
PHD NOTES
Rajie

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INTRODUCTION

1.1 General Overview

The widespread occurrence of Salmonella contamination represents an ongoing public health challenge, causing numerous foodborne illnesses globally. Traditional Salmonella detection methods have significant limitations that prevent quick and precise pathogen identification.

Established techniques like culture-based approaches and PCR are resource-demanding, slow, and sometimes lack the necessary sensitivity for early detection. These methods typically require specialized equipment and trained technicians, restricting their use across different environments, particularly in settings with limited resources or during emergency situations.

There is a clear demand for Salmonella detection systems that are quick, economical, and simple to use, considering the serious health implications of delayed identification. Conventional methods often result in extended response times, potentially facilitating infection spread and hampering effective public health responses.

Additionally, as food supply chains become increasingly interconnected worldwide, there is a growing need for detection technologies that can be readily implemented in various contexts, including laboratories, agricultural settings, and field locations.

To overcome these obstacles, a fundamental shift toward innovative detection approaches is necessary with respect to methods that allows in-situ real-time verification,enhance sensitivity and specificity while also improving accessibility and cost-effectiveness.

Developing a versatile and compact Digital Microfluidics Platform (DMF) with integrated electrochemical (EC) sensors could help identify specific Salmonella bacterial species responsible for outbreaks.

This initiative seeks to enhance public health strategies and enable prompt Salmonella identification, thereby reducing the global impact of foodborne outbreaks.

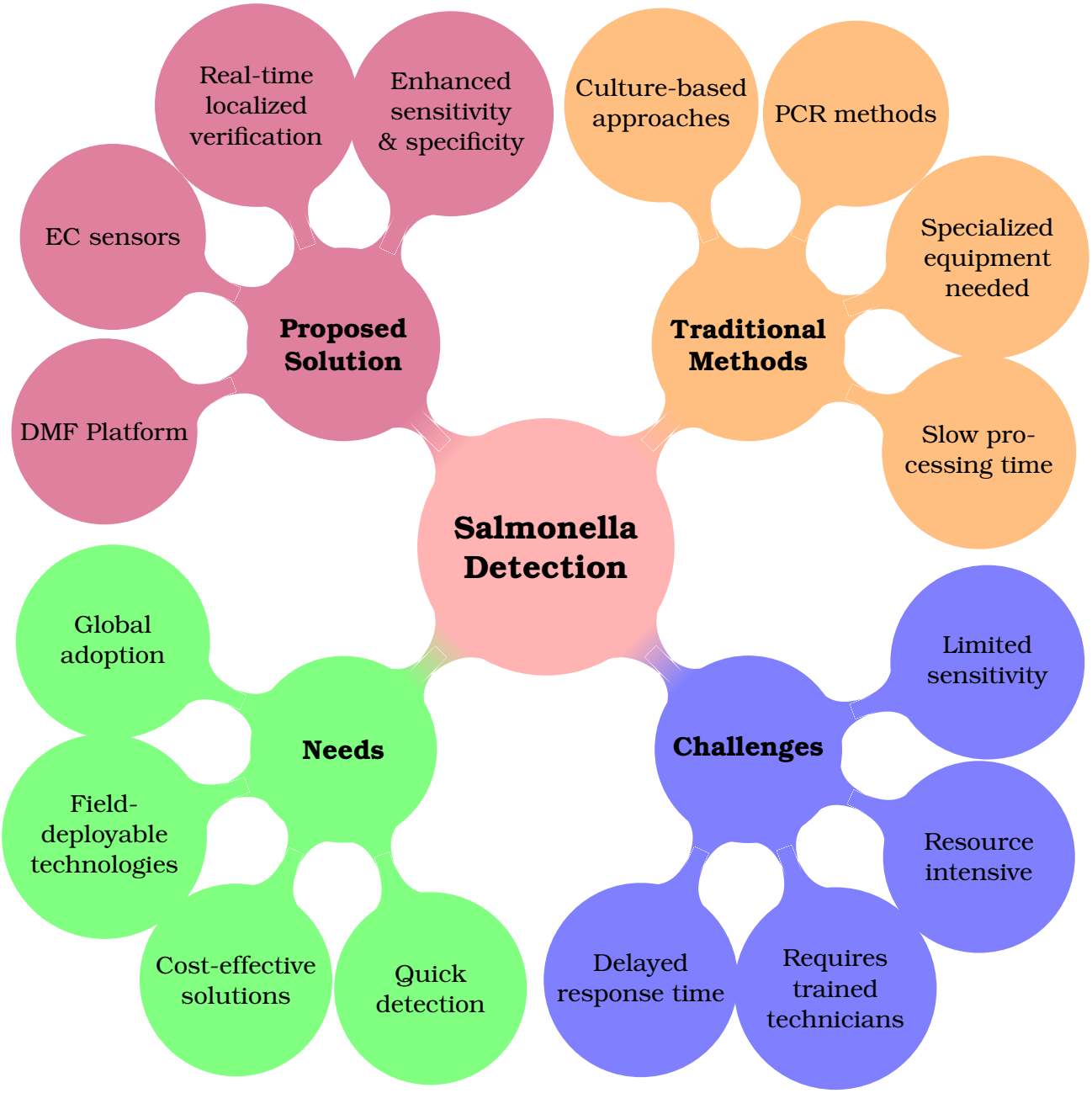


Figure 1.1: Mind map illustrating the challenges of traditional Salmonella detection methods and the proposed Digital Microfluidics Platform solution with integrated electrochemical sensors.

1.2 Problem Statement

The persistence of salmonella contamination poses a threat to public health, leading to a significant number of foodborne illnesses worldwide. Identifying which salmonella species contributed to an outbreak are often impeded by the inherent limitations of the conventional methods employed for detecting this pathogen such as time-consuming and labor-intensive procedures [1].

These techniques also require specialized laboratory equipments, trained staff and large volumes of samples, which limits their usefulness in a variety of contexts, particularly those with low resources or in times of emergency [2, 3].

To address these issues, microfluidic techniques have been employed to reduce the volume of samples and reagents needed for bacteria sample preparation, fast reaction and automatic operation [4, 5] prior for detection procedures via electrochemical (EC) or optical sensors.

Microfluidics techniques however involves in designing complex geometries, inclusion of micropumps and microvalves to prepare the samples and produce the necessary results which can be time-consuming [6]. Therefore, it's necessary to find a different approach when creating a platform that can prepare samples and identify *Salmonella* species without relying on extra hardware or intricate geometries.

DMF is a liquid handling technology which manipulates fluids into discrete droplets on a surface of an array of electrodes through non-contact forces such as electrical, magnetic or thermal [4, 5].

Compared to the microfluidics approach, this method would enable sample volumes to be lowered to nanoliters. Additionally, because the generated sample droplets can be precisely manipulated, samples in droplet form can be further divided into smaller droplets for parallel detection.

For bacteria samples identification, optical sensors are often used as compared to EC sensors when combined with DMF-based platform due to the EC sensor lifetime contributed by the rapid dissolution of reference electrode (RE) made up of silver chloride (AgCl) [7].

These systems are, however, expensive and not portable for conducting sample preparation and identification for on-site detection [8]. Hence, this study would investigate and develop a DMF-based platform to be integrated with an array of improved EC sensors to perform *Salmonella* bacteria species detection.

Previous works are concentrated on detecting a specific pathogen from a droplet [8–11] whereby a singular peak detected during the identification process or changes in colour signifies the pathogen existence in the sample. There are ,however , less reports on detecting multiple species of a targeted pathogen such as *Salmonella* bacteria.

To address this, a probability method such as Fuzzy Logic can be introduced to help in detecting the probability of *Salmonella* bacteria species extisted within the prepared sample. The capability of Fuzzy Logic in determining the pathogen species should be investigated in terms of the sample concentration and voltage resulted from the EC sensor read-out.

1.3 Research Objectives

The main objective of this research is to develop a portable and scalable DMF platform for multiple Salmonella bacteria species detection. Specific objectives are as follows:

1. To develop a DMF platform for move, merge, split and dispense Salmonella bacteria sample in microliter (μL)
2. To integrate the developed DMF platform with an array of EC sensors
3. To develop and evaluate Fuzzy Logic in determining the presence of Salmonella species within a sample.

1.4 Notes Organization

The organization of these notes are structured as below;

1. Chapter 1: Introduction
   Provides a general overview on salmonella and the detection method, its limitations and how DMF integrated with EC and Fuzzy Logic could provide a solution.

2. Chapter 2: Literature Review
   Provides a review on the three topics and critically review the concepts, keypoints, pros and cons, approach and the technology

3. Chapter 3: Methodology
   Presents the methods for the project and milestones

4. Chapter 4: Results and Discussion
   Presents the findings and discuss the relevancy and whether its corroborated with the literature

5. Chapter 5: Conclusion
   Presents whether the objectives has been met

LITERATURE REVIEW

2.1 General Overview

Salmonella bacteria are the pathogens that are capable to cause health related problems such as gastroentritis and enteric fever, in which an infection normally be caused by contaminated water and food [12–15].

Current Salmonella detection methods that are available are listed in Figure 2.1 [16]. Most of these methods required expensive equipments, well-trained personal and no *in-situ* testing [2, 3]. Furthermore, the time needed to detect the pathogen ranged between several hours to 4 hours [16].

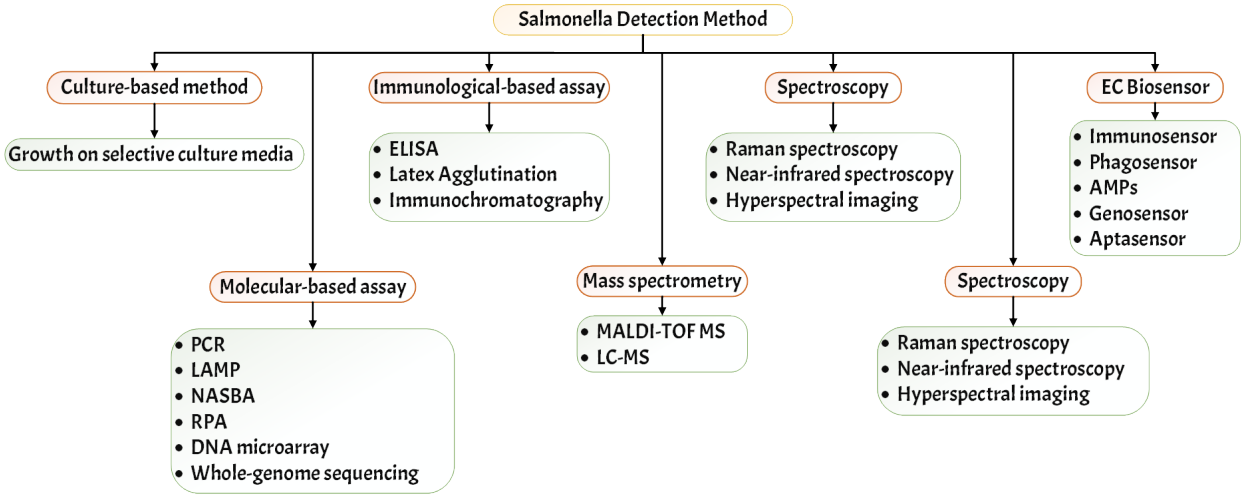


Figure 2.1: Current available methods for Salmonella detection (adapted from [16])

Due to these limitations, developments of rapid and sensitive detection methods of this particular pathogen has been researched to prevent another outbreak from occuring. The main concern is the modularity and compatibility with other techniques to have the sample to be tested on site and provide prelimninary finding before the sample is sent to the laboratory for more detail analysis.

One of the rapid detection methods in the biomedical space is DMF whereby droplets were generated from liquids in microliter (µL) and manipulated on an array of electrodes to perform protocols before the droplets are actuated to the sensing block for detection procedures.

This would allow parallel process to be conducted on the same electrodes, which helps in reducing waste and provide economical handling of the samples for on-site testing purposes [17].

The organization of this literature review notes are structured as below:

1. Section 2.1 provides general overview on salmonella bacteria
2. Section 2.2 provides the available methods (conventional/alternative) used for salmonella detection and the advantages/disadvantages of the said methods.
3. Section 2.3 provides DMF background, timeline of DMF research, and key operations of DMF
4. Section 2.4 provides available DMF fabrication, actuation and validation methods
5. Section 2.5 provides the summary of literature review

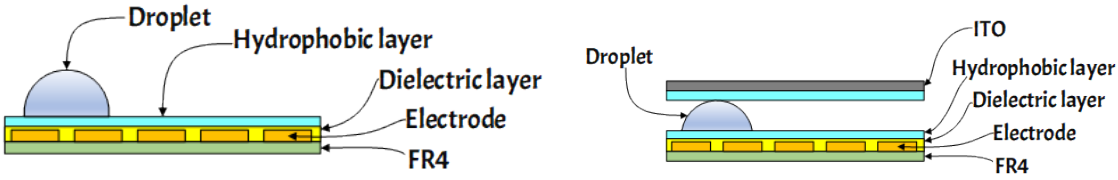
2.2 Digital Microfluidics (DMF)

Digital microfluidics (DMF) represents a significant departure from traditional microfluidic techniques. Instead of relying on continuous flow through intricate channel geometries and external micropumps, DMF controls liquid movement by manipulating individual droplets on a planar surface using localized electric fields applied on an array of electrodes [18, 19].

Due to smaller form factor, DMF allows smaller liquid volume for laboratory processes to be in microliter (µL) and picoliter (pL), thus reducing reagents and samples wastage [20, 21].

Another advantage of DMF have over microfluidics are reduced sample cross-contamination and dispersion. This is due to the droplet served as a self-contained microreactor, there is negligible cross-mixing between different samples or reagents [22, 23].

DMF are categorically divided into two configurations, which are open and closed as illustrated in Figure 2.2.



(a) Open type DMF (b) Closed type DMF

Figure 2.2: Types of DMF configurations

Generally, key operations that can be executed by DMF are shown in Figure 2.3 [24–26]. However, splitting and dispensing operations could not be achieved in open-configuration due to

Figure 2.3: DMF key operations

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