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Molecular and Biochemical techniques for identification of some pathogenic organism.

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Abstract :

Testing for and promptly identifying pathogenic organisms is one of the most crucial steps in preventing and recognizing health, safety, and well-being emergencies. Pathogen detection has become one of the most challenging issues facing the food and water industries because of the high expenses and rapid spread of foodborne and waterborne illnesses in the community. The regulations are already highly stringent in the food industry, where a failure to identify harmful substances could have disastrous consequences, particularly for the elderly, the very young, or people with weakened immune systems. Notwithstanding the need for rapid analytical testing, traditional and conventional bacterial detection procedures might take up to seven days to get a result. Given the advancements in biosensors, chemical knowledge, new technology, and equipment miniaturization, this timetable is untenable. By discussing the main techniques, biology, chemistry, miniaturization, research, and developments for pathogen detection in clinical samples, this review provides an opportunity to fill a knowledge gap in an important area of study.

Keywords: pathogens, Molecular techniques, Surveillance, biochemichal, real-time and PCR, chromogenic agent , fingerprinting DNA.

التقنيات الجزيئية والكيميائية الحيوية لتشخيص بعض الميكروبات المسببة للأمراض

زينب أنس سلمان ، هبه عبدالرحمن جاسم
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مستخلص:

يُعدّ اختبار الكائنات المُمرضة وتحديدها فورًا من أهم الخطوات للوقاية من حالات الطوارئ الصحية والسلامة والرفاهية والتعرف عليها. ونظرًا لارتفاع التكاليف وسرعة انتشار الأمراض المنقولة بالغذاء والماء في المجتمع، برز الكشف عن مسببات الأمراض كواحدة من أصعب المشكلات التي تواجه صناعة الأغذية والمياه. وسيصبح الاختبار ضروريًا لإدارة ومنع تفشي العدوى المميتة المحتملة نظرًا لاحتمالية التوسع السكاني الحتمي وزيادة السياحة إلى بعض المسطحات المائية. في قطاع الأغذية، حيث قد يكون الفشل في اكتشاف المواد المُمرضة مدمرًا، وخاصةً لكبار السن أو صغار السن أو ذوي المناعة الضعيفة، فإن القوانين صارمة للغاية بالفعل. قد تستغرق اختبارات الكشف عن البكتيريا التقليدية والروتينية ما يصل إلى سبعة أيام للحصول على النتيجة، على الرغم من ضرورة وضرورة إجراء اختبار تحليلي سريع. هذا الجدول الزمني غير مقبول في ضوء تطور التقنيات الجديدة، وأجهزة الاستشعار الحيوية، والفهم الكيميائي، وتصغير المعدات. من خلال تناول الأساليب الأساسية، والبيولوجيا، والكيمياء، والتصغير، والبحث، والتقدم المحرز في الكشف عن مسببات الأمراض في العينات السريرية، توفر هذه المراجعة فرصة لسد فجوة المعرفة في مجال دراسي بالغ الأهمية.

الكلمات المفتاحية: مسببات الأمراض، التقنيات الجزيئية، المراقبة، الكيمياء الحيوية، الوقت اللحظي وتفاعل البوليميراز المتسلسل، العامل الجيني، بصمة الحمض النووي.

Introduction

Rapid identification and detection of microorganisms is an important yet challenging part of industry and health. It is well known that standard techniques (such culture media and biochemical tests) are time-consuming and labor-intensive [1]. Molecular methods such as 16S ribosomal RNA gene sequencing based on polymerase chain reaction, however, are necessary for the comprehensive reporting of microorganisms in modern study [2]. while screening techniques necessitate a quick and low-cost classification of isolates of bacteria and fungi [3]. Surveillance studies have helped us better understand the prevalence and spread of foodborne infections, as some foodborne pathogens have become more significant and a public health issue in recent years [4]. The precise epidemiological data provided by suspension studies, which use genetic methods to detect and categorize foodborne pathogens, may be used to discover the root cause of human illnesses [5]. Deoxyribonucleic acid sequencing, polymerase chain reaction multiplex, and random amplified polymorphism , pulsed field gel electrophoresis, multilocus sequence typing, repetitive extra genic palindromic, and many other molecular techniques have been used to detect, characterize foodborne pathogens of significant human importance[6]. According to reports, the main sources of foodborne infections are farm animals, such as pigs, cattle, sheep, goats, and chickens, as well as other animals, including domestic and wild ones[7]. Numerous molecular techniques, including repetitive extragenic palindromic, multilocus sequence typing, pulsed field gel electrophoresis, random amplified polymorphism, deoxyribonucleic acid sequencing, and polymerase chain reaction multiplex, have been employed to identify and describe foodborne pathogens of major human concern [6]. Agriculture animals, including pigs, cattle, sheep, goats, and chickens, as well as other domestic and wild animals, are reported to be the primary causes of foodborne illnesses [7].

Methods to Identify Microorganisms

Foodborne germs are being studied more and more because they can adapt

to a range of environmental and survival conditions. Due to their ability to evolve, these infections have been able to adapt and persist in a range of settings [10]. As illustrated in figure 1, the presence of certain antibodies, virulent genes, and complex defenses further enhances foodborne pathogens' ability to adapt and endure under a range of environmental conditions. For foodborne pathogen monitoring, the ability of the pathogens to persist in a variety of environmental conditions justifies the development and application of dependable, effective methods for separation, detection, and differentiation, classification, and typing procedures [11]. For clinical and therapeutic purposes, surveillance studies provide epidemiological information that can be utilized to pinpoint the infection's origin. They also provide data that helps reduce foodborne pathogen onset and colonization, as well as in adapting appropriate strategies to prevent and control the spread of foodborne illness [12].

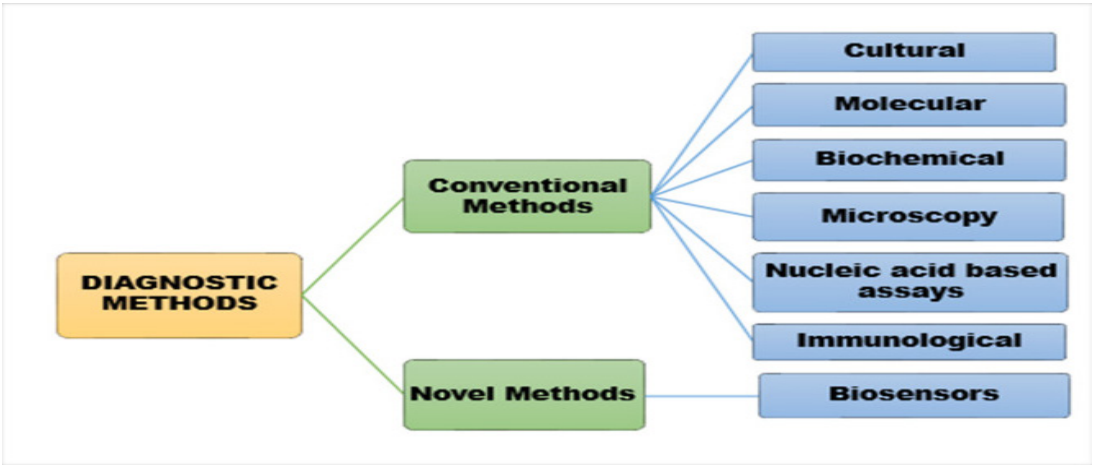

Figure 1: Methods to Identify Microorganisms[13].

Numerous food borne pathogens have been identified in various goods, including Plant, animal, and environmental variables have been implicated in foodborne illnesses, infections, poisonings, and intoxications that occur seldom or are associated with outbreaks [14]. Specifically, handling and consuming contaminated raw chicken goods and meals, such as duck eggs

and meats, has been connected to the majority of outbreaks [15]. A nursery school has linked an outbreak of Salmonella Typhimurium definitive phage type disease to contact with ducklings and young birds [16]. Serum polymerase chain reaction (PCR)-based techniques can be used in conjunction with traditional techniques to efficiently monitor foodborne pathogens. Traditional or culturally accepted methods seem to have been used since the inception of microbiological sampling [17]. Biochemical tests are used in these methods to confirm putative bacterial colonies after enrichment (pre-enrichment and/or selective enrichment), plating onto selective agar, or straight onto selective agar without enrichment [18]. Along with being widely used, they are less costly, only detect living bacteria, and generate isolates that can be further described and investigated, but they are less effective and require more time and work [19].

Pathogen typing using molecular methods

Several molecular techniques have been developed and are frequently used in foodborne pathogen typing. Figure 2 illustrates this.

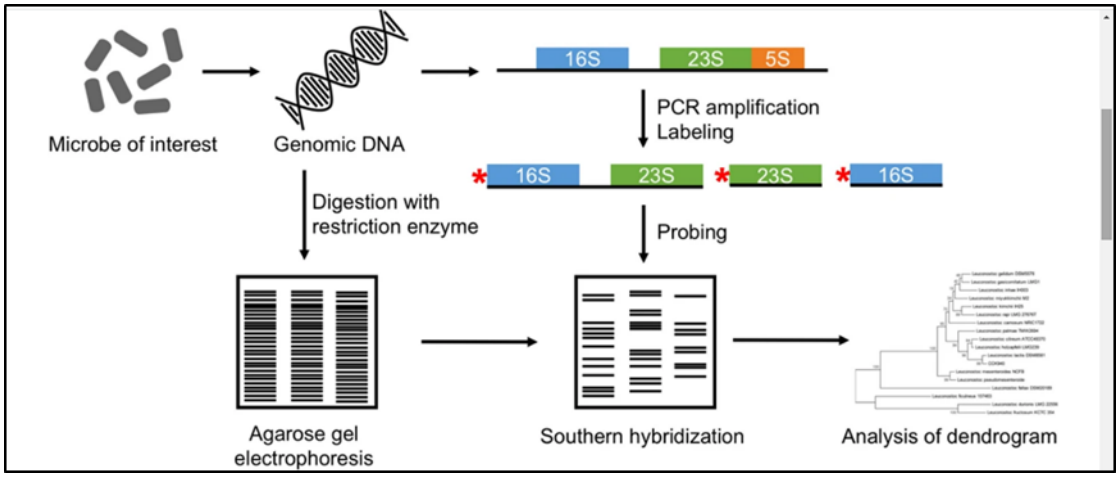

Figure 2: Molecular techniques for typing pathogens[9].

Among the many benefits of typing systems are their ability to help with patient care, enhance our knowledge of disease epidemiology, and make the investigation of foodborne outbreaks easier. Two standards are applied for

assessing typing techniques: Usability (type ability, discriminatory power, repeatability, and agreement between typing techniques) and efficacy (reagent and equipment availability and cost) [20]. Reproducibility is the ability of a typing approach; type ability is the proportion of isolates that can be categorized as belonging to a "type" by a typing process; flexibility, speed, and ease of execution and result interpretation [21]. Discriminatory power is the probability that isolates with similar or closely related profiles are clonal and belong to the same chain of transmission, and it is the ability of a sample to yield consistent findings when examined repeatedly [22]. The ability of a typing technique to type any disease with protocol tweaks is another way they defined its versatility. The degree to which two typing systems group isolates that are highly similar is used to evaluate their agreement [22, 23]. Among these elements, discriminatory power has been highlighted as a crucial element of typing techniques [23].

Pulsed field gel electrophoresis (PFGE)

Several of the most prevalent pathogens isolated from ducks and their environment have been identified using a variety of typing methods, including plasmid profile analysis, random amplified polymorphism deoxyribonucleic acid (RAPD), multilocus sequence typing (MLST), and deoxyribonucleic acid (DNA) sequencing. Additional techniques such as ribotyping, repeated extragenic palindromic (REP), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), enterobacterial repetitive intragenic consensus (ERIC), and others have not yet been documented in relation to duck isolates [25].

Detection methods using polymerase chain reaction (PCR)-based assays

The polymerase chain reaction (PCR), an in situ DNA replication method, allows for the exponential amplification of target DNA in the presence of synthetic oligonucleotide primers and a thermostable DNA polymerase [26]. The bacteria isolated from [27] have been identified or confirmed using a variety of different volumes or units of DNA templates (5–25

ng), Taq DNA polymerase (0.6–1.25 U), primers (0.11–10 μM), and tems perature cycles (45–95.8 °C and 30–40 cycles). Additional PCR reaction ingredients, such as buffer solutions, magnesium (Mg2+), and varying amounts of deoxyribonucleotide triphosphates (dNTPs), have been added to increase detection limits. To identify bacterial isolates, a PCR procedure can employ either a single primer (single PCR) or several primers (multiplex PCR) [28].Real-time PCR, nested PCR, reverse-transcription PCR, and several more forms are examples of further PCR kinds[29].

Mass Spectrometry-Based Methods

The main method for advancing research in microorganism identification has been to shorten the time required to detect a particular bacterium in routine diagnostics [30]. The use of biochemically based semiautomatic and automated systems was a major development in this area. Nowadays, a process needs to be executed for no more than 24 hours to have an impact, and in an emergency, this time frame needs to be much shorter [31]. Due to its ease of use, rapidity, affordability, and compatibility with a wide range of microorganisms, such as bacteria, fungus, and archaea, mass spectrometry (MS)-based methods have grown in popularity as a microbiological typing tool. New methods for data processing, analysis, and visualization have helped us better understand biological systems by allowing us to look at a variety of biomolecules, such as proteins, lipids, carbohydrates, and amino acids, as shown in figure 3 [32]. Several ionization and separation techniques can be applied with MS, including gas chromatography (GC), electromigration techniques, matrix-assisted laser desorption ionization time-of-flight mode (MALDI-TOF), and electrospray ionization (ESI) [33].

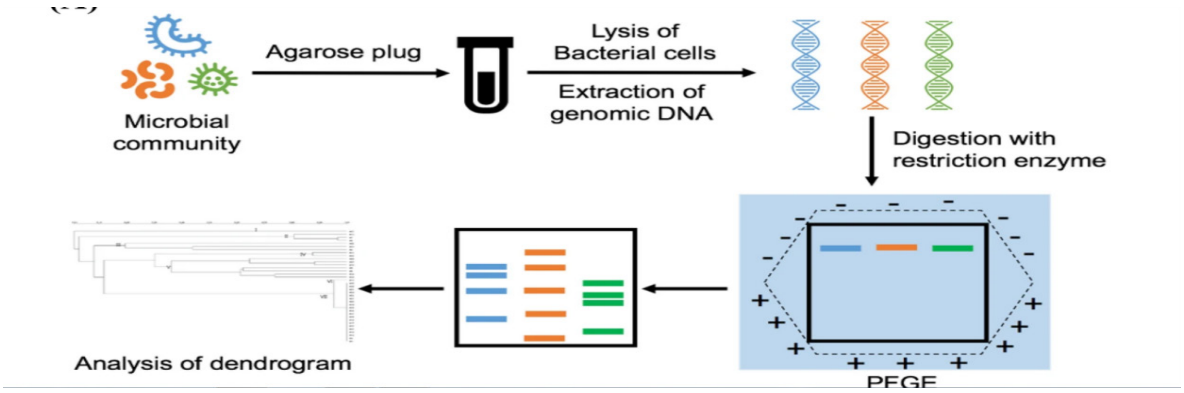

Figure 3: Mass Spectrometry-Based Methods[34].

Liquid Chromatography: High Performance Liquid Chromatography (HPLC)-Based Methods

The combination of liquid chromatography (LC) and mass spectrometry (GC-MS), in spite of initial skepticism, revolutionized the analytical determination of metabolome and, consequently, allowed for the identification of microorganisms by allowing the analysis of high molecular compounds that were either thermally labile or non-volatile in circumstances where GC-MS techniques were not suitable. The majority of metabolites are polar and ionizable, hence ESI-compatible LC partings are required. LC-MS doesn't require sample volatility and requires lower temperatures than GC-MS. This lowers costs and makes sample preparation simpler. Aside from its main application in clinical settings for the detection of microorganisms. Moreover, LC-MS is useful for determining Saccharomyces cerevisiae's complete metabolome coverage and identifying commercially accessible chemicals from Bacillus subtilis and Escherichia coli's in silico metabolome. Using an injector, samples are added to the solvent stream and then separated inside the column to which the stationary phase is chemically connected in LC-MS [35].

Single polymerase chain reaction

One set of primers is used in this polymerase chain reaction (PCR) to identify an organism by targeting a certain gene. When other organisms are present, the target organism can

be identified using the species-specific primer set [36]. Environmental, food, water, or clinical samples can all have bacteria swiftly identified or found using this kind of PCR, with or without pre-enrichment. PCR techniques, which directly detect foodborne pathogens in turbid samples or the environment, may result in false-negative results when used to detect DNA from dead cells. Using enrichment before PCR detection and fluorescent in situ hybridization (FISH) techniques have been suggested as solutions to this issue [37]. Additionally, single PCR can be used to confirm bacterial isolates that were directly picked from agar plates. Recently, the 16S rRNA genes of bacteria have been first amplified using PCR before being sequenced using universal or specialized primers to assist identify new or undiscovered bacterial species.

Multiplex polymerase chain reaction

As seen in figure 4, this variation of the polymerase chain reaction uses a single PCR mixture comprising multiple primers to detect, identify, and discriminate bacteria. Thus, a variety of target sizes that are unique to various DNA sequences can be amplified. Although primer design is crucial to the creation of multiplex PCR, it also lowers costs, decreases sample volume, and enables the quick identification of numerous bacterial species, strains, and other characteristics.

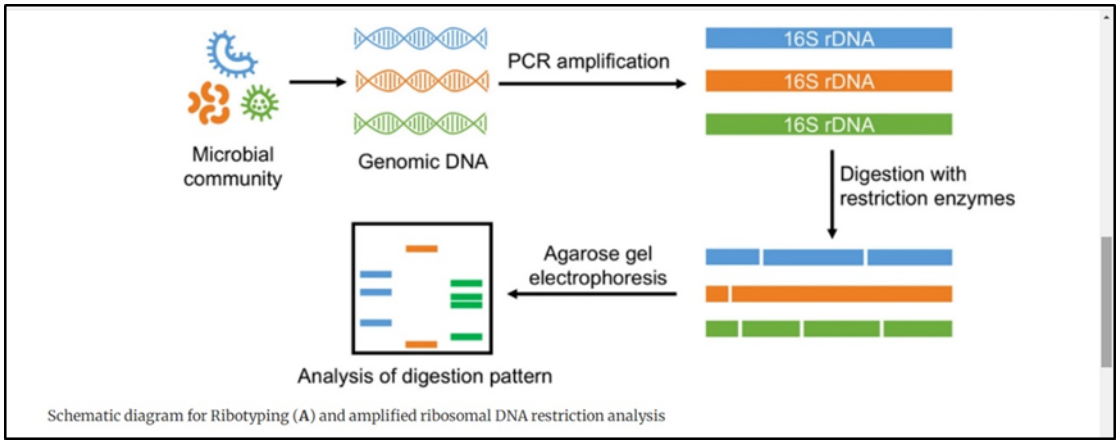

Figure 4: Multiplex polymerase chain reaction[38].

Even though numerous primers may interact throughout the amplification process, all primers must have temperatures that are close to annealing, and the amplicons' sizes must vary significantly. They identified samples and found that 31% of them had multiplex PCR positive results. Twenty percent of the samples had seven C. coli germs, twenty-one C. jejuni, and ten C. coli strains. The environmental elements were separated from the duck-isolated Campylobacter species using multiplex PCR [39].

Other polymerase chain reaction assays

These modified PCR techniques have never been applied before and are readily available for foodborne pathogens that are present in ducks. Among these are reverse-transcription PCR, nested PCR, and real-time PCR. "Real-time PCR" is a polymerase chain reaction technique that amplifies and assays the target DNA in the same step. One or two probes, a specific primer set, and/or fluorescent dye are used in real-time PCR to enhance the detection signals. As opposed to after the reaction is complete, real-time PCR detects the amplified DNA while it is still in progress. In contrast to traditional PCR, real-time PCR reduces detection time and may identify the absolute or relative amount of bacteria in different samples. Post-PCR product processing allows for high throughput while lowering the possibility of amplicon contamination from laboratory conditions. Real-time PCR, however, necessitates costly tools and materials. Reverse-transcription PCR uses RNA as the initial template rather than DNA. After utilizing reverse transcriptase to reverse-transcribe the target RNA into its DNA complement (cDNA), PCR is used to amplify it [40]. RNA is unstable and needs significant skill to manage and quantify for pathogen identification, yet reverse-transcription PCR can detect only living pathogen cells.

Pulsed field gel electrophoresis PFGE

To separate bigger DNA fragments, pulsed field gel electrophoresis (PFGE) uses an electrical current that alternates between three directions in a gel matrix rather than a single path of current flow as in conventional gel electrophoresis. to create a number of

different-sized DNA fragments with strain- or species-specific patterns (also called restriction fragment length polymorphisms, or RFLPs)[41]. assert that restriction endonucleases or restriction enzymes break down whole chromosomes in PFGE. Pulsed field gel electrophoresis is frequently regarded as the "gold standard" typing method for epidemiology and epidemic investigations involving foodborne pathogens. In spite of its limited supply, vulnerability to genetic instability, and minimum turnaround time of three to four days, PFGE provides excellent repeatability, discriminating power, and type ability. Some isolates from human, duck eggs, duck meat, duck liver pate, and/or dead embryos could not be differentiated using PFGE, according to PFGE study of human Salmonella isolates.

Multilocus sequence typing (MLST)

Multilocus sequence typing (MLST), a nucleotide-based, unambiguous, and portable approach for typing bacteria, uses the sequences of internal regions of (usually) seven house-keeping genes. For each house-keeping gene in MLST, different sequences within a bacterial species are identified as unique alleles, and the alleles at the ends of the seven loci determine the allelic profile or sequencing type for each isolate [42,41]. Internal segments of around 450–500 bp are utilized by each gene, and most bacteria possess a sufficient diversity of house-keeping genes to generate multiple alleles per locus, allowing the seven house-keeping loci to discriminate among billions of distinct allelic profiles [41]. By using DNA sequencing, which examines genes directly, multilocus sequence typing (MLST) directly assigns alleles at many housekeeping genes. Multiple locus enzyme electrophoresis (MLEE) is used to indirectly assign alleles at several housekeeping genes by assessing gene expression using electrophoretic mobility. For the majority of bacteria, the typing information offered by MLST is more accurate, discriminating, and readily available.

Random amplified polymorphism deoxyribonucleic acid (RAPD)

Target DNA segments are randomly amplified using a PCR-based method called random amplified polymorphic deoxyribonucleic acid (RAPD),

which uses arbitrary primers, typically 10-mer primers, and low-stringency PCR conditions. Through the amplification of one or more DNA sequences, this technique creates a range of finger printing patterns of different sizes that are specific to each strain. Among RAPD's advantages are its accessibility, quickness, ease of use, and relative price. Because primed sites impact amplification, annealing, and product length efficiency, producing both weak and strong amplicons, interpreting RAPD results can be difficult. RAPD has a type ability of roughly 80%, a middling discriminatory power, and low repeatability [42]. When 58 and 12 RAPD types are employed, the discriminating power of RAPD is enhanced.When the same researchers used RAPD to describe the ambient samples of three isolates from ducks, they likewise discovered a very high degree of heterogeneity among the isolates.

Plasmid profile analysis

One of the first molecular techniques for epidemiology research was plasmid profile analysis. This technique is used to isolate plasmid DNAs from bacteria, and agarose gel electrophoresis is used to separate the DNA. This method is easy to use and the results are easy to interpret because plasmids are mobile extrachromosomal components that bacteria can easily acquire or spontaneously lose. Nonetheless, isolates with different plasmid patterns may be epidemiologically related. Plasmids with a variety of spatial conformations, including linear, nicked, and supercoiled, can affect the technique's dependability by generating various migration velocities when they are subjected to agarose gel electrophoresis [43,42].

Deoxyribonucleic acid (DNA) sequencing techniques

The nucleotide bases' order consisting of adenine, cytosine, guanine, and thymine in a DNA molecule may be ascertained using technology known as DNA sequencing techniques. Many researchers now often and extensively employ DNA sequencing to identify, type, characterize, and/or classify novel or unknown pathogen isolates according to their taxonomic categorization. Traditionally, the target genes have been amplified using PCR prior

to DNA sequencing. The pathogen in question is identified, typed, and/or classified using 15S rRNA, a gene that is frequently amplified for sequencing. Sequencing offers outstanding repeatability, 100% type ability, and high selection power [44].

Mass spectrometry (MS)

For bacterial typing, there are three basic MS systems: targeted LC–MS/MS, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS, and liquid chromatography (LC)–MS/MS [45]. The target microorganisms in MALDI-TOF are treated using laser light. The matrix transmits the radiation. The generated ions go toward a detector at varying rates. By comparing the collected fingerprints, bacterial strains are recognized and described [46]. MALDI-TOF MS is useful for large-scale protein analysis. The LC-MS/MS fingerprinting method relies on peptide analysis. To produce peptides, proteins from the target microbe are separated using LC, ionized using electrospray ionization (ESI), condensed, and transformed into peptides by enzymes like as trypsin. Mass spectrometry (MS) is then used to analyze the samples. Following further fragmentation of the ionized peptides, the sequences obtained are used to identify the bacteria [47]. The target peptide is identified and measured in targeted LC-MS/MS using stable isotope-labeled standards. New amino acid sequence information, peptide mass, and post-translational modifications in proteins can all be gathered using the MS approach [48].

Conclusion

These methods are commonly used to identify, discriminate, type, and categorize diseases. A number of detection and typing techniques have been developed for efficient pathogen identification and outbreak research, therapeutic treatments, and/or epidemiological study. By combining two or more primers and/or protocols and refining those techniques, the detection or typing approach's discriminating strength will be greatly enhanced. The techniques discussed here improve our comprehension of the best detection or typing technique to use and the explanation for the choice. Duck meats, eggs, and products have also been con-

nected to multiple foodborne outbreaks and have been found to be a substantial source of foodborne illnesses. Surveillance research on the identification of foodborne pathogens in ducks and the samples linked to them is, nevertheless, scarce.

Conflicts of Interest

The authors declare no conflict of interest

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