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Microbial Biotechnology in Food Safety: Detection, Control, and Prevention of Foodborne Pathogens

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Abstract

Foodborne pathogens continue to pose a major global public health and economic burden, with traditional detection and control methods increasingly limited by delays, resistance development, and environmental concerns. This review explores the transformative role of microbial biotechnology in modern food safety management. Advanced culture-independent detection technologies including nucleic acid amplification (PCR, LAMP, RPA), next-generation sequencing (NGS)/metagenomics, nanotechnology-integrated biosensors (electrochemical, optical, SERS), and CRISPR-Cas-based diagnostics enable rapid, sensitive, specific, and often on-site identification of pathogens such as Salmonella, Listeria, and E. coli. For control, biologically targeted strategies are gaining prominence, including bacteriophage therapy for pathogen elimination and biofilm disruption, bacteriocins and protective cultures from lactic acid bacteria, and engineered CRISPR-Cas systems for precision microbial elimination. Synthetic biology further advances the field through precision fermentation for sustainable ingredient production and next-generation probiotics with diagnostic and therapeutic functions. Digital integration of artificial intelligence (AI), machine learning, IoT-based real-time monitoring, predictive microbiology, dynamic HACCP, and blockchain traceability shifts food safety toward predictive and preventive paradigms. The review also addresses the growing threat of antimicrobial resistance (AMR) in the food chain and advocates for One Health approaches, reduced antibiotic use, and alternative therapies. Despite technical, economic, regulatory, and societal challenges, microbial biotechnology offers sustainable, high-precision solutions to enhance food safety across the farm-to-fork continuum.

Keywords Microbial biotechnology; Foodborne pathogens; Food safety; Biosensors; CRISPR-Cas; Bacteriophages; Bacteriocins; Precision fermentation; Next-generation probiotics; Artificial intelligence; Antimicrobial resistance; One Health; Predictive microbiology; Blockchain traceability

Introduction

The global food system is currently navigating a period of unprecedented complexity, driven by a convergence of rapid population growth, intensifying climate change, and the persistent, escalating threat of foodborne illnesses (BCC Research, 2024). Conventional approaches to food safety, which have historically relied upon reactive identification and broad-spectrum chemical interventions, are increasingly viewed as insufficient to meet the demands of a high-speed, globalized supply chain (Lavilla, 2025). In this context, microbial biotechnology has emerged not merely as a set of supplementary tools but as a transformative foundation for a new paradigm in food safety management (Aslam et al., 2025). This paradigm leverages the precision of molecular biology, the scalability of synthetic biology, and the analytical power of digital integration to detect, control, and prevent pathogens with a level of accuracy and foresight that was previously unattainable (Kabiraz et al., 2023).

The scale of foodborne disease remains one of the most significant challenges to global public health and economic stability. Estimates indicate that nearly 600 million cases of illness and approximately 420,000 deaths occur annually due to the consumption of unsafe food, resulting in the loss of 33 million disability-adjusted life years (DALYs) (World Health Organization, 2025). The economic ramifications are equally staggering, with the financial impact in the United States alone estimated at approximately USD 75 billion in 2023 (Ioni, 2024). These figures underscore a fundamental reality: microorganisms are highly adaptable and can survive, or even thrive, in harsh food processing environments, facilitating their transfer from farm to table (Vila et al., 2025).

Traditional food safety measures, while essential, face limitations in sensitivity and real-time adaptability. Heat treatments can deteriorate the organoleptic and nutritional properties of food, while the extensive use of chemical sanitizers has contributed to the development of resistant bacterial strains (Fokas et al., 2025). Furthermore, conventional culture-based detection methods, which rely on the ability of organisms to multiply into visible colonies, often require several days to provide actionable results (Kanicheril Ambikalekshmi, 2025). This delay is incompatible with the shelf life of fresh produce and ready-to-eat (RTE) products, which are frequently implicated in outbreaks. Consequently, there is an urgent demand for biotechnological innovations that offer rapid, sustainable, and eco-friendly solutions (MDPI, 2024).

The transition toward microbial biotechnology is supported by its low carbon footprint and independence from cultivable land or climate conditions, as microbial processes can be conducted in controlled bioreactors with high reproducibility (Tan et al., 2022). This shift is reflected in the market, where the global value of fermented ingredients a key output of microbial biotechnology is projected to reach USD 79.3 billion by 2030 (Zhuang et al., 2022).

Figure 1. Conceptual Framework of Food Safety in the Modern Era: Pathogens, Diagnostic Tools, and Control Strategies.

Advanced Detection Technologies: Transitioning to Culture-Independent Paradigms

The primary objective of advanced detection is to achieve high sensitivity and specificity within a timeframe that allows for intervention before contaminated products reach the consumer (Rodriguez et al., 2020). Detection methods are broadly categorized into culture-based and culture-independent techniques, with the latter seeing rapid advancement through molecular biology and nanotechnology (MDPI, 2023).

Table 1. Comparison of Detection Methods for Foodborne Pathogens: Traditional vs. Advanced Biotechnological Approaches

Feature	**Traditional Culture-Based**	**Molecular (PCR/LAMP)**	**Biosensors**	**NGS/Metagenomics**
Detection Time	3-7 days (Kabiraz et al., 2023; Lavilla, 2025b)	1-4 hours (Kabiraz et al., 2023; Sharma et al., 2021)	Minutes to hours (Kabiraz et al., 2023; Lavilla, 2025b)	24-48 hours (Kabiraz et al., 2023; MDPI, 2023)
Sensitivity	High (with enrichment)	Very High	High to Moderate	Very High
Specificity	Phenotypic/Biochemical	Genotypic (Specific)	Recognition-based	Whole Genome/Community
Portability	Low (Lab required)	Moderate (Portable units)	High (On-site/Handheld)	Low to Moderate
Cost per Sample	Low	Moderate	Low to Moderate	High (Decreasing)

Molecular Amplification Foundations

Nucleic acid-based methods have revolutionized pathogen identification by targeting unique genetic signatures. Polymerase Chain Reaction (PCR) and its quantitative variant, qPCR, allow for the exponential amplification of specific DNA fragments, enabling the detection of even a few cells in a complex matrix (Sharma et al., 2021). qPCR, in particular, utilizes fluorescent signals from probes or intercalating dyes to monitor amplification in real-time, allowing for the quantification of colony-forming units (CFU) without the need for subsequent electrophoresis (Liu et al., 2023). Multiplex PCR further increases efficiency by allowing the simultaneous detection of multiple pathogens such as Salmonella, Listeria, and E. coli O157:H7 in a single reaction mixture (Chakraborty, 2024).

However, the requirement for thermal cycling in PCR often necessitates sophisticated laboratory equipment, which can be a barrier for field applications. To address this, isothermal amplification techniques such as Loop-Mediated Isothermal Amplification (LAMP) and Recombinase Polymerase Amplification (RPA) have been developed (Duncan et al., 2019). LAMP operates at a constant temperature (typically 60-65 degrees C), utilizing four to six primers that recognize distinct regions on the target DNA, and ensuring extremely high specificity. The rapid accumulation of DNA in LAMP often leads to visible turbidity or color changes, facilitating simple, equipment-free readouts (Khera et al., 2024).

High-Throughput Sequencing and Metagenomics

Next-Generation Sequencing (NGS) has emerged as a critical tool for comprehensive microbial analysis. Unlike PCR, which requires a pre-defined target, metagenomic NGS allows for the sequencing of all genetic material within a food sample, providing an unbiased view of the microbial community (Meijer & van der Fels-Klerx, 2024). This "shotgun" approach is invaluable for identifying unknown pathogens or characterizing the "resistome" the total collection of antimicrobial resistance (AMR) genes within a food ecosystem (Aulicino et al., 2024).

Recent developments have introduced NGS panel methods, which use specific primer sets to screen for multiple virulence factor genes from various pathogens in a single reaction. For example, studies have successfully used NGS panels to target 13 specific virulence factors from five types of pathogenic E. coli, Listeria monocytogenes, and Salmonella Typhimurium (Naqvi et al 2025). While NGS panel analysis still faces challenges with false positives at lower concentrations (10^5 to 10^6 CFU), its ability to provide high-resolution data on the origin and characteristics of a strain makes it superior for outbreak investigations (MDPI, 2025).

Biosensing Platforms and Nanotechnology Integration

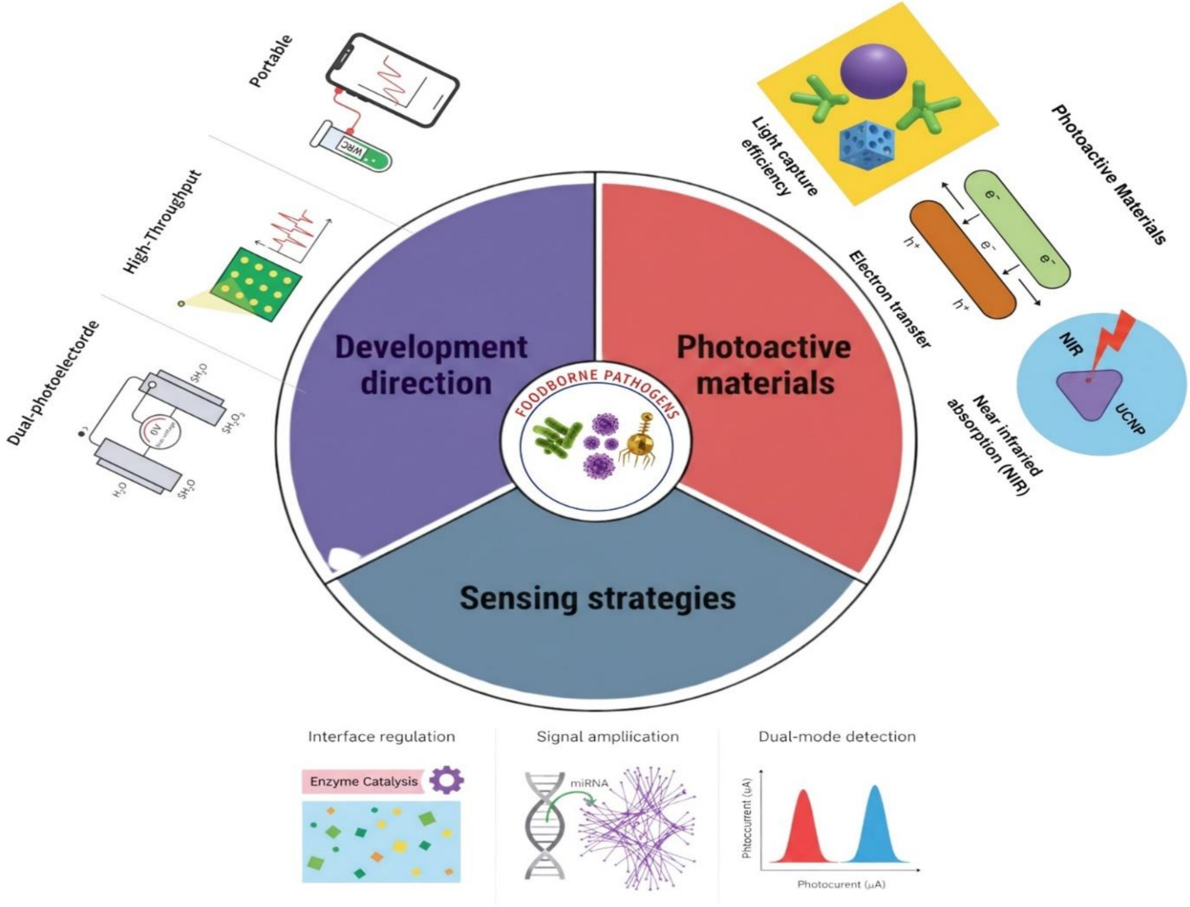
Biosensors represent the intersection of biological recognition and technological transduction. They consist of a biorecognition element such as an antibody, aptamer, or peptide coupled to a transducer that converts a binding event into an electrical, optical, or mass-based signal (Abdel-Karim, 2024).

Electrochemical Biosensors: These are favored for their high sensitivity, low cost, and potential for miniaturization. They detect changes in current, impedance, or potential when a target pathogen binds to the sensor surface (Hemdan et al., 2024). The incorporation of nanomaterials like gold nanoparticles (AuNPs), graphene, and carbon nanotubes has significantly enhanced signal-to-noise ratios and provided more binding sites for bioreceptors (Wu, 2020).

Optical Biosensors: Utilizing Surface-Enhanced Raman Spectroscopy (SERS) or Surface Plasmon Resonance (SPR), these sensors offer rapid, often label-free detection (Pal et al., 2025). SERS, in particular, is being optimized through deep learning algorithms to distinguish between closely related bacterial species based on their unique spectral fingerprints (Rahul et al., 2024).

Aptamer-based Recognition: Aptamers single-stranded DNA or RNA molecules selected through the SELEX process are increasingly replacing antibodies due to their superior stability, low production cost, and high affinity for diverse targets, including toxins and whole bacterial cells (Didarian et al., 2025). Aptamers targeting Salmonella LPS, E. coli O157:H7 outer membrane proteins, and S. aureus enterotoxins have demonstrated nanomolar dissociation constants, highlighting their potential for rapid detection (Zhuo et al., 2017).

Figure 3: Technical Architecture of Advanced Biosensing Platforms and Nanotechnology-Enabled Detection.



CRISPR-Cas Systems in Pathogen Diagnostics

The adaptation of CRISPR-Cas systems for diagnostics has introduced a new level of precision to food safety. Nucleases such as Cas12 and Cas13 possess "collateral cleavage" activity; upon binding to a specific DNA or RNA target sequence, they indiscriminately cleave nearby single-stranded nucleic acids (Mesa-Valle et al., 2023). By introducing fluorophore-quencher labeled probes into the reaction, this collateral cleavage generates a detectable signal within minutes (Li et al., 2022). CRISPR-based assays have achieved rapid detection of Salmonella spp. in food samples within one hour, surpassing the speed and specificity of traditional molecular methods (Suliman Maashi, 2024).

Precision Control: Microbial Antagonists and Bioactive Compounds

The control of foodborne pathogens is shifting from broad-spectrum chemical interventions toward targeted, biologically-based strategies. These "biocontrol" methods utilize natural enemies of pathogens or their antimicrobial secretions to ensure safety without compromising food quality (Vila et al., 2025).

Bacteriophage Biocontrol and Biofilm Disruption

Bacteriophages (phages) are viruses that exclusively infect bacteria. Their high host specificity is a major advantage, as they can eliminate specific pathogens like Listeria or Salmonella without disturbing the beneficial microbiota of the food or the consumer's gut (Imran et al., 2023). Lytic phages are particularly useful because they cause immediate destruction of the host cell (Ranveer et al., 2025).

Phage application is effective across various stages of the food chain, from pre-harvest animal health to post-harvest surface decontamination of meat and fresh produce. One of the most significant challenges in food processing facilities is the formation of biofilms multispecies microbial communities encased in a protective extracellular matrix (Emencheta et al., 2023). Bacteria in biofilms are often 100 to 1,000 times more resistant to traditional sanitizers than their planktonic counterparts (Gummadi, 2024). Phages capable of producing polysaccharide depolymerases can penetrate these matrices, facilitating the disruption and elimination of biofilm-associated pathogens (Fokas et al., 2025).

Bacteriocins and Protective Cultures

Bacteriocins are antimicrobial peptides produced primarily by Lactic Acid Bacteria (LAB). They are ribosomally synthesized and often possess a broad spectrum of activity against gram-positive pathogens like Listeria monocytogenes and Staphylococcus aureus (Mokoena, 2017). Nisin, produced by Lactococcus lactis, is the most prominent example and remains the only bacteriocin widely approved for use as a food preservative by regulatory bodies like the FDA (Sidhu et al., 2022).

Recent research has focused on leaderless and circular bacteriocins, which exhibit enhanced stability against high temperatures and varying pH levels, making them ideal for incorporation into various food matrices. Furthermore, the concept of "protective cultures" the deliberate addition of non-pathogenic, competitive microorganisms to food is gaining traction (Chen et al., 2025). These cultures work through competitive exclusion, nutrient depletion, and the in situ production of organic acids and bacteriocins, providing an additional layer of safety for minimally processed and fermented foods (MDPI, 2024).

CRISPR-Cas as a Tool for Targeted Microbial Elimination

Beyond detection, CRISPR-Cas systems are being engineered for the precision elimination of targeted bacterial populations. By delivering CAS nucleases and guide RNAs designed to target essential chromosomal genes or antibiotic resistance plasmids, it is possible to "program" the death of specific pathogens within a complex community (Benz et al., 2025). Delivery mechanisms for these CRISPR components include bacteriophages (phagemids) and nanoparticles, which can bypass the barriers presented by the biofilm matrix (Gomaa et al., 2014). For example, liposomal CRISPR-Cas9 formulations have demonstrated the ability to reduce Pseudomonas aeruginosa biofilm biomass by over 90% in vitro (Wu et al., 2021).

Synthetic Biology: Re-Engineering the Food Ecosystem

Synthetic biology is redefining the boundaries of food biotechnology by enabling the rational design of microbial pathways for the production of ingredients, additives, and therapeutic probiotics (Aslam et al., 2025).

Precision Fermentation and Biofactories

Precision fermentation utilizes engineered microorganisms as "cell factories" to produce high-purity proteins, enzymes, and vitamins. This technology has successfully commercialized recombinant proteins, such as bovine beta-lactoglobulin, which has received FDA "no questions" GRAS (Generally Recognized as Safe) letters since 2020 (Moualek, 2025). By leveraging promoter libraries and AI-guided metabolic flux analysis, researchers can optimize the production of these ingredients, reducing the environmental impact compared to traditional animal-based sources (Elazzazy et al., 2025).

Table 2. Precision Fermentation Applications Using Engineered Microorganisms in Food Production

Application	Microorganism Platform	Product Example	Regulatory Status
Alternative Proteins	Saccharomyces cerevisiae, K. phaffii	Recombinant whey, leghemoglobin	FDA GRAS (Aslam et al., 2025)
Human Milk Oligosaccharides	Engineered E. coli or Yeast	3'-SL, 6'-SL (Sialyllactose)	Approved in Formula (SciePublish, 2024)
Food Enzymes	Trichoderma reesei, Bacillus spp.	Proteases, amylases, lipases	EFSA/FDA Approved (Aslam et al., 2025)
Flavor Compounds	Lactococcus lactis	Diacetyl (Buttery aroma)	Traditional/Optimized (MDPI, 2024)

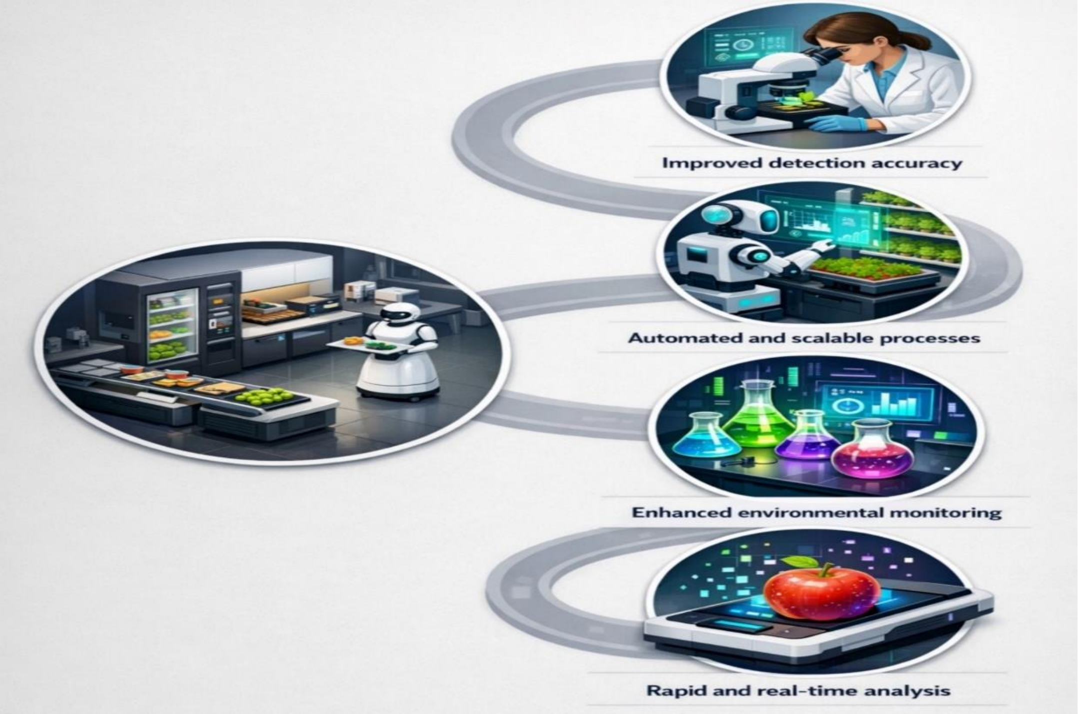
Next-Generation Probiotics (NGPs) for Diagnostic and Therapeutic Use

Synthetic biology is also being used to engineer probiotics with enhanced stability and novel functionalities. These next-generation probiotics can act as "living biosensors," programmed to detect inflammatory markers or metabolic imbalances in the gut and respond by releasing therapeutic molecules (Mimee et al., 2018). For example, Lactobacillus reuteri has been engineered to express phenylalanine ammonia lyase to treat phenylketonuria (PKU), while Lactobacillus plantarum has been modified to reduce dietary oxalate, preventing the formation of kidney stones (Abouelela et al., 2024). These engineered strains provide a bridge between food safety and personalized medicine, moving beyond the prevention of infection to the active management of human health (Jadhav et al., 2026).

Digital Prevention: AI, Machine Learning, and Real-Time Monitoring

The integration of digital technologies Artificial Intelligence (AI), the Internet of Things (IoT),

Figure 2: The Roadmap of Digital Integration: From Improved Detection Accuracy to Real-Time AI Analysis.



and Big Data is transforming food safety from a reactive to a predictive model (Sharma et al., 2021). The AI market for food safety and quality control is projected to experience a compound annual growth rate (CAGR) of 30.9% between 2025 and 2029, reflecting its critical role in the modern bioeconomy (BCC Research, 2024).

AI-Enhanced Diagnostics and Hazard Detection

AI algorithms, particularly deep learning and convolutional neural networks (CNNs), are being integrated with biosensing platforms to handle signal noise and variability in complex food matrices (Banicod et al., 2025). These models can identify patterns in multidimensional data that are invisible to human inspectors, allowing for the accurate classification of pathogens with high sensitivity and stability (Kumar et al., 2025). In industrial settings, AI-powered vision systems are used on production lines to detect even minor physical or microbial contaminants in real-time (Kanicheril Ambikalekshmi, 2025).

Predictive Microbiology and Early Warning Systems

Predictive microbiology involves the use of mathematical models to forecast the growth, survival, and inactivation of microorganisms under various environmental conditions. Systems like ComBase and the Pathogen Modeling Program (PMP) are being updated with AI to process real-time data from IoT sensors monitoring temperature, pH, and humidity throughout the supply chain (Stavropoulou et al., 2019). These models can detect contamination risks up to 30% faster than manual inspections, allowing for proactive interventions such as re-routing or holding potentially compromised batches before they reach retail (Tarlak, 2023). For instance, AI can analyze historical rainfall and temperature data to predict the emergence of harmful algal blooms in seafood or mycotoxin formation in crops (Taiwo et al., 2024).

Dynamic HACCP and Automated Compliance

Traditional Hazard Analysis Critical Control Points (HACCP) plans are often static and manually intensive. AI is enabling the development of "Dynamic HACCP," where safety protocols automatically update in response to changes in the production process, such as new equipment installations or ingredient substitutions (Opara et al., 2025). AI tools can parse standard operating procedures (SOPs) and regulatory databases to suggest critical control points (CCPs) and establish data-informed critical limits, ensuring that food safety management systems remain current and effective (Ioni, 2024).

Blockchain for Traceability and Transparency

Blockchain technology offers a decentralized ledger for recording every transaction and testing result within the food supply chain. By coupling biosensor data with blockchain, companies can ensure that microbial safety records are tamper-proof and accessible to all partners in the chain (Feng et al., 2020). This "farm-to-fork" traceability is essential for rapid source tracking during an outbreak, significantly reducing the scope of recalls and enhancing consumer trust (Doshi et al., 2024).

Antimicrobial Resistance (AMR) in the Food Chain: A Global Public Health Threat

The emergence and dissemination of antimicrobial-resistant foodborne pathogens represent one of the top global public health threats recognized by the World Health Organization (WHO). By 2050, AMR could cause up to 10 million deaths annually if significant action is not taken (Vila et al., 2025).

Mechanisms and Drivers of Resistance

Resistance in foodborne bacteria like Salmonella, Campylobacter, and E. coli is driven primarily by the misuse of antibiotics in human medicine and intensive animal farming. In agriculture, antibiotics are often used sub-therapeutically for growth promotion and disease prevention, creating a selective pressure that favors the evolution of resistant strains (World Health Organization, 2025).

Table 3. Major Foodborne Pathogens with Significant Antimicrobial Resistance Profiles and Key Resistance Mechanisms

Pathogen	Significant Resistance Profile	Key Mechanism
Salmonella enterica	Quinolones, Fluoroquinolones	Efflux pumps, target mutations (World Health Organization, 2025)
Campylobacter jejuni	Multidrug resistance (RE-CmeABC)	Enhanced efflux variant (World Health Organization, 2025)
Escherichia coli	ESBL, Tetracyclines, Sulfonamides	Plasmids, enzymatic degradation (World Health Organization, 2025)
Staphylococcus aureus	Beta-lactams, Carbapenems	Biofilm protection, target modification (World Health Organization, 2025)

Bacteria acquire resistance genes (ARGs) through vertical transfer or horizontal gene transfer (HGT) via conjugation, transformation, and. Mobile genetic elements like plasmids and integrons facilitate the rapid spread of resistance across different bacterial species within the food ecosystem transduction (Swain et al., 2025).

The One Health Approach to AMR Mitigation

Addressing AMR requires a "One Health" framework that considers the interconnected health of humans, animals, and the environment (Sheret al., 2025). Key strategies include:

- **Surveillance:** Implementing comprehensive monitoring of ARGs and the "mobilome" along the entire farm-to-fork continuum (Li et al., 2025).
- **Reduced Usage:** Phasing out the use of medically important antibiotics for non-therapeutic purposes in livestock (Jadhav et al., 2026).

Alternative Therapies: Investing in the research of bacteriophages, antimicrobial peptides, and probiotics as sustainable substitutes for traditional antibiotics (Ranveer et al., 2025).

- **Environmental Management:** Managing water runoff and manure to prevent the environmental amplification and spread of resistant strains (Khera et al., 2024).
- **Regulatory Frameworks and Implementation Challenges**

The rapid pace of biotechnological innovation has challenged existing regulatory systems to adapt while ensuring public safety (U.S. Food and Drug Administration, 2025).

The U.S. and EU Regulatory Landscape

In the United States, the Coordinated Framework for Regulation of Biotechnology (1986) provides a risk-based system involving the FDA, USDA, and EPA. The FDA oversees the safety of human and animal foods derived from genetically engineered plants and microorganisms, often using the GRAS notification system (Mokoena, 2017). Recently, the "Unified Website for Biotechnology Regulation" was established to increase transparency and predictability for stakeholders (Chen et al., 2025).

In contrast, the European Union (EU) often adopts a more process-based and precautionary approach to biotechnology. While precision fermentation enzymes are regulated under established frameworks, the use of certain genome-editing technologies (like CRISPR) in food remains subject to stringent process-based restrictions that can create barriers to international trade (Abouelela et al., 2024).

Barriers to Scalability and Adoption

Despite the potential of microbial biotechnology, several challenges hinder its mainstream adoption:

1. **Technical Scalability:** Many advanced methods, such as CRISPR-based sanitation or synthetic microbial consortia, have only been demonstrated at the bench or pilot scale. Scaling these processes requires significant financial investment and the development of standardized industrial protocols (Taiwo et al., 2024).
2. **Sample Complexity:** The intricate composition of food matrices (fats, proteins, humic acids) can inhibit molecular reactions and interfere with biosensor signals, necessitating robust sample preparation steps (Kabiraz et al., 2023).
3. **Cost and Technical Expertise:** Technologies like NGS and AI-driven monitoring systems can be prohibitively expensive for small and medium-sized enterprises (SMEs) and require specialized training for personnel (Ioni, 2024).
4. **Consumer Perception:** Public acceptance of "genetically modified" or "engineered" microbial solutions remains variable, necessitating transparent communication and education regarding the safety and benefits of these technologies (Benz et al., 2025).

Conclusion

Microbial biotechnology has evolved from a supplementary toolset to a foundational pillar of next-generation food safety strategies. By harnessing molecular precision (CRISPR-Cas, nucleic acid amplification, metagenomics), biological antagonism (phages, bacteriocins, protective cultures), synthetic redesign of microbial cell factories, and digital intelligence (AI-driven prediction, IoT monitoring, blockchain-enabled traceability), the field now offers unprecedented capabilities for rapid detection, targeted control, and proactive prevention of foodborne pathogens. These innovations address critical limitations of conventional approaches such as time delays, broad-spectrum chemical reliance, and emerging antimicrobial resistance while aligning with sustainability goals through lower environmental footprints and reduced resource demands. Nevertheless, successful mainstream adoption requires overcoming persistent barriers: scaling laboratory-proven technologies to industrial levels, mitigating matrix interference in complex food samples, reducing costs for small and medium enterprises, building technical capacity, harmonizing regulatory frameworks across regions (particularly regarding genome-editing tools), and fostering public trust through transparent risk communication. Looking forward, continued interdisciplinary integration combining microbial engineering, nanotechnology, data science, and One Health principles will be essential to stay ahead of evolving pathogen threats, climate-driven risks, and globalized supply chain vulnerabilities. Ultimately, microbial biotechnology promises not only safer food systems but also a more resilient, equitable, and sustainable bioeconomy capable of protecting public health in an era of unprecedented global challenges.

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