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# Research in health surveillance: an approach in the food microbiology

## Pesquisa em vigilância sanitária: uma abordagem na área de microbiologia de alimentos

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## ABSTRACT

Even with all the efforts related to food quality control, foodborne and waterborne diseases (FWD) are important causes of morbidity and mortality worldwide, emerging as a growing economic and public health problem. This debate brings a reflection on the importance of research in Health Surveillance in food microbiology filed, aiming at the prevention and control of FWD. It is discussed the importance of information, data and other technical-scientific products produced from the research to assist in risk analysis, regulation, and actions of Health Surveillance. Also listed are the priority lines of research published by the National Health Surveillance Agency, the financial incentives for research in this field, and the involvement and importance of Graduate Programs. Based on a non-systematic review of the literature, some challenges are pointed out such as: 1) the need for more financial resources for research focused on microbiological control of food; 2) the strengthening of the Notifiable Diseases Information System for directing of the research lines, since it lists the pathogens, foods, places and geographical regions of higher occurrence of FWD; 3) the strategic implementation of genomic sequencing methodologies in the national system of Public Health laboratories, aiming at the molecular characterization of foodborne pathogens; and 4) the encouragement of research with interdisciplinary approaches and the disclosure/publication of these data in scientific journals or other places that increase the scope of information. These were just some of the challenges discussed, but it is known that many others exist for the improvement of microbiological control of food.

**KEYWORDS:** Scientific Research; Health Surveillance; Food Microbiology; Food Safety; Quality Control

## RESUMO

Mesmo com todos os esforços relacionados ao controle da qualidade de alimentos, as doenças de transmissão hídrica e por alimentos (DTHA) são importantes causas de morbidade e mortalidade no mundo, emergindo como um crescente problema econômico e de saúde pública. Este debate traz uma reflexão sobre a importância da pesquisa em vigilância sanitária na área da microbiologia de alimentos, visando a prevenção e o controle das DTHA. É discutida a importância das informações, dados e outros produtos técnico-científicos produzidos a partir da pesquisa para auxílio nas análises de risco, regulação e ações de vigilância sanitária. Também são elencadas as linhas prioritárias de pesquisa publicadas pela Agência Nacional de Vigilância Sanitária, os incentivos à pesquisa nesta área e o envolvimento e a importância dos Programas de Pós-graduação. Com base numa revisão não sistemática da literatura, são apontados alguns desafios como: 1) a necessidade de mais recursos financeiros para pesquisas voltadas ao controle microbiológico de alimentos; 2) o fortalecimento do Sistema de Informação de Agravos de Notificação para direcionamento das linhas de pesquisa, uma vez que lista os patógenos, alimentos, locais e regiões geográficas com maior ocorrência de DTHA; 3) a implantação estratégica de metodologias de sequenciamento genômico no sistema nacional de

laboratórios de saúde pública, visando a caracterização molecular de patógenos de origem alimentar; e 4) o incentivo a pesquisas com abordagens interdisciplinares e a divulgação/publicação destes dados em periódicos científicos ou outros locais que aumentem o alcance das informações. Estes foram apenas alguns desafios colocados em debate, pois sabe-se que muitos outros existem para o aperfeiçoamento do controle microbiológico de alimentos.

**PALAVRAS-CHAVE:** Pesquisa Científica; Vigilância Sanitária; Microbiologia de Alimentos; Segurança de Alimentos; Controle de Qualidade

## INTRODUCTION

It is through the act of eating that all human beings guarantee the body the supply of energy and nutrients essential for the maintenance of life. Adequate food and nutrition contribute to the promotion, protection, and recovery of health, and food, when offered correctly, contributes to full growth and development[1]. In this way, the food supply needs to be adequate in relation to the amount of nutrients and energy, and safe from a hygienic-sanitary point of view[2].

The safety of the food offered is obtained through care at all stages of the production chain, from production to the final consumer. Mechanisms for regulating, controlling, and inspecting the quality of food offered to the population differ between countries, but all aim to build and improve safe food production and distribution systems[2,3,4].

In Brazil, according to Article 6 of Law No. 8080, of September 19, 1990, the identification and dissemination of conditioning factors and determinants of health are included in the field of action of the Unified Health System; such as nutritional surveillance and dietary guidance; and sanitary and epidemiological surveillance actions[5]. Sanitary control of food is a shared responsibility among public administration bodies and entities, such as the Brazilian National Health Surveillance Agency (Anvisa), the Ministry of Agriculture, Livestock and Supply (Mapa), the State, District, and Municipal Health Surveillances, the Central Public Health Laboratories (Lacen), and the National Institute for Quality Control in Health of the Oswaldo Cruz Foundation (INCQS/Fiocruz)[6]

Anvisa coordinates, supervises and controls activities related to registration, inspection, surveillance, and control of food risks, being responsible for establishing norms and standards of quality and identity. Mapa is responsible for regulating and controlling the manufacture of food of animal origin (meat, fish, milk, cheese, eggs, honey), beverages (soda, juice, alcoholic beverage), vinegar, agricultural products *in natura*, and the classification of foods of origin[6,7].

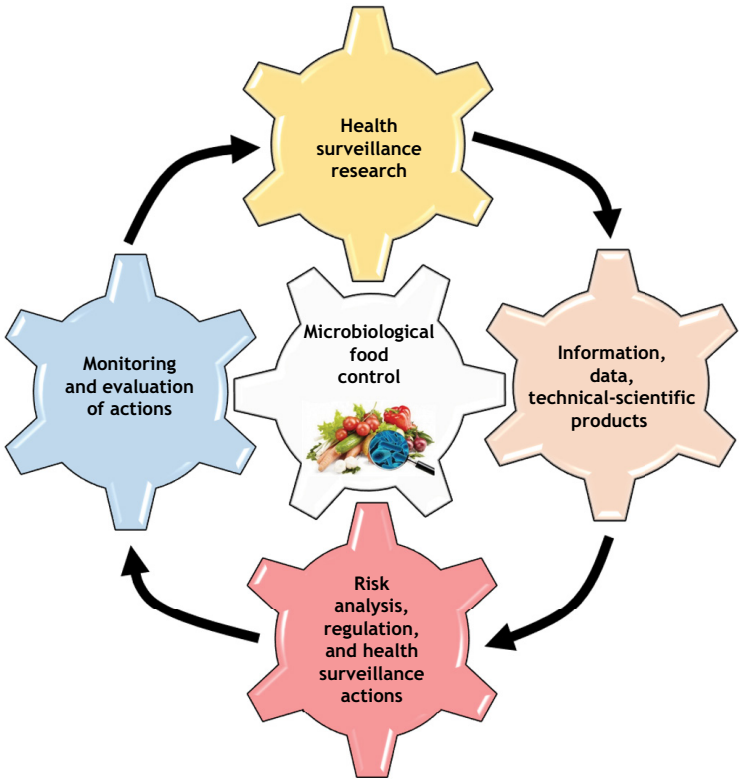
Even with all efforts related to food quality control, water and foodborne diseases (WFD) are important causes of morbidity and mortality worldwide, emerging as a growing economic and public health problem[8,9]. According to Draeger et al.[10], the deficiency in food quality control by public and private agencies has a strong impact on the occurrence of these diseases, as it is necessary to guarantee accurate information for the basis of efficient public policies, which provide greater food security.

Surveillance of WFD outbreaks by the Ministry of Health began in 1999, and they are classified as public health events (PHE). The PHE is compulsorily notifiable in accordance with the National List of Compulsory Notifiable Diseases, Injuries and Public Health Events in Public and Private Health Services throughout the national territory[11]. These data are compiled in the Notifiable Diseases Information System[12] and are of paramount importance to direct the research lines, since it lists the pathogens, foods, places, and geographic regions of greater occurrence of WFD.

Research in health surveillance is located between an innovation system and the social welfare system and must articulate intersectorally to consolidate the area in the production of knowledge and in the search for answers to the numerous problems related to health risks in today's society[13]. In the area of food microbiology, research in health surveillance plays an important role in generating data and other important technical-scientific products for carrying out risk analyzes, that may help in creating and/or changing regulations in the area or more immediate and efficient control and inspection actions, as should occur in cases of WFD outbreaks[9]. Based on the actions taken to comply with regulations (inspections, monitoring programs, among others) and on the data produced by them, new lines of research may be encouraged and/or prioritized over time and the cycle begins (Figure).

In 2011, Anvisa published the National Agenda of Research Priorities in Health Surveillance, which was prepared by a group of specialists and had as assumptions: respecting national and regional health needs and increasing selective induction for the production of knowledge, material and procedural goods in priority areas for the development of social policies. The Agenda comprises four research themes and 119 lines of research. In the theme "2 - Objectives of intervention", item "1.2.3 Food", 14 of these lines are described[13]:

- Studies on family farming and artisanal food production and their impacts on food security;
- Evaluation of the sanitary quality of institutional food;
- Quality control studies, nutritional aspects, and food labeling;
- Assessment of the impact on human health of the use of chemical products (hormones, antimicrobials, and others) in animal husbandry;




Source: Elaborated by the author, 2022.

**Figure.** Cycle of the relationship between health surveillance research and microbiological food control.

- Assessment of safety, impact, and nutritional quality of genetically modified organisms;
- Evaluation of food storage techniques and preservation of nutritional quality;
- Studies on the risk associated with the use of additives in the production of processed foods;
- Studies on street food and impact on health;
- Assessment of contaminants and residues associated with food production;
- Studies on foodborne diseases and emerging pathogens;
- Assessment of the quality of analytical methodologies through

Proficiency Tests;

- Studies on industrial production and its impacts on food security;
- Efficacy, effectiveness, and safety studies of equipment for use in food service units;
- Studies on food and nutrients of interest to food and nutrition policies.

It can be seen that the area of food microbiology is part of several lines of research described in the Agenda. This fact reinforces the importance of encouraging research in this area, in order to guarantee the safety of food offered to the population.

## Incentive and research in the area of microbiological control of food

The *Codex Alimentarius* is an internationally adopted and uniformly presented set of dietary standards. The objectives of the publications of this imported food control body are to protect consumer health and ensure fair practices in international food trade, avoiding unjustified technical barriers to trade[14].

During the review of documents (standards, guides, codes of practice, among others), the *Codex Alimentarius* takes into account data collected by the countries, in addition to scientific literature publications referring to the topic of discussion in question. This data from the scientific literature may have different origins, such as research, development and teaching institutions (universities, technical training schools, foundations), public and private companies, institutes, among others. In this context, the Pos-Graduate Programs that include food quality control in their scope stand out, since they produce course conclusion works, dissertations, theses, scientific articles, technological products (for example: manuals, standardized operating procedures, booklets, and reports), which can be consulted and used in these analyses. According to data from the Sucupira Platform, in the area of Food Science and

Technology, there are 59 Pos-Graduate Programs with different modalities of master's and doctoral courses (Table).

We can see that the number of programs and courses in the academic area is much higher than in the professional area, and there is still no professional doctoral course in the area (Table). However, there are programs from other classification areas of the Coordination for the Improvement of Higher Education Personnel that also operate in food quality control, such as Pharmacy, Collective Health, or Interdisciplinary. The last mentioned is the case of the Graduate Program in Health Surveillance at INCQS/Fiocruz, which has an expressive role in research in the area of food microbiology. This way, this tool is not the most adequate to dimension the graduate programs and courses in Brazil that work in the microbiological control of foods.

Data produced in health surveillance research are extremely important for risk analyses. In the area of food, risk analysis has become "more important than ever" with new modes of food production and processing[15]. Changes in consumption patterns and the expansion of the international market are some of the factors that can contribute to the emergence of new dangers and the urgency of solving known problems[16]. In this way, research in health surveillance is an important tool to know, understand and control these "new" dangers.

For the development of research in health surveillance, investment by the productive sector and inspection bodies is necessary[17]. Another important source of financing also occurs through national and international development agencies. Despite the Universal Public Notices being launched every year by the National Council for Scientific and Technological Development (CNPq), it was only in 2012, in partnership with Anvisa, that the first specific public notice focused on the area of health surveillance was launched. Subsequently, two more public notices were launched and specific actions related to the microbiological control of foods were contemplated in 2012 and 2014, demonstrating the importance of this line of research for the country (Chart).

Even with the launch of these public notices (Table) that contemplated some of the priority areas related to food that were listed in the National Agenda of Priorities for Research in Sanitary Surveillance[13], they are still not enough to meet all research demands. The Agenda aimed to support managers in the areas of health surveillance, research and innovation in order to expand the promotion of prioritized lines of research and contribute to scientific progress in this area and to regulatory processes, supporting all actions relevant to its vast field of action in the protection and promotion of health[13]. Lopes et al.[17] analyzed federal financial transfers for laboratory health surveillance actions in Brazil from 2007 to 2016 and highlighted the need to build a solid and permanent funding policy for public health surveillance laboratories in the country.

## Technical-scientific production of research in sanitary surveillance applied to the microbiological control of food

A priority line of research applied to the microbiological control of food was focused on the development of reference material (RM) production processes for proficiency tests (PT) applied to food microbiology[13]. This initiative aimed to strengthen the quality of laboratories that perform PT in Brazil and reduce external dependence on obtaining RM and participating in PT[18].

Initially, the works were developed using the lyophilization technique as a choice for stabilizing these materials, with the milk matrix being selected due to the intrinsic cryo-protective characteristics of the matrix and because it is liquid, which facilitated the distribution, filling, and subsequent lyophilization of batches[19,20,21,22]. Later, this process was adapted to other matrices such as: cheese[23,24,25,26], beef[27], and chicken[28], chocolate[29], egg[30], rice[30], and water[31]. All these matrices were selected based on the foods most incriminated in cases of WFD in Brazil[12], again highlighting the importance of data from epidemiological surveillance of WFD in directing priority lines of research (Figure).

With the development of these RM, the Proficiency Testing Program for Products Subject to the Sanitary Surveillance Regime (PT/INCQS) can offer 30 rounds of PT in the area of Food Microbiology, strengthening the quality of Brazilian laboratories in the microbiological control of food[30].

Research applied to the production of certified reference materials (CRM) of bacterial strains isolated from food was also carried out. These aimed to reduce dependence on Brazilian laboratories in the acquisition of these materials for use in guaranteeing the validity of the tests carried out. These researches were financed through public notices from CNPq that prioritized this theme (Table) and, subsequently, through a public notice from the Programa Inova Fiocruz[32]. From these works, several CRMs were produced containing different bacterial species of interest in the field of food microbiology[33].

**Table.** Pos-Graduate programs and courses evaluated and recognized in the area of food science and technology registered on the Sucupira Platform.

| Name (Evaluation Area) | Pos-Graduate programs (n = 59) | | | | | | Pos-Graduate courses (n = 91) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AM | AD | PM | PD | AM/AD | PM/PD | AM | AD | PM | PD |
| Food Science and Tecnology (Food Science) | 18 | - | 9 | - | 32 | - | 50 | 32 | 9 | - |

Source: Sucupira Platform. Reviewed and Recognized Courses. Evaluation Area. Knowledge Area. Food Science and Technology [accessed 25 Aug 2022] Available at: https://sucupira.capes.gov.br/sucupira/public/consultas/coleta/programa/quantitativos/ quantitativoAreaConhecimento.xhtml?areaAvaliacao=25.
AM: Academic Master's; AD: Academic Doctorate; PM: Professional Master's; PD: Professional Doctorate; AM/AD: Academic Master's and Academic Doctorate;
PM/PD: Professional Master's and Professional Doctorate.

**Table.** Notices issued by the National Council for Scientific and Technological Development specific to the area of health surveillance.

| Notice | Priority lines related to the microbiological food control | Subtopics | Objectives |
|---|---|---|---|
| Call in Health Surveillance No. 23/2012 | Studies on foodborne diseases and emerging pathogens | Occurrence of foodborne diseases and emerging pathogens | Check the occurrence of foodborne diseases and emerging pathogens (*Trypanosoma. cruzi*, norovirus, and parasites in fruits and vegetables) |
| Call CNPq/Anvisa No. 05/2014 - Health surveillance research | Methodology studies, development of reference materials for evaluating the conformity of products and processes to support the laboratory component of health surveillance actions | Production of reference material | Produce CRMs for use in public laboratories that work with food microbiology, which may include: 1) CRMs for *Salmonella* sp. characterized (identity) for the microorganism and use in qualitative analyzes (presence/absence of the microorganism) or 2) CRM for *Escherichia coli* with determination of the quantitative value for use in quantitative analyzes |
| | Studies on foodborne diseases and emerging pathogens | Prevalence of species of the genus *Campylobacter* in food | To determine the prevalence of species of the genus *Campylobacter* of interest to human health in foods sold in Brazil |
| | | Prevalence of species of the genus enterohemorrhagic *Escherichia coli* in foods | To determine the prevalence of enterohemorrhagic *Escherichia coli* species in foods sold in Brazil |
| Call CNPq/Anvisa No. 17/2017 - Health surveillance research | Not contemplated | Not contemplated | Not contemplated |

Source: Ministry of Science, Technology and Innovation. CNPq. Public Notices. [Access 25 Aug 2022] Available at: http://memoria2.cnpq.br/web/guest/chamadas-publicas?p_p_id=resultadosportlet_WAR_resultadoscnpqportlet_INSTANCE_0ZaM&filtro=encerradas/
CNPq: National Council for Scientific and Technological Development; Anvisa: National Council for Scientific and Technological Development; CRM: Certified Reference Material.

Another line of research in health surveillance applied in the area of food microbiology was that of studies that focused on the evaluation of the microbiological quality of certain food groups in accordance with current national and/or international legislation, associated with any investigation of additional pathogen(s) or groups of microorganisms of public health importance. This type of "experimental design" ended up becoming very common, as the publication of works in scientific journals that only carried out microbiological analyzes provided for in current legislation ended up being seen by editors and/or reviewers as routine activities and without great scientific relevance. In this way, many data referring to microbiological analyzes carried out in food microbiology laboratories, including Lacen, end up not being published and shared with the scientific community. Lopes and Seta[34] carried out an integrative literature review on the theme "public health laboratories and sanitary surveillance" in three databases: 1) Capes Portal - Capes Theses Bank, 2) Scientific Electronic Library Online (SciELO), and 3) Pub-med Central (PMC). The Capes Theses Bank was the one that presented the highest frequency of bibliographic materials in all researched themes, including food. The authors infer that the small number identified in the SciELO and PMC databases seems to be an indication that the production of theses and dissertations does not always result in scientific articles published in indexed journals with full texts and open access.

Researches that evaluated the presence of viruses of interest in food (for example: norovirus and rotavirus) and associated it with the hygienic-sanitary quality foreseen in the legislation were carried out in samples of: lettuce[35], strawberry[36], tomato[37], cheese[38], and natural mineral water[39]. These studies were aligned with Anvisa's priority areas (Chart). Other studies evaluated the presence of bacterial pathogens, such as: *Listeria monocytogenes* in ready-to-eat foods[40], which is currently incorporated as a criterion in current legislation[41,42], and *Cronobacter* spp. in corn-based farinaceous products[43] and natural mineral water[44].

## Characterization studies of pathogens isolated from foods applied to the microbiological control of foods

Another line of research of great importance and explored in the area of food microbiology are studies aimed at the characterization of bacterial pathogens isolated from food samples. In this field, strains isolated in food monitoring studies are collected and used for characterization, focusing on the spread of antimicrobial resistance, virulence factors associated with contamination in the food chain ((for example: biofilm formation, resistance to desiccation, aggregative capacity), molecular genetics, among others.

Research carried out with strains of *Pseudomonas aeruginosa* isolated from samples of mineral water sold in 20-liter gallons demonstrated the ability of most strains to form biofilms[39,45,46]. Extensive studies were also carried out with strains of the genus *Cronobacter*, revealing the circulation of pathogenic clones[44,47,48], the production of cytotoxins[49], antimicrobial resistance[43,47,50,51,52,53], biofilm formation, and aggregative capacity[48].

During the sanitary investigation, it is essential to release results in a short time, in addition to establishing a clonal

relationship between the identified strains, making it possible to investigate the origin of a food outbreak and detect sources of contamination along the food processing chain[54]. Rapid and accurate detection of pathogens is essential during epidemiological investigations into a WFD case or outbreak, and is also critical to preventing foodborne infections and ensuring food safety[55]. In this context, molecular biology applied to the typification of bacteria associated with WFD has been an important tool in the elucidation of outbreaks and understanding of the chain of transmission of these microorganisms[54].

Several techniques can be applied for this purpose, with Whole Genome Sequencing (WGS) being considered the current technique of choice and with the highest investment for this purpose[54,56,57,58,59]. The WGS analyzes the entire sequence present in the genomic DNA of a cell, allowing the identification of mutations that are not possible to visualize in sequencing methods based on small coding regions of the genetic material. The WGS emerged as an efficient technique for tracking microorganism sources in the food industry by adding greater accuracy to the investigation, leading to faster and more efficient decision-making[57,58].

The Food and Drug Administration (FDA), which is the regulatory agency responsible for the safety of food and other products produced and imported in the United States of America, through the Center for Food Safety and Applied Nutrition, established the first laboratory network to use WGS to track foodborne microorganisms, the GenomeTrakr WGS Network. The data is stored in the public database at the National Center for Biotechnology Information (NCBI) and can be accessed by researchers around the world. International PulseNet has also over the years made efforts to implement WGS within the PulseNet network as a routine tool to replace the pulsed field gel electrophoresis technique[58].

To perform the comparison between two bacterial isolates, one of the analyzes that can be used to evaluate the raw data after the sequencing of a bacterial genome is the Multi-locus sequence type (MLST). Three levels of discrimination can be applied: 1) from a scheme that evaluates up to seven constitutive genes, classic MLST; 2) using the central genome MLST (cgMLST) and 3) wide genome MLST (wgMLST)[59].

Despite the benefits of this type of analysis, some factors still limit the use of WGS in the food production chain. Even with the reduction in sequencing costs over the years, the acquisition and maintenance of the equipment, the costs of inputs, and the expertise to perform the procedures and the analysis of the data generated by the professionals still have a higher cost when compared to other molecular methods and limit the use of the WGS technique. In addition, the data may take days for the results obtained to be cured and, to be used routinely, specialized personnel are required who know how to use bioinformatics tools for data processing[54]. In this way, strategies, such as the creation of networks or sentinel laboratories, must be evaluated and implemented so that health surveillance can provide quick responses in order to prevent, minimize, and control outbreaks and sporadic cases of WFD. This was the case of the National Program for Strengthening Health Surveillance Actions in Ports, Airports, and Borders, instituted by Ordinance No. 2,795, of December 6, 2012, which establishes financial incentives for funding for Lacen for actions to carry out laboratory tests[60]. In these actions, it was up to the Lacen of Amazonas, Bahia, Ceará, Pará, Pernambuco, Rio de Janeiro, Santa Catarina, and São Paulo to carry out analyzes for the determination of norovirus in water and food. As the determination of norovirus in water and food requires analyzes that require molecular techniques that are not commonly performed by public laboratories[61], the Lacen of the aforementioned states ended up being chosen for their geographic location closer to the port regions, which would facilitate the effectiveness of the actions.

### Multidisciplinary food research: a challenge to be faced

A challenge to be faced in the area of research applied to food quality control is to carry out work with a multidisciplinary focus. In the past, many works only carried out microbiological or physical-chemical tests individually. This approach ends up weakening the quality control as a whole, as the ideal is increasingly to try to carry out an approach that involves different areas of knowledge, such as label analysis, physical-chemical, microbiological, among others, in order to obtain a more complete panorama of the quality and characteristics of the food, and to allow the correlation of information. An example is the work carried out by Meier et al.[62], which allowed an evaluation of the correlation of physical-chemical parameters of artisanal cheeses with their microbiological quality.

The Resolution of the Collegiate Board of Directors (RDC) No. 724, of July 1, 2022, which provides for the microbiological standards of foods and their application[41], it is a clear example of the need for the convergence of areas of knowledge for the microbiological control of food. This RDC is applied in conjunction with Normative Instruction (NI) No. 161, of July 1, 2022, which establishes microbiological standards[42]. This NI included the quantification of histamine and staphylococcal enterotoxins in certain food groups. To carry out these analyses, immunoenzymatic[63] or physical-chemical[64] techniques are generally used, which are often not implemented in food microbiology laboratories. Furthermore, many professionals do not have the technical qualification to carry out these tests, which would require training for these groups before implementing these methodologies. Another option for resolving this issue is the formation of networks and collaboration between laboratories from different areas of knowledge, in order to enable the resolution of all the tests recommended in the legislation.

## CONCLUSIONS

Research in health surveillance is of great importance for the generation of knowledge, in order to subsidize preventive

actions, regulation of products and services, or even immediate actions to solve detected problems. In the area of food microbiology, it needs more and more encouragement from national and international bodies in order to accelerate the collection of important data for solving current and future problems. As resources are scarce, prioritization within the area is also a challenge, as it depends on WFD epidemiological surveillance data being contemporary, true, and territorial.

With regard to research aimed at the molecular characterization of microorganisms associated with WFD, the WGS is the current technique of greatest choice. Thus, public health laboratories in Brazil will have to adapt to absorb and implement this methodology in the near future.

The sharing of analysis results from food microbiology laboratories through publication in scientific journals, even those that only focus on quality assessment according to legal parameters, also needs to be encouraged.

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## Author's Contributions

Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work. The author approved the final version of the work.

## Conflict of Interests

The author inform that there is no potential conflict of interest with peers and institutions, politicians, or financial in this study