Review

Limits of Detection Analysis of Advanced Technologies for Bacterial Detection in Food Samples: Review & Future Perspective

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Posted Date: 17 January 2024

doi: 10.20944/preprints202401.1268.v1

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Limits of Detection Analysis of Advanced Technologies for Bacterial Detection in Food Samples: Review & Future Perspective

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Abstract: Foodborne illnesses can be infectious and dangerous and most of them are caused by bacteria. Some types of common food-related bacteria exist widely in nature and pose a serious threat to both humans and animals, and can cause poisoning, diseases, disabilities and even death. Rapid, reliable and cost-effective methods for bacteria detection are of paramount importance in food-safety and environmental monitoring. Polymerase chain reaction (PCR), lateral flow immunochromatographic assay (LFIA) and electrochemical methods have been widely used in food-safety and environmental monitoring. In this paper, the recent developments (2013-2023) covering PCR, LFIA and electrochemical methods for various bacteria detection (*Salmonella, Listeria, Campylobacter, Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)*) considering different foods types, analytical performances and the reported limit of detection (LOD) are discussed. It is found that the bacteria type and food sample type contribute significantly to the analytical performance and LOD. Detection by LFIA has higher average LOD (24 CFU/ml) than detection by electrochemical methods (12 CFU/ml) and PCR (6 CFU/ml). *Salmonella* and *E. coli* in the Pseudomonadota domain usually have low LODs. LODs are usually lower for detection in fish and eggs. LFIA with gold and iron nanoparticles are prominent in the majority of articles of 26 CFU/ml and 12 CFU/ml respectively. Electrochemical methods reveal that the average LOD is highest for cyclic voltammetry (CV) of 18 CFU/ml), followed by electrochemical impedance spectroscopy (EIS) of 12 CFU/ml and differential pulse voltammetry (DPV) of 8 CFU/ml. Finally, the review discusses the challenges and future perspectives (including the role of nanomaterials/advanced materials) to improve the analytical performance for bacterial detection.

Keywords: limit of detection; bacterial detection; food sample; LFIA; PCR; electrochemical; multiplexing; food safety

1. Introduction

Foodborne illnesses can be dangerously infectious, and they are predominantly caused by pathogens (e.g., bacteria, fungi, viruses, parasites, etc.) or toxins (e.g. dioxins, heavy metals, mycotoxins, etc.) entering the body through contaminated food [1]. Most of the pathogens that can cause foodborne diseases are bacteria [2]. Bacteria can cause acute poisoning, long-term diseases, serious disabilities and even deaths [3]. Among all, *Salmonella* species causes the most serious illnesses and deaths related to contaminated food [4-6]. *Salmonella* is commonly found in birds, eggs, vegetables and also in natural water. Its symptoms include fever, vomiting, pain and dehydration etc. *Salmonella* can be divided into over 2600 species. Among them, *Salmonella enterica* and *Salmonella*

 doi:10.20944/preprints202401.1268.v1

typhimurium are the most commonly found [7]. *Listeria* usually exists in processed products such as milk, meat, seafood and can grow in the refrigerators [8]. *Listeria* was shown to cause miscarriages in pregnant women or deaths of infants, although the chance is really low [9]. Around 20 species in *Listeria* can cause human diseases and *Listeria* monocytogenes is the type that causes the most harm to humans [10]. Most *Campylobacter* infections in humans are acquired by eating and touching contaminated poultry, seafood and meat [11-12]. More than 20 species of *Campylobacter* have been implicated in human disease, with *Campylobacter jejuni* and *Campylobacter coli* being the most well known ones [13]. The most common symptoms of *Campylobacter* infections are diarrhea, fever, vomiting, and stomach cramps [14-15]. *S. aureus* is normally found in birds, meat and milk [16]. *S. aureus* is one of the common bacteria that display antimicrobial resistance, to antibiotics like methicillin and vancomycin [17-18]. Common symptoms of *S. aureus* are shown on the skin, as painful red welts and sores [19-20]. Generally, *E. coli* can be found in contaminated meat, milk and vegetables [21]. *E. coli* can be divided into 3 main groups-Enteropathogenic, Enteroinvasive and Enterohemorrhagic. A subtype of Enterohemorrhagic *E. coli* is the most toxic variant that is also easily transferred [22-23]. Although *E. coli* does not cause any symptoms in most healthy humans, it can lead to diarrhea, vomiting, and fever [24-25].

As many bacterial species currently pose a major threat to humans, a quick, accurate and cheap method to detect bacteria in the environment is essential, especially for food samples [26]. The traditional method to detect bacteria is through culturing of bacteria, which includes isolating the bacteria and monitoring the growth of the colonies [27]. During the culture process, the bacterial colonies are fixed and stained on a glass slide and confirmed by microscopy observation in order to identify different types of bacteria. This process is usually very time-consuming and labor-intensive [28]. Other methods are more complex and can overcome some limitations of bacteria culture. Another common detection method is high-performance liquid chromatography (HPLC) which has high sensitivity [29]. When the concentration of bacterial colonies is very low but still cannot be ignored for human health, it will be a challenge for these methods [30]. Researchers have developed many alternative methods to overcome these problems [26]. One technology that has been widely used more recently is enzyme-linked immunosorbent assay (ELISA), which is available as a commercial test kit for the detection of bacteria. However, it has disadvantages, some of which include; low sensitivity and necessary cold chain that limits its application range [31]. As a result, it is really difficult to meet the demand for large-scale bacteria detection in food samples with current technologies.

LFIA is a relatively novel method in food safety analysis for the detection of bacteria in food. LFIA is cost-effective, simple to use, and can produce results rapidly with fewer samples [32-33]. It measures the concentration of bacteria by the darkness of color on the strip. Conjugated nanoparticles, dominate the porous membrane as an indicator [34-35]. Gold nanoparticles (GNPs) and iron oxide NP (IONP) are the most used NPs in the LFIA because of their low toxicity, and particles size and shape can be controlled by many factors [7,36]. Another relatively new method-PCR is a widely used technique for the detection of bacteria in food. It can make millions to billions of copies of a DNA sample rapidly so it has a high sensitivity and a relatively better detection limit than other common detection methods [37-38]. An alternative well-known technique is electrochemical analysis methods for the detection of bacteria in food. The electrochemical methods mainly measure the changes in the electronic properties caused by bacteria introduced to the solution [39-40].

In this review, PCR, LFIA and electroanalytical techniques and their efficiency in the detection of bacteria in food samples have been summarized. Recent developments (2013-2023) covering PCR, LFIA and electrochemical methods for detection of various bacterial species (*Salmonella, Listeria, Campylobacter, S. aureus and E. coli*) taking into account the different food types, analytical performances and the reported limit of detection (LOD) are discussed from 150 references. Current challenges and future avenues (including the role of nanomaterials /advanced materials) to further improve the analytical performance for bacterial detection are discussed.

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2. Research Methods

Information was collected from Science Direct with keywords: bacteria, PCR, LFIA, electrochemical method, LOD. In Figure 1, 50 peer-reviewed articles by every detection method from 2013–2023 were compared to identify the limit of detection (LOD) for different bacteria: bacteria in the Pseudomonadota domain which includes *Salmonella*, *E. coli*; the Campylobacterota domain includes *Campylobacter*; and the Bacillota domain includes bacteria *Listeria, S. aureus*. In Figure 1, every type of bacteria by every detection method contains 10 articles and lowest LOD of it is listed. Please note that some papers included the LOD of more than one bacterium or more than one detection method, but only the detection of bacteria with the lowest LOD in that paper is discussed here. The data were collated by bacteria type, year of article, detection type: multiplex or not, food sample type, number of replicates, LOD (CFU/ml) in Table 1-3.

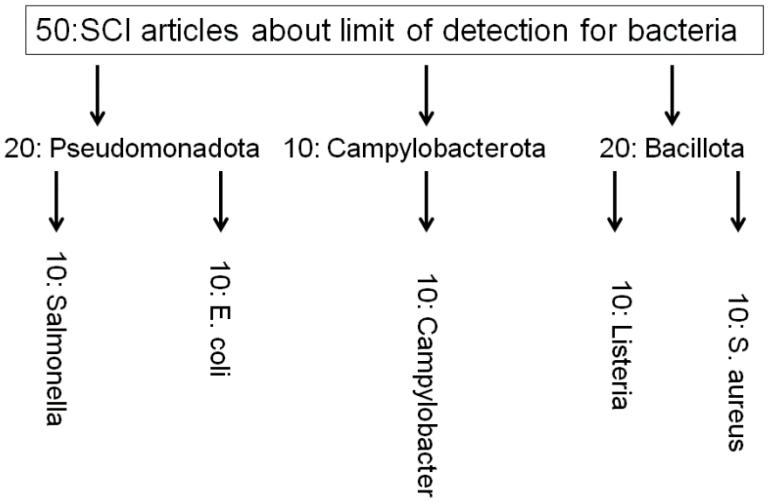

Figure 1. Hierarchy of analysis of papers dealing with limits of detection for different bacteria.

Table 1. PCR: summary of parameters and limit of detection (LOD) for bacteria considered.

Bacteria type	Multiplex?	Food sample	Sample number	LOD(CFU/ml)	Year	Reference
Salmonella	No	Beef	10	0.04	2022	[41]
	No	Chicken	10	0.1	2017	[42]
	Yes	Bacteria Solution	8	0.2	2013	[43]
	Yes	Pork	7	2	2019	[44]
	No	Lettuce	18	2.65	2021	[45]
	Yes	Bacteria Solution	6	3	2022	[46]
	Yes	Natural Water	8	3	2020	[47]
	Yes	Chicken	6	4	2018	[48]
	No	Sheep	7	9	2020	[49]
	No	Chicken	6	10	2017	[50]
Listeria	Yes	Fish	9	0.2	2022	[51]
	Yes	Egg	50	0.2	2014	[52]
	Yes	Duck	160	0.48	2022	[53]
	No	Soybean	20	4	2019	[54]
	No	Milk	35	5	2017	[55]
	No	Milk	6	5	2022	[56]

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	Yes	Pork	5	9	2013	[57]
	Yes	Milk	13	10	2023	[58]
	Yes	Lettuce	14	10	2022	[59]
	Yes	Lettuce	21	10	2016	[60]
Campylobacter	No	Pork	8	0.3	2014	[61]
	No	Milk	5	1	2023	[62]
	No	Milk	8	1	2020	[63]
	Yes	Chicken	9	1	2017	[64]
	No	Sheep	41	4.3	2013	[65]
	Yes	Pork	30	10	2013	[66]
	No	Pork	54	10	2020	[67]
	Yes	Chicken	40	10	2018	[68]
	No	Chicken	6	10	2013	[69]
	No	Milk	12	13	2020	[70]
S. aureus	No	Milk	24	0.25	2019	[71]
	Yes	Milk	46	0.48	2017	[72]
	No	Fish	8	1.2	2018	[73]
	No	Egg	50	3.8	2020	[74]
	Yes	Pork	51	9.6	2014	[75]
	Yes	Milk	9	10	2022	[76]
	Yes	Rice	8	19	2016	[77]
	Yes	Egg	12	20	2022	[78]
	No	Milk	5	28	2018	[79]
	Yes	Beef	9	42	2016	[80]
E. coli	No	Natural Water	6	0.04	2018	[81]
	Yes	Fish	180	0.12	2016	[82]
	Yes	Beef	32	0.14	2020	[83]
	Yes	Cabbage	25	1	2018	[84]
	No	Milk	5	1.03	2021	[85]
	No	Natural Water	7	1.2	2015	[86]
	Yes	Apple	22	2	2020	[87]
	No	Milk	7	4.4	2020	[88]
	No	Beef	12	10	2018	[89]
	Yes	Milk	8	10	2015	[90]

Table 2. LFIA: summary of parameters and limit of detection (LOD) of for bacteria considered.

Bacteria type	Multiplex ?	Food sample	Sample number	Particle	Size(nm)	LOD(CFU/ml)	Year	Reference
Salmonella	No	Orange	5	Gold	20	1	2023	[91]
	No	Chicken	5	Gold	40	1	2019	[92]
	No	Chicken	6	Gold	NA	1	2018	[93]
	No	Egg	11	Gold	15	1.05	2017	[94]
	No	Milk	7	Gold	20	1.6	2017	[95]
	Yes	Grape	9	Iron	40	8	2022	[96]
	No	Milk	7	Gold	15	8.6	2021	[97]
	No	Chicken	5	Iron	150	16	2019	[98]
	No	Lettuce	6	Gold	NA	17	2023	[99]
	No	Milk	5	Iron	NA	34	2019	[100]

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Listeria	Yes	Beef	6	Europium	NA	7	2021	[101]
	No	Pork	30	Gold	20	8	2023	[102]
	No	Milk	12	Manganese	200	9.2	2021	[103]
	No	Lettuce	5	Iron	189	10	2022	[104]
	No	Milk	8	Gold	50	10	2017	[105]
	No	Pork	6	Gold	28	11	2022	[106]
	Yes	Egg	9	Gold	10	19	2017	[107]
	No	Lettuce	6	Gold	10	30	2017	[108]
	No	Lettuce	5	Palladium	NA	48	2020	[109]
	Yes	Milk	6	Gold	NA	75	2019	[110]
Campylobacter	No	Milk	7	Iron	NA	3	2022	[111]
	No	Poultry	60	Gold	50	10	2018	[112]
	Yes	Poultry	9	Iron	NA	10	2018	[113]
	Yes	Poultry	8	Cobalt	50	10	2018	[114]
	No	Fish	105	Iron	NA	10	2014	[115]
	No	Milk	6	Gold	15	50	2019	[116]
	No	Chicken	6	Gold	2	100	2020	[117]
	No	Pork	112	Gold	30	100	2018	[118]
	No	Chicken	7	Gold	33	131	2019	[119]
	No	Beef	5	Gold	40	150	2016	[120]
S. aureus	No	Egg	6	Gold	40	1.6	2022	[121]
	No	Pork	9	Gold	NA	2	2017	[122]
	No	Milk	80	Silicon	NA	3	2014	[123]
	No	Lamb	36	Gold	40	5.96	2021	[124]
	Yes	Fish	80	Gold	NA	10	2022	[125]
	No	Milk	30	Gold	10	10	2013	[126]
	Yes	Milk	32	Gold	50	18	2023	[127]
	Yes	Pork	6	Gold	15	35	2015	[128]
	No	Turkey	6	Carbon	NA	40	2017	[129]
	No	Milk	6	Silicon	NA	100	2023	[130]
E. coli	No	Pork	50	Europium	NA	1	2020	[131]
	No	Milk	7	Gold	NA	1	2016	[132]
	No	Pork	8	Gold	NA	2.2	2023	[133]
	No	Milk	5	Gold	NA	2.7	2019	[134]
	No	Apple	7	Gold	NA	3	2020	[135]
	No	Chicken	7	Iron	NA	10	2022	[136]
	No	Beef	10	Gold	36	10	2020	[137]
	No	Milk	5	Gold	38	12.5	2020	[138]
	Yes	Milk	6	Gold	2	20	2019	[139]
	Yes	Milk	8	Palladium	35	34	2017	[140]

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Table 3. Electrochemical methods: summary of parameters and limit of detection (LOD) for bacteria considered.

Bacteria type	Multiplex?	Food sample	Sample number	Electrochemical technique	Sensor material	Linear range (CFU/ml)	LOD (CFU/ml)	Year	Reference
Salmonella	Yes	Milk	8	DPV	Polymer Mn-MOF on gold	10-10^8	2.6	2022	[141]
	No	Apple	7	EIS	screen-printed gold	100-10^8	3	2016	[142]
	No	Pork	8	CV	gold, graphene on glassy carbon	24-2400	3	2014	[143]
	No	Egg	10	EIS	Hechtia argentea lectin on gold	15-2.57*10^7	5	2020	[144]
	No	Milk	5	CV	chitosan hydrogel, and glassy carbon	10-10^5	5	2015	[145]
	No	Milk	5	DPV	CoFe-MOFs-graphene on gold	10-10^5	6	2021	[146]
	No	Milk	9	EIS	DNA-AuNPs	24-2.4*10^8	6	2014	[147]
	No	Chicken	8	DPV	ssDNA/ rGO-CNT/ GCE	10-10^8	10	2020	[148]
	No	Chicken	7	DPV	DNA/ rGO-TiO_2/ GCE	10-10^8	10	2019	[149]
	No	Apple	8	EIS	diazonium layer onto SPEs	10-10^8	10	2016	[150]
Listeria	No	Milk	13	SWV	Methylene blue	NA	1	2023	[151]
	No	Lettuce	12	CV	Aptamer Pt/HCNs nanozyme	NA	2	2023	[152]
	Yes	Milk	11	EIS	AuNPs-MWCNTs	10-10^7	3.22	2021	[153]
	No	Pork	6	EIS	DNA modified ferrocene	14-1.4*10^6	4	2020	[154]

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	No	Tomato	6	EIS	gold	NA	4	2013	[155]
	No	Milk	8	EIS	paper-based with tungsten disulfide	10-10^8	4.5	2022	[156]
	No	Chicken	6	CV	ALG-thiomer/Pt	10-10^6	5	2022	[157]
	No	Milk	5	EIS	gold interdigitated	100-2200	5.5	2018	[158]
	No	Pork	25	DPV	ZnO-3DNGHSi@MB/AuNP	15-1.5*10^7	6.8	2022	[159]
	No	Lettuce	5	DPV	modified glassy carbon	100-10^7	10	2021	[160]
	No	Beef	31	EIS	DNA modified gold	NA	8	2023	[161]
	No	Poultry	118	EIS	glassy carbon	NA	10	2021	[162]
	No	Chicken	156	DPV	DNA modified gold	10-1000	10	2020	[163]
	No	Poultry	100	SWV	thin-film gold	NA	11	2015	[164]
	Yes	Chicken	36	DPV	Ag/AgCl	NA	13	2018	[165]
Campylobacter	No	Poultry	7	ASV	graphene modified glassy carbon	50-500	15	2015	[166]
	No	Chicken	50	EIS	AuNPs on glassy carbon	NA	50	2019	[167]
	No	Milk	6	EIS	interdigited platinum	NA	100	2020	[168]
	No	Milk	5	CV	TiO_2	NA	100	2020	[169]
	No	Milk	5	CV	DNA modified gold	NA	100	2019	[170]
S. aureus	No	Apple	9	CV	Au/nitrogen doped carbon	10-10^8	1	2022	[171]

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	No	Apple	7	CV	AuNPs@ Fe_3O_4/GCE	10-10^7	1	2022	[172]
	No	Milk	6	CV	Au modified paper-carbon	NA	2	2022	[173]
	No	Pork	7	EIS	Stamp imprinted gold	NA	3	2021	[174]
	No	Milk	7	EIS	AuNP modified GCE	10-10^7	3.3	2020	[175]
	No	Orange	9	CV	Ag-Cs-GrQDs/NTiO_2 SPEs	10-5*10^8	5	2022	[176]
	No	Milk	6	DPV	carboxylated MWCNTs	NA	5	2020	[177]
	No	Fish	7	EIS	AuNPs-rGO-ssDNA	10-10^6	10	2014	[178]
	No	Milk	7	DPV	Ab-SWCNT	10-10^7	13	2017	[179]
	Yes	Milk	7	EIS	BSA on platinum	100-10^6	15.9	2021	[180]
	No	Milk	7	DPV	ZnO-CuO on gold	1000-10^6	2	2021	[181]
	No	Egg	6	CV	Ferrocene SPCEs	10-10^8	3	2022	[182]
	No	Milk	6	DPV	CdS@ZIF-8	10-10^8	3	2019	[183]
	No	Milk	8	EIS	cysteamine/ ferrocene-modified	NA	3	2018	[184]
E. coli	No	Milk	5	CV	AuNPs modified SPCEs	7-7*10^6	3.5	2019	[185]
	No	Milk	9	EIS	rGO-CysCu on gold	10-10^8	3.8	2017	[186]
	No	Fish	5	CV	AuNPs on glassy carbon	15-1.5*10^8	4	2022	[187]
	Yes	Lettuce	12	EIS	ZIF-8 decorated ferrocene	10-10^7	5	2023	[188]
	No	Apple	9	CV	silver	10-10^8	10	2020	[189]

	No	Milk	5	DPV	gold	50-5*10^7	10	2019	[190]

3. Results

Table 1 provides a breakdown of the analysis of 50 peer-reviewed articles used in this review, based on the type of bacteria, multiplexing capability, the type of food sample, replicates and, reported LOD. The limits of detection are further shown in Figure 2, according to (a) multiplex detection capability and (b) food sample types, in an attempt to and highlight the analytical capabilities of the various techniques/methods considered. Since there are too many food samples in Table 1, they are divided into eight types: mammals (including beef, pork and sheep), birds (include chicken and duck), fish, egg, milk, plants (including lettuce, soybean, rice, cabbage and apple), natural water and bacterial solution for easier analysis.

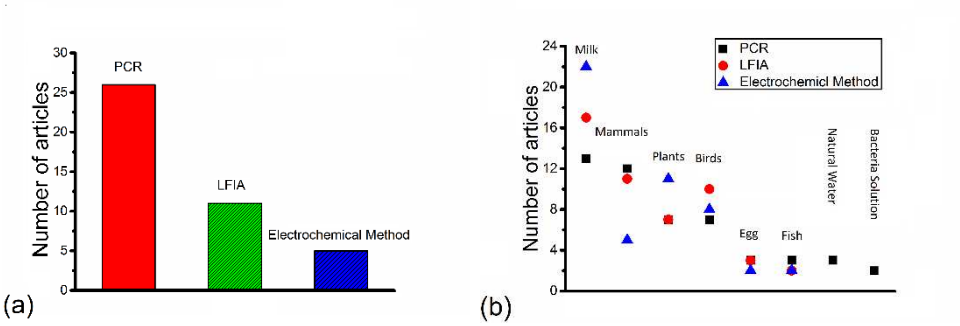

Figure 2. Number of articles (a) with multiplex detection of bacteria simultaneous and (b) food samples by different detection methods.

Figure 2a indicates that the percentage of articles include multiplex detection of bacteria simultaneously by PCR, LFIA and electrochemical methods. It is a little more than half (52%) in PCR, but only around one fifth (22%) for LFIA, and one tenth (10%) for electrochemical methods. Figure 2b shows the percentage of articles according to food sample type with varying detection methods. Although milk was always included in most articles (13, 17, 22 in PCR, LFIA, and electrochemical methods respectively), the second is mammals in PCR and LFIA (12 and 11 respectively) but plants mainly feature for electrochemical methods (11). In addition, more common food samples in every detection method accounts for more than half of the articles together. On the other hand, articles about natural water and bacterial solution only appear in PCR, not LFIA or electrochemical methods.

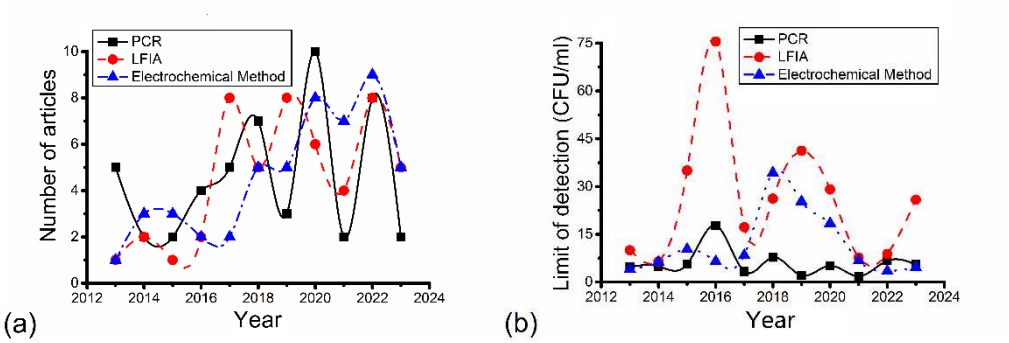

Figure 3. Timeline of the annual number of articles collected and the annual average limit of detection in different years by different detection methods. (a) Number of articles. (b) Average limit of detection.

Figure 3a presents the annual number of articles and average limit of detection over the years 2013 to 2023 by different detection methods. There was at least one article published every year for every detection method from 2013 to 2023. Articles published in 2019 to 2023 are always higher than

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articles published in 2013 to 2018 in every detection method indicating increased research interest. For PCR, the annual number of articles published was 5 in 2013, then it decreased to 2 in 2014. It increased gradually to 7 in 2018 and decreased to 3 in 2019. After 2019, fluctuations were observed every year. It reached the highest point of 10 in 2020 and decreased to the bottom at 2 in 2021. In the case of LFIA, it was only 1 in 2013. It increased gradually to 8 in 2018 and highly fluctuated every other year. It reached 8 again in 2019 and decreased to the lowest level at 4 in 2021. In the case of electrochemical analysis, it also started with 1 in 2013. It increased sharply to 3 in 2014 and decreased to 2 in 2016. Then it increased gradually to 9 in 2022, followed by a large decline to 5 in 2023.

In Figure 3b, the annual average LOD was usually the highest in LFIA, except for the electrochemical methods in 2018. Overall, the LOD was usually the lowest in PCR, except for the electrochemical methods in 2016 and 2022. In PCR, the average LOD was about 4 CFU/ml in 2013. Then it increased gradually to around 18 CFU/ml in 2016, and was followed by a large drop to roughly 3 CFU/ml in 2017. After 2017, fluctuations were observed every year and decreased in an overall trend to about 2 CFU/ml in 2021 (also the lowest for all detection methods in every year). It increased to about 6 CFU/ml in 2022. In LFIA, it was around 10 CFU/ml in 2013, it decreases to roughly 7 CFU/ml in 2014 and increased tremendously to about 75 CFU/ml in 2016 (also the highest for all detection methods in every year). Then it decreased sharply to about 17 CFU/ml in 2017 and was followed by an increase to nearly 40 CFU/ml in 2019 again. After that, it decreases gradually to approximately 8 CFU/ml in 2021 and increased again to roughly 25 CFU/ml in 2023. By the electrochemical method, it was about 4 CFU/ml in 2013, and it increased gradually to around 10 CFU/ml in 2015. After a decrease to about 7 CFU/ml in 2016, it increased again to around 35 CFU/ml in 2018. Then it decreases gradually to roughly 3 CFU/ml in 2022.

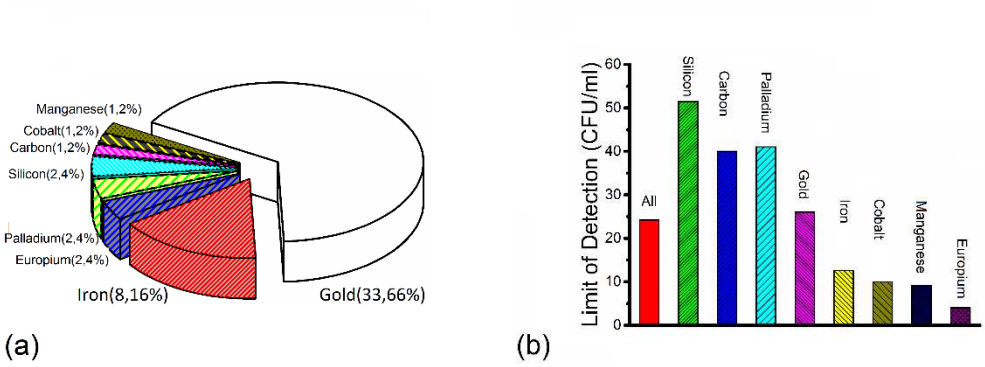

Figure 4. Detection of bacteria with LFIA with different nanoparticles (white: Gold, red: Iron; dark blue: Europium; green: Palladium; light blue: Silicon; pink: Carbon; yellow: Cobalt; brown: Manganese). (a) Number of articles with different nanoparticles. (b) Average LOD with different nanoparticles.

Figure 4a shows that gold is the most studied nanoparticles in articles with LFIA involving 33 articles, and it is followed by iron involving 8 articles. These two nanoparticles account for more than four fifths of the articles on LFIA. Only 2 articles involving europium, palladium, and silicon. Only 1 article involve carbon, cobalt, and manganese. Figure 4b illustrates that the average LOD is the highest for articles involving silicon (little over 50 CFU/ml), followed by carbon and palladium of roughly 40 CFU/ml. The average LOD for gold is about 26 CFU/ml, which is a little higher than the average LOD for all articles with LFIA (24 CFU/ml). It is followed by iron of around 12 CFU/ml, cobalt of 10 CFU/ml, Manganese of about 9 CFU/ml and Europium of only 4 CFU/ml.

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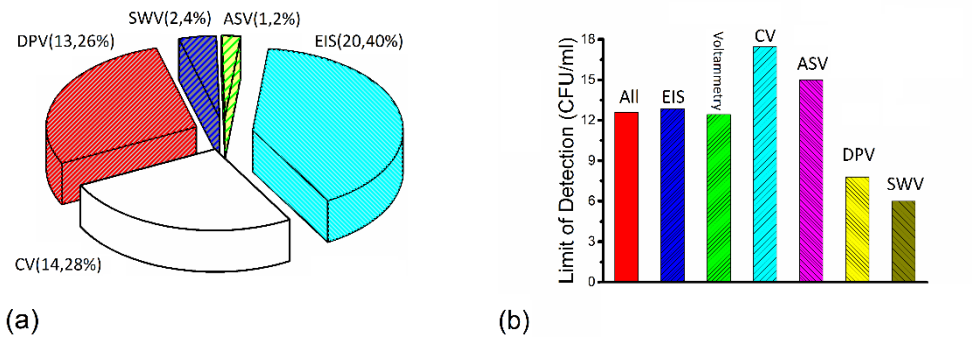

Figure 5. Detection of bacteria by electrochemical method with different techniques (white: cyclic voltammetry (CV); red: differential pulse voltammetry (DPV); dark blue: square wave voltammetry (SWV); green: anodic stripping voltammetry (ASV); light blue: electrochemical impedance spectroscopy (EIS)). (a) Number of articles by different techniques. (b) Average LOD by different techniques.

Figure 5a shows that electrochemical impedance spectroscopy (EIS) accounts for the highest number of articles of 20, and it is followed by cyclic voltammetry (CV) of 14, differential pulse voltammetry (DPV) of 13, and these techniques occupy the majority (more than nine tenths) of articles in electrochemical methods. In addition, only 2 articles involve square wave voltammetry (SWV) and only 1 article involves anodic stripping voltammetry (ASV). Figure 5b illustrates that the average LODs for all articles, articles involving EIS and voltammetry have similar LOD at around 12 CFU/ml. The average LOD for CV is the highest at roughly 18 CFU/ml. It is followed by ASV of 15 CFU/ml, DPV of around 8 CFU/ml, and SWV of only 6 CFU/ml.

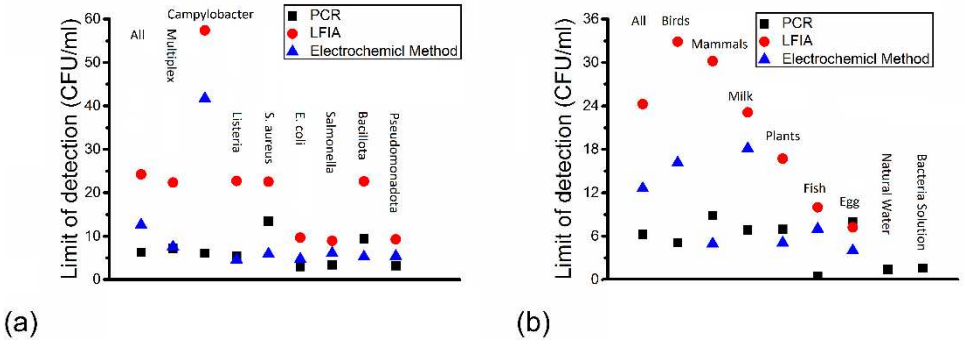

Figure 6. Average limits of detection of different bacteria and food samples by different detection methods. (a) Different bacteria. (b) Different types of food samples.

Figure 6 presents the average LOD of different (a) types of bacteria and (b) types of food/ water samples by different detection methods. Figure 6a shows that the overall average LOD is lowest for PCR, and highest for LFIA. The average LOD is higher for multiplex detection of bacteria simultaneous in PCR than single detection ones, but lower in LFIA and electrochemical methods. PCR has the lowest average LOD among all detection methods for *Salmonella*, *Campylobacter* and *E. coli* and these bacteria are all gram negative (-). In addition, electrochemical methods have the lowest average LOD among all detection methods for *Listeria* and *S. aureus* and these bacteria are all gram positive (+). On the other hand, LFIA always has the highest average LOD for every type of bacteria. The average LOD for bacteria in the Pseudomonadota domain are usually lower than bacteria in the Bacillota domain by PCR and LFIA, but similar to the latter by electrochemical method. For bacteria in the Pseudomonadota domain, the average LOD for *E. coli* is lower than it is for *Salmonella* by PCR and electrochemical methods, but higher than the latter by LFIA. For bacteria in the Bacillota domain, the average LOD for *Listeria* is lower than it for *S. aureus* by PCR and electrochemical method, but higher than the latter by LFIA. The average LOD of *Campylobacter* is usually the highest among all

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bacteria by every detection method, except that it is lower than *S. aureus* by PCR. In addition, only average LODs of *Campylobacter* LFIA and electrochemical methods are over 30 CFU/ml by all detection methods and among all bacteria.

Figure 6b shows that PCR has the lowest average LOD among birds, fish, milk, while the electrochemical methods show the lowest average LOD among mammals, egg and plants. LFIA always has the highest average LOD among all food samples, except that it is highest by PCR in egg. Among all food samples, egg has the lowest average LOD by LFIA and electrochemical methods among all food samples, while fish has the lowest average LOD by PCR (the lowest among all foods samples by all detection methods, also the only average LOD lower than 1 CFU/ml). In contrast, the data for birds demonstrated the highest average LOD by PCR, which is followed by mammals and milk (only these three have average LODs over 20 CFU/ml by all detection methods and among all bacteria). In addition, mammals exhibited the highest average LOD by PCR and milk the highest average LOD by PCR by electrochemical method among all food samples. Natural water and bacterial solution only show in detection by PCR, and their average LODs are lower than all other food samples by all detection methods except fish by PCR.

EU limits for *Salmonella*, *Listeria*, *Campylobacter*, *S. aureus* and *E. coli* in food for most people are 100, 100, 1000, 1000, 100 CFU/mL, respectively [21]. All the LODs in this review are far lower than the EU limits stipulate. Although some food intended for special groups such as infants, elderly people and patients with certain diseases require no presence of these bacteria at all, at least one article with LOD within 0.3 CFU/ml is included in every type of bacteria by PCR [21]

4. Discussion

The review of the LODs for PCR, LFIA and electrochemical methods has revealed trends in this research area that will inform food safety and public health experts. Figure 2a illustrates that the percentage of articles with multiplex is the highest in PCR, followed by LFIA and electrochemical method. That is the main reason that PCR is considered as a reliable standard detection method in the detection of bacteria under many circumstances. However, PCR does have disadvantages of high cost, time-consuming and complex procedure. As a result, PCR cannot replace LFIA and electrochemical method in the detection of bacteria completely. Milk is the most popular food sample for bacteria detection by every detection method. In addition, many articles involve mammals, plants and birds in every detection method (Figure 2b).

The results in Figure 3a show that more articles were published in 2019-2023 than in 2013-2018 by every detection method. In addition, the number of articles every year by every detection fluctuates highly. While the number of articles in LFIA and electrochemical method reached the peak in 2022 (8 and 9 articles respectively), articles in PCR reached the peak in 2020 of 10 articles. While the number of articles in LFIA and electrochemical method reached the lowest with only 1 article in 2013, articles in PCR reached the lowest in 2014 and 2015 in every year of 2 articles.

In Figure 3b, the annual average LOD was usually the highest by LFIA, and the lowest by PCR in every year. While the annual average LOD for PCR and LFIA reached the first peak in 2016 (about 75 CFU/ml and 18 CFU/ml respectively), the LOD for electrochemical method reached the first peak in 2015 of around 10 CFU/ml. Later, the annual average LOD for PCR and electrochemical method reached the second peak in 2018 (around 8 CFU/ml and 35 CFU/ml respectively), the LOD for LFIA reached the second peak in 2019 of around 40 CFU/ml. And the annual average LOD by PCR reached the lowest of only about 2 CFU/ml in 2019, and it was around 7 CFU/ml in 2014 by LFIA, and 3 CFU/ml in 2014 by detection method. The relationship between the annual average LOD and the year by every detection method is still unclear. Figure 3 shows that although the detection of bacteria has attracted more attention from researchers in recent years, LOD in PCR, LFIA and electrochemical method did not a decreasing trend. The main reason is that LODs in these articles are all lower than the EU limits, so the main purpose in many articles may not be lowering the detection limit.

Figure 4a shows that only 3 articles by LFIA involve nonmetal nanoparticles (silicon: 2, carbon: 1). The majority of articles with LFIA involve metal nanoparticles. Figure 4b illustrates that average

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LODs for nonmetal nanoparticles are usually higher than metal nanoparticles, except that the average LOD of palladium is little higher than carbon.

Figure 5a shows that EIS, CV, DPV account for the most articles by electrochemical method. Figure 5b illustrates that among these three main techniques in electrochemical method, average LOD is highest for articles with CV (18 CFU/ml), followed by EIS (12 CFU/ml) and DPV (8 CFU/ml). The main reason is that 2 articles with LODs of 100 CFU/ml for the detection of *Campylobacter* involve CV, and 1 article with a LOD of 100 CFU/ml for the detection of *Campylobacter* involve EIS. It also shows that few articles for the detection of *Campylobacter* of extremely LODs increase average LODs of different techniques by electrochemical methods.

In Figure 6a, the average LOD was usually the highest for LFIA in bacteria investigated. The average LOD was the lowest by PCR in gram (-) bacteria, and by electrochemical method in gram (+) bacteria. *Campylobacter* is gram (-), and its average LOD is usually the highest among all bacteria by every detection method, except it was lower than *S. aureus* by PCR. The recommended EU limits for *Salmonella*, *Listeria*, *Campylobacter*, *S. aureus* and *E. coli* in food for most people are 100, 100, 1000, 1000, 100 CFU/ml, respectively. All the LODs in this review are far lower than these EU limits. A possible reason for the relative higher average LOD of *Campylobacter* and *S. aureus* is that EU limits for them are relatively high. The average LOD for *Salmonella* and *E. coli* (both gram (-)) in the Pseudomonadota domain are usually lower than *Listeria* and *S. aureu*s (both gram (+)) in the Bacillota domain by PCR and LFIA, but similar to the latter by electrochemical method. The difference between 2 bacteria in the same domain is much smaller than the difference between 2 different domains.

This review also shows that the average LOD for multiplex ones is higher than non-multiplex ones by PCR, but lower than them by LFIA and electrochemical methods. One of the main possible reasons is that PCR usually has lower LOD than LFIA and electrochemical method. It is difficult to keep both detection efficiency and sensitivity at the same time when LOD is already really low, but not that difficult when LOD is relatively high. This could be a promising focus for the development of bacteria detection in the future. This review also indicates that fish and egg have the lowest average LOD among all types of food samples. One of the main reasons is that bacteria in fish and egg can be distinguished FROM nearby animal cells. The complexity of food sample composition can lower the performance of detection and makes LOD higher. To address such limitations and challenges, sample enrichment and improvement in device properties of detection are needed. PCR, LFIA and electrochemical method have been used in detection of different bacteria, and many of them involve multiplex detection. It is often observed that different types of bacteria coexist in a single food sample. As a result, a multiplex detection that can fulfill the requirements of a low detection limit and high efficiency is necessary for food safety. These detection methods can also be combined with other technologies to obtain a better detection performance.

Challenges and Future Perspectives

Sensitivity & Specificity: Enhancing the sensitivity and specificity poses a significant challenge. For LFIA, lateral-flow design and integration of monoclonal antibodies and nanomaterials seems crucial for enhancing specificity and LODs. For electrochemical methods, electrode modification with diverse nanomaterials has emerged as a prevalent technique, amplifying signals and improving sensitivity. Despite the high specificity of monoclonal antibodies, their production remains intricate and expensive. Microfluidic platforms offer a seamless integration with LFIA and electrochemical approaches. The integration of specific aptamers or DNA strands enhance the PCR-based bacterial detection in terms of sensitivity and specificity.

Sample Complexity: Addressing the challenges related to sample complexity and matrix effects and cost is crucial for the development of efficient bacterial detection systems. Complex biosensing systems necessitate pretreatment of food samples, with different food types requiring varied sample treatments and techniques. Achieving data under similar sample treatment and identical testing conditions is challenging but important.

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Analysis Time: The total time required for analysis varies across different bacterial detection methods including LFIA, electrochemical, PCR-based systems. LFIA and electrochemical sensors are well known for their rapid analysis speed and are capable of multiplexing.

Role of nanomaterials and advanced materials for future developments: The integration and successful utilization of various materials and nanomaterials for bacterial detection in food samples is well reported in recent years. Nanomaterials offer unique properties including high surface area, tunable physicochemical characteristics and enhanced reactivity which makes them ideal candidates for improving sensitivity and detection limits, specificity, and overall performance [193-196]. Figure 7 shows some commonly used materials/nanomaterials for various sensing fabrication which can be employed for sensitive bacterial detection.

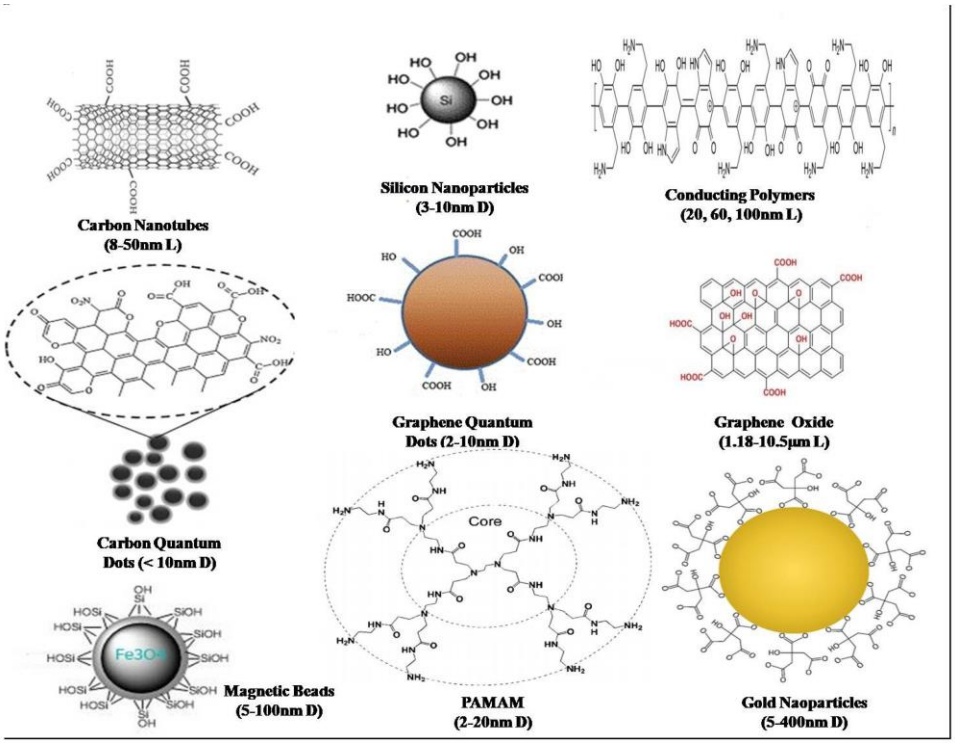

Figure 7. Commonly used nanomaterials in various kind of sensors fabrication with their sizes. L: length; D: Diameter. Reproduced under the terms of the CC-BY license from Ref. [196], Copyright 2020, The Authors, published by MDPI.

LFIA: Nanomaterials play a crucial role in enhancing the performance of LFIA for bacterial detection in food samples. Gold nanoparticles (AuNPs), carbon nanotubes, magnetic nanoparticles, and quantum dots are among the commonly utilized nanomaterials. These materials are employed for conjugation with antibodies specifically related to the targeted species. Nanomaterials are normally integrated into the test strip e.g., AuNPs are frequently utilized as labels for bacterial detection (due to their distinct color change properties). The immobilization of antibodies on the surface of these nanoparticles facilitates the specific binding to bacterial antigens, thereby enabling the qualitative or quantitative detection of the target bacteria. Moreover, the use of nanomaterials in LFIA is reported to help in signal amplification and improved sensitivity (and lower detection limit) [191].

PCR: Nanomaterials find major application in PCR-based bacterial detection methods, contributing to the sensitivity and efficiency of the amplification process. Nanoparticles, such as AuNPs, silicon and magnetic nanoparticles, are often utilized in PCR assays. One significant application is in the extraction/purification of nucleic acids from bacterial samples. Magnetic nanoparticles coated with specific ligands can bind to bacterial DNA or RNA selectively, enabling the isolation from complex food matrices. This enhances purity and subsequently improves the reliability of PCR amplification. Additionally, nanoparticles, as labels for detection can help in facilitating the visualization of PCR products. Quantum dots, for instance, provide a fluorescent

 doi:10.20944/preprints202401.1268.v1

signal which can be quantified, enhancing the sensitivity and specificity of bacterial detection in food samples via PCR [192].

Electrochemical methods: Nanomaterials play a crucial role in enhancing the performance of electrochemical methods for bacterial detection. Carbon-based nanomaterials, metal nanoparticles and nanocomposites are commonly integrated onto the electrode surfaces to improve the response and signal amplification. Nanomaterials provide improved surface area for the immobilization of specific recognition elements (antibodies or aptamers) which ensures efficient capture of the target bacteria, thereby improving the sensitivity. In addition, nanomaterials modify the electrode surface to promote electron transfer kinetics and hence result in rapid and reliable electrochemical signals and detection. The unique properties of nanomaterials, such as size, structure, conductivity, and catalytic activity, contribute to the overall performance of electrochemical biosensors for bacterial detection [193-196].

In summary, the integration of nanomaterials in LFIA, PCR, and electrochemical methods for bacterial detection in food samples represents a promising strategy to overcome the challenges associated to sensitivity, specificity, overall performance and LODs. The exploration of novel nanomaterials and their tailored applications would help to further improve the detection limits (LODs) and advance the capabilities of bacterial detection technologies in the food safety realm.

5. Conclusions

The development of detection technology for monitoring the quality and safety of foods has provided promising tools for improved quantitative performance. In order to improve the accuracy and precision of different detection methods (PCR, LFIA and electrochemical method), different parameters such as bacteria type, year of article, detection type: multiplex or not, food sample type, number of replicates have been considered as determinants of LOD. The results show that bacteria type and food sample type strongly contribute to predict the LOD. Average LOD is the highest for the detection by LFIA (24 CFU/ml), followed by electrochemical method (12 CFU/ml) and PCR (6 CFU/ml). *Salmonella* and *Escherichia coli* in the Pseudomonadota domain usually have lower LODs than other bacteria. LODs are usually lower for detections in fish and egg than in other food samples analyzed. Most articles by LFIA involve metal nanopaticles-especially gold and iron. The average LOD of articles involving gold (26 CFU/ml) is higher than it of iron (12 CFU/ml). EIS, CV and DPV are three major techniques among articles by electrochemical method. CV has the higher average LOD (18 CFU/ml) than EIS (12 CFU/ml) and DPV (8 CFU/ml). Sample enrichment and improvement in device properties of detection and the possibility of combination with other detection technologies are needed to lower LOD and improve performance of detection further. This review provides guidance for future developments of bacteria monitoring technologies, based on the enrichment of bacteria from samples, and the development of multiplex detection methods that can increase the detection efficiency but also keep the detection limit low. The integration and exploration of novel nanomaterials will help to further improve the detection limits (LODs) and advance the capabilities of bacterial detection technologies in the realm of food safety.

Author Contributions: Conceptualization, X.Z. and F.T.; methodology, A.B. and B.S.; software, A.B.; validation, B.S. and F.T.; formal analysis, X.Z.; investigation, B.S.; resources, F.T.; data curation, X.Z.; writing—original draft preparation, X.Z. and B.S.; writing—review and editing, C.C. and F.T.; visualization, B.S.; supervision, B.S., J.C. and C.C.; project administration, F.T.; funding acquisition, F.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by TU Dublin Postgraduate Research Scholarship Program.

Conflicts of Interest: The authors declare no conflict of interest.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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 doi:10.20944/preprints202401.1268.v1

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