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Microbiological quality of ready-to-eat street foods in Lisbon, Portugal

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Abstract
Street food sector is an important component of the food distribution system in many cities. However, foods exposed for sale on the roadside are generally associated with conditions that favors its potential microbiological contamination. In this study, the microbiological quality of 118 ready-to-eat street foods sold in the metropolitan area of Lisbon, Portugal was evaluated. Mesophilic aerobic bacteria, yeasts, moulds, *Enterobacteriaceae* and *Escherichia coli* were used as spoilage/hygiene indicators. *Listeria monocytogenes, Salmonella* spp., coagulase-positive staphylococci (CPS), *Clostridium perfringens*, *Bacillus cereus* and *E. coli* pathotypes implicated in gastrointestinal disease were used as food safety/hygiene indicators. Thirty-five (29.7%) of the samples were classified as satisfactory, 29 (24.6%) as questionable and 51 (43.2%) as unsatisfactory. Three samples (2.6%) were unsatisfactory/ potentially dangerous, due to the presence of the *L. monocytogenes* > 10^2 cfu/g and/or *B. cereus* > 10^5 and/or CPS > 10^4. *B. cereus* genes encoding causative toxins were detected in the strains isolated from two samples containing > 10^5 cfu/g. *Salmonella* spp., *Clostridium perfringens* and pathogenic *E. coli* were not detected in any of the examined samples. CPS was detected in 26.3% of the samples. A significant relationship between microbiological quality and food groups, with the increase of questionable and unsatisfactory classifications for those samples including raw components, like fruits and vegetables, was observed (Fisher-Freeman-Halton = 29.01, $p < 0.001$). The obtained data highlights for the need of education and training of street-food vendors to improve the food safety management system in the place of sale.

Keywords Ready-to-eat foods · Street food · Food safety management system · Microbiological quality · Microbiological guidelines · Portugal

1 Introduction

The definition of street food was proposed by FAO and WHO as "foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption, or consumption at a later time without further processing or preparation" [1, 2].

Maria J. Barreira and Silvia Marcos have contributed equally to this work.

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Street foods are linked to urban lifestyles and, because of that, are usually commercialized in areas of constant, or intermittent, passage of people, such as near workplaces, schools, public transport stops, and sporadic short-term festive events such as football games, concerts, fairs and summer or Christmas festivals [3]. Although consumers are aware of the existence of some limitations associated with the premises conditions in which ready-to-eat street foods are prepared [4–6] sometimes they choose these foods because of its easy access, lower price, characteristic taste, variety and fast service [4–6].

Street food vending equipment include bicycles, mobile homes, food trucks or food stalls. In many of these small facilities, where food can be prepared, sold and consumed the "Five keys to safer food": keep clean; separate raw and cooked; cook thoroughly; keep food at safe temperatures; and use safe water and raw materials [7] may not be followed. Moreover, the usual accumulation of garbage in these places attracting insects, rodents and/or birds, may naturally, contribute to the contamination of the foodstuff there prepared.

Street food facilities in the European Union (EU) must comply with the requirements of European Commission (EC) Regulation 852/2004 concerning food safety. In this regulation, there is a special provision (in Annex II, chap. III) that sets down the requirements for street food vendors [8]. Street food vendors must follow very strict hygiene rules in order to prevent food contaminations and ensure the safety of the product.

However, according to the European Food Safety Authority [9] between 2018 and 2022, 738 strong evidence outbreaks and 12555 human cases were associated with "restaurant, pub, street vendors, takeway etc.". Also, "restaurant, pub, street vendors, takeway etc." were the main places of exposure reported for strong evidence general outbreaks (*i.e.* outbreaks involving cases from more than one household) (709 outbreaks, 42.1% of the strong evidence general outbreaks). *Salmonella spp*, *Staphylococcus aureus* toxins, *Clostridium perfringens* and *Bacillus* toxins were the most common causative agents found in outbreaks associated to this place of exposure between 2018 and 2022 [9].

Most of the research on street food microbiological quality has been performed outside Europe, in developing countries [10–16], where living and food production conditions are not of a high standard and where, in many places, there is a shortage of potable water. However, with a growing interest on this type of food in European countries, it will be crucial to increase the number of studies evaluating the microbiological quality of street food samples sold in Europe, as these results may contribute to a better understanding of the risks associated with the consumption of this type of food.

It is then the main objective of this work to contribute with scientific data regarding the microbiological contamination of ready-to-eat street foods sold in the metropolitan area of Lisbon, Portugal.

2 Methodology

2.1 Sampling

One hundred and eighteen (118) ready-to-eat street food samples were collected by convenience sampling in seven areas of the Lisbon district (Fig. 1), between March 2019 and December 2022. Samples were purchased in 39 different street food selling points, transported to the laboratory, in the provided packaging, under controlled refrigeration conditions (1 ºC–8 ºC), kept refrigerated (1 ºC–5 ºC) in laboratory and analyzed within a maximum of 24 h after collection.

2.2 Samples classification and characterization

The different samples were classified according to the National Institute of Health Doctor Ricardo Jorge (INSA) guidelines 2019 [17]. They were grouped into groups and subgroups based on the type of preparation (with/without heat treatment), necessity for manipulation or presence of raw components or those with their own flora that had not undergone heat treatment (Table 1):

2.3 Microbiological analysis

ISO 7218:2007/Amd1:2013 [18] and ISO 6887-1:2017 [19] were followed for all microbiological examinations.

All 118 samples were tested for the enumeration of mesophilic aerobic bacteria, *Enterobacteriaceae*, *Escherichia coli*, *Bacillus cereus*, coagulase positive staphylococci, *Listeria monocytogenes*, *Clostridium perfringens*, moulds and yeasts. They

Fig. 1 Street food sampling zones

were also tested for the presence of *Salmonella* spp. and *Listeria monocytogenes* in 25 g. Detection of intestinal pathogenic *E. coli* in 25 g was performed whenever the presence of *E. coli* was detected in the enumeration.

Given our experience, and the available resources, the chosen microbiological tests were those we considered more relevant for the microbiological quality assessment of the ready-to-eat street food samples here tested.

2.3.1 Sample preparation and pre-enrichments

For enumeration tests and *Salmonella* spp. detection a test portion of 26 g of food sample was used to prepare a 1:10 homogenized initial suspension using Buffered Peptone Water (BPW- Oxoid, Basingstoke, Hampshire, UK) in a stomacher bag (bioMérieux, Marcyl l'Etoile,France). For enumeration tests (mesophilic aerobic bacteria, *Enterobacteriaceae*, *Escherichia coli*, *Bacillus cereus,* coagulase positive staphylococci, *Listeria monocytogenes* and *Clostridium perfringens*), subsequent suitable, decimal dilution series of the initial suspension, with tryptone salt diluent (Biokar Diagnostics, Pantin, France) were prepared, according to the expected microbial concentration. For these examinations, about 10 ml of the initial suspension was used. The remaining 250 ml was used as the non-selective pre-enrichment for the detection of *Salmonella* spp, and pathotypes of intestinal pathogenic *E. coli* and incubated at 37 °C ± 1 °C for 18 h ± 2 h, as described in ISO 6579-1: 2017/Amd1:2020, and ISO/TS 13136:2012 [20, 21].

For *Listeria monocytogenes* detection, a 1:10 homogenized initial suspension was prepared using a test portion of 25 g and 225 ml of Half Fraser broth (Biokar Diagnostics). The initial suspension was subsequently incubated at 30 °C ± 1 °C for 24 to 26 h, as described in ISO 11290-1:2017 [22]- primary enrichment.

2.3.2 Mesophilic aerobic *bacteria*, *Enterobacteriaceae*, *Escherichia coli*, *Bacillus cereus* and coagulase positive staphylococci (CPS) enumeration

Enumeration of mesophilic aerobic bacteria, *Enterobacteriaceae*, *E. coli*, *B. cereus* and coagulase positive staphylococci were performed by the AFNOR or MICROVAL validated TEMPO® AC, TEMPO® EB, TEMPO® EC, TEMPO® BC and TEMPO® STA automated methods (bioMérieux), respectively, following the manufacturer instructions.

2.3.3 *Clostridium perfringens* enumeration

Clostridium perfringens enumeration was performed according to ISO 15213-2:2023 [23]. Briefly, black characteristic colonies of *C. perfringens* were enumerated after one milliliter of the initial suspension and subsequent decimal dilutions being incorporated in Tryptone Sulfite Cycloserine (TSC, Biokar Diagnostics) agar and incubated under anaerobic conditions at 37 °C ± 1 °C, for 20 h ± 2 h. GEN box anaer, CO_2 generator sachets (bioMérieux) were used for the culture, in jar, of anaerobic bacteria. The confirmation was performed using lactose fermentation and sulfite reduction tests, at 46 ºC ± 0,5 ºC.

Table 1 Samples classification into groups and subgroups [17]

Group	Subgroup	Food category
Group 1	1A (n = 22)	Fully cooked food not handled after heat treatment, and food reconstituted from dehydrated products, except powdered infant formulas (PIF)
	1B (n = 27)	Fully cooked foods handled after heat treatment not served hot
	1C (n = 3)	Fully cooked foods added with pasteurized components that were storage under refrigeration during shelf-life
Group 2	2A (n = 2)	Mixed fully cooked foods added with small quantities of raw fruits/ vegetables or frozen raw fruits/vegetables
	2B (n = 2)	Mixed fully cooked/pasteurized foods added with raw fruits with, or without, sauces
	2C (n = 38)	Mixed fully cooked/ pasteurized foods added with raw vegetables and may include raw fruits
	2D (n = 9)	Mixed foods, meat, fish or seafood (that may be raw/marinated/smoked or salted) or raw milk cheeses, added, or not, with fully cooked foods or raw fruits/vegetables/seaweeds
Group 3	3A (n = 15)	Raw fruits and/or raw vegetables, cut or prepared for consumption on the same day, with or without sauces, added, or not, with small quantities of fully cooked food
	Total (n = 118)	

Group 1 samples (n = 52) consisted mainly of savory snacks or appetizers that are usually served cold (e.g. patties, salt cod fritters), pastry products (e.g. croissants, Portuguese donuts) and sandwiches (e.g. hot dogs and hamburgers)

Group 2 samples (n = 51) included mainly sandwiches containing fresh vegetables (e.g. hot dogs or hamburgers containing lettuce, tomato and raw carrot) and mixed salads consisting of cooked food and raw vegetables

Group 3 samples (n = 15) included fruits and juices with, or without, vegetables

2.3.4 Yeasts and moulds enumeration

Yeasts and moulds enumeration was carried out in accordance with ISO 21527-1:2008 [24]. Yeasts and moulds were enumerated after 0.1 ml of initial suspension and subsequent decimal dilutions being spread on Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Biokar Diagnostics), and incubated at 25 °C ± 1 °C during 72 h to 5 days.

2.3.5 *Listeria monocytogenes* detection and enumeration

Listeria monocytogenes detection was carried out in accordance with VIDAS-LMO (BIO 12/11–03/04) (bioMérieux) procedure following the technical data sheets of the manufacturer. The positive results were confirmed by isolation in COMPASS® Listeria agar (Biokar Diagnostics) and Oxford agar (bioMérieux) after incubation at 37 °C ± 1 °C, during 48 h ± 2 h. Sheep blood agar plates (COS; bioMérieux) were used to isolate typical colonies of presumptive *L. monocytogenes*. This agar was also used to confirm the β-hemolysis of *L. monocytogenes* isolates after incubation at 37 °C ± 1 °C for 24 h ± 2 h. VITEK 2 compact system (bioMérieux) biochemical tests confirmed the identity of the isolates, which were subsequently stored at – 20 °C in Tryptone Soy Broth (TSB, Biokar Diagnostics) with 20% glycerol.

Enumeration of *Listeria monocytogenes* was performed following ISO 11290-2:2017 [25]. Briefly, *Listeria monocytogenes* colonies were enumerated after 1 ml of the initial suspension (1:10 sample/BPW homogenized dilution) being spread on the surface of three COMPASS® Listeria agar (Biokar Diagnostics) plates.

2.3.6 *Salmonella* spp. detection

The Enzyme immunoassay VIDAS Easy SLM AFNOR (BIO-12/16-09/05) was used for the detection of *Salmonella* spp. antigens using the ELFA (Enzyme Linked Fluorescent Assay) method. The manufacturer`s instructions were followed.

2.3.7 Detection of *Bacillus cereus* toxins

For those samples that presented *Bacillus cereus* levels > 10^5 cfu/g (Supplementary Table 1), genes encoding hemolysin BL (*hbl)*, non-hemolytic enterotoxin (*Nhe*), enterotoxins cytotoxin K1 (*CytK1*) and cytotoxin K2 (*CytK2*) and emetic toxin cereulide (*ces*) were investigated. The presumptive *Bacillus cereus* strains were isolated on *Bacillus cereus* Mannitol Egg Yolk (MYP) agar (Biokar Diagnostics) and confirmed according to ISO 7932:2004 [26].

DNA was extracted using the BioRobot EZ1 automated extraction method (Qiagen, Hilden Germany) and the kit procedure for Gram-positive bacteria (EZ1&2 DNA Tissue Kit, Qiagen).

Detection of *cytK-1*, *cytK-2* and *ces* genes was carried out using the methodologies described in ISO 7932:2004/Amd.1:2020 [26].

Detection of the *nheABC* and *hblCDA* genes, was carried out using the methodologies described in Yang et al. [27] and Ngamwongsatit et al. [28], respectively.

2.3.8 Pathogenic *Escherichia coli* detection

The presence of potentially pathogenic *E. coli* was investigated by the detection of eight pathotype-specific virulence genes (*stx1, stx2, eae, aggR, aaiC, astA, estA* and *eltB*). From the 17 *E. coli* positive samples (≥ 10 cfu/g) (see Supplementary Table 1), DNA of 1 ml of the BPW enrichment was extracted using the BioRobot EZ1 automated extraction method (Qiagen). The kit procedure for Gram-negative bacteria (EZ1&2 DNA Tissue Kit) was followed.

The presence of Shiga toxin-producing *Escherichia coli* (STEC) characteristic genes (*stx1*, *stx2* and *eae)* was investigated by real-time PCR, using the methodologies described in the ISO/TS 13136:2012 [21].

The detection of enterotoxigenic *E. coli* (ETEC) characteristic genes (*eltB* and *estA*) was carried out by multiplex PCR using the primers described by Nguyen et al. [29], methodology adapted from Campos et al. [30], Fujioka et al. [31] and Nguyen et al. [29].

In order to characterize enteroaggregative *Escherichia coli* (EAEC), the *astA* gene was detected by simple PCR using the methodology proposed by Boisen et al. [32] while the *aggR* and aaiC genes were detected by the methodology of Boisen et al. [33].

2.4 Interpretation of the microbiological results and statistical analysis

Guidelines for the interpretation of microbiological results were based on INSA Guidelines [17]. INSA Guidelines are based on international legislation, on several international guidelines for ready-to-eat foods and on the experience of more than 40 years of the National Institute of Health Doctor Ricardo Jorge (INSA). INSA is a centenary institution and the Portuguese Health reference laboratory. These guidelines, are adapted to Portuguese gastronomy, and were established to be applied in microbiological surveillance programs within the implemented food safety systems or in monitoring studies. INSA guidelines apply to single samples ($n = 1$) and the objective is not batch evaluation or official control, because they are not associated with a formal sampling plan.

The microbiological results in ready-to-eat foods were based on the number of colony-forming units (cfu) per gram of the analysed sample and/or on the detection of pathogenic microorganisms and/or toxin(s) (compilation of the used criteria on Supplementary Table 2). The results were classified in 3 levels:

Satisfactory—the analytical result is within the expected values, i.e. less than the Maximum Reference Value (MRV);

Questionable—the analytical result is higher than or equal to the MRV and less than or equal to the Maximum Admissible Value (MAV).

Unsatisfactory and unsatisfactory/potentially dangerous—the analytical result is higher than the MAV or at levels indicating a potential health hazard.

A sample was satisfactory when the level of the analytical results of all the tested microbiological parameters were satisfactory; questionable when at least one of the analytical results had a questionable level and none of them was unsatisfactory or unsatisfactory/potentially dangerous; and unsatisfactory or unsatisfactory/potentially dangerous when at least one of the analytical results was higher than the MAV or at potentially dangerous levels considered for that classification.

Fisher- Freeman- Halton's exact test of independence, with a 99.9% degree of confidence, was performed, using IBM SPSS Statistics 27.0.1 software, to determine if there was a significant relationship between the microbiological quality and the attributed food group.

2.5 *Listeria* spp. whole-genome sequencing (WGS) and in silico typing

Genomic DNA was extracted from fresh cultures of all *L. monocytogenes* isolates, using the ISOLATE II Genomic DNA Kit (Bioline, London, England, UK), and quantified in the Qubit fluorometer (Invitrogen, Waltham, MA, USA) with the dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions. DNA was then prepared using the NexteraXT library preparation protocol (Illumina, San Diego, CA, USA) and then cluster generation and sequencing (2×150 bp) on either a MiSeq, a NextSeq 550 or NextSeq 2000 instrument (Illumina) were performed.

Listeria monocytogenes in silico 7-gene Multilocus Sequence Typing (MLST) was performed using EFSA One Health WGS System Portal, where *Listeria monocytogenes* Fastq sequence files were uploaded (EFSA-S-2023-001192 (sample 20); EFSA-S-2023-001191 (sample 108)). *Listeria monocytogenes* sequence reads were also deposited in the European Nucleotide Archive (ENA) under the bioproject PRJEB31216 (ERR12308493 (sample 20); ERR12308498 (sample 108)).

3 Results

In this study, microbiological analyses were carried out on 118 samples of ready-to-eat foods, purchased in street vending equipment, in various locations in the district of Lisbon. Samples classification into groups/subgroups as well as interpretation of the microbiological results were based on INSA guidelines [17].

Table 2 presents the results regarding the assessment of the level of microbiological quality of the analyzed samples.

Of the 118 tested samples 35 (29.7%) were satisfactory, 29 (24.6%) questionable, 51 (43.2%) unsatisfactory and 3 (2.6%) unsatisfactory/ potentially dangerous (Table 2).

The level unsatisfactory/potential dangerous in three samples (all of subgroup1A) was due to: presence of *L. monocytogenes* above 100 cfu/g (sample 108); concomitant presence of high levels of *B. cereus* ($> 10^5$ cfu/g) and coagulase positive staphylococci ($> 10^4$ cfu/g) (sample 109); and presence of *B. cereus* above 10^5 cfu/g (sample 104) (Supplementary Table 1). *L. monocytogenes* below 100 cfu/g was also detected in another sample from subgroup 1A (sample 20) (Supplementary Table 1). The two *L. monocytogenes* isolates were typed and belong to two different sequence

Table 2 Results by parameter and by group/subgroup of the street-food samples according to INSA Guidelines [17]

Microbiological parameter/ microbiological quality	Group 1			Group 2				Group 3	
	1A total = 22	1B total = 27	1C total = 3	2A total = 2	2B total = 2	2C total = 38	2D total = 9	3A total = 15	Total (*n* = 118) (%)
Meso aero bac									
Sat	12	21	1	2	0	14	5	8	63 (53.4)
Quest	3	2	0	0	0	10	2	6	23 (17.8)
Unsat	7	4	2	0	2	14	2	1	32 (27.1)
Enterobacteriaceae									
Sat	16	21	3	0	0	10	5	5	60 (50.9)
Quest	1	3	0	0	2	9	2	4	21 (17.8)
Unsat	5	3	0	2	0	19	2	6	37 (31.4)
Escherichia coli									
Sat	19	26	3	2	2	27	9	13	101 (85.6)
Quest	–	–	0	0	0	8	0	1	9 (7.6)
Unsat	3	1	0	0	0	3	0	1	8 (6.8)
Moulds									
Sat	22	27	3	2	2	32	9	7	104 (88.1)
Quest	0	0	0	0	0	1	0	3	4 (3.4)
Unsat	0	0	0	0	0	5	0	5	10 (8.5)
Yeasts									
Sat	19	24	2	0	0	34	6	11	96 (81.4)
Quest	3	3	0	0	1	4	0	4	15 (12.7)
Unsat	0	0	1	2	1	0	3	0	7 (5.9)
C. perfringens									
Sat	22	27	3	2	2	38	9	15	118 (100)
Unsat	0	0	0	0	0	0	0	0	0 (0)
Pot dang	0	0	0	0	0	0	0	0	0 (0)
Bacillus cereus									
Sat	19	27	2	2	2	34	9	15	110 (93.2)
Unsat	1	0	1	0	0	4	0	0	6 (5.1)
Pot dang	2	0	0	0	0	0	0	0	2 (1.7)
CP staphylococci									
Sat	16	22	2	1	2	31	6	7	87 (73.7)
Quest	2	4	1	1	0	7	3	7	25 (21.2)
Unsat	3	1	0	0	0	0	0	1	5 (4.3)
Pot dang	1	0	0	0	0	0	0	0	1 (0.8)
L. monocytogenes									
Sat	20	27	3	2	2	38	9	15	116 (98.4)

Table 2 (continued)

Microbiological parameter/ microbiological quality	Group 1			Group 2				Group 3	
	1A total = 22	1B total = 27	1C total = 3	2A total = 2	2B total = 2	2C total = 38	2D total = 9	3A total = 15	Total (*n* = 118) (%)
Unsat	1	0	0	0	0	0	0	0	1 (0.8)
Pot dang	1	0	0	0	0	0	0	0	1 (0.8)
Salmonella spp.									
Sat	22	27	3	2	2	38	9	15	118 (100)
Unsat	–	–	–	–	–	–	–	–	0 (0)
Pot dang	0	0	0	0	0	0	0	0	0 (0)
Total (%)									***n* = 118**
Sat	12	16	0	0	0	5	2	0	**35 (29.7)**
Quest	3	6	1	0	0	10	3	6	**29 (24.6)**
Unsat	4	5	2	2	2	23	4	9	**51 (43.2)**
Pot dang	3	0	0	0	0	0	0	0	**3 (2.6)**

Compilation of the used criteria on Supplementary Table 2

Meso Aero Bac Mesophilic aerobic bacteria, *CP staphylococci* Coagulase positive staphylococci, *Sat* Satisfactory, *Quest* Questionable, *Unsat* Unsatisfactory, *Pot Dang* Unsatisfactory/ potentially dangerous, *NA* Not applicable, *ND* Not detected, *D* detected in 25 g

types, ST 121 and ST 87. Regarding the genes encoding *B. cereus* toxins, two strains were isolated from sample 109, both having *ces* and *nheA, nheB* and *nheC* toxin genes. Additionally, one of the isolates also had the *cytK-2* gene and the other had the *hblA*, hb*lC*, and *hblD* genes. In *B. cereus* isolate from sample 104 were also detected *nheA*, *nheB* and *nheC* genes. *Salmonella* spp., *Clostridium perfringens* and pathogenic *E. coli* were not detected in any of the samples.

Coagulase positive staphylococci was detected in 31 (26.3%) of the samples. In 25/31 samples the results were higher than the MRV and lower than or equal to the MAV. Twenty-eight percent of these samples (7/25) belonged to group 1, 44% (11/25) to group 2 and 28% (7/25) to group 3. Coagulase positive staphylococci results $\geq 10^2$ cfu/g and $< 10^4$ cfu/g were obtained in 5 (4.3%) samples of which 4 (80%) belonged to group 1 and 1 (20%) belonged to group 3. One sample from subgroup 1A was considered unsatisfactory/ potentially dangerous (Table 2).

The level of unsatisfactory (51 samples) was related with several microbiological parameters, being the most frequent the *Enterobacteriaceae* above the MAV (37/51, 72.5%). Levels above MAV of mesophilic aerobic bacteria, moulds, *E. coli* and yeasts were also enumerated in several unsatisfactory samples (32, 10, 8 and 7 samples, respectively) (Supplementary Table 1).

Most of the unsatisfactory samples (30/51, 58.8%) presented levels above the MAV for at least two of the tested parameters and, within the remaining 21, 13 (61.9%) presented also questionable levels for at least one of the tested parameters (Supplementary Table 1).

Concerning the 29 samples with questionable level, the *Enterobacteriaceae*, with an enumeration between MRV and MAV, was the most frequent result (14/29, 48.3%). Moreover, 12/29 (41.4%) of the samples with questionable level had two or more parameters with results between MRV and MAV (Supplementary Table 1).

When looking at the obtained results, considering also the group of the tested samples, it was possible to find a significant association between microbiological quality and food group (Fisher-Freeman-Halton = 29.01, $p < 0.001$). There is a clear decrease in the number of satisfactory samples and a consequent increase of questionable and unsatisfactory samples, as we go from group 1 to group 3 samples (Table 3).

This significant relationship occurs between microbiological quality regarding moulds count and food group (Fisher-Freeman-Halton = 25.79, $p < 0.001$) and microbiological quality regarding *Enterobacteriaceae* at 37 °C count and food group (Fisher-Freeman-Halton = 26.02, $p < 0.001$) (Table 3).

Table 3 Fisher's exact test results-relationship between microbiological quality and sample group

	N	MQ	M		Moulds		Yeasts		EB		EC		Total	
			Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp (%)	Obs (%)
Group 1	52	Satisfactory	27.8	34b	45.8	52b	42.3	45a	26.4	40b	44.5	48b	15.4 (29.6)	28b (53.8)
		Questionable	10.1	5a	4.4	0a,b	6.6	6a	9.3	4a	4.0	0a	12.8 (24.6)	10a (19.2)
		Unsat or pot dang	14.1	13a,b	1.8	0a	3.1	1a	16.3	8a	3.5	4b	23.8 (45.8)	14a (26.9)
Group 2	51	Satisfactory	27.2	21a	44.9	45a	41.5	40a	25.9	15b	43.7	40b	15.1 (29.6)	7b (13.7)
		Questionable	9.9	12a	1.7	1a	6.5	5a	9.1	13a	3.9	8a	12.5 (24.5)	13a,b (25.5)
		Unsat or pot dang	13.8	18a	4.3	5a	3.0	6a	16.0	23a	3.5	3a,b	23.3 (45.7)	31a (60.8)
Group 3	15	Satisfactory	8.0	8a,b	13.2	7b	12.2	11a	7.6	5a	12.8	13a	4.4 (29.3)	0b (0.0)
		Questionable	2.9	6a	0.5	3a	1.9	4a	2.7	4a	1.1	1a	3.7 (24.7)	6a (40.0)
		Unsat or pot dang	4.1	1b	1.3	5a	0.9	0a	4.7	6a	1	1a	6.9 (46)	9a (60.0)
Results		Fisher-freeman-halton	12.68		25.79		6.88		26.02		9.72		29.01	
		p value	0.01		**$2e^{-6}$**		0.11		**$2e^{-5}$**		0.03		**$4e^{-6}$**	

The observed values (Obs) are the number of samples that belong to a category. The expected values (Exp) are the number of samples that we would expect to belong to each category, on average, if the proportions of samples classified as satisfactory; questionable; unsatisfactory or unsatisfactory/ potentially dangerous, were independent of the attributed food group (1, 2 or 3). *p*-values in bold (< 0.001) means there is a significant relationship between microbiological quality and sample group. Different letters means significantly statistically different microbiological quality classification proportions inside each food group (z test with adjusted $p < 0.05$)

MQ Microbiological quality, *M* Mesophilic aerobic bacteria, *EB Enterobacteriaceae*, *EC Escherichia coli*, *Unsat* Unsatisfactory, *Pot Dang* Unsatisfactory/potentially dangerous

4 Discussion

Microbiological and chemical contamination of street foods is believed to be a significant contributor to foodborne diseases [34, 35] being the poor environmental sanitation, inadequate infrastructure and improper food handling, pointed out as the main risk factors associated with street foods [34].

In Europe, there is clearly a lack of studies assessing the hygiene conditions and practices, as well as the microbiological quality of street foods offered for sale in mobile premises [16].

However, the few existent studies in Europe clearly show lack of hygiene conditions and practices in some street food facilities and confirmed that ready-to-eat street foods may be potential vehicles of foodborne diseases. An exploratory study in Oporto, Portugal, reported unsatisfactory microbiological quality for all street food samples tested, and point these foods as potential vehicles of clinically relevant *L. monocytogenes* serotypes and/or *E. coli* carrying clinically relevant virulence/antibiotic resistance features as well as food-handlers as a critical risk factor for foodborne illness [30]. Giammanco et al., in Italy [36], demonstrated that 54% of the 50 "primosale" cheese samples bought in historical street markets of Palermo were of unsatisfactory or borderline quality according to EC regulation 2073/2005/EC criteria. These authors also found a correlation between poor microbiological quality and some retail sale conditions. Still with regard to the microbiological quality of street foods sold in Europe, Lozano-León et al. [37] identified a thermostable direct hemolysin- positive *Vibrio parahaemolyticus* from an outbreak (64 cases of ilness) associated with the consumption of live oysters from a typical outdoor street market in Galicia, Spain.

A study conducted by Czarniecka-Skubina et al. [38], concerning the hygiene practices of European street food vendors, concluded that none of the tested 120 street food facilities, in Paris, was fully compliant with the hygiene requirements.

In our study, INSA Guidelines [17] were used to indicate whether the results of the performed microbiological tests were within acceptable levels, thus making possible to identify situations that were potentially out of control, and to alert to the need of implementing corrective adequate measures. These guidelines allow the assessment of the microbiological quality of processes, defining indicative values, which compliance is not mandatory. They can be used as a tool for evaluating the production and conservation processes of ready-to-eat foods, making possible to check if Good Hygiene Practices (GHP) and the dates stipulated for the consumption of products are under control from a microbiological point of view.

The results obtained in this study, confirm a low compliance with GHP along the different processes. The detection of *E. coli* and coagulase positive staphylococcus (hygiene indicators) above the MRV in 17/118 (14.4%) and 31/118 (26.2%) samples, respectively, indicates the need to encourage food business operators to review or implement Good Hygiene Practices.

The significant association between microbiological quality and food group (Fisher-Freeman-Halton = 29.01, $p = 4e^{-6}$), with a decrease in the percentage of satisfactory samples as we move from group 1 to 3 samples (Table 3), may be related with the type of components included. An inadequate selection and washing of raw components, time/temperature storage abuse and/or the incorrectly setting of product shelf-life may justify these results.

The enumeration of *Enterobacteriaceae* above the MVA was the most common parameter among the unsatisfactory samples, regardless the food group. This microbiological parameter is a good indicator of the compliance with GHP at the end of the process of heat-treated foods. It can also be used as an indicator of adequate washing, hygienization, and time control in products containing fruit, vegetables and other ready-to-eat raw components. However, the levels detected in products with long refrigeration storage periods should be analyzed as indicators of spoilage and not indicators of hygiene.

In this study, the classification in the level unsatisfactory/potential dangerous was only attributed to samples of group 1. However, it is important to notice that these 3 samples are all of the same type of food-coxinhas de frango (chicken croquettes). Although fully cooked, this is a multi-component ready-to-eat food that requires a lot of manipulation to prepare. Moreover, despite being fried before serving, as it is a breaded preparation, with some thickness, it is possible that the temperature reached inside was not enough for the elimination of microorganisms.

The identification of *B. cereus* isolates containing causative toxin genes in two samples with high levels of *B.cereus* is of concern, since the presence of these toxins is associated to gastrointestinal (GI) diseases [39] and intoxications with fatal outcomes continue to be reported [9]. The cause of the high number of *B. cereus,* (8 samples $\geq 10^3$ cfu/g), may be related to temperature and time control problems, mainly in the preservation of cooked foods, the use of contaminated raw materials or contamination through the food processing/preservation environment.

L. monocytogenes, ST 121 and ST 87 clonal complexes, were detected in this study by whole-genome sequencing. These sequence types were already reported in human clinical *L. monocytogenes* isolates whole-genome sequenced in the context of a cross-sectional survey of *Listeria monocytogenes* in ready-to-eat foods and human clinical cases in Europe [40]. ST87 was also reported in a study performed in Gipuzkoa in Northern Spain, aiming to describe the clinical features and the molecular epidemiology of human listeriosis over the 2010–2020 period [41] and ST 121 was reported in a study investigating the population diversity of *Listeria monocytogenes* isolates from diverse sources and geographical locations (mainly Europe and North America) [42]. The integration of both *L. monocytogenes* isolates in the global WGS *L. monocytogenes* Portuguese National Institute of Health database, allowed to verify that ST121 isolate integrated a large cluster of strains isolated from foodstuffs (with strains since 2009), highlighting for the wide-range transmission of *L. monocytogenes* strains.

5 Conclusions

Despite the existence of European regulation that sets down the requirements covering street food vendors [8], the microbiological quality level detected in the convenience samples examined, in this study, highlights the need for food business operators to improve the implemented food safety systems. Programs to motivate and raise awareness, among this specific group of food business operators, about the importance of compliance with the rules of good personal hygiene practices, good manufacturing practices and how incorrect handling (cross-contamination) can influence the safety and quality of the finished products and in consequence the consumer`s health, as well as the assignment/determination of an adequate shelf-life to prepared street foods, should be encouraged.

The data obtained provide insight to the need for periodic/regular microbiological surveillance by food business operators and monitoring studies by official entities, on ready-to-eat foods and surfaces in contact with foods and in the assessment of the hygienic and structural conditions of the premises in these specific units that sell/serve street foods. Moreover, data generated may be of great value for the development of quantitative microbiological risk assessment models using geographical specificities.

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Data availability All supporting data and protocols have been provided within the article or through Supplementary data files. *Listeria monocytogenes* sequence reads were deposited in the European Nucleotide Archive (ENA) under the bioproject PRJEB31216 (ERR12308493 (sample 20); ERR12308498 (sample 108)).

Declarations

Ethics approval and consent to participate Not applicable.

Competing interests Authors disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

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