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Review

Applications of biosensors for bacteria and virus detection in food and water–A systematic review

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ABSTRACT

Biosensors for sensitive and specific detection of foodborne and waterborne pathogens are particularly valued for their portability, usability, relatively low cost, and real-time or near real-time response. Their application is widespread in several domains, including environmental monitoring. The main limitation of currently developed biosensors is a lack of sensitivity and specificity in complex matrices. Due to increased interest in biosensor development, we conducted a systematic review, complying with the PRISMA guidelines, covering the period from January 2010 to December 2019. The review is focused on biosensor applications in the identification of foodborne and waterborne microorganisms based on research articles identified in the Pubmed, ScienceDirect, and Scopus search engines. Efforts are still in progress to overcome detection limitations and to provide a rapid detection system which will safeguard water and food quality. The use of biosensors is an essential tool with applicability in the evaluation and monitoring of the environment and food, with great impact in public health.

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Introduction

Pathogens in food and water threaten animal production and the food supply, with severe financial impacts. The consumption of unsafe food products contaminated by bacteria, viruses, parasites or chemicals responsible for foodborne diseases is the one of the major burdens on public health and presents a significant obstacle to socioeconomic development all over the world. According to WHO, unsafe food and water containing harmful bacteria, viruses, parasites, or chemical substances cause more than 200 diseases – ranging from diarrhea to cancers. Also, it is estimated that 600 million people fall ill because of contaminated food, with detrimental effects on human health. More than 70% of all emerging diseases come from animal sources (Kuiken et al., 2005).

A crucial issue in controlling easily transmitted diseases is to achieve a rapid, selective and specific method of assessment for pathogen detection. At present, efforts are needed to elaborate new tools that can be practically applied for the discrimination of pathogens and to trace their spread to avoid global threats in the environmental and healthcare sectors.

Efforts toward establishing standards in developing countries with limited resources are of great importance to public

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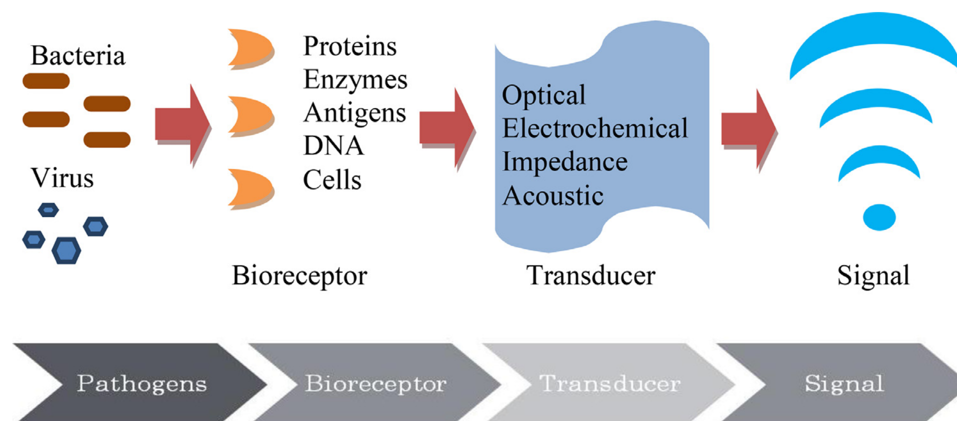

Fig. 1 – Principles of biosensor usage.

health. Conventional and official methods for the detection and identification of pathogens principally rely on specialized molecular and culture methods. The culture methods are very effective and sensitive, but they have high cost and take a long time. On the other hand, molecular methods focusing on an amplification of a targeted gene are more descriptive and specific, and can be completed in a shorter time. Unfortunately, they need more expensive consumables for extraction and to achieve accuracy. Nevertheless, they can be used in the field, although they require costly equipment and trained personnel to perform. Thus, there is still a need for rapid and affordable detection and identification tools with high sensitivity and selectivity (Vidic et al., 2017; 2019).

Biosensors have raised great interest in recent years. They are considered as powerful emerging tools for the detection of various biomarkers for both healthcare and environmental monitoring (Kotsiri et al., 2019; Manzano et al., 2018; Vidic et al., 2017). A biosensor is a small device that transforms the recognition of a biological molecule (DNA/RNA, antibody, protein, whole cell etc.) into a signal (electrochemical, optical, piezoelectric, nanomechanical, mass-sensitive, etc.) (Choi et al., 2018). Biosensors can provide rapid response in a short time, ultrasensitive detection of biomolecules, and have the potential to be miniaturized for portable use, requiring minimal sample processing compared to conventional analytical methods. A schematic figure of a typical biosensor is shown in Fig. 1.

Biosensors can potentially be used in the food industry, healthcare sector, farming, water treatment and water analysis. Biosensors have been utilized in several real-time applications. In particular, lab-on-a-chip devices give the possibility to perform multiple laboratory processes in a semi-automated, miniaturized format. A lab-on-a-chip integrates one or several laboratory functions in a single integrated system that allows sample preparation and detection on the same chip. A lab-on-a-chip requires an extremely small sample volume to detect microorganisms (Liu et al., 2014). This device takes advantage of the basics of the physics, manipulation and study of minute amounts of fluids. The rapid development of microfluidics, biotechnology and nanotechnology has raised a lot of interest in the development of new biosensors for the detection of foodborne pathogens.

Biosensors can be categorized based on the type of transducers and operating principles utilized, as an effective transducer can provide repeatable and reliable signals. Optical biosensors take advantage of optical characteristics such as absorbance, fluorescence and chemiluminescence. The recognition elements are primarily enzymes and antibodies, while devices are fabricated using fiber optics and optoelectronic transducers. Plasmonic biosensors, utilizing methods such as surface plasmon resonance, localized surface plasmon resonance, and surface-enhanced Raman scattering, are based on optical phenomena generated by light, which interact with conducting interfaces in thin films and nanoparticles that have smaller sizes than the incident wavelength. Optical sensing systems still need to be digitized or displayed as an electronic signal.

Acoustic biosensors based on piezoelectric crystals operate by detecting the binding of the analyte (target) by its modulation of the crystal oscillation frequency. The physical properties of the acoustic wave are then correlated with the amount of bound analyte. However, this type of biosensor has problems with the detection of substances in viscous liquids because the crystals may cease to oscillate.

The fundamental principle of an electronic-based biosensor is that it immobilizes the biomolecules on solid electronic supports and activates electronic coupling and electronic circuity that assures effective signal transference from the active biomolecules. The degree of electronic coupling between the biomolecules and electronics depends mainly on the properties of the electrode surface. A range of methods has been developed to optimize both the selectivity and strength of the generated electronic signals (Willner and Katz, 2000). The chemical composition of the biomolecules and electrode material and biochemistry dictate the success of the sensor's measurement. Similarly, electrochemical biosensors (amperometric, potentiometric, conductometric, and impedimetric) rely on current, voltage, capacitance or impedance measurements to detect the binding between the receptor and the target, which converts the biochemical reaction into a measur-

able electrical signal. Impedance biosensors exploit the interactions of biomolecules with a conductive or semiconducting transducer surface to observe the resultant current (Knopf and Bassi, 2018).

The limit of detection refers to the lowest analyte concentration that can be reliably distinguished from the assay background. The permissible limits of microorganisms present in food and water differ according to the type of matrix and the effect of the organism on human health. For instance, ISO :2017 defines the absence of *Salmonella* from food and therefore the use of a method with low limits of detection is important to ensure public health protection. In contrast, according to European guideline 2006 7 /EK, the presence of 500 CFU of *E. coli* and 200 CFU of enterococci in 100 mL of seawater permits the description of recreational water as "Good quality". Therefore, for recreational water analysis a method with a higher detection limit could be safely used. To improve biosensor sensitivity and reach low limits of detection, many strategies can be employed; notably, utilization of nanomaterials for signal enhancement or improvement of sensor surface functionalization (Cui et al., 2020; 2019; Farre et al., 2020; Vidic et al., 2019; Vizzini et al., 2020; 2019). Other strategies to increase detection sensitivity include pathogen magnetic preconcentration prior to analysis, using for instance magnetic beads decorated with aptamers that bind specifically to the target (Kotsiri et al., 2019), or coupling an enzymatic reaction to the recognition event, using for instance antibodies conjugated with horseradish peroxidase enzyme (Vizzini et al., 2020). Additionally, pathogen pre-concentration prior to analysis enables separation of the target from the food and in that way eliminates molecules from the matrix that may interfere with the recognition event.

This paper summarizes an overview of the latest commercialized and technological advances in the biosensors for the detection of bacteria and viruses in water and food matrices. To provide a better understanding of the appropriate sensing mechanisms, each section is categorized based on the target organisms and their applications. The important issues in water and food environment are identified, and the perspective of biosensor-based strategies for water and food environment monitoring is discussed.

1. Materials and methods

1.1. Search strategy

A systematic review of the literature was done by searching articles (i.e., journal articles, magazine articles, proceedings of conferences and extended abstracts published) during the period from 1st of January 2010 to 31st of December 2019, in databases like Pubmed, ScienceDirect, and Scopus corresponding to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Liberati et al., 2009). The search strategy was based on electronic data as shown above: ("biosensor") and ("bacteria" or" virus") and ("food" or "water") and ("lab on a chip").

The search focused on researched published articles with no exclusion in terms of language in peer-reviewed journals or international conference proceedings. Detailed information

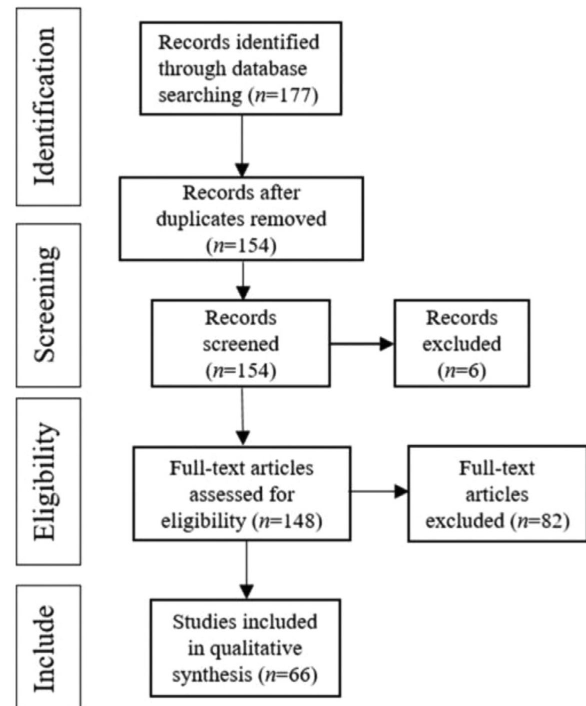

Fig. 2 – Flowchart for literature review.

about the biosensors, the applied method, the target microorganism, the matrix, and the limit of detection were reported. The initial search yielded 177 studies. Each record's title and abstract were screened and subjected to inclusion criteria, shown in **Appendix A Table S1**, and duplicates were removed, resulting in 154 studies. Finally, sixty - six studies met all inclusion criteria and were analyzed. The remaining articles were evaluated for relevance. The documents that had passed the initial screening were read to determine the inclusion and exclusion criteria. The process followed is reported in Fig. 2.

1.2. Statistical analysis

Statistical analyses and graphical presentations were performed with the IBM SPSS Version 25.0 statistical software package and Microsoft Excel to correlate the microorganisms and applied biosensing methods. A significance level was adopted ($p<0.05$). A chi-square statistic was calculated to examine if there is a correlation between the applied methods and microorganisms.

2. Results

This systematic review retrieved the applications of biosensors on food and water samples. The final selected articles and information on the authors, publication year, microorganisms, type of growth, method of determining the presence of the microorganisms and limit of detection are presented in Table 1 with their corresponding references. The European Food Safety Authority (EFSA) and WHO reported that *Campylobacter, Salmonella, Listeria*, Enteropathogenic *E. coli* and *Yersinia* are the five most widespread bacterial pathogens,

Table 1 – Total records for biosensor applications in food and water.

Microorganism	Matrix	Method	Limit of Detection (LOD)	Reference
S. enterica	Culture	Microcantilever	10^3 CFU/mL	Ricciardi et al., 2010
E. coli K12	Cultured bacteria	Electrochemical biosensor	10^3 CFU/mL	RoyChaudhuri and Dev Das, 2010
E. coli	Fresh spinach	Surface plasmon resonance (SPR)	10^3 CFU/ mL	Linman et al., 2010
HAV, HEV	Culture	Electrochemical immunosensor array	0.5 ng/mL, 1.0 ng/mL	Tang et al., 2010
E. coli	Apple juice, orange juice	Magneto-elastic (ME) resonant μ-diver system (MER-μds) is proposed and prototyped	10^2 CFU/mL	Xue et al., 2012
S. enterica serovar *Enteritidis*, *S. pneumoniae*, *E. coli* O157:H7	Mineral water, raw cow milk, frozen ground meat	SPR	2.8 CFU/mL	Bouguelia et al., 2013
E. coli	Culture	Electrochemical detection of microbial 16S ribosomal RNA	1 CFU/mL	Heidenreich et al., 2013
E. coli	Culture	Plate count technique using arrayed microelectrodes	Not mentioned	Bajwa et al., 2013
C. jejuni	Culture	Microresonator	Not mentioned	Poshtiban et al., 2013
E. coli	Culture	Optical transmission (EOT) phenomenon in plasmonic nanohole	$<10^2$ cells	Kee et al., 2013
E. coli	Culture	Optical-immunoassay	5.7×10^1 CFU/mL	Ma et al., 2013
S. typhimurium	Chicken breast	Microfluidic nano-biosensor	10^3 CFU/mL	Kim et al., 2014
V. parahaemolyticus	Aquatic sewage water, oyster	FMN:NADH oxidoreductase	10 CFU/mL	Peng et al., 2014
S. typhimurium	Culture	Electrical biosensor	10^3CFU/mL	Nguyen et al., 2014
S. agalactiae	Milk	Lab-on-a-chip magnetoresistive cytometer, microfluidic	10 CFU/μL	Fernandes et al., 2014
S. mutans, *P. aeruginosa*	Culture	Impedance biosensor	10^5 CFU/mL	Lillehoj et al., 2014
L. pneumophila	Water	Optical microfluidic devices	4×10^4 cells/mL	Pires and Dong, 2014
Norovirus	Lettuce	Electrochemical biosensor	6×10^1 copies/mL	Hong et al., 2015
Planktothrix agardhii NIVA-CYA116	Freshwater	Nanoparticles based amperometric biosensor	6×10^{12} mol/L target DNA	Ölcer et al., 2015
Campylobacter spp.	Poultry meat	Microscale electrodes	9×10^{-11} mol/L	Morant-Miñana and Elizalde, 2015
L. monocytogenes	Lettuce	Impedance biosensor	3×10^2 CFU/mL	Chen et al., 2015
E. coli O157, *E. coli*	Culture	Optical biosensor	5×10^2 cells	Tachibana et al., 2015
E. coli	Culture	Optical biosensor	10^3 - 10^7 CFU/ mL	Tang et al., 2015
E. coli	Water, milk, blood, and spinach samples	Visual detection using a smartphone	10^1–10^3 CFU/mL	Choi et al., 2016
Norovirus	Culture	Electrochemical aptasensor	Not mentioned	Kitajima et al., 2016
S. typhimurium	Culture	Acoustic wave	10^2 BCE/sample	Kordas et al., 2016
L.monocytogenes	Culture	Latinum interdigitated array microelectrodes	5.39 CFU/mL	Sidhu et al., 2016
H5N1	Poultry	Aptasensor fluorenscence	0.4 HAU	Xu et al., 2016
E. coli O157:H7, *S. typhimurium*, *V. parahaemolyticus*	Culture	Optical biosensor- LAMP & Eriochrome Black T (EBT)	3.8×10^2 copies	Oh et al., 2016
Bacillus. anthracis	Culture	SPR	10^5 -10^7 spores/mL	Adducci et al., 2016
L. monocytogenes	Culture	Colorimetric sensing nanoparticles	2.17×10^2 CFU/mL	Alhogail et al., 2016
Tobacco mosaic virus	*E. coli*	Optical biosensor- LAMP	9.1 ng/mL	Zang et al., 2016
L. monocytogenes	Culture	Electrochemical impedance analysis	1.6×10^2 CFU/mL	Chen2016
S. agalactiae	Milk	Lab-on-a-chip magnetoresistive cytometer, microfluidic	10^2 CFU/mL	Duarte 2016

(*continued on next page*)

Table 1 (*continued*)

Microorganism	Matrix	Method	Limit of Detection (LOD)	Reference
Salmonella spp., *E. coli, S. aureus, Campylobacter* spp. and *Listeria* spp.	Culture	Protein chip, antibody; biosensors;	40 CFU/mL	Poltronieri et al., 2016
V. parahaemolyticus	Oyster	Immunoassay	10 cells	Park et al., 2017
monoplex *S. typhimurium, S. typhimurium* and *V. parahaemolyticus*	Water, milk	Optical LAMP	5×10^1 CFU	Park et al., 2017
S. Enteritidis, S. typhimurium	Culture	Supercritical angle fluorescence	1.6 copies/μL	Hung et al., 2017
E. coli O157:H7	Culture	Graphene-interfaced electrical biosensor	10^1–10^2 cells/mL	Pandey et al., 2017
L. monocytogenes	Culture	Ortable electronic andmicrofluidic setups	Not mentioned	Sharma et al.,2017
Salmonella spp.	Culture	Optical biosenor	10^6 Refractive Index Unit (RIU)	Nguyen et al., 2017
Norovirus	Culture	Electrical aptasensor	10^{-10} mol/L	Chand and Neeethirajan, 2017
S. typhimurium	Culture	QCM-based aptamer selection	10^3 CFU/mL	Wang et al., 2017
S. aureus	Culture	Phage peptide	Phage	De Plano et al., 2017
S. aureus	Milk;	Lab-on-a-chip magnetoresistive cytometer, microfluidic	10^2 CFU/mL	Duarte et al., 2017
L. monocytogenes	Tuna, salmon and shrimp, frosen seafood	Optical LAMP Lab-on-paper chip	10^2 copies of L. *monocytogenes* DNA per 50 g of sample	Pisamayarom et al., 2017
E. coli, Salmonella spp, *V. cholerae*	Chicken	Optical LAMP Lab-on-paper chip	3×10^{-5} ng/μL	Sayad et al., 2017
S. typhimurium	Beef	Electrochemical process	10 CFU/mL	Thiha et al., 2018
E. coli	Culture	SPR	3×10^2 CFU/mL	Galvan et al., 2018
E. coli	Culture	Microfluidic device encapsulating	Not mentioned	Li et al., 2018
E. coli NCTC 13,441	Culture	Electric-dielectrophoretic impedance measurement (DEPIM) of the microbeads	5×10 copies/reaction	Nakano et al., 2018
Salmonella spp.	Milk	Acoustic	2 cells/μL	Papadakis et al., 2018
S. typhimurium	Culture	Spiny gold nanoparticles joined with aptamer	4 CFU/mL	Ma et al., 2018
E. coli	Culture	Supercritical angle fluorescence	5×10^4 Refractive Index Unit (RIU)	Ferdman et al., 2018
E. coli, S. mutans, B. subtilis	Autoclave water, tap water, water lake, milk	Electrically-receptive thermally-responsive (ER-TR)	5 cells/mL	Khan et al., 2018
E. coli, P. putida	Culture	Optics-free platform	10 CFU/mL	Koo et al., 2018
S. enterica serovar *Enteritidis, E. coli*	Water, milk	Electrochemical DNA analysis	1.2×10^8 copies/mL, 9.6×10^6 copies/mL	Park et al., 2018
S. enterica, L. monocytogenes, E. coli	Juice	Optical biosensor	10 CFU/mL	Wei et al., 2018
Salmonella serogroups	Chicken, ready to eat turkey	Impedance biosensor	7 cells/ mL	Jasim et al., 2019
S. enterica	Vegetable salad, egg yolk, egg white, whole egg and minced pork meat	Antibody, immobilized on protein AG-magnetic beads direct PCR	2 CFU/mL	Vinayaka et al., 2019
Salmonella spp., *S. aureus, E. coli*	Culture	Colorimetric detection of LAMP	3×10^1 CFU/sample, 3×10^2 CFU/sample, 3×10^1 CFU/sample	Trinh et al., 2019
E. coli O157:H7, *S. typhimurium, V. parahaemolyticus* and *L. monocytogenes*	Mik	Colorimetric detection of LAMP	10^3 bacterial cells/mL	Oh and Seo, 2019
Salmonella typhimurium	Culture, apple juice	Immunomagnetic separation, fluorescence labeling	5.8×10^1 CFU/mL	Wang et al., 2019

(*continued on next page*)

Table 1 (*continued*)

Microorganism	Matrix	Method	Limit of Detection (LOD)	Reference
S. typhimurium	Milk samples	Antibodies magnetic nanoparticles (MNPs) - PSs and electrical voltage change	3.3×10^1 CFU/mL	Hou et al., 2019
S. typhimurium	Chicken	Magnetic nanoparticle (MNP) colorimetric biosensor	1.4×10^1 CFU/mL	Zhang et al., 2019
E. coli O157:H7	Culture	Heat killed FITC-labeled fluorescence aptamer	10^2 cells/mL	Hao et al., 2019

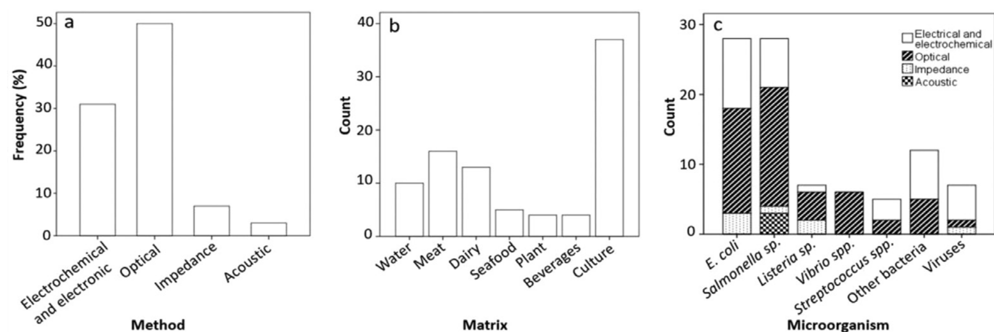

Fig. 3 – Research methods used in this study: (a) Percentage of methods used in sensors in the eligible papers; (b) Percentage of articles with biosensors according to matrix; (c) method usage according to microorganism.

while Norovirus is the most widespread foodborne virus (EFSA, 2018; Vossen, 2001). The majority of the biosensors presented in **Table 1** target these pathogens. The detection of some non-prevalent pathogens such as *Listeria* is also frequently elaborated because of the high mortality rate of listeriosis. The biosensors were individually analyzed using the biological system and the matrix and allowing comparison of their limits of detection. Currently developed methods are based on nucleic acid amplification, biosensors, flow cytometry, spectrometry techniques and multi-sensor systems. Papers were organized according to the methods applied on the biosensors (**Fig. 3a**) and the matrix (**Fig. 3b**). A bar chart was used for displaying target organisms and applied methods (**Fig. 3c**). The impedance biosensors were categorized separately from electrical biosensors due to the large number of studies employing these methods. The figure presents the application of the methods on the target microorganism.

A chi square test of independence was performed to examine whether the relation between various microorganisms and the applied methods are independent of one another. The results of the statistical analysis suggest that the relation between the variables was marginally significant ($N = 93$), $p = 0.056$. The results are presented in **Appendix A Table S2**.

2.1. Escherichia coli

One of the most common bacteria associated with foodborne and waterborne illnesses is *E. coli*. The necessity of handling outbreaks like a triggering event with spinach led scientists to develop analytical tools (Linman et al., 2010). The methods have been focused on the recognizable elements of the bacterial surface. The methods included the use of aptamers (Wei et al., 2018) and antibodies (Kee et al., 2013) to achieve immobilization on nanoparticles (Khan et al., 2018). Various strategies have been applied including Polymerase Chain Reaction (PCR) (Poltronieri et al., 2016; Tachibana et al., 2015), loop mediated isothermal amplification (LAMP) (Oh et al., 2016), surface plasmon resonance (SPR) (Bouguelia et al., 2013; Galvan et al., 2018; Xue et al., 2012), fluorescence (Ferdman et al., 2018), spectroscopy (Tang et al., 2015) and electrochemistry (Koo et al., 2018; Pandey et al., 2017). Other integrated microfluidic systems included provision of the washing solution, elution solution, a channel for DNA extraction as well as the detection method (Bajwa et al., 2013; Oh and Seo, 2019).

Surveys were conducted concerning the need for quantification. A very interesting approach proposed amplification and visual detection or quantification using a smartphone (Choi et al., 2016). The method was tested successfully in a number of matrices such as drinking water, milk, blood, and spinach. Another researcher (RoyChaudhuri and Dev Das, 2010) developed a biomolecule-compatible electrical model which quantifies the number of immobilized antibodies. Focusing on the results reported in **Table 1**, a method was developed focused on the detection and specificity of microbial 16S ribosomal RNA of *E. coli* cells in meat samples (Heidenreich et al., 2013). There was a stage of short pre-enrichment followed by an RNA isolation and biochip measurement. The procedure was able to detect cells (about 1 CFU/mL) within 7 hr.

Li et al. (2018) demonstrated a microfluidic device encapsulating a coplanar waveguide for the discrimination of live and heat-killed *E. coli* cells suspended in culture media us-

ing microwave signals over the frequency range of 0.5–20 GHz. Detection was accomplished by measurement of the reflected microwave signals, which were caused by the reduction of the cytoplasm conductance and permittivity upon cell death.

Recent technological advances have allowed researchers to develop a centrifugal microfluidic automatic wireless endpoint detection system integrated with LAMP that can perform 30 reactions at the same time (Sayad et al., 2018). Similar works have assessed microfluidic devices using the LAMP method (Trinh et al., 2019; Oh and Seo, 2019).

Using a microfluidic system, Hao et al. (2019) suggested a combination of dendrimers and aptamers as recognition elements for *E. coli* O157:H7 detection. This strategy enhanced the sensitivity due to the multiple binding sites on the targeted cells.

2.2. Salmonella *spp.*

Salmonella spp. constitutes one of the primary risks for foodborne disease and is a threat to public health. It is among the most frequently reported causes of foodborne disease. Even though the classic culture media technique is the gold standard testing method, it is time consuming and 2–5 days are necessary to confirm a diagnosis.

Many works have focused on meat products, dairy, vegetables, beverages and cultures. A large number of studies have used nanoparticles to concentrate bacteria, such as quantum dots (Kim et al., 2015), magnetic beads (Wei et al., 2018) and quartz crystals (Wang et al., 2017). Binding was also achieved with beads functionalized with specific aptamers (Ma et al., 2018), conjugated with an electrode (Thiha et al., 2018) or antibodies (Ricciardi et al., 2010). Methods applied included LAMP (Oh et al., 2016), PCR (Hung et al., 2017; Poltronieri et al., 2016), impedimetric sensors (Jasim et al., 2019; Nguyen et al., 2014), acoustic wave sensors (Kordas et al., 2016), and SPR (Bouguelia et al., 2013). The limits of detection ranged from 2 to 10^3 CFU/mL with results obtained within a minimum of 30 min. The 20% of papers dealing with *Salmonella* detection applied multiplex methods for simultaneous detection of several bacteria (Sayad et al., 2018) or viruses (Sharma et al., 2017). Efforts were made for the development of an integrated microfluidic sensor that gave precise results by image analysis performed with a smart phone application (Zhang et al., 2019).

A microfluidic device that took advantage of centrifugal forces was integrated in a system to detect four bacteria (Oh and Seo, 2019). The system included DNA extraction filled with silica micro-beads and LAMP cocktails for specific bacterial genes amplification. The multiplexed LAMP allowed the simultaneous analysis of 18 samples with the limit of detection of 10^3 CFU/mL.

A micro-nanotechnology method was applied in milk (Papadakis et al., 2018). This acoustic biosensor used magnetic beads combined with antibodies to capture cells after a pre-enrichment step. In the assay, the LAMP method was applied for DNA amplification. The results showed a limit of detection of about 2 cells/μL.

Despite the ongoing research in the biosensor field, there is still a need to evaluate and improve biosensor devices, to decrease the procedure time, and to reduce the cost as well as to improve the limit of detection. Recently, in an attempt to address the aforementioned issues, many different biosensor systems have been reported. A combined system was developed with an integrated rotary microfluidic system appropriate for DNA extraction, LAMP and a colorimetric lateral flow strip (Park et al., 2017) for the detection of monoplex *S. typhimurium* and multiplex *S. typhimurium* and *Vibrio parahaemolyticus*. The process was based on a centrifugal microfluidic platform on a single disk. The limit of detection was at 50 CFU/sample.

An interesting study was presented by Nguyen et al. (2017) who combined the PCR technique with SPR into one device that was able to achieve fluorophore-free detection of amplicons. This method managed not only to amplify the DNA of *S. typhimurium* but also to give a precise measurement using the SPR fiber sensor part. The SPR fiber sensor was reusable and showed advantages for diagnostic applications.

A pathogen evaluation system based on microfluidics using a simple film was presented for the detection of *S. enteritidis* (Park et al., 2018). The device was capable of simultaneous gene amplification, solution mixing and electrochemical detection through the use of polyimide and polyester films deposited onto a polycarbonate housing chip. The process lasted about 10 min and detected 9.6×10^6 copies/mL of *S. enteritidis* in milk samples.

Another method for detection used antibodies immobilized on magnetic beads for binding (Vinayaka et al., 2019). Then, direct PCR was performed without requiring DNA extraction and the associated product loss. A limit of detection of 2 CFU/mL was observed in a variety of samples such as vegetable salad, egg yolk, egg white, whole egg and minced pork meat.

A lab-on-a-chip method focused on microdevices was able to perform all necessary procedures for *Salmonella, E. coli* O157:H7 and *S. aureus* detection in food samples (Trinh et al., 2019). A paper-embedded device was used for complete DNA extraction and amplification by the LAMP method. Colorimetric changes provided a response within 75 min. The microdevice was able to detect 3×10^2 CFU/sample.

Wang et al. (2019) developed an online process for the detection of *Salmonella*. The device concentrated bacteria with immune magnetic nanoparticles to increase the specificity. The magnetic bacteria were labeled with the immune fluorescent microspheres, which allowed fluorescence emission. A smart phone application permitted quantification of the fluorescence. The biosensor achieved a lowest detection limit of 58 CFU/mL, with dynamic range from 1.4×10^2 to 1.4×10^6 CFU/mL.

A rapid screening method was proposed for high sensitivity *Salmonella* detection. Magnetic nanoparticles were modified with anti-*Salmonella* monoclonal antibodies to bind bacteria cells, and polystyrene microspheres (PSs) were modified with anti-*Salmonella* polyclonal antibodies and catalases. The magnetic nanoparticles were transported to the PSs via a polymer tube and the electrical voltage change was monitored. The biosensor achieved a detection limit as low as 33 CFU/mL (Hou et al., 2019).

2.3. *Listeria monocytogenes*

L. monocytogenes is one of the most important food pathogens. Its presence has been investigated in seafood and beverage

matrices and cultures. For its detection, studies have mainly focused on optical and impedimetric methods. These have been based on antibodies (Poltronieri et al., 2016), nucleic acids (Alhogail et al., 2016), modified magnetic nanoparticles (Chen et al., 2015; Wei et al., 2018) or platinum microelectrodes (Sidhu et al., 2016). The detection methods were LAMP, PCR and impedimetric measurement (Chen et al., 2016). Multiplex PCR has also been evaluated (Sharma et al., 2017).

Another study reported a colorimetric assay for *L. monocytogenes* detection with magnetic nanoparticles. A D-amino acid was used as a substrate because it could bind with *Listeria* protease and provide color changes in contaminated solutions containing gold nanoparticles and some reagents. The method could identify the microorganisms at 2.17×10^2 CFU/mL.

An interesting study used an optical biosensor, based on a lab-on-paper chip, for the identification of the hly gene of *L. monocytogenes* in raw and frozen seafood (Pisamayarom et al., 2017). The assessment was integrated with LAMP and the fluorescence signal was provided by SYBR dye, all in a single chip platform. The analysis provided high sensitivity (100%), with a detection limit of 100 copies of the target hly gene.

Lastly, an integrated automatic molecular sensor used a centrifugal microfluidic device for the detection of *Listeria* among three non-related bacteria (Oh and Seo, 2019). The device contained positions for sample loading, washing solution, an elution solution and a LAMP mix. As the device rotates, the reagents are released. The procedure was completed in 65 min time and enabled the processing of 18 samples and two controls at the same time. The limit of detection for Listeria was 10^3 CFU/mL in milk samples.

2.4. Vibrio *spp.*

Cholera is responsible for 1.3 to 4.0 million cases and causes 21,000 to 143,000 deaths annually worldwide (Ali et al., 2015). About 7% of the selected articles have involved *Vibrio* spp. (**Fig. 3c**). The methods have been applied on meat, seafood, dairy products and bacterial cultures. Preconcentration is achieved with a bacteriophage coupled with luciferase, a bioluminescent system (Peng et al., 2014) and particles labeled with antibodies. Overall, the recognition of *Vibrio* spp. was visualized using optical sensing, LAMP (Oh and Seo, 2019) and luminescence.

A microfluidic device was developed that enabled control with a zigzag-shaped microchannel sand RPM (Oh et al., 2016). An intriguing technique was applied for detection of *Vibrio parahaemolyticus* based on a stationary liquid-phase lab-on-a-chip (Park and Choi, 2017). This technique was used to capture particles functionalized with an antibody and labeling particles functionalized with horseradish peroxidase and an antibody. The device included a sample chamber, a washing chamber and a detection chamber connected by two channels. In the sample chamber, the two types of particles were mixed with the sample and the agglomerate was transported to the detection chamber containing a chromogenic substrate solution. *V. parahaemolyticus* in contaminated oyster samples was detected with a limit of detection of 10 cells.

A centrifugal microfluidic device was developed containing Bluetooth® wireless technology to send results to a smartphone. The detection was confirmed by LAMP (Sayad et al., 2018). The microdevice could perform 30 reactions at the same time for *V. cholerae* strains and other species in a 60 min analysis.

In addition, Park et al. (2017) worked on a combined system that was developed with an integrated rotary microfluidic system appropriate for DNA extraction, LAMP and a colorimetric lateral flow strip. The multiplex assay included *S. typhimurium* and *V. parahaemolyticus* detection based on a centrifugal microfluidic platform on a single disc. The limit of detection achieved was 50 CFU/sample.

2.5. Streptococcus *spp.*

Among the other bacteria, *Streptococcus* spp. is a major cause of human and veterinary morbidity and mortality worldwide. Biosensors have been proposed to screen milk and water samples for *Streptococcus*. Increased specificity was obtained with magnetically labeled specific antibodies and peptides (Duarte et al., 2016). The detection comprised SPR (Bouguelia et al., 2013), an impedance array (Lillehoj et al., 2014), a cytometer and an electrical sensor (Fernades et al., 2014). Multiplex analysis has been also tested (Lillehoj et al., 2014).

A tool was assessed for diagnosis of bovine mastitis in the agricultural sector caused by *Streptococcus agalactiae*. Milk samples were selected and mixed with a solution that combined specific antibodies and magnetic nanoparticles to achieve rapid and accurate detection by using a lab-on-a-chip magnetoresistive cytometer (Duarte et al., 2016). This method of immunological detection permitted the recognition of 100 CFU/mL.

Khan and colleagues (Khan et al., 2018) worked to optimize the limit of detection and response time for the detection of bacterial strains within a single sensor chip. They suggested an electrically-receptive thermally-responsive sensor conjugated with a composite polymer with evaporated Au electrodes for capturing both Gram-positive and Gram-negative bacteria in real time. The samples matrices were tap water, lake water, autoclave water and milk. The obtained limit of detection was 5 cells/mL for *E. coli, B. subtilis* and *S. mutans*.

2.6. *Other bacteria*

One of the most common foodborne and waterborne illnesses in the European Union is campilobacteriosis. A microresonator (Poshtiban et al., 2013) based on specific phage biorecognition probes immobilized on the sensor surface showed promise for *Campylobacter jejuni* detection. It enabled the use of high resonance frequencies. Various bacterial cells were tested to reinforce the specificity of detection and to decrease signals from nonrelated bacteria. The first attempted electrochemical genosensor based on thin-film gold electrodes deposited onto a Cyclo Olefin Polymer substrate was utilized for the detection of *Campylobacter* spp. in poultry meat, with a limit of detection of 9×10^{-11} mol/ L (Morant-Miñana and Elizalde, 2015). The electrode surface required electrochemical activation, which points to the excellent performance of the device towards acids.

The immediate need for the detection of a variety of microorganisms in the food industry led to development of a biosensor with antimicrobial peptides with species-specific targeting and binding capabilities (Lillehoj et al., 2014). *Streptococcus mutans* and *Pseudomonas aeruginosa* interacted with specific peptides immobilized on a gold surface through cysteine-gold chemistry, which generated an electrical signal. The minimum concentration detected was 10^5 CFU/mL within a period of 25 min. Another work used a conductometric sensing device containing a sub-micron-thick glass membrane for *Pseudomonas putida* (Koo et al., 2018). The device had attached oligonucleotide probe-polystyrene beads which became electrophoretically mobile with an observable step decrease in ionic current with the binding of target 16S rRNA.

There was a need to develop portable, user-friendly biosensors for use in water treatment and environment control. For this purpose, an integrated passive-flow optical microfluidic device was designed. Ring-shaped organic photodiodes were integrated into a capillary-induced flow microfluidic channel for monitoring chemiluminescent sandwich immunoassays enhanced by gold nanoparticles (Pires and Dong, 2014). The method allowed the detection of 4×10^4 cells/mL of *Legionella pneumophila* in water samples.

A few studies have been carried out on the identification of cyanobacteria that produce protease inhibitor oligopeptides such as cyanopeptolins, which cause drinking water contamination. The biochip consists of Au electrode arrays and a microfluidics system which allows a controlled reagent flow for fast DNA detection (Ölcer et al., 2015). The results suggested binding of the avidin and enzyme-modified Au nanoparticles to the biotinylated detection probe, which uses amperometric measurement and gives a limit of detection of 6×10^{12} mol/ L target DNA.

The difficulty typicaly encountered in spore detection was overcome with a portable SPR biosensor using antibodies (Adducci et al., 2016). According to the authors, this was the first report of the differential detection of *Bacullus globigii* with the above method in a mixed sample containing at least one additional *Bacillus* spp. The limit of detection was 10^5 spores/mL with antibody injection and 10^7 spores/mL with direct capture.

In the agricultural sector, bovine mastitis is one of the most costly diseases for dairy farmers. The illness is frequently caused by *Staphylococcus aureus*. To detect the bacterium, milk samples were selected and mixed with a solution that combined specific antibodies and magnetic nanoparticles and analyzed using a lab-on-a-chip magnetoresistive cytometer (Duarte et al., 2017). This method of immunological detection permitted the recognition of 100 CFU/mL of *S. aureus*.

An interesting work presented a use of a phage clone (De Plano et al., 2017) that is able to create a specific complex with the *S. aureus* cell surface. Biosensors based on bacteriophages represent many advantages because of their high specificity for a targeted bacterium, robustness and easy and inexpensive fabrication. The phage clone was immobilized onto a mica surface for selective bacterial detection. The physiosorbed phage permitted the recognition of about 50% of cells in a few minutes.

The development of an integrated biosensing system permitted the detection of three strains of pathogens at the same time (Trinh et al., 2019), utilizing a microdevice able to perform paper-embedded DNA extraction after a 30-min incubation and hydration. Amplification was carried out by the LAMP method and the colorimetric response was observed with the naked eye. The device was simultaneously used to detect the pathogens *E. coli* O157:H7, *Salmonella* spp, and *S. aureus*. The detection assay was completed in 75 min with a limit of detection of 30 CFU/sample.

2.7. Viruses

To determine the presence of hepatitis viruses, an integrated automatic electrochemical immunosensor array has been designed (Tang et al., 2010). In the device, virus antibodies were immobilized on an electrochemical sensor array that captured antigens from the solution. The model has been applied for the detection of $\leq$1.0 ng/mL of viral proteins. A suggestion was proposed for detection of both viruses and bacteria (Sharma et al., 2017). Assays for the detection of natural DNA extracted from Hepatitis A (HAV), Hepatitis E (HEV), *Listeria* and *Salmonella* were developed and tested via conventional fluorescence together with a magnetic tunneling junction. The DNA probes permitted the assessment of multiple pathogens simultaneously in a single assay.

Recent studies focused on the detection of a foodborne pathogen that causes sporadic and epidemic gastrointestinal diseases, norovirus. An electrochemical biosensor was developed with a nano-structured gold electrode conjugated with concanavalin A (Hong et al., 2015). The system could detect the presence of low concentrations of 60 copies/mL.

An innovative idea was implemented with the application of miniaturized microelectromechanical systems and an aptamer to develop a portable electrochemical sensor (Kitajima et al., 2016). A specific aptamer for Norovirus was immobilized on a gold working electrode to bind with the virus, which was measured by means of cyclic voltammetry and fluorescence observation.

A study on the identification of norovirus used a polydimethylsiloxane microfluidic chip incorporated with screen-printed carbon electrodes (Chand and Neethirajan, 2017). The chip was covered with packed silica microbead zones to achieve filtration. The capture was achieved by a carbon electrode with a viral capsid-specific aptamer conjugated with graphene-gold nanoparticles. Modulation of the electrochemical signal revealed the presence or absence of virus, with a detection limit of 1×10^{-10} mol/ L.

Quantum dots (QDs) were used as fluorescence reporters at the 3′ and 5′ terminal of an aptamer for the capture of avian influenza virus (AIV) H5N1 (Xu et al., 2016). The response of the aptamer upon target binding of the virus was demonstrated by a quartz crystal microbalance. The microstructure of the hydrogel in the sensor was characterized by scanning electron microscopy. The spectrophotometric results showed emission peaks whose intensities were correlated to the titer of virus.

For the detection of tobacco mosaic virus-like particles, an impedimetric microsensor platform was applied (Zang et al., 2016). The method used genetic modification of the tobacco mosaic virus-like particle coat proteins, which enabled the autonomous formation of specific virus-like particle nanosensing probes carrying cysteines. The monitoring was assessed by

impedance changes at 100 Hz.The detection limit of the target antibody was 9.1 ng/mL.

3. Discussion

Biosensors are considered to be powerful tools for the detection of target molecules or their intermediates in environmental and food samples (Khan et al., 2018). The performance of a biosensor is affected by a variety of parameters, such as the type of transducer, the affinity of recognition elements to the analyte and the sample matrix. This tool outmatches the traditional analytical methods like PCR and ELISA in terms of short assay time and cost-effectiveness. Biosensors have received considerable interest in clinical diagnosis, industry, the military and environmental science.

This systematic review analyzed one hundred seventy-seven articles to assess various identification systems assisted by different biosensing techniques. As indicated, a significant number of studies focused on the development of biosensors with applicability to food and water samples, as useful tools for industry and in field work. Further research is required to lower the time response and limit of detection and to miniaturize the whole device to a handheld format that can be used at the point-of –care

Optical and electrochemical detection methods are the most commonly used technologies on biosensors because they are able to achieve higher sensitivity than other sensing methods. Among the target organisms, *E. coli* and *Salmonella* spp. constitute the most common pathogenic bacteria associated with foodborne diseases. It appears that biosensors targeting gram negative bacteria use more optical detection while those targeting gram positive bacteria use electrochemical technology. Moreover, only a few studies have been applied in environmental water samples, suggesting operational difficulties for the current methods (Kotsiri et al., 2019). One of the main problems in water microbiological analysis is the high pathogen dilution in samples, as they usually range from 1 to 10 CFU/mL.

Regarding the developed devices, the majority of studies have involved the most common pathogens *S. enterica, E. coli*, and *L. monocytogenes*. Continued studies are taking place to improve the microfluidics of the devices in order to translate the expensive molecular methods into a low cost and portable tool.

More studies have used antibodies for the capture of pathogens than aptamers. That can possibly be explained by aptamers status as an emerging methodology requiring specific nucleotides for each microorganism. Aptamers selected by the cell-SELEX procedure are frequently chosen, as they capture bacterial cells with high affinities without the need for complicated sample preparation (Majdinasab et al., 2018). Only a minor proportion of studies used bacteriophages for bacterial recognition or capturing.

The chi square test was used to assess the differences in the relationships between the target microorganisms and the performed methods. The relation between these variables was marginally significant, as shown in **Appendix A Table S2**.

Biosensors for virus detection have been less fully assessed compared with bacterial detection, which can be explained by the difficulty in concentration taking into account the size and large amount of the matrix that is required in testing water and food samples.

There is still room for optimization of pathogen detection because two of the most challenging issues remain to be solved: limitations linked to food sample preparation for analysis and the low sensitivity of detection methods.

The possibility of applying novel technological achievements for in-field diagnostics is expected to allow control and elimination of health and environmental threats caused by pathogens (Manzano et al., 2015). This could eliminate foodborne diseases, accelerate production rates, and reduce economic losses for the food industry. Some new applications show nanomaterials as promising methods for fast detection, especially when their properties are combined in order to increase the sensitivity. Carbon dots are considered to be appropriate for monitoring pathogenic bacteria and their use combined with aptamers has made significant progress, with low limits of detection (Cui et al., 2019, 2020). In future years, more diagnostic tools will become widely available. Emerging concepts will be created to assure the market's needs. The development of common platforms with disposable components, such as sensing chips and cartridges, which can be customized for multiplex tests and applications, will dominate the biosensor field in the near future.

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Supplementary materials

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