# Microbiological Contamination in Different Food Service Units Associated with Food Handling

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**Abstract:** Background: A microbiological analysis of samples taken from hands of food handlers and utensils/crockery in food service units was used to assess the risk of food contamination. We aimed to assess indicators of microbiological contamination in different food service units. Methods: We used data for an official inspection proposal (2018–2019) in different food service units serving a district in Northeast Portugal. We showed the proportion of positive samples for each microorganism assessed in samples taken from hands and kitchen utensils/crockery. Results: Out of 471 samples taken from hands of food handlers, 26.8% (n = 126) were classified as "unsatisfactory" due to the presence of at least *Escherichia coli*, *Staphylococcus aureus* and/or total and fecal coliforms. Such a proportion varied according to the type of food service unit with a higher proportion being observed in Canteens (40.0%) and in Retirement Home/Day Care Centers (37.8%). More than one third of 649 samples taken from kitchen utensils/crockery were classified as "unsatisfactory" (n = 181; 27.9%) or "bad" (n = 49; 7.6%). There were significant differences in these proportions across food service units, with a higher proportion of "unsatisfactory" samples being observed in Fishmongers and Butcher Shops/Charcuteries (50.0% and 45.1%, respectively), while a higher proportion of "bad" samples was observed in both Pastry/Bakery and Fishmonger establishments (33.3%), followed by Butcher Shops/Charcuteries (14.1%). Conclusions: Our results emphasize the need of awareness of hygiene practices among food handlers of different food service units, particularly in Fishmongers, Pastry/Bakery establishments and Butcher Shops/Charcuteries, highlighting the need of a periodic hygienic evaluation.

**Keywords:** food contamination; microbiological evaluation; food handlers' hands; utensils; hygienic indexes

## 1. Introduction

Over the centuries, although they remain underreported and their incidence unknown, foodborne diseases are the ones that most affect the human population. These diseases are due to several microorganisms which are influenced by climate change, changes in food technology, social, economic and demographic habits, as well as the globalization of the food trade [1]. According to the World Health Organization (WHO), foodborne diseases cause 600 million cases and 420,000 deaths annually and the most frequent causes of foodborne illness were diarrheal disease agents [2]. There has been an increasing concern about food quality and safety in order to prevent foodborne diseases. Therefore, the

surveillance of products provided by food service units has been an emergent issue in the field of public health.

Microbiological contamination is one of the major challenges when food safety is under discussion. Indeed, the majority of foodborne illnesses has been associated with bacterial contaminants [3,4]. Certain groups, such as elderly, young children, pregnant women or immuno-compromised people, have a high risk of having food-related diseases that can lead to death, by pathogen mechanisms that take advantage of a weak immune system [2,3].

Some foodborne illnesses due to microbiological contamination are associated with poor handling practices during meal processing in food units or food service establishments [5]. Poor hygiene conditions to which food is exposed during processing, storage and distribution contribute to increasing foodborne risk [3]. In addition, cross-contamination is one of the main factors associated with microbiological food contamination before the final preparation step [3,5]. Cross-contamination due to the transfer of microorganisms from objects or surfaces to the food is an important issue. Indeed, a pathogenic microorganism can remain viable in cutting boards and food contact surfaces [6–8] demanding the rigorous cleaning and disinfection of kitchen utensils, equipment and surfaces [9,10]. Hands of food handlers have also been considered an important determinant of microbiological contamination of food and washing hands plays a valuable role in preventing the transmission of foodborne illnesses [4,11,12]. *Staphylococcus aureus* colonizes the nasopharynx of asymptomatic carriers leading to the contamination of the hands of food handlers [13,14]. Antimicrobial resistant strains of *S. aureus* have been detected in food [12,15,16] and foodborne outbreaks of *S. aureus* have been related with post-process food contamination by the hands of food handlers [17].

The increasing concern about food safety and food quality demands the compulsory implementation of hygiene procedures to control the microbiological contamination of food in food units and food service establishments. The European Union (EU) has adopted a regulation for improving the quality of food [18]. The training of all those involved in the preparation, processing and distribution of food in order to prevent the incorrect handling and, consequently, the spread of food-borne diseases is fundamental to the application of good food handling practices [5]. However, monitoring the hygienic conditions of food service units is an excellent tool to know if food handlers in food establishments or food units comply with hygiene procedures and to understand which food establishments deserve special attention from public health authorities [18–20].

Hygienic conditions are assessed by the index of total and fecal coliforms, belonging to the *Enterobacteriaceae* family, which are usually found in the intestinal tract of humans and other warm-blooded animals. The presence of fecal coliforms is an indicator of fecal contamination allowing to assess poor hygienic sanitary conditions. Fecal coliforms do not indicate a health risk, but are a premise for the possible existence of other microorganisms considered dangerous for consumer health [21–23]. Bacteria belonging to the *Enterobacteriaceae* family are the main agents of intestinal infection, standing out as the most interesting microorganism in food *Escherichia coli*. Food associated outbreaks have been particularly associated with verocytotoxin producing *E. coli* (VTEC), which the strain O157:(H7) has been recognized as a very important cause of food-borne illness [22,23]. This bacterial species is one of the most important indicators of fecal contamination of meat products, vegetables, fruits, juices, milk, drinking water [22,23], as well as fecally contaminated hands of food-handlers [21].

The presence of a large microbial population responsible for food contamination has been assessed by the counts of mesophilic aerobic bacteria (colonies growing at 30 °C). Although there is no distinction between pathogenic microorganisms and microorganisms causing changes in food, the determination of mesophilic aerobic bacteria gives a general indication of the contamination of food or utensils used during storage or processing [8,24]. Therefore, the aerobic mesophilic bacteria, total and fecal coliforms, *E. coli* and *S. aureus*

have been considered as microbial indicators and have been used for the assessment of a poor hygienic and sanitary food quality [4].

In Portugal, there are public health units working at a local level and they are constituted by the public health doctoral and sanitary staff. These teams are responsible for tasks related with public health surveillance [25]. One of these tasks is the inspection of food units for the assessment of the hygiene status of hands of food handlers and surfaces. By using data collected for official inspection purposes, we aim to evaluate the hygiene level of different food services units serving a district in Northeast Portugal. The main reason for carrying out this type of assessment in food units is to minimize the risk of microbial contamination associated with food unities, leading to an intervention by public health units whenever the results are not satisfactory. This intervention is based on official Portuguese regulations [26] and European laws [27].

## 2. Materials and Methods

### 2.1. Study Setting and Data Collection

This is a descriptive study based on data collected between January 2018 and December 2019 for audit proposal in regard to the hygiene and safety in food service units, aiming specifically at the assessment of hygiene status of hands of food handlers and surfaces. Data collection was carried out by Environmental Health Technicians from a Public Health Unity serving a district in Northeast Portugal. Technicians went to different food service units in this geographic area for collecting samples from the hands of the food handlers and from kitchen utensils and crockery used in food handling. Samples were taken by using swabs moistened with tryptone saline solution and placed at 30° angle with the surface from which the sample should be taken.

The microbiological analyses were carried out in Public Health Laboratory (PHL) by microbiological techniques using appropriate culture media for plate counts of coliforms, *E. coli*, *S. aureus* and mesophilic microorganisms at 30 °C, in accordance with the sanitary surveillance program at regional level, proposed by the local public health authorities. Out of all samples collected during the time period studied, samples from cheese factory (n = 6) were excluded because there were few to be analyzed according to the type of food service unit. The data analyzed correspond to 1120 samples taken from the surface of the hands of the food handlers (n = 471) and from utensils/crockery used in food handling (n = 649).

### 2.2. Microbiological Parameters Assessed

In regard to the samples taken from kitchen utensils/crockery, the microbiological parameters of interest were the number of colony forming units (CFU) of mesophilic microorganisms per swab/plate and the presence (positive sample that means 1 CFU or more) of total coliforms, fecal coliforms, *E. coli,* using an adapted method previously described [26]. In regard to the number of CFUs of mesophilic microorganisms, samples were classified into three groups (<100, 100–999 and ≥1000 CFUs) while the presence of coliform bacteria and *E. coli* allowed to classify the samples as positive (at least 1 CFU). Briefly, for total coliforms, fecal coliforms and *E. coli* evaluation, samples were cultured in MacConkey broth and incubated at 37 °C/48 h. Next, bacterial suspensions were inoculated in Brilliant Green Bile medium (2%) at 37 °C and 44 °C/48 h, and Buffered Peptone Water medium at 44 °C/48 h, followed by indole detection.

The microbiological parameters of interest in samples taken from the hands of food handlers were the presence (positive sample that means 1 CFU or more) of total coliforms, fecal coliforms, *E. coli* and *S. aureus* according to an adapted procedure described before [26]. For total and fecal bacteria, as well as *E. coli*, samples were cultured in MacConkey broth and incubated at 37 °C/48 h, followed by incubation in Brilliant Green Bile medium (2%) at 37 °C and 44 °C/48 h, and Buffered Peptone Water medium at 44 °C/48 h and indole detection. For *S. aureus* determination, the samples were inoculated in Mannitol Salt Agar at 37 °C/48 h and bacteria suspension was tested for coagulase positivity [28,29].

The hygiene status of kitchen utensils/crockery was classified as "satisfactory" if there were less than 100 CFU of mesophilic microorganisms at 30 °C per swab/plate [26] and the detection of coliforms was negative; as "unsatisfactory" if the number of CFU at 30 °C was greater than 100 per swab/plate or if the presence of total coliforms was positive; as "bad" if the detection of fecal coliforms or *E. coli* was positive [28,29]. The hygiene status of the hands of food handlers was considered "unsatisfactory" if there was sample positivity for at least one type of microorganisms assessed and "satisfactory" otherwise.

### 2.3. Data Processing

The data obtained were processed using Microsoft Office Excel (Microsoft Office 365 Pro PLUS). We obtained the proportion (%) of "unsatisfactory" samples among the total of samples taken from hands of food handlers and of "unsatisfactory" and "bad" samples among the total of samples taken from kitchen utensils/crockery. Among samples taken from hands, the proportion of positive samples for total coliforms, fecal coliforms, *E. coli* and *S. aureus* were computed. The samples taken from kitchen utensils/crockery were classified according to the number of mesophilic microorganisms at 30 °C per swab/plate into less than 100, 100–999 and 1000 CFUs or more and the proportion of samples in each group was obtained. We also computed the proportion of samples taken from kitchen utensils/crockery that were positive for total coliforms, fecal coliforms and *E. coli*.

All results were stratified according to the type of food service categorized into the following groups: Canteen, School/Kindergarten, Retirement Home/Day Care Center, Pastry/Bakery establishment, Fishmonger, Restaurant/Coffee Shop/Snack Bar, Supermarket/Grocery Store and Butcher Shop/Charcuterie. Differences between the type of food service units in regard to the microbiological parameters were assessed by using the qui-square test ($\chi^2$) or the likelihood-ratio chi-square test (LR) when the expected value of the number of sample observations in each group of the variable was less than five. The significance level was set at 0.05.

### 3. Results

Table 1 shows the distribution of samples taken from hands of food handlers and from crockery/utensils according to the type of food service, as well as, the proportion of samples classified as "unsatisfactory" and "bad". There were 1120 samples analyzed, the majority of them were obtained from Restaurants/Coffee Shops/Snack Bars (n = 457), followed by Retirement Homes/Day Care Centers (n = 248), Butcher Shops/Charcuteries (n = 141), Schools/Kindergartens (n = 131), Supermarkets/Grocery Stores (n = 53), Pastry/Bakery establishments (n = 41), Fishmongers (n = 26) and Canteens (n = 23).

**Table 1.** Distribution of samples taken from hands of food handlers and from crockery/utensils according to the type of food service.

| Type of Food Service | Samples Taken from Kitchen Utensils/Crockery n = 649 | | | | Samples Taken from Hands of Food Handlers n = 471 | | |
|---|---|---|---|---|---|---|---|
| | Total | Unsatisfactory | Bad | *p*-Value | Total | Unsatisfactory | *p*-Value |
| | n | n (%) | n (%) | | n | n (%) | |
| Canteen | 13 | 3 (23.1) | 1 (7.7) | | 10 | 4 (40.0) | |
| School/Kindergarten | 73 | 17 (23.3) | 3 (4.1) | | 58 | 14 (24.1) | |
| Retirement Home/Day Care Center | 147 | 31 (21.1) | 13 (8.8) | | 101 | 38 (37.6) | |
| Pastry/Bakery | 24 | 6 (25.0) | 8 (33.3) | <0.001 * | 17 | 5 (29.4) | 0.026 ‡ |
| Fishmonger | 12 | 4 (50.0) | 6 (33.3) | | 14 | 4 (28.6) | |
| Restaurant/Coffee Shop/Snack Bar | 282 | 75 (26.6) | 8 (2.8) | | 175 | 48 (27.4) | |
| Supermarket/Grocery | 27 | 11 (40.7) | 2 (7.4) | | 26 | 3 (11.5) | |
| Butcher Shop/Charcuterie | 71 | 32 (45.1) | 10 (14.1) | | 70 | 10 (14.3) | |

* likelihood-ratio chi-square test; ‡ chi-square test.

Out of 471 samples taken from hands of food handlers, 26.8% (n = 126) were classified as "unsatisfactory" due to the presence of at least one type of the microorganisms assessed. Such proportion varied according to the type of food service unit from 11.5% in Supermarket/Grocery Stores to 40.0% in Canteens ($\chi^2$ = 15.94; *p* = 0.026). Instead, more than one third of 649 samples taken from kitchen utensils/crockery were classified as "unsatisfactory" (n = 181; 27.9%) or "bad" (n = 49; 7.6%). There were significant differences in this proportion across food service units (LR = 65.01; *p* < 0.001), such that the proportion of "unsatisfactory" samples was higher in Fishmongers and Butcher Shops/Charcuteries (50.0% and 45.1%, respectively), while a higher proportion of "bad" samples was observed in both Pastry/Bakery and Fishmonger establishments (33.3%), followed by Butcher Shops/Charcuteries (14.1%) (Table 1).

Of all samples taken from kitchen utensils/crockery, 14.0% (n = 91), 7.4% (n = 48) and 2.9% (n = 19) were positive samples for total coliforms, fecal coliforms and *E. coli*, respectively. Figure 1 presents the proportion of samples taken from kitchen utensils/crockery, presenting one CFU or more of total coliforms, fecal coliforms or *E. coli,* according to the type of food service unit (samples could be positive for all microorganisms assessed). There were significant differences in the frequency of positive samples for total coliforms, varying from 7.8% in Canteens to 50% in Fishmonger establishments (LR = 28.29, *p* < 0.001), for fecal coliforms varying from 2.8% in Restaurants/Coffee Shops/Snack Bars to 33.3% in Pastry/Bakery and Fishmonger establishments (LR = 35.57, *p* < 0.001) and for *E. coli* varying from 0.0% in Schools/Kindergartens and Supermarket/Grocery Stores to 25.0% in Fishmonger establishments (LR = 24.90, *p* < 0.001).
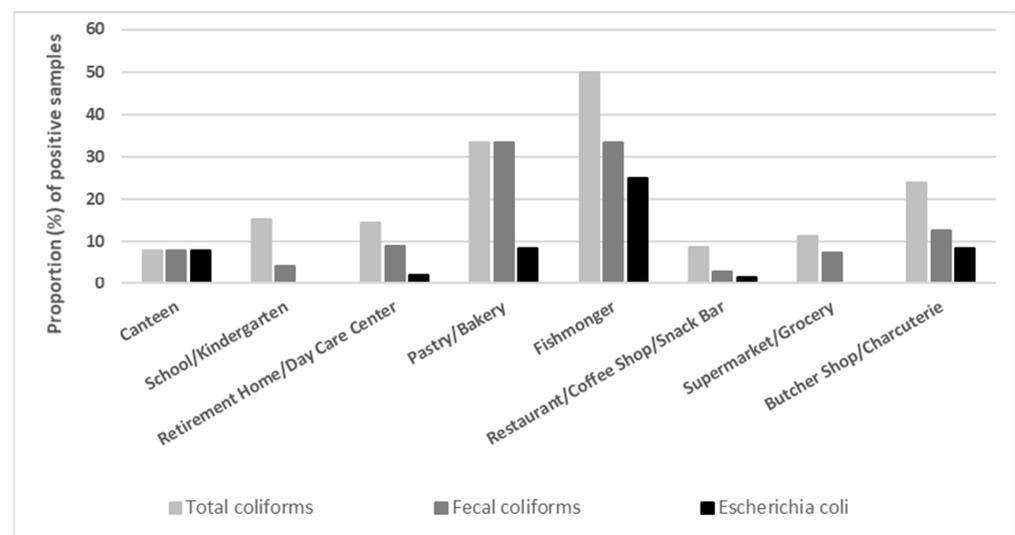
**Figure 1.** Proportion of positive samples for each group of microorganisms assessed in samples taken from kitchen utensils/crockery.

Figure 2 presents the proportion of samples taken from kitchen utensils/crockery, presenting less than 100 CFUs, 100–999 CFUS and 1000 or more CFUs of mesophilic microorganisms at 30 °C per swab/plate according to the food service unit. Concerning this parameter, 17% (n = 109) of samples taken from kitchen utensils/crockery presented between 100 and 999 CFUs and the same proportion of samples presented 1000 or more CFUs. The higher proportion of samples presenting 1000 or more CFUs of mesophilic microorganisms were observed in Fishmonger establishments (75.0%; n = 9), Butcher Shops/Charcuteries (41.0%; n = 29) and Supermarket/Grocery Stores (37.0%; n = 10). Otherwise, the results showed that a higher proportion of samples with the lowest level of mesophilic microorganisms (<100 CFU/utensil) were harvested in Schools/Kindergartens (76.7%), followed by Restaurants/Coffee Shops/Snack Bars (72.7%), Retirement Homes/Day Care Centers (71.4%) and Canteens (69.2%).
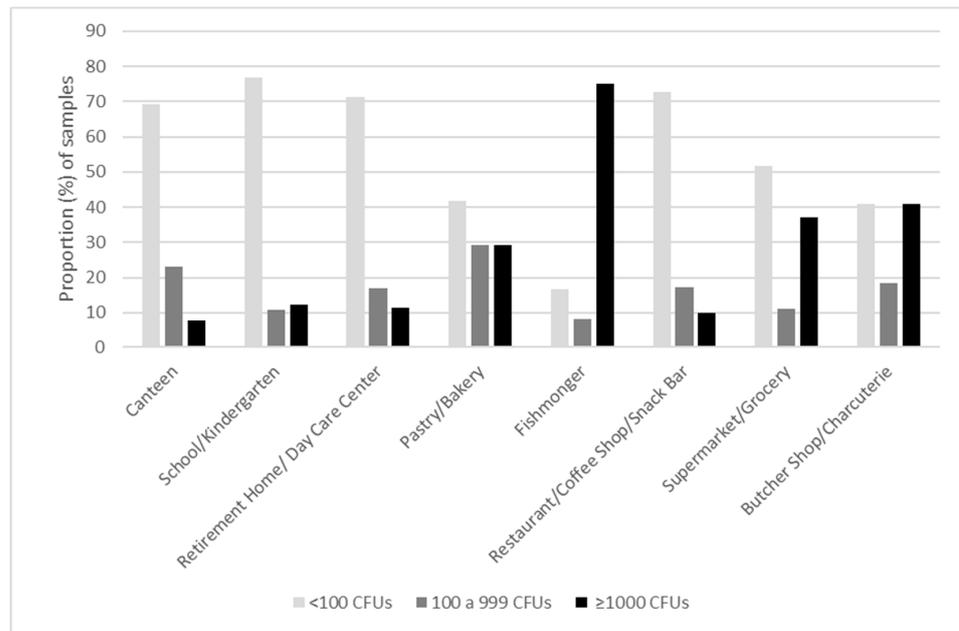
**Figure 2.** Proportion of samples taken from kitchen utensils/crockery according to the number of CFUs of mesophilic microorganisms at 30 °C per swab/plate.

In regard to the samples taken from hands of food handlers, there were 21.2% (n = 100), 11.5% (n = 54), 8.9% (n = 42) and 3.6% (n = 17) of positive samples for total coliforms, fecal coliforms, *S. aureus* and *E. coli*, respectively. Figure 3 presents the proportion of positive samples taken from hands according to the type of food service unit. There were significant differences in the frequency of positive samples for total coliforms, varying from 7.7% in Supermarket/Grocery Stores to 40% in Canteens (LR = 21.64, *p* = 0.003) and for fecal coliforms varying from 0.0% in Supermarket/Grocery Stores to 20.0% in Canteens (LR = 20.46, *p* = 0.005). Positive samples for *E. coli* were observed in Schools/Kindergartens (n = 2; 3.4%), Retirement Homes/Day Care Centers (n = 7; 6.9%) and Restaurants/Coffee Shops/Snack Bars (n = 8; 4.6%).
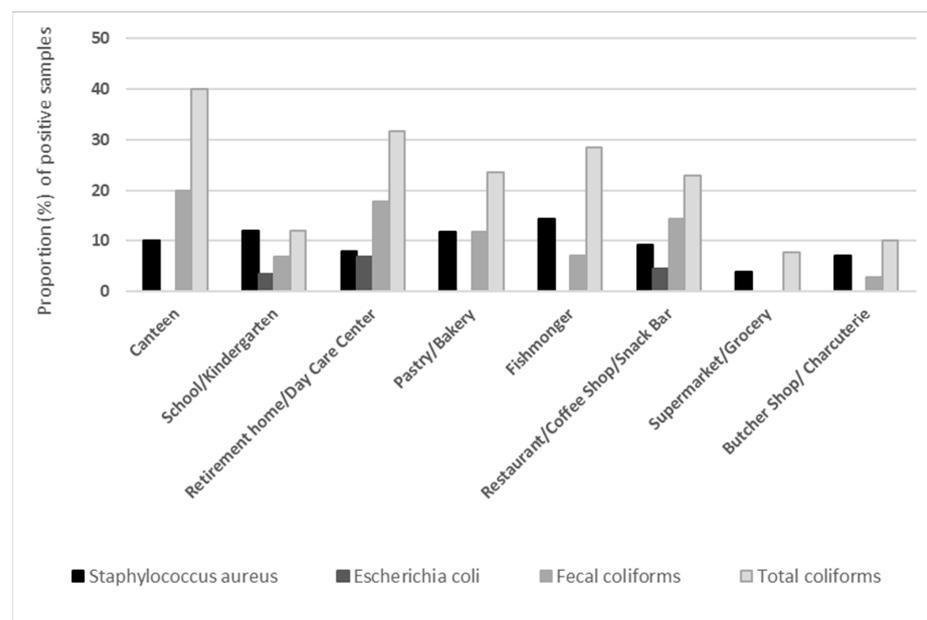
**Figure 3.** Proportion of positive samples for each group of microorganisms assessed in samples taken from hands of food handlers.

## 4. Discussion

This analysis was based on data collected for official inspection proposal in order to assess the hygiene level of food service units based on the detection of microbial indicators. According to our results, there were differences in the hygienic level according to the type of food services. Fishmongers, Butcher Shops/Charcuteries and Pastry/Bakery Shops presented the worst profiles in regard to the microorganisms control in utensils/crockery. Instead, among samples taken from hands of food handlers, a higher proportion of "unsatisfactory" samples were observed in Canteens and Retirement Homes/Day Care Centers.

Since animals slaughtered for human consumption are reservoirs of microorganisms [4,30], presenting a high load in the digestive tract, the evisecretion has been associated with contamination of carcasses and cuts of sheep, cattle and poultry that will be butchered [30–32]. In addition, it has been described that Butcher Shop operators often used the same chopping board and knife to split different kinds of meat, increasing the bacterial count during this food preparation process [33]. Thus, the butchering process of contaminated carcasses and cuts of animals is likely to contaminate the utensils used for butchering [6]. Additionally, fish used for human consumption can harbor several pathogenic microorganisms [34]. Therefore, the evisecretion and preparation of fish for sale in Fishmongers can be a strong determinant of microbiological contamination of utensils used in this process. Additionally, Fishmongers require the use of utensils that contact directly with the food, during the scaling and/or evisecretion of the fish [35]. These issues could explain the high proportion of "unsatisfactory" and "bad" samples taken from utensils used in Fishmongers and Butcher Shops/Charcuteries we observed in our setting.

Our results also revealed a worrisome proportion of samples taken from utensils/crockery in Pastry/Bakery establishments classified as "bad" (33%). Because Pastry/Bakery establishments usually serve ready-to-eat foods such as sandwiches, which are not exposed to a high temperature before consumption, our findings posed added concerns in regard to the microbiological quality of these foods. Indeed, foodborne pathogens have been detected in sandwiches pastries and desserts partially explained due to the cross-contamination and mishandling during the food preparation [36].

According to our findings, a higher proportion of positive samples for *E. coli*, fecal and total coliforms were taken from utensils/crockery in Fishmongers, Pastry/Bakery and Butchery/Charcuterie establishments. The coliform group is part of the family of *Enterobacteriaceae* which includes microorganisms sensitive to heat treatment [4] and that are usually destroyed by most disinfectant agents used in food production for the cleaning of surfaces and equipment [37]. In this sense, the food units with a high proportion of samples taken from utensils/crockery that were positive for fecal coliforms and/or *E. coli* reflect poor hygiene conditions.

Our data also revealed that Canteens and Retirement Homes/Day Care Centers had a high proportion of samples taken from hands of food handlers classified as "unsatisfactory", denoting less adherence to handwashing practices. Indeed, it has been reported that a reduction occurs in counts of *Enterobacteriaceae,* including *E. coli* after hand washing, particularly when food handlers use antimicrobial hand soap [11,20]. Additionally, the evaluation of hygienic patterns of the hands of manipulators in different food units also showed that more than 10% of positive samples for *S. aureus* were found in Fishmongers (14.3%), Schools/Kindergartens (12.1%) and Pastry/Bakery establishments (11.8%). This should be considered worrisome because in Fishmonger and Pastry/Bakery establishments there is a great need for the manipulation of food, exposing it more easily to microbial agents present on the hands [14,35].

Otherwise, our results showed that Schools/Kindergartens, Restaurants/Coffee Shops/Snack Bars, Retirement Homes/Day Care Centers and Canteens presented a low level of mesophilic microorganisms (<100 CFU/utensil). It is possible that these food units are more prone to comply with guidelines to control poor surface hygiene, cross-contamination, insufficient heat treatment and the regulation of the time/temperature of food storage [26]. It is important to highlight that inadequate cooking, cross-contamination and inadequate

storage contribute to the foodborne disease burden when foods are contaminated before the final preparation step [5]. Our results also emphasized that Schools/Kindergartens have patterns of good hygiene practices associated with utensils, supported by the absence of *E. coli* and the highest proportion of samples with mesophilic microorganisms below 100 CFUs. A recent study reported that appropriate food hygiene interventions among child caregivers of weaning infants, focused on safe hand hygiene, safe food preparation and storage, as well as safe feeding, can reduce foodborne exposure to enteric pathogens [38].

The classification of samples taken from utensils/crockery as "unsatisfactory" or "bad" was based on a positivity for the coliforms bacterial group and a high load of mesophilic microorganisms (>100 UFC). According to this classification, Fishmongers, Butcher Shops/Charcuteries and Pastry/Bakery establishments were the food unities with a higher risk of contamination and, therefore, foodborne diseases are more likely to be associated with these food units.

There is a body of research highlighting the association between bacterial counts in utensils and hands during the process of food preparation and the microbiological contamination of food. It has been reported that an increase in bacterial count occurs in meat samples when Butcher Shop operators use the same chopping board and knife to split different kinds of meat [32,33]. In addition, the evisecretion and preparation of fish for sale in Fishmongers can be a strong determinant of microbiological contamination of fish [35]. Moreover, foodborne pathogens detected in sandwiches, pastries and desserts are due to cross-contamination and mishandling during the food preparation [36]. However, to the best of our knowledge, there is no published studies based on the same classification of samples we used, preventing comparisons with previous research.

There is a lack of data about the hygiene practices in food units included in this study, namely the hygiene behavior of the staff and compliance with appropriate cleaning procedures. Data about these issues could give insights about hygiene practices in order to change guidelines and to design training aimed at the hygiene practices among food handlers. Although this limitation, the results here reported provide information on the hygienic status in different food units and, to the best of our knowledge, there is no published studies based on data collected by public health authorities for the assessment of the health status in different types of food units. Based on such information, health authorities can implement strategies to sensitize workers about good hygienic practices in the food sector, in order to prevent possible food outbreaks and to minimize the onset of foodborne diseases [18]. Our results alert the health authorities in regards to some type of food units where it is important to improve training of staff to ensure food safety. In accordance with the legislation, the results were still far from what was desired, although periodic analyses were carried out in samples taken from the hands of food handlers and the utensils used in food preparation [26].

## 5. Conclusions

Based on data collected for an official inspection proposal of food service units between 2018 and 2019 in different food service units serving a district in Northeast Portugal, we found that a high rate of contamination of utensils/crockery was observed in Fishmongers, Pastry/Bakery establishments and Butcher Shops/Charcuteries, while a high rate of "unsatisfactory" samples taken from hands of food handlers were observed in Canteens and Retirement Homes/Day Care Centers. Samples taken from utensils/crockery were positive for total coliforms, fecal coliforms, *E. coli* and colonies of mesophilic microorganisms.

The analysis of the data presented in this study emphasizes the need of awareness of hygiene practices among food handlers of different food service units, particularly in Fishmongers, Pastry/Bakery establishments and Butcher Shops/Charcuteries. The design of training for staff adapted to each type of food unit seems be an important issue in improving food safety. These interventions should sensitize workers and inform them about the mandatory hygiene procedures such as frequent hand washing, the use of gloves

or correct storage of kitchen utensils, to improve the hygiene status of these food units. In this sense, it would be important to carry out studies, particularly in food units where the results were worse, in order to verify if the implemented actions by the local public health entities were effective.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Ethics Committe of the Unidade Local de Saúde do Nordeste (number 27/2020, at 24-09-2020).

**Informed Consent Statement:** The patient's consent was waived because data was collected during official inspection of food service units. Researchers received anonymized data, there is no way of identifying these food service units.

**Data Availability Statement:** Data isn't publicly available. Data is available only for research purpose restricting access to authorized researchers.

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

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