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Meat Technology — Special Issue 66/3

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 615.33.015.8:579.842.1/.2

ID: 179474441

https://doi.org/10.18485/meattech.2025.66.3.86

Review Paper

Enterobacteriaceae in the food chain – modern strategies for detection and control

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A R T I C L E I N F O

Keywords:
Foodborne illness
Enterobacteriaceae
Antibiotic resistance
Pathogen control
PCR
Biosafety

A B S T R A C T

Members of the *Enterobacteriaceae* family, such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Yersinia* spp., are significant contributors to foodborne diseases worldwide. This paper presents a comprehensive overview of their transmission through the food chain, resistance profiles, and modern strategies for detection and control. Particular emphasis is placed on advanced diagnostic approaches, including PCR-based techniques, isothermal amplification, and nanotechnology-driven methods. Control interventions ranging from physical decontamination (e.g., irradiation, pulsed light) to the use of natural antimicrobials such as bacteriophages and algal compounds are also discussed. The review underlines the necessity of integrated approaches in reducing the prevalence of foodborne pathogens and improving public health outcomes.

1. Introduction

Foodborne diseases are a global concern, with *Enterobacteriaceae* often implicated as primary agents. Due to poor hygienic practices and insufficient control measures, particularly in low-resource environments, the transmission of these bacteria through contaminated food continues to pose significant health risks. This work aims to consolidate current knowledge about transmission routes, genetic adaptability, resistance trends, and available tools for detection and control (*Eng et al.*, 2015).

2. Routes of transmission of foodborne pathogens in the food chain

The dissemination of foodborne pathogens within the food chain occurs through various pathways, including insect vectors, faecal-oral routes, contaminated food and water, and direct animal-to-human transmission. Some pathogens, such as *Escherichia coli* and *Salmonella* Enteritidis, are zoonotic and can be transmitted from animal reservoirs to humans. In contrast, *Salmonella* Typhi lacks an animal host and is particularly pathogenic to humans (*Mezal et al.*, 2014).

Insects are significant vectors of foodborne pathogens due to their habits and ecological niches. Their attraction to decaying organic matter, frequent

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Paper received August 15th 2025. Paper accepted August 19th 2025.
The paper was presented at the 63rd International Meat Industry Conference "Food for Thought: Innovations in Food and Nutrition" – Zlatibor, October 05th-08th 2025.
Published by Institute of Meat Hygiene and Technology – Belgrade, Serbia.

contact with human environments, and physiological features enable them to act as mechanical carriers of microbial agents. Studies have shown that ants in a Brazilian hospital carried *E. coli* and *Salmonella*, while cockroaches sampled from hospitals, restaurants, and retail environments in Spain harboured *E. coli*, *Salmonella*, and other *Enterobacteriaceae* (*Mezal et al.,* 2014). In the South Canary region of India, over 4% of cockroaches collected from various sites tested positive for *Salmonella* spp. Despite these evidences, the role of flies in foodborne pathogen transmission remains under-researched. Houseflies are particularly risky due to their frequent defecation, approximately every 4–5 minutes, which facilitates the spread of pathogens through body hairs, feet, regurgitation, and faecal excretion (*Song et al*., 2021). Research suggests that flies may also transmit pathogens vertically to their offspring. A study by Alexandre Lamas found that Australian bush flies from cattle farms carried a significantly higher bacterial load compared to those collected in urban areas (*Lamas et al*., 2019). Alarmingly, 94% of *Salmonella* and 87% of *Shigella* strains isolated from these flies exhibited multidrug resistance, indicating that flies could serve as vectors for antimicrobial resistance dissemination (*Steele & Odumeru*, 2004).

Water plays a crucial role in the food production continuum and also poses a substantial risk as a medium for microbial contamination. The survival and transmission of pathogens via irrigation water depend on the level of contamination. Pathogens such as *E. coli* and *Salmonella* can be transported via water or soil to plant surfaces, and some strains, like *E. coli* O157:H7, can even penetrate plant tissues, including the stomata of radish seeds and lettuce leaves. Manure-derived *E. coli* has been shown to persist in soil or grassland environments for up to six months, creating long-term reservoirs for contamination (*Avery et al*., 2004).

Animals are also direct sources of foodborne pathogens. Livestock, such as pigs, cattle, and poultry, frequently harbours *E. coli*, including Shiga toxin-producing strains (STEC), which can be transmitted to humans through undercooked meat or poor handling procedures. In a South Korean study, 131 *E. coli* strains were isolated from 334 pork samples obtained from slaughterhouses and markets (*Todd et al*., 2009). Additionally, a UK supermarket survey reported that 23 out of 99 chicken samples contained *E. coli*, emphasizing the ongoing risk at the consumer level (*Heo et al.*, 2020).

Finally, food processing environments and kitchen hygiene practices are critical control points for pathogen entry and spread. Contaminated raw materials, insufficient cleaning of food-contact surfaces, and improper storage or thermal treatment can facilitate cross-contamination and allow pathogens to persist throughout the food supply chain (*Hall et al.,* 2008).

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

The *Enterobacteriaceae* family includes several critical foodborne pathogens, notably *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Yersinia pseudotuberculosis*. These organisms not only contribute significantly to gastrointestinal infections globally but also represent a growing threat due to their contribution to increasing antimicrobial resistance and their high genetic variability, which complicates detection, treatment, and prevention strategies.

Escherichia coli is commonly found in the gut microbiota of humans and animals. While non-pathogenic strains are usually harmless, pathogenic variants can lead to serious illnesses, ranging from gastroenteritis to haemolytic uremic syndrome. Initially susceptible to most antibiotics, *E. coli* has developed resistance mechanisms, including the production of extended-spectrum β-lactamases (ESBLs) and carbapenemases, which hydrolyse a wide range of β-lactam antibiotics. Resistance genes, such as *aadA1, aadA2, mcr-1, blaTEM-1,* and *crf*, are frequently reported in *E. coli*. Molecular typing techniques, such as multilocus sequence typing (MLST), reveal distinct sequence types (STs) among isolates from poultry, cattle, and humans, highlighting the complex epidemiology of foodborne *E. coli* transmission (*Ramadan et al*., 2020). *Salmonella* spp. are also significant foodborne pathogens, with both typhoidal and non-typhoidal strains presenting considerable health burdens. Multidrug resistance is common in *Salmonella*, with resistance genes like *macB, macA,* and *ampE* identified through comparative genomic approaches. Molecular epidemiological tools, including serotyping and MLST, are employed to trace the genetic diversity of *Salmonella* strains. For instance, over 300 isolates have been classified into more than 25 distinct STs. Outbreak investigations emphasize the importance of plasmid profiling in linking clinical cases to contaminated food products, particularly raw meat (*Zhang*, 2021).

Shigella species, especially *S. dysenteriae* and *S. boydii*, are major contributors to childhood diarrhoea, particularly in developing regions. High levels of resistance to fluoroquinolones and third-generation cephalosporins have been observed, with *blaTEM-1*, *blaCTX-M*, *blaOXA-1*, and *blaSHV-12* genes implicated in resistance phenotypes. Serotyping and pulsed-field gel electrophoresis (PFGE) have shed light on outbreak dynamics and strain evolution, while longitudinal studies indicate shifts in dominant serotypes and the emergence of less virulent, sporadic strains (*Wang et al.*, 2016).

Yersinia pseudotuberculosis, though less commonly reported, is a notable enteropathogen with rising antimicrobial resistance. Isolates from both environmental and clinical sources have shown resistance to tetracyclines, beta-lactams, and sulphonamides, driven by genes such as *tetA, tetD, blaTEM-1B,* and *sul2.* Virulence is closely linked to the presence of a 70-kb plasmid and chromosomally encoded toxins like YPMs, as well as high-pathogenicity islands (HPIs), which enhance its infectivity and survival (*Fukushima*, 2003).

4. Emerging technologies for detection and control of *Enterobacteriaceae* in food

Advancements in analytical technologies have significantly improved the speed and accuracy of detecting foodborne pathogens. Among the most promising approaches are those utilizing nanomaterials, nucleic acid-based amplification, and hybridization techniques.

- Nanotechnology-Based Detection: Nanomaterials offer enhanced biosensing capabilities due to their high surface area and unique physicochemical properties. Gold and silver nanoparticles (5–110 nm) are commonly employed to amplify detection signals through optical changes, such as color shifts induced by aggregation. For instance, gold nanoparticles (AuNPs) have been used to detect *Salmonella* and *E. coli* O157:H7 at concentrations as low as 98.9 CFU/mL and 1–10 CFU/mL, respectively. Magnetic nanoparticles (e.g., iron, nickel) and quantum dots also contribute to sensitive pathogen detection via magnetic and fluorescent signals. Additionally, carbon nanotubes (SWNTs and MWNTs) demonstrate excellent conductivity and can be used in field-effect transistors to detect bacteria at extremely low levels, down to 1 CFU/mL. Despite their sensitivity, cost and functionalization challenges limit widespread application (*Gilmartin & O'Kennedy*, 2012).
- PCR-Based Methods: PCR remains a cornerstone of molecular diagnostics. Its capacity to amplify pathogen-specific DNA sequences with high specificity enables rapid identification. Variants such as loop-mediated isothermal amplification (LAMP) and multiplex PCR (mPCR) further extend its utility. LAMP offers high sensitivity, rapid amplification at a constant temperature (60–65°C), and visual result interpretation. However, the risk of aerosol contamination and complex primer design are limiting factors. Multiplex PCR enables simultaneous detection of multiple pathogens by targeting different genes, enhancing diagnostic throughput and cost-efficiency. Technologies like the GeXP system integrate chimeric primers and capillary electrophoresis for high-throughput, fluorescent detection (*Chen et al*., 2012).
- Nucleic Acid Hybridization: Fluorescence in situ hybridization (FISH), using fluorescently labelled rRNA probes, enables direct visualization of pathogens under a fluorescence microscope. Variants such as line probe assays (LIPA) and peptide nucleic acid (PNA) probes have further refined specificity. Due to their uncharged and hydrophobic nature, PNA probes exhibit greater stability and cell permeability than DNA probes, making them suitable for improved analytical performance (*Rho et al*., 2009).

5. Innovative strategies for controlling *Enterobacteriaceae* foodborne pathogens

Food contamination and poisoning caused by *Enterobacteriaceae* pathogens have gained increasing attention in food safety management. Modern control strategies include a range of physical, chemical, and biological methods designed to ensure microbial safety while maintaining product quality.

Irradiation: Ionizing radiation is widely used for food decontamination, particularly in meat and poultry processing. It disrupts microbial DNA, induces protein denaturation, and damages cell membranes. A 4 kGy dose has been shown to eliminate *Salmonella* and significantly reduce *E. coli* and other *Enterobacteriaceae*, without compromising sensory properties, thereby extending shelf life (*Bard*, 2004).

Pulsed Light (PL) Treatment: PL induces DNA damage through photochemical reactions, forming pyrimidine dimers that inhibit replication. Studies have demonstrated reductions of up to 4.5 log CFU/g in *Salmonella* and *E. coli* on fresh produce. However, the effectiveness of PL decreases with uneven surfaces and extended pulse intervals and may cause browning in fruits due to polyphenol oxidase activation (*Ares et al*., 2014).

Microwave Sterilization: Microwave energy disrupts cell membrane permeability and causes protein denaturation, leading to microbial death. Water-assisted microwave treatment at 63°C effectively inactivates *Salmonella* on leafy vegetables. The technique's effectiveness varies depending on the food matrix, and it can complement other physical treatments like cold plasma and cavitation jets for enhanced control (*Shahla et al*., 2013).

Slightly Acidic Electrolyzed Water (SAcEW) and Fumaric Acid: SAcEW, especially when combined with 0.6% fumaric acid and mild heating (40°C, 5 min), significantly reduces *E. coli* and *Salmonella* in fresh meat. This combined treatment improves bactericidal efficacy and extends the shelf life of meat stored at refrigeration temperatures by up to 8 days (*Ahmad et al*., 2015).

Emerging Biocontrol Approaches: Biological agents, such as antimicrobial peptides from *Bacillus* spp. and algae-derived bioactives, offer promising alternatives. These substances demonstrate inhibitory effects against *E. coli* and other pathogens, although further studies are required to understand their mechanisms, effective dosages, and interactions in complex food systems. Algal compounds like fucoidans and ulvans also contribute to antimicrobial defence and may enhance overall food safety and nutritional value (*Neetoo et al*., 2010).

6. Conclusion

The persistence and transmission of *Enterobacteriaceae* foodborne pathogens and their resistance genes throughout the food production continuum, from animal husbandry environments to human consumption, pose a serious threat to global public health. Innovative detection technologies, including molecular and immunoaffinity-based approaches, provide increased sensitivity and specificity for early pathogen identification. At the same time, advanced control measures such as irradiation, pulsed light, microwave sterilization, slightly acidic electrolyzed water, and bioactive compounds from natural sources show significant potential in reducing microbial contamination while maintaining food quality. Integrating these emerging technologies into comprehensive food safety management systems is essential. To effectively mitigate risks, coordinated global efforts through regulatory frameworks, policy implementation, and continuous monitoring are crucial. A multidisciplinary approach that combines scientific innovation, regulatory oversight, and industry application will be vital in ensuring food safety and public health.

Disclosure Statement: No potential conflict of interest was reported by authors.

Funding: This paper was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (No 451-03-136/2025-03/200030)

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