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REVIEW ARTICLE

Microfluidics for detection of food pathogens: recent trends and opportunities

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Abstract
Safe and healthy food is the fundamental right of every citizen. Problems caused by foodborne pathogens have always raised a threat to food safety and human health. Centers for Disease Control and Prevention (CDC) estimates that around 48 million people are affected by food intoxication, and 3000 people succumb to death. Hence, it is inevitable that an approach that is efficient, reliable, sensitive, and rapid approach that can replace the conventional analytical methods such as microbiological and biochemical methods, high throughput next-generation sequence (NGS), polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA), etc. Even though the accuracy of conventional methods is high, it is tedious; increased consumption of reagents/samples, false positives, and complex operations are the drawbacks of these methods. Microfluidic devices have shown remarkable advances in all branches of science. They serve as an alternative to conventional ways to overcome the abovementioned drawbacks.
Furthermore, coupling microfluidics can improve the efficiency and accuracy of conventional methods such as surface plasma resonance, loop-mediated isothermal amplification, ELISA, and PCR. This article reviewed the progress of microfluidic devices in the last ten years in detecting foodborne pathogens. Microfluidic technology has opened the research gateway for developing low-cost, on-site, portable, and rapid assay devices. The article includes the application of microfluidic-based devices to identify critical food pathogens and briefly discusses the necessary research in this area.

Keywords Foodborne pathogens · Food safety · Immunoassays · Molecular diagnostic techniques · Microfluidic device

Introduction

Access to hygienic and wholesome food is a fundamental right for all citizens to make a healthy and efficient society. The risk of food contamination extends from farm to fork, making it imperative to maintain food safety at every level. However, the surging popularity of street foods poses a significant challenge for regulatory bodies striving to ensure food safety. Sometimes, inadvertent consumption of contaminated food happens due to human errors. Notable pathogens behind foodborne outbreaks include *Staphylococcus aureus, Bacillus cereus, Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes,* and *Clostridium perfringens* (Paniel & Noguer 2019; Ma et al. 2020; Shang et al. 2020; Zhong et al. 2018).

Traditional culture-based methods involve growing microorganisms in various media with long turnaround times (18–24 h) (Paniel and Noguer 2019). Immunological assays offer faster detection, but false-positive concerns limit their use. Molecular diagnostic techniques like polymerase chain reaction (PCR), mass spectrometry, high throughput sequencing, etc., provide rapid and specific detection. PCR revolutionized scientific methods but has non-specific binding issues. SDS-PAGE protein profiling in proteomics faces challenges like a lack of databases and the inability to distinguish closely related species (Canciu et al. 2023; Grace 2023). Table 1 discusses the existing techniques for detecting pathogens, and Fig. 1 shows the characteristics of the conventional techniques used for pathogen detection. Microfluidics has driven the development of point-of-care (POC) devices, revolutionizing

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Table 1 Existing techniques for the detection of pathogens in food

Pathogen	Method	Drawback	References
Escherichia. coli	Culture based	Time consuming	Deisingh et al. (2004)
	PCR based	• Produces false positives • Require enrichment of samples • Time consuming	Deisingh et al. (2004)
	Immunological detection	• Lengthy enrichment procedure • Cannot be applied to all samples	Song et al. (2020) and Deisingh et al. (2004)
Listeria	Culture based	• Time consuming • May produce false positives	Chen et al. (2017)
	PCR based	• DNA- based identification can produce false-positive results	Chen et al. (2018)
	Immunological detection	• Only the antigenicity is determined (may vary from toxicity) • False positive results	Saravanan et al. (2021)
Norovirus	PCR based	• Difficult to differentiate between infectious and inactivated virus	Terio et al. (2020)
Campylobacter	Culture based	• Lengthy and Time consuming	Thornvalet al. (2021)
	DNA sequencing based	• Expensive • Time consuming	Thornvalet al. (2021)
Salmonella typhimurium	Culture based methods	• Interpretation of results are tedious and laborious • Low sensitivity • Microbial contamination	Lee et al. (2015) and Sullivan et al. (2020)
	Immunoassays	• False positive results due to contaminants	Tegtmeyer et al. (2022)
	PCR based methods	• Require pre-enrichment of samples	Bailey (1998)
	DNA sequencing	• Need skilled personnel	Wilson et al. (2016) and Diep et al. (2019)

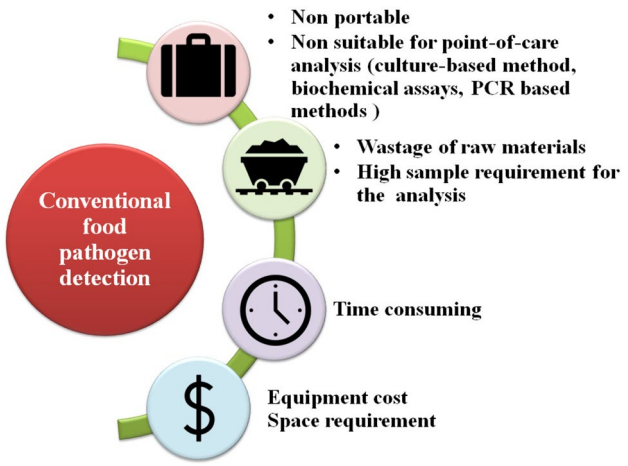

Fig. 1 Characteristics of conventional techniques used for food pathogen detection

the biomedical field. Recently, there has been a surge in the use of point-of-care testing (POCT) in medical diagnostics, particularly for quickly detecting and monitoring infectious diseases. In food safety, many people prefer POCT for finding pathogens because it allows easy on-site testing, gives quick results right where the samples are taken, and is especially useful in places with limited resources (Helmy et al. 2023).

Microfluidics

Microfluidics, a cost-effective solution in scientific fields, manipulates fluid flow through micro-channels (10^{-9} to 10^{-8} L). "Lab-on-a-chip" (LOC) handles µL to pL volumes, integrating electronic and fluidic components. Microfluidic devices, distinct in structure and design from macroscale counterparts, provide fast processing, reduced reagent consumption, quick reactions, point-of-use production, point-of-care diagnostics, efficient heat transfer, and easy integration with other units. Microchannels, microvalves, and advanced fluid mechanics enable controlled sample flow, fostering on-chip sensing across various scientific branches such as chip sensing (Gileberman et al. 2019), optical sensing (Chen et al. 2022b, a), organ-on-chip (Dornhof et al. 2021), drug discovery and analysis (Ma et al. 2020), cell manipulation, single cell analysis (Willner et al. 2018), reactor technology (Narayanamurthy et al. 2020), etc. Microfluidic devices with aqueous micro-reactors support cell propagation, allowing precise monitoring of cellular responses to biochemical stimuli and weak signals. They aid cell screening

by separating cells based on size, which helps distinguish microparticles from blood cells, bacteria, spores, circulating tumor cells, parasites, and nanoparticles. Though microfluidic technology has been developed academically, the significant technical challenges include usability limitations, a lack of standardization, and difficulty scaling up from a laboratory to a commercial level (Gurkan et al. 2024). Figure 2 shows the characteristics of lab-on-a-chip technology used for food pathogens.

Microfluidics integrating with different technologies

By integrating immunoassay into microfluidics, disease diagnosis is notably improved. Moreover, microfluidics transforms various aspects of drug discovery, local drug delivery, cell encapsulation, culture, and the creation of artificial organs (Shao et al. 2020). The progress in Micro Electromechanical System (MEMS) technology enables portability, miniaturization, integration, and automation.

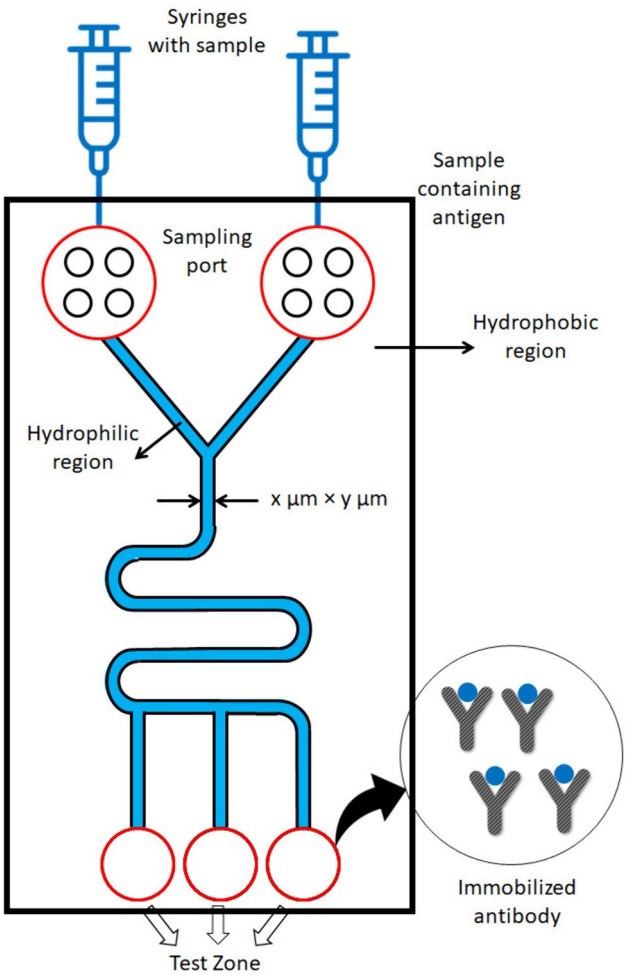

Fig. 2 Characteristics of lab-on-a-chip technology used for food pathogens

Cost-effective fabrication techniques, including laser cutting, photolithography, and polydimethylsiloxane (PDMS) printing, produce microfluidic devices from materials like paper, glass, silicon, and polymers (Tsao 2016). Fluid behavior differs at the microscale, enabling control of properties like temperature, pH, and concentration. Droplet and T-junction microfluidic devices are designed for chemical reactions. Droplet-based microfluidic devices are ideal for conducting separate reactions with minimal samples. T-junction microfluidics is employed for immiscible fluids. A typical T-junction microfluidic device for droplet generation is depicted in Fig. 3. LOC integrates various laboratory processes onto a single platform, enhancing the surface-area-to-volume ratio (Chen et al. 2022b, a; Sackmann et al. 2014).

Achieving sensitivity and precision involves integrating established techniques such as electrochemical methods, Raman spectroscopy, fluorescence microscopy, and mass spectrometry. Electrochemical techniques stand out for their insensitivity to detector miniaturization, facilitation of monitoring via digital systems, and cost-effective fabrication. Therefore, paper-based microfluidics extensively utilize electrochemical detectors (Christopher et al. 2020). The remarkable sensitivity and spatial resolution of surface-enhanced Raman scattering (SERS) are utilized in automated microfluidic devices to detect two biomarkers, enabling dynamic liquid measurements concurrently. With the use of metal nanoparticles, SERS has become renowned for its ability to multiplex detection (Zhong et al. 2018). Coupling SERS with droplet microfluidics holds promise for advancing our understanding of cellular systems, potentially lowering the detection limit to 10 pm or beyond. Willner et al. (2018) developed a SERS droplet microfluidic device to analyze single cells with 85–100% sensitivity and 90% efficiency.

With the advancement in the fabrication of microfluidics, it is now possible to make "chip calorimeters" that can assay sample volume with much smaller amounts of cells (1×10^3 cells/µL). Based on molding techniques,

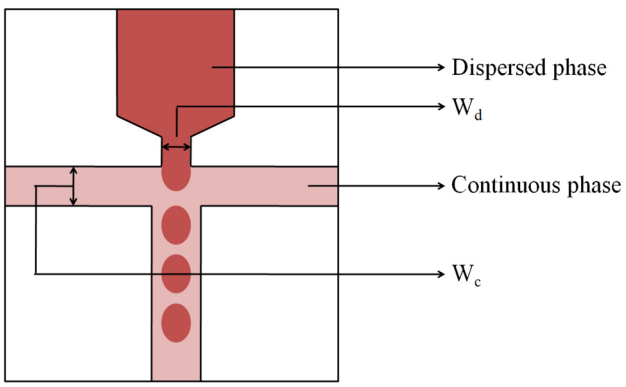

Fig. 3 Typical T junction microfluidic device for droplet generation

microfluidic fabrication is divided into three types: soft lithography (Graham et al. 2021), hot embossing (Jiang et al. 2021), and injection molding (Ma et al. 2020). These three methods offer cost-effective solutions with excellent throughput. As the name implies, soft lithography is ideal for soft polymers, organic monolayers, and gels. This technique is commonly used for PDMS microfluidic devices. Injection molding and hot embossing are closely related techniques. The melted thermoplastic is injected through the mold cavity at a specific rate, cooling the mold and removing the cast from the mold (Burklund et al. 2020). With the help of a graphic model, it is now possible to print the three-dimensional form of the device that one is intended to make. The digital model of the device is sliced into layers and reproduced using a 3D printer. Inkjet 3D printing, fused deposition molding, multi-jet molding, stereo-lithography, and two-photon polymerizations are the few technologies involved in 3D printing. However, the resolution of the 3D printers available in the market could not produce microfluidic devices as precise as the traditional lithography technique. Figure 4 shows different methods for the fabrication of microfluidic devices.

Microfluidics in the detection of food pathogens

In response to the growing global population, advanced food processing technologies have emerged to meet market demands. One-quarter of the world's population is at risk of foodborne illness due to inadequate food inspection and the rapid growth of the economy. Microfluidics has garnered considerable attention from researchers for producing various food structures, including emulsion-based and self-assembled structures. In the dairy industry, microfluidic technology disperses stabilizers and gums in ice cream mixes or dairy-based products, ensuring adequate mixing. It can seamlessly integrate with food processing machinery to create highly concentrated nano-emulsions, nano-encapsulations, and nano-suspensions. The satiety index of food is significantly influenced by its microstructure, which additives, fibers, particles, or bubbles can control. Porous calcium alginate beads can be produced using a T-junction microfluidic device to enhance food products' energy content ratio and sample homogeneity (Burklund et al. 2020).

People are increasingly aware of health issues as the economy grows and living conditions improve. The current approach to identifying toxins such as aflatoxin and botulinum neurotoxin relies on mouse bioassays, which are slow, costly, and involve sacrificing many mice. Microfluidic-based devices offer a solution to the limitations of traditional methods. Major pathogens causing food poisoning include *E. coli* O157:H7, *Listeria monocytogenes*, *Campylobacter jejuni*, *Bacillus cereus*, and *Clostridium perfringens* (Tsao et al. 2016). Approximately one-quarter of the global population faces the risk of foodborne illness due to inadequate food inspection and rapid economic expansion.

Consequently, there is a need for new technologies and strategies to ensure food safety. Microfluidics is employed to achieve rapid and precise results in this regard. This review discusses the recent advancements in microfluidic technology for detecting food pathogens (Table 2). Figure 5 describes the application of microfluidics in the food industry.

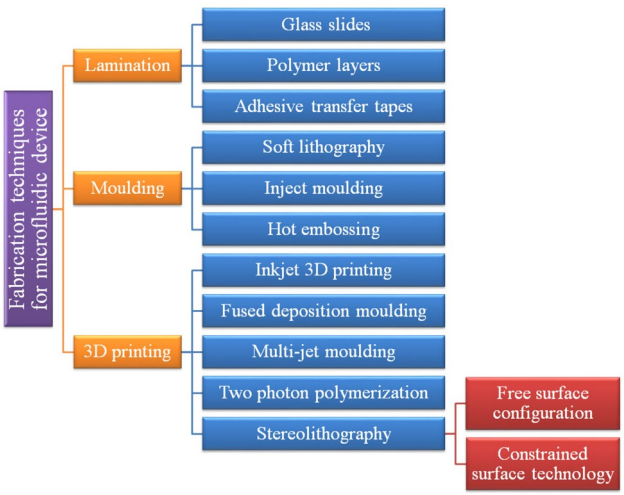

Fig. 4 Different techniques for the fabrication of a microfluidic device

Detection of *Salmonella* spp

Centers for Disease Control and Prevention (CDC) reports indicate that Salmonella infection is the most prevalent among foodborne illnesses in developed and developing countries. Individuals affected by this infection commonly experience symptoms such as dehydration, diarrhea, fever, and stomach cramps. Salmonellosis can manifest as either typhoid or non-typhoid salmonellosis, with the latter typically transmitted through consumption of food or water contaminated with various zoonotic serovars of *Salmonella* Salmonella. Among reported cases, *S. enteritidis* and *S. typhimurium* are frequently associated with severe outbreaks. The primary sources of *Salmonella* contamination include uncooked meat and poultry products and cross-contamination with other foods.

Almalaysha et al. (2024) introduced a disposable MEMS biosensor based on impedance for detecting *S. Typhimurium* in spiked raw chicken samples. By combining focusing and trapping capabilities, the device enhanced the concentration of *Salmonella*Salmonella, which is available for binding with antibodies, thereby increasing sensitivity. The limit of

Table 2 Recent developments in the field of microfluidics for detection of pathogens in food

Microfluidic device	Fabrication method	Limit of detection (LOD)	Channel size	Time taken for analysis	Demonstrated food sample	Features/ advantages	Remarks	Reference
Salmonella typhimurium								
PDMS microfluidic impedance-based device	Photolithography process	3×10^3 CFU/mL	2.5 mm × 0.5 mm	30–45 min	No actual food sample used to demonstrate the device	• High signal-to-noise ratio • Impedance directly proportional to cells bound with antibodies • Reusable	The design is suitable only for the analysis of one sample	Ghosh et al. (2015)
Polyester sheet based microfluidic device	Non-lithographic rapid prototyping	7.7 cells/mL	55 mm × 5 mm	1.2 h	Spiked milk sample	• The potential generated is proportional to the S. *typhimurium* concentration • Multiple samples can be analysed at a time • Easily disposable	Cannot use a high flow rate. Hence it is fixed to 100 µL/min	de Oliveira etl al. (2018)
PDMS based microfluidic nanobiosensor	Photolithography	10^3 CFU/mL	0.4 mm × 0.05 mm	40 min	Chicken extract	• The fluorescence is directly proportional to cell concentration • LOD of the device is better than the other methods, including like lateral flow strip, which can detect 10^6 CFU/mL in 30 min(5)	High flow rate cannot be used because two inlet ports must ensure the proper mixing of the samples	Kim et al. (2015)
PDMS based Microfluidic biosensor	3D printing	33 CFU/mL	2 mm × 8 mm	2 h	Spiked milk samples	• It is specific and sensitive to S. *typhimurium* • Simple fabrication • qPCR and RT-PCR which have 10 detection limit of 10 CFU/mL, but need 24 h (4)	Analysis of one sample is possible	Hou et al. (2019)

Table 2 (continued)

Microfluidic device	Fabrication method	Limit of detection (LOD)	Channel size	Time taken for analysis	Demonstrated food sample	Features/ advantages	Remarks	Reference
PDMS based microfluidics	Photolithography	58 CFU/mL	31 mm × 50 µm	1.5 h	Spiked apple juice	• Online monitoring of pathogen is possible	The optimum flow rate is 20 µl/h to avoid the processing limitation of the smartphone It has a complex detection system that includes fluoresce microscopy	Wang et al. (2019)
PDMS/glass microfluidic device	Photolithography	37 CFU/mL	5 mm × 300 µm	40 min	Pork sample	• Analysis of multiple samples is possible • The presence of baffles ensures efficient mixing	NA	Wang et al. (2019)
Polycarbonate microfluidic	Micro-milling equipment	10 CFU/mL	NA	10 min	Water from chicken package	• Hand held optical detection system • The sample does not require filtration, culturing, and/or isolation • Preloaded reagents • Suitable for field assay	NA	Fronczek et al. (2013)
PDMS based droplet microfluidic device	Photolithography	50 CFU/mL	NA	5 h	Spiked milk sample	• Single cell level detection • Label free detection • Fluorescence imaging-based system	NA	Ng et al. (2021)
PDMS based microfluidic device	Soft lithography	30 CFU/mL	50 µm × 30 µm	1 h	Spiked apple juice	• Shorter result time • Easier extension due to only one antibody and one label • Lower cost due to less expensive materials	Need to integrate magnetic separation of the target bacteria from sample and colorimetric detection to achieve fully automatic detection	Yao et al. (2020)

Table 2 (continued)

Microfluidic device	Fabrication method	Limit of detection (LOD)	Channel size	Time taken for analysis	Demonstrated food sample	Features/ advantages	Remarks	Reference
PDMS based microfluidic device	Soft lithography	300 cells/mL	NA	1 h	Ready-to-eat turkey breast	• It has specificity for different *Salmonella* serotypes • Selectivity on different types of bacterial cells • Distinguish between live and dead cells	NA	Almalaysha et al. (2024)
PDMS based microfluidic device	Soft lithography	168 CFU/mL	NA	25 min	NA	• Colorimetric determination • On-site detection	NA	Jin et al. (2023a)
PES based microfluidic device	Dip method	260 CFU/mL	NA	20 min	Lettuce, chicken breast, and milk	• High sensitivity	NA	Chen et al. (2023)
PDMS based microfluidic device	Soft lithography	3.7×10^1 CFU/mL	NA	30 min	Skim milk	• Equipment free • Visual quantitative detection	NA	Man et al. (2023)
PDMS based microfluidic device	Photolithography	5.0×10^4 cells/mL	NA	6 h	Egg yolk	• It offers a rapid and economic formula to detect viable *S. enterica* in eggs	Detection time is high	Kubo et al. (2020)
Escherichia coli								
PDMS biosensor	Soft lithography	10^3 cells/mL	24 mm × 300 µm	30 min	NA	• Alternative to animal produced anti-bodies • Rapid testing • Detection efficacy will increase for pathogenic E. *coli*	Nonspecific binding caused by interspaces between the beads as well as beads and the microchannel wall	Jeong et al. (2013)
PDMS	3D printing	12 CFU/mL	2.5 mm × 1 mm	1 h	Milk sample	• Possible to detect E. *coli* from large samples • Reaction volume is as low as traditional PCR	Ct value close to the real-time PCR method	Zhang et al. (2018)
PDMS	Photolithography	39 CFU/mL	10 mm × 33 µm	2 h	NA	• Excellent antibody coverage for the electrode surface	NA	Ghosh Dastider (2018)

Table 2 (continued)

Microfluidic device	Fabrication method	Limit of detection (LOD)	Channel size	Time taken for analysis	Demonstrated food sample	Features/ advantages	Remarks	Reference
PDMS	Photolithography	10^2 cells/mL	50 mm × 100 µm	NA	NA	• Sensitive • Does not require PCR	NA	Jiang et al. (2017)
PDMS	Soft photolithography	0.35 CFU/mL	NA	10 h	Spiked Mango juice	• Easy to fabricate • Cost effective	NA	Fande et al. (2023)
Double sided tape	Laser cutting	NA	60 mm × 50 µm	NA	NA	• Silver doped fibre improved the rate of bacteria capture by 100 times	E. *coli* binding to the surface lowers binding efficiency Works with similar efficiency for S. *aureus*	Smith et al. (2020)
PMMA	Lamination using glass slides	10^5 CFUs/mL to 3.2×10^7 CFUs/mL	12 mm × 50 µm	NA	spiked PBS and PD fluid	• No auto fluorescence	NA	Tokel et al. (2015)
Device based on GMI sensor	MEME technology	50 CFU/mL	5 mm × 60 µm	NA	NA	• Reliable, high capture efficiency	NA	Yang et al. (2015)
PDMS	Photolithography	10^7 CFU/mL	-	NA	NA	• Measured by voltage fluctuation by motile bacteria (only viable bacteria considered)	Preferred small population samples	Vural et al. (2018)
PDMS	3D printing	12 CFU/ml	3 mm × 100 µm	5 min	NA	• Online impedance measurement	Linear relationship exist between the impedance measure and bacterial concentration	Yao et al. (2018)
PDMS	Soft lithography	10 CFU/ml (spiked water) and 40 CFU/ml (spiked milk)	NA	2 h(spiked water) and 18 h (spiked milk)	Spiked milk and water samples	• High sensitivity with single molecule resolution	NA	Bian et al. (2015)
Listeria monocytogenes								
Cloth-based microfluidic device	Wax screen printing	1.1 fM	10 mm × 0.4 mm	NA	Spiked milk sample	• Cost effective • Device fabrication does not require trained personnel • High detectability and specificity for the target DNA	NA	Qiuping et al. (2020)

Table 2 (continued)

Microfluidic device	Fabrication method	Limit of detection (LOD)	Channel size	Time taken for analysis	Demonstrated food sample	Features/ advantages	Remarks	Reference
PTFE	NA	0.copies/µL	70 mm × 15 µm	42 min	NA	• Highly sensitive to pathogen detection • Minute quantities of sample is required	Fluorescence detection method used in the study is semi-quantitative or qualitative	Shu et al. (2013)
PDMS	Soft-lithography	NA	9 mm × 100 µm	100 s	NA	• Distinguish between L. *monocytogenes* and L. *innocua*	It can expand for the detection of any bacteria	Rodriguez et al. (2019)
Thermoplastic elastomer (TPE)	Hot embossing	10 CFU/mL	NA	17 min	Beef extract	• Customizable • Low cost	NA	Malic et al. (2015)
Campylobacter jejuni								
PDMS/glass hybrid colorimetric based microfluidic device	Photolithography	1×10^2 CFU/mL for milk 1×10^4 CFU/mL for chicken breasts	40 mm × 500 µm	24-48 h	Spiked samples of milk and chicken breasts	• Simplified sample preparation • Useful for rapid screening and surveillance study • Cross contamination is prevented by zigzag channels	A lag-phase of 8 h experienced for the growth of C.*jejuni* in the device	Ma et al. (2020)
Hybrid PMMA and PDMS microfluidic device	Injection moulding and spin coating	1×10^5 cells/mL	NA	35 min	Spiked drinking water	• Can detect waterborne E. coli and adenovirus • Require low cost of monitoring devices • Rapid detection of up to 16 analytes are possible	Use large volume of expensive chemiluminescence reagents	Matos Pires and Dong (2013)
Norovirus								
Paper based microfluidics	Manually patterned	3.3–4.4 ng/mL	3 mm × 500 µm	10 min	Spiked mussel samples	• Easy fabrication • Low cost	Since nitrocellulose membrane is used the device must be stored in a dry atmosphere away from the sunlight	Weng and Neethirajan (2017)

Table 2 (continued)

Microfluidic device	Fabrication method	Limit of detection (LOD)	Channel size	Time taken for analysis	Demonstrated food sample	Features/ advantages	Remarks	Reference
Paper based microfluidic	Wax printing	1 copy/μL in DI water and 10 copies/μL in reclaimed water	21 mm × 2.4 mm	Assay time is less than 1 min once the images are obtained	DI and reclaimed waste water	• It uses a smartphone- based fluorescence microscope • Nucleic acid amplification is not necessary because of low concentration	Difficult to image non-aggregated particles	Chung et al. (2019)
PDMS-PMMS microfluidic device	Mono-lithography	10^2 PFU/singles oyster	NA	< 4 h	Oyster sample spiked with murine norovirus	• Automated cell concentration, RNA extraction, nucleic acid amplification and detection • Separate chambers for each step • 50% of sample recovery from the sample	NA	Chung et al. (2015)
Staphylococcus aureus								
PDMS microfluidic device	Photolithography	1.5 × 10 CFU/μL	200μm × 100μm	4 min	Spiked drinking water	• Use of immunospheres increase the capture area • Higher the sample flow rate lesser the time for analysis • Capture rate of microspheres decreaseswith decreasein concentration	High flow rate (> 10μL/min) affect the stability of fluorescence signal High level of immunospheres result in multilayerarrangement	Song et al. (2020)
PDMS microfluidic device	Soft lithography	NA	NA	< 30 min	NA	• Deposition of nanoparticles are avoided by segmented flow • Utilize SERS biosensor	NA	Xiaonan et al. (2013)

Table 2 (continued)

Microfluidic device	Fabrication method	Limit of detection (LOD)	Channel size	Time taken for analysis	Demonstrated food sample	Features/ advantages	Remarks	Reference
Paper based microfluidic device	CO_2 laser engraver	80 CFU/mL	600 µm × 600 µm	60 min	Orange juice, milk	• Human serum or food can be used as sample • Method of fabrication is easy	Optimal reaction time is high	Azam Bagheri and Hosseini (2020)
Clostridium perfringense								
PDMS microfluidic device`	Soft lithography	100 cells/L	NA	1 h	Spiked water sample	• Possible to quantify multiple food and water pathogens in environment • It is possible for the multiple sample analysis during routine monitoring	Optimal reaction time is high	Ishi et al. (2013)
Bacillus subtilis								
Paper based microfluidic device	Wax printing	10 pg/µL	NA	min	NA	• Inexpensive • Quantify nucleic acid	NA	Roy et al. (2017)
Legionella pneumophila								
PDMS microfluidic device	Soft lithography	10^4 cells/ mL	500 µm × 15 µm	1.5 h	Spiked fresh water	• Portable device • On-site and good for initial screening	NA	Yamaguchi et al. (2017)
Digital microfluidics (DMF)	Standard lithography	0.5 µM to 122 pM	100 µm × 10 µm	30 min	DI water	• Fast and quick analysis	NA	Foudeh et al. (2015)

NA, Not applicable

Fig. 5 Applications of microfluidic technology in the food industry

detection (LOD) was established as 1–2 cells/mL by analyzing poultry samples with varying concentrations across a frequency range of 100 Hz to 10 MHz.

A novel colorimetric biosensor was developed using a microfluidic chip for rapid, sensitive, and on-site detection of *Salmonella* Salmonella. This biosensor utilizes immune gold@platinum nanoparticles (Au@Pt NPs) for specific bacterial labeling, a finger-driven mixer with two air chambers for efficient immunoreaction, and a nuclear starstruck membrane for effective bacterial isolation. This simple biosensor achieves *Salmonella* detection as low as 168 CFU/mL within 25 min (Jin et al. 2023a). Chen et al. (2023) developed an origami microfluidic device for rapid *S. enterica* detection, from sample preparation to endpoint detection, featuring nucleic acid extraction on a paper dipstick, isothermal recombinase polymerase amplification (RPA), and lateral flow assay for results. They achieved nucleic acid amplification in 20 min with 100% *S. enterica* specificity. The Polyethersulfone (PES) based device's LOD was 260 CFU/mL. Compared to chromatography paper, PES showed superior sensitivity, detecting *S. enterica* in lettuce, chicken breast, and milk at 6 CFU/g, 9 CFU/g, and 58 CFU/mL, respectively, after 6 h enrichment. PES displays excellent compatibility with isothermal nucleic acid amplification, holding promise for integrated sample-to-answer microfluidic devices in pathogen detection across various food products. A portable microfluidic bio-aptasensor, SSBP-NCMS, incorporating horseradish peroxidase as detection probes, was utilized to detect *S. typhimurium*. This aptasensor can detect concentrations as low as 3.7×10^1 CFU/mL, demonstrating excellent specificity and reliability (Man et al. 2023).

A novel figure-actuated microfluidic biosensor was created to sensitively detect *S. Typhimurium* with immunomagnetic separation, isolate the target bacteria, and combine rolling circle amplification (RCA) with CRISPR/Cas12a to enhance the detection signal. It has a 1.93×10^2 CFU/mL LOD and approximately 106% mean recovery in spiked milk samples (Xiang et al. 2023). A versatile integrated microfluidic biosensing system was developed to rapidly and sensitively detect *S. typhimurium.* It features a microfluidic chip with adaptable valves, RPA for nucleic acid detection, and a custom real-time fluorescence detection system. It has a LOD of 1.0×10^2 copies/μL within 30 min, aligning with results from real-time quantitative polymerase chain reaction (qPCR) tests. With its adaptable valve, this system holds promise for on-site foodborne pathogen detection (Jin et al. 2023a, b). A portable detection device was created for rapid, sensitive, and straightforward detection of viable *SalmonellaSalmonella.* It utilizes a finger-actuated microfluidic chip and an enhanced recombinase-aided amplification (RAA) assay. The fluorescent signal from RAA amplification is captured using a USB camera and analyzed through a mobile application for quantitative bacterial concentration determination. It has an LOD of 1.3×10^2 CFU/mL to 1.3×10^7 CFU/mL within two hours (Qi et al. 2023). A sophisticated colorimetric biosensor was developed for swift and automated detection of *Salmonella*Salmonella within a sealed microfluidic chip. It quantitatively detects *Salmonella*Salmonella within 30 min, with an LOD of 10^1 CFU/mL (Lu & Wang 2023). The VETPOD is a ready-to-use cartridge with a LAMP-based polymer chip designed to address contemporary challenges in swiftly detecting *Salmonella* spp. This has a LOD of 1.38 CFU/25 g, slightly surpassing the reference method by 1.17 times (Vinakaya et al. 2023).

Oliveira et al. (2018) developed an easily disposable microfluidic device (DμFD) using a non-lithographic rapid prototyping method in which multiple samples can be analyzed simultaneously. Unlike other microfluidic devices, the microchannel is made of carbon ink. Similarly, Kim et al. (2015) demonstrated the detection of *Salmonella*Salmonella labeled with antibody conjugated with fluorescent Quantum Dots using a magnetic bead in a PDMS-based microfluidic device. The fluorescence that develops thus can be directly correlated with cell concentration. Interdigitated electrode arrays (IDM) are used to measure any sample in which cells are suspended. Gosh et al. (2015) developed a gold IDM array biosensor using photolithography. The impedance is directly proportional to the concentration of bacteria bound to the antibodies. The high signal-to-noise ratio, re-usability of the device, and short detection time are the salient features of the device. The developed device has a lower detection level of 3×10^3 CFU/mL and is comparable with other impedance biosensors (Wang et al. 2020; Huang et al. 2021). A microfluidic biosensor was engineered to detect SalmonellaSalmonella with a biosensor employs a metal–organic framework (MOF) known as NH2-MIL-101(Fe), which

exhibits peroxidase-like activity to amplify biological signals with LOD 14 CFU/mL (Qi et al. 2021).

A microfluidic biosensor was designed for *Salmonella* detection, employing viscoelastic inertial microfluidics to separate magnetic bacteria from unbound magnetic nanoparticles (MNPs). It utilizes enzyme-catalytic colorimetry to amplify biological signals. This detects *SalmonellaSalmonella* at concentrations as low as 30 CFU/mL within one hour (Yao et al. 2020). A detection method for *S. enterica* using PCR on a microfluidic disc device with a fluorescent probe was developed. *S. enterica* was detected at concentrations as low as 5×10^4 cells/mL or higher concentrations in egg yolk within six h, including the sampling time (Kubo et al. 2020). Wang et al. (2019) developed a similar but different detection system which consists of a light source, fluorescent microscope, and smartphone. The sensitivity of this study can be improved by using brighter fluorescent material. The only limitation of this study is the video processing speed and image capturing quality of the smartphone used. The use of a higher-performance camera/smartphone can overcome it. Fronczek et al. (2013) developed a handheld microfluidic device for Mie scattering detection of *S. typhimurium* in meat or water from poultry packaging. The complex chicken samples can be directly pipetted to the vacuum-dried reagents device. Ng et al. (2021) developed a single-cell droplet microfluidic system to detect viable *Salmonella* as an alternative. The fluorescent imaging system is used to distinguish between encapsulated single cells. After droplet generation, each contains pL to nL, collected for culturing them. A noticeable change in the microdroplet's fluorescence can be observed in less than five hours. In short, this droplet microfluidics can be used for single-cell-level analysis with a LOD of 50 CFU/mL, which helped to curtail detection time from 24 to 5 h.

However, Salmonella Shigella (SS) agar can provide results within 24 h, offering a faster alternative (Dekker and Frank 2015). Furthermore, the evolution of microfluidic technology has revolutionized pathogen detection, offering miniaturized handheld devices with enhanced sensitivity. Integrating microfluidic devices with impedimetric biosensors, PCR, enables real-time monitoring and assessment of pathogens, significantly reducing time and workforce requirements.

Detection of *Escherichia coli*

Escherichia coli is one of the critical microorganisms of concern regarding food safety. They are gram-negative and non-spore-forming bacteria which is found in our intestines. Consumption of food contaminated with the feces of animals or infected humans is the primary source of *E. coli* infection. Other standard vehicles of contamination are raw milk, cross-contamination, uncooked ground meat, manure from cattle, or other animals as fertilizer.

A novel dual-mode hydrogel array biosensor was developed to quickly screen and accurately quantify virulent *E. coli* O157:H7 using a DNA-modified phage probe specific to *E. coli*. These probes are hybridized with complementary DNA (cDNA) to create double-stranded DNA fragments (phage@RCA-dsDNA), which are visually detectable through SYBR green dye fluorescence and smartphone analysis for rapid screening. Additionally, unreacted cDNA in the supernatant was quantitatively measured via microfluidic chip electrophoresis (MCE), with signal reduction correlating to bacterial concentration with LOD 6 CFU/mL. This also detects *S. typhimurium* using *the S. typhimurium* phage@RCA-DNA probe (Xu et al. 2024). Antimicrobial peptides (AMPs) are gaining popularity due to their ability to bind effectively to multiple target microorganisms due to their cationic and amphiphilic nature. Jeong et al. (2013) replaced antibodies with Magainin-1, which has superior antimicrobial properties. The device can identify *E. coli* concentrations of 10^3 cells/mL under 30 min. The non-specific binding was eliminated by converting fluorescence images into black-and-white photos and subtracting the background images. To attain rapid detection, a higher flow rate of 4 µL/mL should be applied to achieve a saturation detection level in 30 min. The detection efficiency and time required were not dependent on the bacteria concentration till 10^3 cells/mL (Yoo et al., 2014). Another study used an electrochemical sensing system to detect *E. coli*. The immunosensor biochip integrated with nanoporous alumina membranes was immobilized with antibodies using a GPMS silane monolayer. The setup consisted of upper and lower compartments with nanoporous alumina membranes in the middle. When the target bacteria enter the upper compartment, they get anchored by the antibody covering the nanopores, thus generating the electrolyte current, which the impedance spectrum can monitor. PDMS-based microfluidic devices for digital droplet PCR were used to detect *E. coli* O157:H7 and *L. monocytogens* simultaneously. Droplet digital PCR (ddPCR) amplifies the target DNA into a different droplet, which helps the user quickly quantify DNA copy numbers (Bian et al. 2015).

Plasmonic sensor technologies were explored using surface plasmonic resonance (SPR) technologies. Protein-G-based surface chemistry with anti-LPS antibodies was exploited to construct a gold-coated device that is disposable, rapid, and selective. The device is equipped with a single micro-channel and custom-made SPR platform based on the principle of Kretschmann configuration, which uses prism coupling to satisfy momentum conservation for plasmon excitation by an external light source (Tokel et al. 2015). Yang et al. 2015 detected *E. coli* using a Giant Magneto-Impedance (GMI) sensor in conjugation

with a gold-coated open-surface microfluidic device. Magnetic beads with streptavidin monolayer covalently coupled to the hydrophilic bead surface were also incorporated. The principle was that detecting the fringe field (Hf) of the dynabeads using magnetic sensors could detect the presence, content, or absence of *E. coli*. This technique can detect concentrations ranging from 50 to 1000 CFU/mL with high capture efficiency. Jiang et al. (2017) used immobilized poly-(amidoamine) dendrimer on PDMS microchannels to develop a fluorescence intensity-based microfluidic detection platform. The microchannels were modified with DNA aptamers, and the fluorescence signal was enhanced up to 50 times by Rolling Circle Amplification (RCA). The LOD of the dendrimer-Aptamer-RCA system is 10^2 cells/mL. Zhang et al. 2017 developed a coaxial channel-based DNA extraction in conjugation with microfluidic PCR. Dastider et al. (2018) conducted another interesting study with MEMS biosensors for rapid and accurate detection of *E. coli* O157:H7 with a LOD value of 39 CFU/mL. The focusing area directs the flow towards the channel's centerline using p-DEP force and fluid drag flow. The change in impedance caused due to antigen–antibody binding is measured using an impedance analyzer. This device requires no enriching step and takes approximately two hours to process.

Alternatively, the *E. coli* inactivation kinetics was determined using a novel microfluidic mixer via chlorine solution in the sub-second range due to the high mixing rate and the ability to control the flow rate in microfluidic chambers. The design incorporated three mixing designs: "Y" inlet junction, Dean's vortex mixer, and chaotic mixer with three inlets for *E. coli*, chlorine solution, and dechlorinating agent. The contact time was quantified using the CFD model, and pathogen survival was measured using the most probable number (MPN) method (Yao et al. 2018).

A rapid and sensitive microfluidic-based electrochemical device was developed to detect *Escherichia coli*. It demonstrated a linear range of bacterial concentrations from 0.336×10^{12} to 40×10^{12} CFU/mL, with a LOD of 0.35 CFU/mL and a quantification limit of 1.05 CFU/mL (Fande et al. 2023). In conclusion, studies suggest that microfluidic platforms designed for detecting *E. coli* eliminate the need for a large and tedious experimental setup and reduce detection time from 48–72 h to 0.5–5 h. This provides a broad application not only in ensuring food safety but also in disease control.

Detection of *Listeria monocytogenes*

Listeria monocytogenes, a gram-positive, is the second most common foodborne disease, causing 30% fatality. The major reservoirs include unpasteurized or raw milk and their products, raw fruits and vegetables, and uncooked meat products. Immunoassays are widely used for rapid on-site detection, but their poor affinity and low detectability make them less reliable. Shang et al. 2020 developed an ultrasensitive cloth-based microfluidic device to detect *L. monocytogenes* using chemiluminescence methods. Coupling microfluidics with PCR has benefits like consuming fewer reagents/samples, portability, and miniaturization of the whole process, which helps in the rapid on-site detection of the pathogen. Strohmeier et al. (2014) developed a highly sensitive microfluidic single-phase continuous flow nested PCR device for the detection of food pathogens, which can amplify and detect the genomic DNA of *L. monocytogenes* within 42 min. Microfluidic devices helped to miniaturize the nucleic acid amplification setup, such as PCR, recombinase polymerase amplification, hybridization chain reaction (HCR), and loop-mediated amplification (LAMP), which require several days. Rodriguez et al. (2019) developed a LOC device with gold nanostars as an SERS tag to detect *Listeria* spp. within 100 s. The device can also distinguish between *L. monocytogenes* and *L. innocua*. Malic et al. (2015) developed a high gradient magnetic separation (HGMS) microfluidic device with immune-magnetic nanoparticles and a 3D magnetic capture chip. This capture region dramatically depends on the magnetic shell, the magnetic field's strength, and the pillar's geometry. Within 17 min, a three-fold increase in concentration efficiency is achieved, which makes this device ideal for ensuring food safety and can be expanded as a point-of-care device.

The conventional culture method necessitates at least five days of ascertaining the absence of *Listeria* in food samples. If *Listeria* spp. or *L. monocytogenes* are present, an additional ten days are required to identify their presence. With the help of microfluidic-based detection devices, food safety was revolutionized by swiftly assessing the quality of food products in less than 4 h. This remarkable advancement accelerates the detection of harmful microorganisms, enhancing the monitoring process significantly.

Detection of *Campylobacter jejuni*

The development of antibiotic-resistant strains of *Campylobacter* to clinically essential drugs such as tetracycline, fluoroquinolones, and ampicillin is a daunting concern to the health sector. The increasing rate of bacterial gastroenteritis in humans is *caused by Campylobacter*-borne food infections. The primary sources of *Campylobacter* infections are contaminated poultry meat and unpasteurized milk. The standard approach has several drawbacks. A minimum of 7–9 days is required for the analysis, which is a laborious process biased towards *C. jejuni.*

Ma et al. (2020) developed a rapid testing polymer-based microfluidic device for *Campylobacte*r identification using chromogenic changes. The device chamber had two primary reagents, a chromogenic medium, and antibiotics, which

successfully detected multiple species of *Campylobacter* in 24 h. This technique finds application in complex food matrices such as milk and chicken breast with identification under 60 h. The device is also capable of identifying *Campylobacter* in a complex bacterial cocktail. This device is far superior to the conventional method due to its rapid detection, less sample consumption, and user intervention. In another study, a hybrid PMMA-PDMS-based multiplexed microfluidic biosensor was integrated into an array of poly-(2,7-carbazole/fullerene organic blend) heterojunction photodiodes (PHPDs) were used for the chemiluminescent detection of *C. jejuni* with LOD of 1×10^5 cells/mL with an assay time of 30 min.

Yunxuan et al. (2023) developed a convenient, hybrid microfluidic device incorporating paper and polymer components. This cutting-edge technology seamlessly combines paper-based DNA extraction, isothermal nucleic acid amplification, and lateral flow detection, providing a user-friendly solution for molecular analysis. The study for detecting *C. jejuni* using microfluidics in recent years is low; its edge over the conventional methods unfolds a rapid, convenient, and on-site detection tool.

Detection of Norovirus

Norovirus is a prevalent cause of gastroenteritis. Current detection methods involve immunoassays and RT-PCR. Conventional techniques like ELISA and nucleic acid-based assays are resource-intensive. Using 6-carboxyfluorescein aptamer and nitrocellulose, paper-based microfluidics offer cost-effective, easy-to-make alternatives to PDMS, silicon, or glass devices (Weng and Neethirajan 2017).

In another study, antibody-conjugated, submicron, fluorescent (yellow-green) polystyrene particles and the norovirus were "visualized" down to the single virus copy level on the microfluidic paper analytic devices (μPAD). The antibody-conjugated particles and norovirus could flow (capillary action), and only the aggregated particles were isolated through image processing and a smartphone-based fluorescence microscope. This rapid technique does not require washing, sample concentration, or nucleic acid amplification steps (Chung et al. 2019). Detecting norovirus in agriculture, marine, and foods is as essential as other food pathogens. Detection of viruses in any samples involves tedious sequential steps such as target concentration, cell lysis, amplification, and detection. Since norovirus is an RNA virus, only 3–30% of sample recovery is achieved in 24 h. Chung et al. (2019) developed a microfluidic device with switchable charge microbeads that can automatically perform the previously mentioned processes in separate chambers. These microbeads adsorb the norovirus particle from the sample and lyse via bead beating. The standard procedure for norovirus detection takes around 24–48 h; automation made it less than 4 h.

Thus, fabricating microfluidic devices with reduced cost of production, assay time to less than 5 min, and sensitivity to exceedingly low LOD for detection of norovirus from samples such as food, fomites, and water is essential, which can eliminate the requirement of nucleic acid amplification used in PCR methods and in-vitro cell culture to detect viruses. Figure 6 depicted different foodborne microorganisms and microfluidic technologies.

Other pathogens and toxins

S. aureus is the most common food pathogen, a gram-positive, rod-shaped bacterium. The toxin, coagulase, causes infection in both humans and livestock. Like all other food pathogens, the microbial culture method is considered the golden standard. To date, rapid detection technique for detecting and identifying bacteria includes PCR, LAMP, ELISA, quartz crystal microbalance (QCM), electrochemical

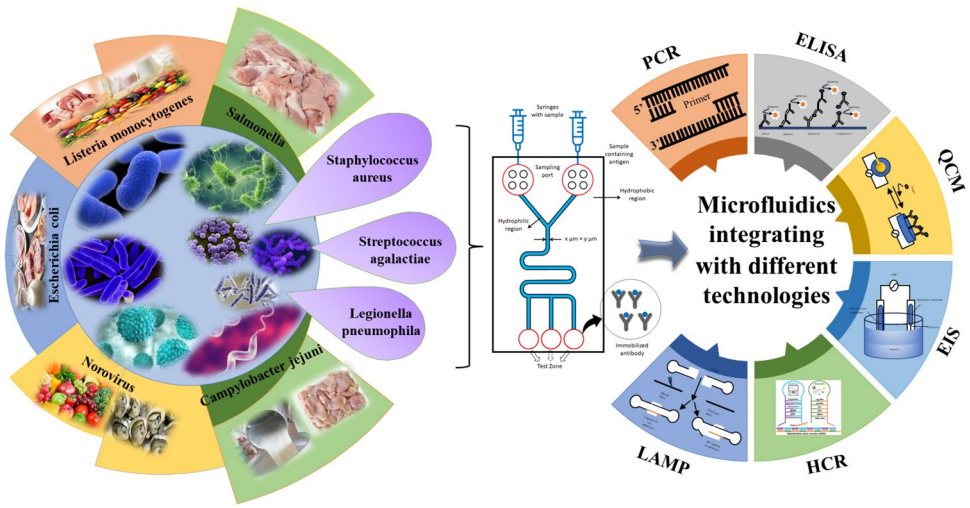

Fig. 6 Different foodborne microorganisms and microfluidic technologies. PCR, Polymerase Chain Reaction; ELISA, Enzyme-linked Immunosorbent assay; QCM, Quartz Crystal Microbalanace; EIS, Electrochemical Impedance Spectroscopy HCR, hybridization chain reaction; LAMP, Loop-mediated Amplification

impedance spectroscopy (EIS), and fluorescence microscopy. Song et al. (2020) developed a microfluidic platform immobilized with antibody-coated microspheres. It has a 1.5×10 CFU/μL LOD, whereas the traditional method has an LOD of 0.5 MCF. They reduced the detection time to 4 min from 18–24 h. Xiaonan et al. (2013) developed a rapid detection of *S. aureus* in the food sample. This device has coupled two different confocal micro-Raman spectroscopic systems to microfluidic platforms for recording the SERS spectral features of *S. aureus*. Azam Bagheri and Hosseini (2020) developed a paper-based microfluidic colorimetric nano biosensor device for rapid, easy, and sensitive detection of *S. aureus*. They use ultra-small nanoclusters (NC) using DNA as a template in a one-pot chemical process and set as a bio-receptor conjugation for the detection of whole-cell bacteria. The linear dynamic range of the colorimetric Au/Pt/NC-based optical sensor covered a range of *S. aureus* concentrations from 10^8–10^2 CFU/mL with a detection limit of 80 CFU/mL. A PDMS microfluidic impedance immunosensor for *E. coli* O157:H7 and *S. aureus* detection via antibody-immobilized nanoporous membrane was developed by Tan et al. (2011). The impedance spectrum was recorded for bacteria detection ranging from 1 Hz to 100 kHz. For both pathogens, the maximum impedance reported for the device was around 100 Hz. The detection time for the device is within two hours with LOD 10^2 CFU/mL.

C. perfringens is usually found on raw meat and poultry, in the intestines of animals, and the environment. The direct quantification of multiple pathogens has been desired for diagnostic and public health purposes for a long time. Ishi et al. (2013) applied microfluidic quantitative PCR (qPCR) technology to detect and quantify multiple food- and waterborne pathogens. They have quantified *E. coli*, *Salmonella* spp., and *Campylobacter* spp. Roy et al. (2017) have developed calorimetric nucleic acid detection on paper microchips using loop-mediated isothermal amplification and crystal violet dye. DNA extracted from the food sample was amplified using LAMP. *Legionella pneumophila*, a gram-negative human pathogen, has an outbreak rate lower than that of sporadic cases. Many Legionnaires' disease (LD) outbreaks have been linked to various sources of contaminated water in hospitals, industrial facilities, hotels, family residences, and cruise ships. Yamaguchi et al. (2017) developed an on-site monitoring of *L. pneumophila* in cooling tower water using a portable microfluidic system. The LOD was 10^4 cells/mL with a detection time of 1.5 h. It is portable for on-site measurement and can be used to initially screen for *Legionella* contamination in freshwater. Foudeh et al. (2015) have developed rapid and multiplex detection of Legionella's RNA using digital microfluidics with a limit of detection of 0.5 μM to 122 pM within 30 min. Similarly, the detection of various toxins can be determined using microfluidic devices. L-glutamate, an amino acid, is considered for food safety in consideration of the Chinese restaurant syndrome, Alzheimer and Parkinson's disease. On-chip bead-based microfluidic devices provide 91% selectivity for the determination of L-glutamate in food samples. *S. aureus* produces staphylococcal enterotoxin B (SEB), which is commonly associated with food poisoning. Yang et al. 2015 developed a high throughput optical carbon nanotube (CNT) based microfluidic device for detecting SEF with LOD 0.1 ng/mL. Aflatoxins, the most predominant and toxic mycotoxins, are often detected in commodities like grains, nuts, beer, and coffee. Ma et al. (2020) developed a portable visual, quantitative detection of aflatoxin B_1 using a distance readout microfluidic chip with LOD 1.77 nm (0.55 ppb). Liu et al. (2013) reported a microfluidic chip-based nano-LC coupled to a triple quadrupole mass spectrometer (QqQ-MS) system for the quantitative determination of aflatoxin in food samples. The samples can be assayed in a single test with LOD 0.004 to 0.008 ng/g. Hu et al. (2013) also developed a microfluidic smectite polymer nanocomposite sensor for aflatoxin detection. Spiked corn extraction solution with aflatoxin was tested on this chip and completed in 10 min with LOD 5–80 ppb. Olcer et al. (2014) developed a real-time microfluidic electrochemical method for the detection of deoxynivalenol (DON) in wheat with a limit of detection of 6.25 ng/mL. Zhang et al. (2015) developed a PDMS microfluidic passive pumping array chip with a real-time biosensor for marine toxin detection with LOD 200 μg/L. Botulinum, the main toxin responsible for foodborne botulism, was also detected on a Forster resonance energy transfer (FRET)-based microfluidic device (Bae et al. 2015). Babrak et al. (2016) also developed a 96-well microfluidic immunoassay device with a spiral microchannel in each well to detect botulinum toxin with a detection limit of 0.9 ng/mL in less than 30 min. Wang et al. (2024) developed portable, integrated microfluidics for rapid and sensitive diagnosis of *Streptococcus agalactia*. It was a susceptible and specific device.

Future scope and challenges

Ensuring food safety remains a paramount concern for humanity, presenting a formidable challenge to the food industry. Conventional analytical methods for detecting food pathogens are often time-consuming and labor-intensive, underscoring the need for a more efficient, reliable, sensitive, and rapid approach. Microfluidics emerges as a transformative solution, enabling the creation of miniature laboratories on a chip. Leveraging advancements in microfabrication technology and discovering novel receptors and detectors, microfluidic-based food analysis devices are poised to revolutionize food safety practices shortly.

Microfluidics offers a compelling alternative to traditional detection methods, significantly reducing time, reagent usage, and workforce. With a focus on common foodborne pathogens like *Salmonella* and *E. coli*, microfluidic platforms enable handheld, rapid, portable, and on-site pathogen detection. These devices can be enhanced with PCR, ELISA, SPR, and LAMP techniques to enhance detection efficiency. However, successful integration with rapid detection methods necessitates careful consideration of various aspects, often overlooked in current endeavors. Smartphone integration holds promise for empowering consumers to conduct competent food examinations before consumption, contributing to enhanced food safety.

Despite recent strides in user-friendly and cost-effective fabrication methods, challenges persist in large-scale production and integration with other technologies. Parallelization emerges as a favored scale-up strategy, while advancements in three-dimensional channel design offer promising avenues for scaling up droplet microfluidics. However, obstacles such as low spatial resolution and high production costs still need to be solved. Although microfluidics holds immense potential, particularly in creating complex emulsions, it is still in its nascent stages, leaving ample room for improvements in mass production techniques. Thus, microfluidics incorporating immune-based techniques is a cornerstone in realizing the "farm-to-fork" food safety vision.

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