Chapter

Foodborne Pathogens of Enterobacteriaceae, Their Detection and Control

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Abstract

Foodborne pathogens of *Enterobacteriaceae* including *Escherichia coli*, *Salmonella*, *Shigella, Yersinia*, etc., causes a great number of diseases and has a significant impact on human health. Here, we reviewed the prevalence, virulence, and antimicrobial susceptibility of *Enterobacteriaceae* belonging to 4 genera: *E. coli*, *Salmonella*, *Shigella,* and *Yersinia*. The routes of the pathogens' transmission in the food chain; the antimicrobial resistance, genetic diversity, and molecular epidemiology of the *Enterobacteriaceae* strains; novel technologies for detection of the bacterial communities (such as the molecular marker-based methods, Immunoaffinity based detection, etc.); and the controlling of the foodborne pathogens using chemical/natural compounds or physical methods (such as UV-C and pulsed-light treatment, etc.), is also summarized.

Keywords: foodborne pathogens, *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia*, detection and control

1. Introduction

Foodborne illness is the biggest health problem in the world. Due to unsanitary food processing methods, this situation is very serious in developing countries. Approximately 70% of diarrhea cases in developing countries are related to the consumption of contaminated food. An estimated 3.5 billion people have been infected, with 450 million people affected, most of them children [1]. There are many causes of foodborne illness, among which the most important are foodborne pathogens, including *E. coli (E. coli), Salmonella, Shigella,* and *Yersinia*. They can cause many diseases and have a significant impact on people's health and finance. *E. coli* is considered one of the main human foodborne pathogens. It is linked to a variety of acute and invasive human illnesses, and it is easy to spread across different ecosystems. *Salmonella* is a gram-negative, rod-shaped, flagellar facultative anaerobic bacteria belonging to the *Enterobacteriaceae* [2, 3]. *Salmonella* is divided into two categories: *Salmonella enterica* and *Salmonella bangri* [2, 3]. For *S. enterica*, more than 2600 sera have been isolated and described, many of which are pathogenic to humans and animals [2–4]. And *Shigella* is

the third most common foodborne bacterial pathogen, according to the CDC. *Yersinia* also causes a range of foodborne illnesses with distinct characteristics in humans, ranging from asymptomatic carriers to hemorrhagic colitis and fatal typhoid fever.

In recent years, the detection of foodborne pathogens has developed rapidly. Many techniques such as PCR, nanotechnology, nucleic acid hybridization are widely used [5]. There are also many control methods for foodborne pathogens. In the present paper, we summarized the transmission, antimicrobial resistance, genetic diversity, and molecular epidemiology of the *Enterobacteriaceae* strains, and also novel technologies for detection and the controlling of the foodborne pathogens.

2. Transmission of pathogens in the food chain

Foodborne pathogens are transmitted through the food chain in many ways, such as insect transmission, fecal-oral transmission, food and water transmission, animals transmission, and so on. Some pathogens, such as *E. coli* or *Salmonella enteritidis* can be passed from animal hosts to people, but *Salmonella typhi* has no animal host and is highly harmful to humans.

Insects are considered to be carriers of foodborne pathogens. Their association with degradable substances and their endogenous and coexistence (with humans) are behavioral patterns that are particularly important for the ability of flies, cockroaches, and ants to transmit foodborne diseases. A study conducted in an ant colony in a Brazilian hospital found that several bacteria, including *E. coli* and *Salmonella,* were related to ants. Another study found cockroaches and several cockroach-related bacteria in several buildings in Spain, including *Salmonella* (hospitals), *E. coli* (hospitals, restaurants, companies, and grocery stores), and *Enterobacteria* (shops and food industry factories). In addition, an assessment of cockroaches gathered from hospitals, houses, grocery shops, and restaurants in the South Canary region of southwest India revealed that more than 4% of cockroaches tested positive for several *Salmonella* strains [6]. But existing understanding about the health dangers posed by flies and food is inadequate currently. Flies are at risk of transmitting foodborne pathogens because they have a bowel movement every 4 to 5 minutes during the day [7]. In general, houseflies can promote the spread of pathogens in four different ways: through body hair and surface, through the glandular hair on the feet, through the regurgitant rumen itus, and through the digestive tract [7]. Recently, some researchers have claimed that adult houseflies can spread their eggs and bacteria to food, so that these bacteria could be retransmitted to the first generation of adult flies [8]. Alexandre Lamas studied the bacterial populations of the Australian bush flies in three diverse places: cattle farms parking lots, metropolitan shopping malls, and a barbecue spot [9]. In the agricultural setting, the number of bacterial per fly was highest, whereas, it was lowest in the city [9]. Furthermore, multi-drug resistance was found in 94% of *Salmonella* isolates and 87% of *Shigella* isolates, suggesting that these flies might operate as food carriers for antimicrobial resistance transmission [10].

Water is well-known for its importance in the production, processing, and preparation of food. It is also a medium for the transmission of pathogens during food manufacturing [11]. The quantity of contamination in irrigation water determines pathogen survival, and the higher the degree of contamination, the better. They may survive outside of their human hosts for months to years before being transmitted to humans through water [12]. *E. coli* and *Salmonella* can leach through water or soil to the plant surface [13] and even *E. coli* O157:H7 can be absorbed by lettuce leaves. In

addition, *E. coli* from livestock manure may persist for at least 5–6 months on soil or grassland, giving pathogens an excellent chance to infect other sources. In another research, *E. coli* O157:H7 could not only attach to the outer surface of radish seeds but also invade the inner tissues and stomata [14].

Many microorganisms that cause foodborne diseases can be transferred directly from animals to people. Mammals such as pigs and cattle are thought to host many foodborne pathogens, which are transmitted to humans either through direct contact with humans or by being processed into food for human consumption. *E. coli* is a typical element of the gut flora of humans and animals, and it is commonly found in poultry and wild animals. As a result, *E. coli* is one of the most likely infections to spread through food. The Shiga toxin-producing *E. coli* (STEC) strain is a serious foodborne pathogen that may be transmitted by consuming pig chow. From 334 pork samples collected from a South Korean slaughterhouse and retail market, 131 strains of *E. coli* were identified [15]. Simultaneously, *E. coli* was discovered in chickens. According to the Daily Mail, a food safety survey conducted in a supermarket in the UK found that 23 out of 99 chicken samples were infected with *E. coli*.

There are many key points where pathogens can infiltrate and jeopardize human food safety, such as the food itself, the surfaces of food preparation tools or food processors [16]. At each food processing or preparation facility location, a variety of factors may impact contamination and transmission. For example, microbial pathogens can be brought into the kitchen environment through commercial foods, cross-contamination of foods via kitchen equipment, or be reused due to inadequate cooking or storage [17, 18].

3. Antimicrobial resistance, genetic diversity and molecular epidemiology of the *Enterobacteriaceae* foodborne pathogens

3.1 *E. coli*

E. coli is one of the most common food-borne pathogens and may spread a variety of diseases through the food chain in different ecosystems. There are pathogenic and non-pathogenic strains of *E. coli*. Of these, pathogenic strains can cause a variety of intestinal diseases.

The original *E. coli* was sensitive to almost all antibacterial drugs [19], but multi-resistance of *E. coli* is now increasingly common. The resistance mechanism of *E. coli* includes the acquisition of encoding ultra-broad-spectrum β-lactamase (resistance to broad-spectrum cephalosporin), carbapenase (resistance to carbapenems), et al. The most common mechanism for the development of resistance in *E. coli* is the production of β-lactamase hydrolyzing β-lactamase antibiotics [20]. Ultra-broad-spectrum β-lactamases (ESBLs) are produced by mutations in β-lactamases and could be encoded by genes that effectively hydrolyze third and fourth-generation cephalosporins as well as monoclonal antibodies. However, β-lactamase inhibitors like clavulanate and tarmacadam can stop them [21]. Genes such as *aadA1*, *aadA2*, *mcr-1*, *crf*, and $bla_{\text{TEM-1}}$ are related to the drug resistance in *E. coli* (**Table 1**) [19].

The genetic diversity of *E. coli* is reflected not only at the individual level but also at the molecular level. Ramadan et al. [22] used Multilocus sequence typing (MLST) to explore the genetic diversity in *E. coli*, as indicated by the various distribution of *E. coli* lineages among different sources. It was found that a wide range of STs was found in chicken, human and beef isolates. And the most common STs isolated from chicken

Strain	Resistant phenotype	Resistance genes
Escherichia coli	Streptomycin/spectinomycin resistance	*aadA1, aadA2*
	Polymyxins resistance	*mcr-1*
	Fluorinated and nonfluorinated phenicols resistance	*crf*
	β-lactams resistance	$bla_{\text{TEM-1}}$
Salmonella	Beta-lactam resistance	*ampE*
	Macrolide resistance	*macB, macA*
	Aminoglycoside resistance	*aac6-I, acrD, acrD*
	Amidoalcohol (chloramphenicol) resistance	*mdfA, rarD*
	Amido alcohol (chloramphenicol) resistance	*gyrA, gyrB, parC, parE*、
	Other	*nfsA*
Shigella	Cephalosporins and Fluoroquinolones resistance	$bla_{\text{TEM-1}}$, $bla_{\text{CTX-M}}$, $bla_{\text{OXA-1}}$, $bla_{\text{SHV-12}}$
Yersinia	Tetracycline and minocycline resistance	*tetD, tetA*
	Ticarcillin and amoxicilin resistance	$bla_{\text{TEM-1B}}$
	Trimethoprim resistance	*dfrA14, drfA1*
	Sulfonamide resistance	*sul2*
	Chloramphenicol resistance	*catA2*

Table 1.
Resistance phenotype and resistance genes of the strain.

isolates differed significantly from human and beef isolates, which was consistent with previous research.

The genetic diversity of *E. coli* causes changes at the molecular level. Findlay et al. [23] revealed the cause of Urinary Tract Infection (UTI) was the direct sharing of *E. coli* between local farms and the local population. They found that the $bla_{\text{ctX-M}}$ or $bla_{\text{CMY 2}}$ plasmid isolated from the farm *E. coli* isolates was almost identical to one of the three plasmids isolated from the urine of local people, and these three plasmids are found in almost all humans and animals on earth.

3.2 *Salmonella*

Salmonella is gram-negative bacteria. Based on the clinical presentation of the patient with their *Salmonella* infection, we usually identify them as typhoidal *Salmonella* and non-typhoidal *Salmonella* (NTS).

Salmonella has multidrug resistance because it is resistant to a variety of first-line antibiotics such as ampicillin, chloramphenicol and methicillin/sulfamethoxazole. Lu et al. [24] classified gene products by direct homology through functional annotation of the COG database. COG functional annotation was performed on 13 drug resistance genes of *Salmonella*, such as beta-lactam resistance and macrolide resistance. Also, they found that genes like *ampE*, *macB,* and *macA* are drug resistance genes in *Salmonella* (**Table 1**).

Salmonella is an important foodborne pathogen and its genetic diversity is of great significance for the prevention and control of the disease. Methods commonly used in genetic diversity research include serotyping and pulse electrophoresis typing, which are time-consuming and have poor traceability [25]. Zhang et al. [26] conducted multilocus sequence typing of 311 *salmonella* strains, and MLST typing results were divided into 26 ST types.

Molecular epidemiology has been used to document vector to human transmission and to investigate outbreaks of *Salmonellosis* in hospitals. *Salmonella* typing is

epidemiologically important because it provides correlations between cases, foci, and between cases and food or other vectors, animals, regions, and periods. Riley et al. [27] studied an outbreak of enteritis in the northeastern United States in late 1981 caused by *Salmonella* Newport through commercially available raw beef. The outbreak strain is of the same serotype and is sensitive to most antibiotics. Plasmid analysis revealed two plasmids (3.7 and 3.4Md) of strains isolated from raw beef and patients with identical restriction profiles. Meanwhile, 45 percent of intestinal strains from New Jersey and Pennsylvania had the same plasmid profile. Through follow-up of patients, it was also found to be related to raw beef. Without molecular biological analysis, these cases would not be considered part of the outbreak.

3.3 *Shigella*

Shigella is the most common cause of diarrhoeal disease in humans worldwide, and its drug resistance is already a major public health burden. *Shigella* resistance tests have been reported in some areas of Shanxi Province, China. Of 474 strains, only 2 strains (0.5%) were sensitive to all 21 antimicrobial agents [28], 14 strains (3.0%) were co-resistant to the third-generation cephalosporins and fluoroquinolones. Wang et al. [29] found that $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M}}$, $bla_{\text{OXA-1}}$, $bla_{\text{SHV-12}}$ are Cephalosporins and Fluoroquinolones resistance genes (**Table 1**).

Shigella is a common cause of diarrhea and death, particularly in children under the age of five. It is critical to investigate the genetic diversity of *Shigella.* Ei-Gendy et al. [28] isolated a total of 70 strains of *Shigella* from children younger than 5 years of age in Egypt, including 40 *Shigella dysenteriae* and 30 *Shigella boydii*. Among them, serotypes 7(30%), 2(28%), and 3(23%) accounted for the majority of *S. dysenteriae* isolates and 50% of *S. boydii* isolates were serotype 2.

Shigella is a common foodborne pathogen, and its molecular epidemiology is of great significance for the prevention and control of *Shigella*. Chen et al. [30] collected and typed 161 *Shigella* isolates obtained from Renai and adjacent townships from 1997 to 2000 using serological and PFGE techniques. The finding showed that the strain giving rise to foodborne illnesses remained the most common cause of *Shigellosis* during 4 years. Chen found that the percentage of these outbreak strain isolates among *Shigella flexneri* serotype 2a isolates recovered each year dropped. During this time, although several closely similar strains resembling outbreak strains have also emerged, they are far less transmissible and pathogenic than outbreak strains.

3.4 *Yersinia*

Yersinia pseudotuberculosis is the enteropathogen that causes gastrointestinal illnesses in people. Antibiotics that target gram-negative bacteria are typically effective against this species. However, the resistance to *Yersinia* is becoming more widespread. Three multi-drug-resistant (MDR) strains of *Y. pseudotuberculosis* were recovered from the environment in Russia and patients in France [31]. The resistance genes in *Yersinia* include *tetD*, *tetA* $bla_{\text{TEM-1B}}$, *dfrA14*, *drfA1, sul2* and *catA2,* etc., which are related to the tetracycline, minocycline, ticarcillin, amoxicillin and Trimethoprim resistance (**Table 1**).

The genetic diversity of *Yersinia pestis* is still mainly studied by typing. There have been many studies on the genetic diversity of *Yersinia*. Xu et al. [32] screened 102 *Y. pestis* isolates from Qinghai and 16 genotypes were identified by CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat).

Yersinia is considered to be the pathogen of human intestinal diseases, and its molecular epidemiology is the focus of current research. The presence of a 70-kb virulence plasmid was required for the pathogenicity of *Y. pseudotuberculosis*, which was necessary for virulence. According to Fukushima [33], *Y. pseudotuberculosis* could produce a novel super antigenic toxin by chromosomal encoding, known as YPMa, YPMb or YPMc. It could also produce a pathogenicity island termed as HPI (high-pathogenicity island) or R-HPI (a right-hand part of the HPI with truncation in its left-hand part). All of these can contribute to its pathogenicity.

4. Novel technologies for detecting the pathogens

In recent years, the rapid detection of foodborne pathogens has developed rapidly. Molecular biology, nucleic acid hybridization, and other technologies have been highly valued and widely used in laboratory or factory production.

4.1 Nanoparticles in pathogen detection

Substances are manipulated at atomic, molecular, and supramolecular scales through nanotechnology ("nanotech"). Advances in manipulating these nanomaterials allow specific or non-specific binding of different biomolecules. The large specific surface area allows more biomolecules to be immobilized, thereby increasing the number of reaction sites that can be used to interact with the target species, which is one of the main advantages of biosensing using nanomaterials. In addition, nanomaterials have been widely used in 'label-free 'detection due to their excellent electronic and optical properties, and biosensors with enhanced sensitivity and improved response time have been developed [34].

Metal nanoparticles, especially gold and silver (5–110 nm in size) exhibit excellent properties, such as signal amplification, have potential application in various areas such as variable optical and electrical determinations. Gold nanoparticles (AuNPs) change the color aggregation from blue to red with the ability to scatter light, showing excellent chemical stability and electrical conductivity. AuNPs were used to detect Salmonella and *E. coli* O157: H7 organisms at 98.9 CFU/mL and 1–10 CFU/mL, respectively. Magnetic nanoparticles such as iron, nickel, and cobalt (size range of 1–100 nm) with electrical conductivity properties for utilization as a detection mean. Quantum dots (2–10 nm) were detected in *E. coli* O157:H7 10^3 CFU/mL through a semiconductor material consisting of semiconductor fluorescent nanonuclei (typically cadmium mixed with selenium or tellurium). Carbon nanotubes are formed by anisotropies of carbon-containing cylindrical graphene sheets. Multiwalled nanotubes (MWNTs, 2–100 nm) with photoluminescence and excellent electrical properties are composed of many concentrated single-walled nanotubes (SWNTs, 0.4–3 nm). A half conductance apparatus was used to monitor *E. coli* o157:h7 at 1 cell/mL restriction [35]. Thiol modified oligonucleotides covalently bound-based methods to gold nanoparticles are used as probes in various rapid detection ways. Due to its cost, functional chemistry is not so widespread. This method employs nonfunctional AuNPs to detect dsDNA and ssDNA [36].

4.2 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) plays an important role in molecular methods in detecting foodborne pathogens. As early as 30 years ago, PCR, which was invented

for the detection of single bacterial pathogens present in food by identifying specific target DNA sequences [37]. PCR works by amplifying specific target DNA sequences in a three-step cycle [38]. Firstly, single-stranded DNA was obtained from target double-stranded DNA by high-temperature denaturation. Then, deoxyribonucleic acid was lead on the backbone of DNA by adding specific primers and heat-resistant DNA polymerase in the polymerization process of DNA, so a new double-stranded DNA was synthesized. The amplified products of PCR were stained by ethidium bromide on electrophoretic gels [39]. PCR such as loop-mediated isothermal amplification (LAMP), multiplex PCR (mPCR) and RT-PCR, etc. is used to detect foodborne pathogens, including *E. coli* 157: H7, *S. aureus*, *Campylobacter jejuni*, *Salmonella* and *Shigella* [40]. Because of the advantage of high specificity, efficiency and easy operation, LAMP and mPCR are used quite frequently [41–47].

4.2.1 Loop-mediated isothermal amplification (LAMP)

Now, molecular diagnostic technologies based on nucleic acid amplification have been applied extensively in the detection regions, such as Loop-mediated isothermal amplification (LAMP) developed by Notomi [41–45]. Various confirmatory studies have been used to evaluate the feasibility of LAMP technology for microbial identification and diagnosis [42]. LAMP kits for detecting *Salmonella*, *E. coli,* and *Listeria monocytogenes* have been commercialized in the initial phase of development.

The loop-mediated isothermal amplification method offers several advantages: high sensitivity (2–5 orders of magnitude higher than conventional PCR methods); short reaction time (30–60 min can complete the reaction); no special instrumentation is required for clinical use; the operation is simple (whether DNA or RNA, the detection step is to mix the reaction liquid, enzyme, and template in a reaction tube, place in a water bath pot or incubator at 63°C for about 30 to 60 minutes, observe the results by the naked eye) [42–44]. There are also some disadvantages of the loop-mediated isothermal amplification method: high sensitivity, easy to form aerosol pollution once the lid is opened, combined with the current majority of domestic laboratories can not strictly partition, false-positive problems are relatively severe, so we strongly recommend using real-time turbidimeter during the development of the kit, do not open the reaction tube after the reaction. Primer design is more demanding, and some disease genes may not be amenable to the use of loop-mediated isothermal amplification methods [41–43].

4.2.2 Multiplex PCR (mPCR)

mPCR technology is more new-fashioned, which can simultaneously detect more pathogens than before, up to four or more pathogens [45–47]. Chen et al. simultaneously detected *S. enteritidis*, *S. flexneri*, and *E. coli* 157:H7 using five pairs of primers for invading protein (invA), 16S rDNA, invading plasmid antigen H (IPAH), Listeria hemolysin o (HlyA), and immunoglobulin (EAEA) genes [45]. The mPCR detection limit of mixed genomic DNA was 7.58×10^4 copies. Further improvements to mPCR by Gilmartin and O'Kennedy [46] promoted the process of a new GeXP PCR detection of four foodborne bacterial pathogens: *Salmonella*, *Yersinia*, *E. coli* 157:H7, and *Shigella*. The genome lab gene expression profiler (GeXP) gene analysis system can detect multiple pathogens in a single reaction with high throughput. Chimeric primers, universal primers and capillary electrophoresis with PCR products rather than agarose gel electrophoresis were involved in GeXP multiplex PCR amplification.

Synthesis of amplicons with universal tags by chimeric primers containing gene-specific sequences with universal tags at the 5′ end. Then, a universal primer will drive the remaining PCR reaction, which contains the same sequence of universal tags used by chimeric primers. Forward universal primer was covalently labeled with fluorescent dyes at the 5′ end for detection during capillary electrophoresis [47]. This method has higher sensitivity and is suitable for high-throughput analysis. Detection limits of Grignard PCR for *Salmonella*, *Yersinia*, *E. coli* 157:H7, and *Shigella*.

The characteristics of multiplex PCR are high efficiency, systematic and economic simplicity. High efficiency: a variety of pathogenic microorganisms in the same PCR reaction tube can be detected simultaneously, or multiple pathogens can be detected with multiple types of genes of interest. Systematic: mPCR is suitable for the detection of grouped pathogens. Economic simplicity: this will greatly economical of time, reagent and cost, and provide more accurate diagnostic information for clinical practice, because multiple pathogens are detected synchronously.

4.3 Nucleic acid hybridization technologies in pathogen detection

A general method of fluorescence in situ hybridization (FISH) using oligonucleotide probes of rRNA for nonmolecular technology. Probe lengths of 15 to 25 nucleotides labeled at the 5′ end were used for FISH. The specifically labeled cells were detected by an apparent fluorescence microscope. Rapid culture and independent detection of *Salmonella* were successfully performed using FISH combined with flow cytometry [48–50].

Line probe analysis (LIPA) is composed of oligonucleotide probes with specific oligonucleotides and nitrocellulose bands, which are connected by parallel lines along with the bands and discrete lines. The color change of hybridization results can be detected by vision. Innogenetics has produced several line probes for bacterial detection, such as *Escherichia coil*. The test results are consistent with those of antibiotics. Recently, 599 strains of *Escherichia coil* were improved and evaluated, and the sensitivity and specificity of the method were proved [51, 52].

Nielson et al. found a DNA analog called peptide nucleic acid (PNA) for detecting foodborne pathogens. This probe is more stable because PNA is not charged. In addition, PNA has a greater advantage in that it is relatively hydrophobic and easier to enter non-bacterial cells. PNA has higher specificity than DNA oligomer because the TM of the PNA probe is higher than that of its DNA probe. Theoretically, in addition to PNA and FISH, PNA can also replace DNA oligonucleotides to improve analytical performance [53, 54].

5. Controlling of the *Enterobacteriaceae* foodborne pathogens

At present, food pollution and poisoning caused by foodborne pathogens have attracted extensive attention. In the food industry, technologies such as irradiation, pulsed light treatment, microwave sterilization, slightly acid electrolytic water and fumaric acid treatment, algae extract treatment, *Bacillus* antimicrobial peptide treatment is usually used to control foodborne pathogens.

5.1 Irradiation

In more and more countries, ionizing radiation processing is the most common method of food purification, and in the short run, a growing number of

radiation-purified foods are presumed to be approved for production. It is a secure, smart, environmentally clean, and energy-efficient process, and it is especially valuable as a purification process for the final product. Due to the availability of irradiation in handling packaged foods, irradiation is regarded by most food safety officers and scientists as an effective critical control point in the processing of meat and poultry hazard analysis and critical control point (HACCP) system.

The high-energy photons or free radicals generated by ionizing radiation can break the DNA chain and generate reactive oxygen free radicals, and can also cause protein denaturation and cell membrane damage. Hesham reported that an irradiation dose of 4 kGy can effectively control the bacterial pathogens in meat by destroying *Salmonella*, significantly reducing *E. coli* [55]. They found the number of *Enterococcus faecalis* and *Enterobacteriaceae* was reduced by more than 1.8 log units and 5 log units, respectively, when treated with 4 kGy of irradiation, and no *Salmonella* was detected in the meat samples [55], which could prolong the cold storage shelf life without any significant impact on the sensory quality of meat.

5.2 Pulsed-light treatment

Nucleic acids are easily destroyed by pulsed light (PL). Pyrimidine bases form dimers the DNA of bacteria, viruses, and other pathogens through photochemical intervention and block DNA replication, and if there is not enough repair mechanism, it will ultimately lead to the death of microorganisms [56]. Xu et al. [57] investigated the inactivation effect of PL on *Salmonella* and *E. coli* in fresh raspberries. It was found that the pulsed light treatment of 28.2 J/cm^2 for 30 s could reduce them by 4.5 and 3.9 lgCFU/g, respectively. However, considering the adverse effects on raspberry color and ground, the recommended dosage of PL is 5.0 J/cm^2. Rajkovic et al. [58] found that PL can kill *E. coli* in meat products, but the sterilization effect becomes worse with the extension of pulse interval. Ozer et al. [59] used pulsed ultraviolet light to treat *E. coli* on the surface of seafood. The results showed that the irradiation distance was 5 cm and the treatment time was 30 s, reducing 0.86 lgCFU/g; When the irradiation distance was 8 cm and treated for 60 s, 1.09 lgCFU/g was reduced [60]. This shows that under the condition of a long irradiation distance, the sterilization rate can be improved by prolonging the treatment time, but the surface temperature of the sample increases significantly with the extension of the treatment time.

However, in the sterilization process of fruits and vegetables, if the PL intensity is too high, due to the effect of PL on protein structure, it will improve the activity of polyphenol oxidase (PPO) to a certain extent and cause browning [61]. In the process of meat sterilization, PL has a poor sterilization effect on uneven surfaces [62], and the sterilization only stays on the surface.

5.3 Microwave sterilization

Microwave sterilization is that microwave constantly changes the direction of electromagnetic field, changes the ion and electron density around microbial cell membrane, destroy the permeability of cell membrane, lead to protein degeneration in cells, destroy cell metabolism, and microbial death [63].

De La Vega-Miranda observed that under 950 W water-assisted microwave treatment, *Salmonella typhimurium* on pepper and coriander foliage decreased by 5.12 log and 4.45 log after being treated at 63°C for 25 s and 10 s, respectively, and finally reached 3× 10^8 CFU/g [64]. The sterilization effect of microwave sterilization under

the same conditions (power and temperature) varies due to different objects. The high-voltage pulsed electric field sterilization technology to treat liquid food shows that it can effectively eliminate *E. coli*, *Salmonella*, *E. coli* O157:H7, et al. reaching the level of pasteurization. The cold source plasma has a significant sterilization effect on *Salmonella* and *B. subtilis* in pepper, and the cavitation jet technology also has a significant sterilization effect on *E. coli* and *K. pneumonia*.

5.4 Slightly acidic electrolyzed water and fumaric acid

Slightly acidic electrolyzed water (SAcEW) is a type of EW and promising sanitizer for food products. Effects of SAcEW combination with other chemical disinfectants on the ideal bactericidal efficacy of foods. Organic acids can inactivate foodborne pathogens, and show stronger bactericidal effects in organic acids used in meat antibacterial agents.

Ahmad found that a single treatment and combined treatment of fresh meat with micro-electrolyzed water or fumaric acid can reduce *E. coli* and *S. Typhimurium* in meat [65]. The efficacy of *Salmonella* and study the quality guarantee period and organoleptic quality of the meat during conserve at 5°C and 12°C. The inoculated meat samples were soaked for 5 min in each treatment, with or without gentle heating. Compared with other treatments, SAcEW +0.6% FA 40°C 5 min had a stronger bactericidal effect on fresh meat and significantly lessened *E. coli* and *Salmonella* respectively reduced 2.34 and 2.88 logCFU/g. This combined treatment made the natural bacteria (TBC) lag time of meat stored at 5°C longer. Compared with the untreated meat, the treatment of combined extended the quality guarantee period of meat by 8 days and 6–7 days when respectively stored at 5°C and 12°C. The study has shown that the combined treatment of SAcEW +0.6% FA has the potential as a new way to improve the microbial security and quality of fresh meat [65].

5.5 Other technologies for controlling the *Enterobacteriaceae* foodborne pathogens

Recent studies have shown that some biological macromolecules can also be used to control foodborne pathogens of *Enterobacteriaceae*, such as *Bacillus* antimicrobial peptides and algae extracts. Chen et al. [66] found that *Bacillus* antimicrobial peptides can be applied to the control of food-borne pathogens in seafood, but there are still many key issues that need to be further studied, especially the effect of *Bacillus* antimicrobial peptides and their main active ingredients on common foodborne pathogens in seafood antibacterial effect; the relationship between the dose of *Bacillus* antimicrobial peptides and the survival and production of toxins in complex food environments; key issues such as the mode of action of bacillus antimicrobial peptides at the cellular and molecular levels on pathogenic bacteria.

Algae is a multifaceted natural substrate that contains a wide range of bioactive compounds. Antibacterial, analgesic, and antioxidant properties of phytosterols isolated from different algae have been demonstrated. Brown algae fucoidans and green algea ulvans both have antibacterial capacities. The most potent chemicals against *E. coli* are carvacrol and thymol [67]. Algae and alga extracts have also been reported as having the ability to enhance food quality when used as feedstock, as well as assisting in the management of microbial contamination in fish farms [68]. Nowadays, algae-rich foods have emerged, food safety, functional food, and non-traditional diet are worthy of attention [69–71]. Algae are a kind of available resource for new bioactive molecules. Therefore, Algae have great potential for application in

Foodborne pathogens	Treatments	Results/Activity	Reference
Escherichia coli	4 kGy dose of radiation	Reduce >5 log units	[66]
	Slightly acidic electrolyzed water and fumaric acid	Reduce 2.34 log CFU/g	[65]
	Brown Algae Methanol Extract	Sensitive	[67]
	Phage cocktail	Spraying the phage mixture resulted in a 4.5 log CFU reduction after 2 h	[72]
	Phage DT1 and DT6	100% reduction in CFU/ml within an hour	[73]
	Lactobacillus acidophilus A4	Anti-adhesive/ Antibiofilm	[74]
	L. acidophilus La-5	Anti-quorum sensing	[75]
	Carvacrol, thymol, trans-cinnamaldehyde	Antibiofilm Reduced expression of virulence genes	[76]
	Surface-layer protein extract	Anti-adhesive	[77]
	Resveratrol	Antibiofilm	[78]
	Microwave radiation	Elimination of the superficial	[79]
Salmonella	4 kGy dose of radiation	Not detected	[55]
	Water-assisted microwave heating	5.12 log reduction	[64]
	slightly acidic electrolyzed water and fumaric acid	Reduce 2.88 log CFU/g	[65]
	Brown Algae Methanol Extract	Sensitive	[67]
	Phage cocktail	Using MOI 5 leads to about 4.4 log reductions	[60]
	Phage F01-E2	The CFU of turkey cooked meat and chocolate milk was reduced by 5 log, and the CFU of hot dog was reduced by 3 log	[80]
	Phage cocktail PC1	More than 99% reduction in CFU at MOI 10 or above	[81]
	Bifidobacterium lactis Bb12/*Lactobacillus rhamnosus* LGG	Anti-adhesive	[82]
	E. coli Nissle	Anti-invasive	[83]
	T315 compound	Antibiofilm	[84]
	Methylthioadenosine	Reduced motility Anti-invasive	[85]
	Microwave radiation	Theoretical complete inactivation	[86]
Shigella	Phage cocktail	About 4 log reduction	[87]

Foodborne pathogens	Treatments	Results/Activity	Reference
	Containing six novel *Shigella* specific phages	About 99% decrease	[88]
Yersinia	*Yersinia enterocolitica* phages	Decreasing by 1–3 logs on food samples	[89]
	Bacteriophage specific to serotype O1 *Yersinia ruckeri* (φNC10)	Polysaccharide Depolymerase activity capable of degrading *Y. ruckeri* O1-LPS	[90]

Table 2.
Controlling of the Enterobacteriaceae *foodborne pathogens.*

controlling foodborne pathogens [70]. Algae may be used as fresh food preservatives, active packaging, or antifouling and biofilm inhibitors based on the above advantages. To maximize the advantages of algae and algae compounds in food safety, attractive sensory characteristics should be pursued shortly (**Table 2**).

6. Conclusion

A plenty number of studies have been confirmed that foodborne pathogens of *Enterobacteriaceae* and their resistance genes can not only remain in animal husbandry and related environment but also transmitted to human beings through the food chain or other ways, causing a major threat to public health. Also, it has been highlighted how much important are novel technologies for the detection of foodborne pathogens (such as molecular marker-based methods, immunoaffinity-based detection, etc.). In addition, chemical/natural compounds or physical methods (such as UV-C and pulsed-light treatment, etc.) play key roles in the prevention of foodborne pathogen growth and diffusion. As one of the causes of foodborne diseases of global concern, foodborne pathogens should be controlled by countries and organizations around the world through the establishment of policies and food safety management systems.

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