence of Deoxynivalenol lowers the grain quality and has harmful impacts on human health [29]. Raman spectroscopy is used along with Infrared spectroscopy for the detection of Deoxynivalenol. Investigations were made using FT-Raman spectroscopy to characterize and categorize ground wheat and barley that had been contaminated with various proportions of Deoxynivalenol. The detections were made in the spectral regions of 1800–800 cm^{-1}. The final quantities of Deoxynivalenol were measured and the wheat was separated into high and low-based quantities of Deoxynivalenol [30].

Another investigation emphasized the qualitative as well as quantitative identification of Aspergillus-produced aflatoxin in maize. Raman bands varied on the amount of aflatoxin present in the samples. Raman spectroscopy accompanied by FT-IR and FT-NIR was used for the detection of varying concentrations of aflatoxins. After analysis and identification, the aflatoxins were categorized based on their concentrations and it was found that Raman spectroscopy yielded better results as compared to FT-NIR.

Various chemical agents present in food can also be detected by Raman spectroscopy. Coumarin is a substance that is naturally present as a constituent in various plants such as tonka beans and sweet clover and was used as a flavoring agent until its hepatotoxic activity was reported. By monitoring the interaction of p-cresol with 4-bromoethyl acetoacetate, the IR and Raman spectra of 6-methyl-4-bromomethyl coumarin were obtained [31].

Surface Enhanced Raman Spectroscopy (SERS)

Infrared and Raman spectroscopy techniques were among the first vibrational spectroscopy techniques to be developed and were rapidly used as methods of analysis broadly. Later in1974, during the analysis of the pyridine molecule, it was observed after proper experimentation and computation that many orders of magnitude were present which was quite different from the normal Raman spectroscopy and led to the identification of SERS [32]. Since then, SERS technology has been used in food detection which was primarily driven by the demand for quick and accurate methods to identify food contaminants. SERS has emerged as a diagnostic tool for the identification of foodborne pathogens such as *Salmonella*, *Staphylococcus aureus*, and *E.coli*. [33]. Due to the ability of SERS to identify the molecular and cellular mechanisms of the bacterial cell and give a clear vibrational spectrum of the same, the use of SERS gas increased extensively. SERS's strong Raman signal and ability to identify single molecules make it particularly useful for detecting microorganisms.

A study of the origin of the band at approximately 730 cm-1 was performed in the SERS spectra for bacteria which were attributed to adenine-related chemicals, using a stable isotope method in conjunction with SERS. In another study, the differences in the Raman spectra of bacteria at 785 nm related to adenine, hypoxanthine, xanthine, guanine, uric

acid, and AMP were examined [34]. The first study using SERS for the detection of bacteria involved the use of silver colloid substrate for the identification of E.coli. The primary bands obtained were peptides and polysaccharides present in the cell wall and membrane. SERS primarily refers to SERS substrates because they are required for SERS measurements and their SERS activity. To increase the Raman signal of the surface or nearby molecular level, SERS primarily refers to the usage of rough metal surfaces or metallic nanoparticles through special preparation. In a particular excitation region, the sample interacts with the special preparation of well-performing SERS substrates [35].

The ability of SERS to detect Bacillus in food is because of its high efficiency in identifying dipicolinic acid (DPA) which serves as a biomarker for Bacillus in vivo. Peaks were obtained for different species of pathogen but the detection of Gram-negative and Gram-positive was found difficult to identify. To differentiate the foodborne pathogens, different batches of gold colloid were combined with seven different bacteria. According to the findings, the gold colloid is useful for accurately and quickly detecting food borne pathogens using the SERS. The use of the gold colloid in the SERS is advantageous for the quick and accurate detection of food borne pathogens. An active substrate that could be subjected to surface-enhanced Raman Scattering was formed by combining Ag nanoparticles and Ag nanospheres. The nanoparticles enabled the detection of three microbial strains, that is, E. coli, *Staphylococcus aureus*, and *Salmonella typhimurium* [36].

Although Raman emissions can be used to detect bacteria by identifying the differences in the various cell types by chemometric and statistical analysis, the use of biochemical tools is still highly advised.

Colorimetric Methods

One of the simple stand most useful detection methods is colorimetric pathogen detection. The presence or absence of microorganisms can typically be determined by using colorimetric detection instruments or a straight forward ultra violet spectrophotometer. According to numerous researches, greater sensitivity of microbial strains can be attained through quick detection using nanoparticles and colorimetric tests. Commonly used nanoparticles are Cerium oxide (Ceo2), Gold (Au), Platinum (Pt), Ferric oxide (Fe_3O_4), and many more according to their strength and affinity.

Gold nanoparticles were used, and the catalytic ability of gold nanoparticles was utilized to report the presence of E. coli [36]. To work as an enzyme-nanoparticle biosensor for bacteria detection, Beta-galactosidase was additionally linked to cationic Au nanoparticles and functionalized with amine head groups. Enzymatic activity can be recovered when the negatively charged surface of E.coli attaches to the cationic nanoparticle, allowing Beta-galactosidase to be released. By suspending the E. coli in a phosphate buffer solution, its concentration can be determined [37]. According to a study, the Au nanoparticle's colorimetric sensing system allowed the naked-eye detection of *Bacillus subtilis* when suspended in a 3-(N- morpholino)-propanesulfonic acid (MOPS) buffer. Additionally, it was concluded that the colorimetric sensor is appropriate for the quick and accurate microbiological detection assay of complicated materials.

Several organizations with a strong focus on food safety have established sampling techniques for establishing food homogenates to find harmful bacteria in environmental and food samples.

Analytical Techniques for the Detection of Mycotoxins

Thin Layer Chromatography

It is an easy, inexpensive, and quick analytical procedure that produces results based on qualitative or semi-quantitative estimation. TLC is a potent tool for the simultaneous examination of several mycotoxin-contaminated samples. This technique provides a wide variety for the stationary phase, mobile phase, and detecting reagents. TLC was used extensively in the early research on mycotoxins, and it has turned out to be more fruitful for the detection of colored or fluorescent compounds as mentioned in **Table 2 [**38, 39].

Table 1: Classification of Food borne Diseases

Pathogen	Diseases caused	Main Symptoms	References
1. *Bacillus cereus*	Food Poisoning	Watery diarrhea and abdominal cramps, nausea and vomiting	[11], [12]
2.*Campylo bacterjejuni*	Campylo bacteriosis	Diarrhea, cramps, fever and vomiting, diarrhea may be bloody.	[11], [12]
3.Clostridiumbotulinum	Botulism	Blurred vision, drooping eyelids, lethargy, constipation, difficulty swallowing, dry mouth, and muscle weakness.	[11], [12]
4.Escherichia coli	Hemorrhagic colitis	Decreased urine production, dark or tea-colored urine, and losing pink color in cheeks and inside the lower eyelids.	[11], [12]
5. Norovirus	Viral gastroenteritis	Nausea, vomiting, diarrhea, Myalgias and abdominal pain	[11], [13]
6. HepatitisA	Hepatitis	Nausea, joint pain, dark-coloredurine, pale stools, anorexia and abdominal discomfort	[11], [13]
7.Salmonella	Salmonellosis	Diarrhea, fever, stomach cramps, vomiting	[11], [12]
8. Aspergillus flavus	Aspergillosis	Fever, cough, shortness of breath	[14]

Table 2: Mycotoxins detected in food by TLC

Mycotoxin	Food Matrix	Detector	Spraying Reagent
Patulin	Apple juice	TLC plate	Ammonia fumes
Citrinin	Corn and Barley	Fluorescent TLC plate	Ammonium Chloride

Gas Chromatography

It is a conventional and analytical method that is used for detecting and separating gaseous and volatile compounds. Components are separated based on their affinity with the stationary phase or mobile phase. This technique can be used for both Qualitative and Quantitative analysis. In this technique, the mobile phase is a chemically inert gas that carries the molecules of the analyte through a column. The gases usually used as mobile phases are helium, nitrogen, etc. Gas Chromatography coupled with a Flame Ionization Detector (FID), Electron Capture Detector (ECD), and Mass Spectroscopy is mainly used for the detection of trichothecenes mycotoxins. To increase mycotoxins' volatility and reduce their polarity, most researchers prefer to derivatize them [40].

The mycotoxins determined using Gas Chromatography with the assistance of various detectors are mentioned in **Table 3[**41].

Table 3: Mycotoxins detected in food by GC

Mycotoxin	Food Matrix	Detection
Deoxynivalenol	Cereals, barley	GC-MS
Trichothecenes	Cereals, wheat	FID GC-MS
Zearalenone	Poultry food	GC

High-Performance Liquid Chromatography (HPLC)

HPLC is one of the most popular methods for determining the presence of mycotoxins in food when HPLC is coupled with a diode array detector (DAD), ultraviolet (UV), or fluorescence detector (FD). Most of the mycotoxins can be analyzed in approximately less than 20minutes after being injected into the column [42].

The major mycotoxins detected and identified by HPLC as established by standard committees are aflatoxin B1, aflatoxin M1, ochratoxin A, and fumonisins B1 and B2. There has been extensive use of HPLC for the determination of Deoxynivalenol, zearalenone, and aflatoxins in cereal products, maize, and hazel nut respectively [43].

To derivatize non-fluorescent mycotoxins into fluorescent derivatives using the High-Performance Liquid Chromatography-Fluorescent Detector (HPLC-FD) method, the use of specific label in reagents is done, and the results obtained by this technique are highly sensitive, selective, and reproducible. Another approach created for the investigation of distinct mycotoxins is High Performance Liquid Chromatography- Ultra Violet (HPLC-UV) which uses a diode array UV detector for identification of mycotoxins with variable structures. Mycotoxins detected by HPLC are mentioned in Table 4[44].

Table 4: Mycotoxins detected byHPLC [44]

Mycotoxins detected	Detection Methods	Food material used
Aflatoxin B1	Fluorescent	Grapes and must
Ochratoxin	Fluorescent	Coffee, wheat, figsand
Citrinin	Fluorescent UV	Soft cheese, corn and cereals
Patulin	UV and fluorescent UV diode array detector	Apple juice
Zearalenone	Fluorometric array	Cereals

Liquid chromatography/mass spectrometry (LC/MS)

The most effective method for concurrently screening, detecting, and quantifying many mycotoxins is liquid chromatography combined with mass spectrometry (LC-MS).This technique is of great use for toxins that show very little or negligible sensitivity for UV absorption or fluorescence. The technique works by combining electrospray (ESI) ionization or atmospheric pressure chemical ionization (APCI) with mass spectrometers (**Table 5**)

Table No. 5 Mycotoxins detected by LC-MS

Mycotoxins detected	Detection Methods	References
Fumonisin	ESI(+) with Mass Spectrometry	[45]
Ochratoxin A	Fluorescence detectors, APCI	[46]
Aflatoxin	ESI ionization, Fluorescence detectors	[47]
Zearalenone	Ionization with APCI and ESI	[48]

Infrared Spectroscopy (IR)

This technique helps in the structural elucidation of a molecule and for the identification of functional groups present in the molecule.

Near Infrared (NIR) spectra of various foods have broad bands resulting from overlapping absorptions primarily related combinations of vibrational modes involving 'C-H', 'O-H', and 'N-H' chemical bonds. The transmittance is measured in the spectral range of 800-100 nm for samples such as wheat, whole grains, and cheese. Another spectral region from wavelength 100-2500 nm is usually used for measuring the quality of fruits and wheat powder [49]. It is a potential method for the quick and non-destructive identification of mycotoxins in grains.

Fourier Transform Infrared Spectroscopy- Following are the mycotoxins identified by IR and FTIR mentioned in **Table 6 [**50].

Table 6: Mycotoxins identified by FTIR

Mycotoxin	Detection Method	Food Matrix
Fumonisin	NIR Spectroscopy	Corn and Maize
Deoxynivalenol	Mid IR spectroscopy and NIR transmittance	Wheat and Maize
Ochratoxin A	FTIR	Dried Grapes

Analytical Methods for Detection of Chemicals in Food

UV spectrophotometry

This technique was frequently employed for the identification of pesticide residues in environmental samples. The method was used to detect pesticides such as dicamba and atrazine. The limit of detection was found to be 0.1µg/ml and 0.2 µg/ml for atrazine and dicamba respectively. Additionally, spectrophotometric detection techniques were found to be effective for detecting organo-pesticides including 'malathion', 'phorate', and 'dimethoate'in food samples.The process involved oxidizing organo-phosphorous insecticides with little excess of N-bromosuccinimide. Rhodamine B reacted with the left amount of N-bromosuccinimide and the color change at 550 nm was measured spectrophotometrically after that [51].

Chromatographic Methods

One of the first few methods for pesticide detection to be used was chromatography. The fundamentals of chromatography have under gone numerous changes as technology advances.

Thin Layer Chromatography is used to detect pesticides in many modified ways. To detect fungicides, the TLC bioassay uses a TLC plate that has been treated with *Cadosporium cladosporioides* spores. The absence of fungal growth around the sample area indicated that the pesticide was present.

Gas Chromatography method uses a stationary solid or liquid phase and a gaseous mobile phase to detect the analyte from the sample. For both qualitative and quantitative analyses of pesticides, gas chromatography (GC) is frequently employed. The various detectors used in GC are Electron Capture Detector (ECD), Flame Photometric Detector (FPD), Flame Ionization Detector (FID), Infrared detector, and Massspectrometer (MS).

High-Performance Liquid Chromatography when compared to other types of chromatography, this type has a stronger advantage. This method can be used to detect the analytes at higher pressure as compared to other techniques. By using both normal phase and reverse phase columns, HPLC coupled with a diode array detector (DAD) was used to investigate β-cyfluthrin [52].Other pesticides such as Malathion, dalazion, and sumuthion have been detected by using acetonitrile as themobile phase in reverse-type HPLC.

3. ANALYTICAL METHODS FOR DETECTION OF WATER BORNE PATHOGENS

Chromatography-This technique is technically complex and restrained from being used generally due to its large equipment requirements, more consumption, and thermostating systems which are possible to maintain with in a laboratory only. On the contrary, a quick and precise approach was developed which was Gas Chromatography - Differential Mobility Spectrometry (GC-DMS) which was used for the detection of E. coli.

The commonly found bacteria to form colonies in water are the Pseudomonas species. Liquid Chromatography when combined with time-of-flight mass spectrometry (LC–TOF-MS) was used to detect the presence of Pseudomonas putida on water supply biofilms made from samples from water supply pipelines [53].

MALDI-TOFMS-MS in combination with methods like matrix-assisted laser desorption ionization-time off light (MALDI-TOF) enables the detection of biocomponents via ions produced during the analysis. Another technique that makes use of MS is electro spray ionization mass spectroscopy (ESI-MS).These techniques provide information about microbial identification and detection in complicated samples such as surface water and waste water. They also produce fast and accurate results for analysis. MALDI-TOF MS was used to identify Legionella species in drinking water and the results obtained were truly convincing.Therapidandpreciseidentificationofcoliformsinwastewater, river water, and groundwater using MALDI-TOF MS technology was also beneficial. MALDI-TOF analysis was used for the identification of approximately 100 isolates of bacteria in different water samples and the obtained results were as demonstrated in **Figure No. 2[**54].

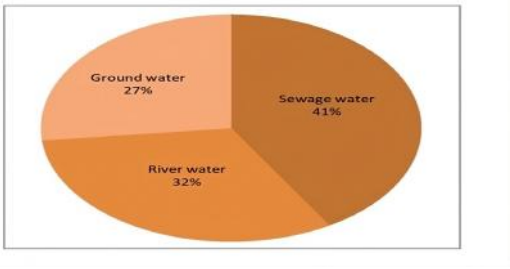

Fig 2: Percentage of bacterial contamination in different water sample

4. CONCLUSION

The need for quick detection techniques has grown significantly as the world has become more concerned about the effects of food on human health, its safety, and other issues. To preserve food safety and prevent harmful food poisoning, it is crucial to rapidly identify microorganisms in food. Although traditional pathogen detection methods are sensitive, they are tedious and time-consuming for the detection and identification of microbial contamination in food, placing them behind analytical methods in terms of detection time. The conventional methods are time-consuming and tedious in comparison to modern analytical methods. The benefits of sophisticated methods also include quick data analysis, low cost, strong intensity, and quick response time. Pathogens can be found in samples even in

extremely low concentrations, therefore analytical procedures must be acceptable for in-situ real-time monitoring as well. The analytical methods are used in combination with various detectors and biosensors which provide rapid and real-time detection of pathogens in food samples. Moreover, the type of food and nutritional elements (protein, fat, fiber, and carbohydrates) present in food determine the development of new tools for detecting hazardous microorganisms. To detect the pathogens in each food product, certain sample preparation techniques and analytical equipment are required. Numerous biosensors can concurrently detect numerous analytes with the fewest possible interferences and have excellent applications in medical diagnostics, food quality control, environmental monitoring, and other industries. The analytical methods provide potential advantages such as validation, precision, accuracy, cost-effectiveness, and are liable method for the detection of microorganisms.

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