Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00379


# Microbiological assessment of a hospital kitchen before and after Good Manufacturing Practices' intervention

Dionice Capistrano da SILVA[1], Janaina Prieto de OLIVEIRA[1], Emanoelli Aparecida Rodrigues SANTOS[1], Leonardo Ereno TADIELO[2], Fábio Sossai POSSEBON[1,3], Juliano Gonçalves PEREIRA[1]*

**Abstract**

The aim of this study was to investigate the impact of Good Manufacturing Practices (GMP) on the identification and prevention of bacteria that could pose a risk to patient meals. The study used two GMP checklists and microbiological sampling of bacteria, performed six months apart, to assess the facility's ability to reduce bacterial counts. The results showed hygiene deficiencies in all areas of the hospital. However, there was a significant improvement in GMP compliance following the implementation of an action plan ($p = 0.0234$). Before the intervention, 55.55% of the criteria were compliant and after the intervention, 69.63% were compliant. Surfaces involved in raw material handling were identified as the most critical in terms of contamination, with high levels of aerobic mesophilic count, Enterobacteriaceae, and Listeria monocytogenes. The intervention significantly reduced the number of bacteria on these surfaces ($p < 0.05$). Before, the aerobic mesophilic ranged from 0.00 to 6.34 log CFU/cm², and after, from 0.00 to 4.31 log CFU/cm². Additional testing of incoming materials, food, and hands showed no significant risk. The study highlighted the importance of good hand hygiene, which improved after extensive staff training. The GMP guideline and microbiological characterization of the production areas in the hospital kitchens were essential for understanding and acting on bacterial contamination..

**Keywords:** patients; foodborne disease; surfaces; HACCP.

**Practical Application:** GMP improves hospital hygiene and reduces bacterial contamination on critical surfaces.

## 1 INTRODUCTION

Unsafe food poses a significant threat to human health, with approximately 600 million cases of foodborne disease (FD) occurring annually (FAO, 2022). Numerous outbreaks associated with hospital food have been reported worldwide (Cokes et al., 2011; Gaul et al., 2013; Najjar et al., 2015; Russini et al., 2021; Shetty et al., 2009). The epidemiology of FDs is changing with the emergence of new and unexpected pathogens on a national or global scale. The presence of *Listeria monocytogenes* (LM) in food may be due to the contamination during production, processing, and storage of raw materials and finished products because this microorganism adapts to different environmental conditions, variations in pH, temperature, salt, and ability to adhere and form biofilms on different surfaces (Cordero et al., 2016; Shetty et al., 2009; Wiktorczyk-Kapischke et al., 2021).

Several studies have shown that hospital meals have caused poisoning, abdominal pain, and diarrhea. The most common factors contributing to nosocomial food poisoning include inappropriate handling practices, undercooking, storage at room temperature, cross contamination of raw materials, and the transfer of contaminants from raw materials to cooked meals (Al-Abri et al., 2011; Altekruse & Swerdlow, 1996; Argaw & Addis, 2015; Evans et al., 1996; Naranjo et al., 2011; Regan et al., 1995; WHO, 2002; Wu et al., 2018). Foodborne outbreaks in hospitals can cause high morbidity and mortality, especially in vulnerable patients (Banna et al., 2022). Certain groups of patients exhibit an increased susceptibility to foodborne pathogens which can be attributed to several host factors, such as age, metabolic diseases, primary immunodeficiencies, and immunocompromised persons (Lund & O'Brien, 2011). Large-scale food production coupled with inadequate manufacturing practices coupled with the susceptibility of hospitalized patients can lead to outbreaks of foodborne illnesses in hospitals (Assanasen & Bearman, 2018). Foodborne pathogens can reach patients through contaminated food, food handlers, or improper food handling. Nosocomial bacteria can also come from hospital staff and equipment (Russini et al., 2021; WHO, 2002).

The history of hospital-acquired food poisoning dates back to the late 1950s. McKillop (1959) studied cases of food

*Received: 31 July, 2024.*
*Accepted: 22 Aug., 2024.*
[1]*Universidade EStadual Paulista "Júlio de Mesquita Filho", School of Veterinary Medicine And Animal Science, Botucatu, SP, Brazil.*
[2]*Universidade do Estado de Santa Catarina, Departamento de Produção Animal e Alimentos, Lages, SC, Brazil*
[3]*Universidade EStadual Paulista "Júlio de Mesquita Filho", Institute for Biotechnology, Botucatu, São Paulo, Brazil*
*Corresponding author: juliano.pereira@unesp.br
Conflict of interest: nothing to declare.
Funding: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

poisoning in hospitalized patients with diarrhea relating to ingestion of contaminated raw meat products and ready-to-eat foods with *Staphylococcus aureus* and *Salmonella* species. After preparation, 173 foods tested positive for *Staphylococcus aureus* and *Salmonella* sp. In 1973, a *Salmonella* sp. outbreak in a Maine hospital was initially linked to contaminated eggnog and human-to-human transmission continued even after the source was removed (Steere et al., 1975). A study of foodborne outbreaks in hospitals in Scotland (1973–1977) identified 50 incidents involving more than 1,530 people, mostly from psychiatric and geriatric wards. *Clostridium perfringens*, *Salmonella* sp., and *Staphylococcus aureus* were the main microorganisms identified (Sharp et al., 1979). In 1989, a *Salmonella* sp. outbreak in a university hospital cafeteria affected 19.6% of the population. Contaminated mashed potatoes were identified as the source, transmitted by the hands of food handlers (Khuri-Bulos et al., 1994). *Salmonella* Typhimurium contamination of preheated food samples was found in the UK hospital cook-and-chill system. Contamination of kitchen surfaces led to outbreaks of gastroenteritis in children's hospitals, highlighting the need for surveillance and hygiene practices (Lacey, 1993). Pinto et al. (2004) found *Salmonella* sp. and LM in hospital enteral feeds and food preparation areas. Roy et al. (2005) found bacterial contamination in pediatric hospital enteral feeds due to cross contamination during food preparation. A comprehensive review of the literature concerning healthcare-associated foodborne outbreaks (HA-FBO) in 37 member countries of the Organization for Economic Co-operation and Development (OECD) identified 85 outbreaks that took place from 2001 to 2018. These outbreaks were primarily linked to the consumption of food tainted with *Salmonella* sp. (24 outbreaks), norovirus (22 outbreaks), and LM (19 outbreaks). Among the reported causes, 46% of HA-FBOs showed inadequate time or temperature control during food preparation; carriers among kitchen personnel or food handlers were reported in 23%; insufficient hygiene practices in handling raw food or cleaning the kitchen, equipment, or environment were reported in five cases, involving pathogens like *Salmonella* sp. and LM (Boone et al., 2021). Recently, Hobbs et al. (2023) investigated two listeriosis outbreaks that occurred in 2018, both linked to a food establishment within an Ontario cancer center. Samples of deli meats and environmental swabs (mainly from a meat slicer) taken from the food premises were genetically related to the confirmed cases. On 23 May 2019, the same strains of LM from an inpatient with listeriosis at a different hospital in Northwest England were identified, pointing to a common source of exposure. Traceback investigations identified chicken sandwiches from two producers implicated with the outbreak. Isolates of LM from unopened packs of cooked duck, chicken, and ham, sampled from the food environment, were detected (Public Health England, 2020).

The survey of foodborne epidemiology in hospitals, therefore, suggests that food handlers play a critical role in ensuring safe food production for consumption. Ayçiçek et al. (2004) collected 180 samples from the hands and gloves of staff in a hospital kitchen before and during food preparation. Out of 60, 51 (85%) gloves sampled during work and 57 (95%) hands sampled before work tested positive. They isolated 16 different types of bacteria, the most common being *Staphylococcus aureus* (126/180; 70%), coagulase-negative *Staphylococcus* (102/180; 56.7%), *Bacillus* spp. (19/180; 10.5%), and *Escherichia coli* (14/180; 7.8%).

To prevent FD, hospital kitchens must be vigilant in food preparation, especially given the increased susceptibility of individuals purchasing food in healthcare facilities (Food Standards Australia New Zealand, 2002). This requires careful standardization of microbiological criteria for patient food and regular evaluation to identify, control, and minimize health risks. In Brazil, Law No. 9,431/1997 requires hospitals to have a nosocomial infection control program. Hospital-acquired infections, also known as nosocomial infections, are defined as infections acquired after a patient's admission to the hospital, manifesting during or after discharge, and associated with hospitalization (Brasil, 1997). However, this legislation does not cover hospital food and nutrition units (UANH), which are subject to specific good practice regulations but are not mandatorily included in the epidemiologic investigation of hospital outbreaks (Brasil, 1976, 1990, 2004, 2020a, 2020b, 2020c).

To ensure high standards of GMPs, it is necessary to regularly evaluate the effectiveness of the program and validate control measures. However, retail and food service operations present unique challenges due to the variety of meals and incoming raw materials (Lahou et al., 2015). The objective of this study was to evaluate the effectiveness of the GMP program in a hospital kitchen using a checklist and microbiological sampling. The goal was to identify and control sources of bacteria that could compromise patient meals and to reduce the number of bacteria on kitchen surfaces through an action plan.

## 2 MATERIAL AND METHODS

### 2.1 Experimental design

The study was carried out in a hospital kitchen that serves two large hospitals and seven health units in the state of São Paulo, Brazil. The study was divided into two moments for evaluation; in the first evaluation, the initial diagnosis of the situation was carried out, through sampling on surfaces of equipment and utensils, raw materials, ready-to-eat foods, water supply, and hands of food handlers. Also, at this time, a checklist was created, with questions that evaluate good food manufacturing practices (Suppl. Data A). Based on the data obtained in this first evaluation, training was carried out with the team and 6 months later, aiming to evaluate the implementation of the correct functioning of good handling practices; the checklist (Suppl. Data A) was repeated, and new collections were carried out to evaluate microbial contamination. In the first assessment, the facility produced an average of 3,650 meals per day, which increased to 6,800 meals per day in the second assessment.

### 2.2 First good manufacturing practices' checklist

Compliance with 136 checklist questions was evaluated according to the GMP criteria (Suppl. Data A) established by Resolution RDC nº 216/2004 and CVS nº 5/2013 of the state of São Paulo (Brasil, 2004; Governo do Estado de São Paulo, 2013).

### 2.3 Initial microbiological evaluation

Swabs were taken from food contact and nonfood contact surfaces (Table 1).

The swab samples were collected in areas of 100 cm² (one mold measuring 10 cm × 10 cm) of equipment, containers, and packaging; 400 cm² (four molds measuring 10 cm × 10 cm) of environment such as window, floor, worktop, and wall; and the entire side of utensil's food-contact surface. Each sterile cotton swab with plastic stick was moistened with 0.85% saline solution and rubbed 10 times horizontally and 10 times vertically inside the area of the sterile mold. The tips of the swabs were cut with sterile scissors and placed in tubes containing 10 mL of 0.85% saline solution. The tips were tested after the cleaning procedures and during the work shift for Aerobic Mesophilic Count (AMC) and *Enterobacteriaceae* (EB). For AMC, after serial dilutions, 1 mL aliquots of the dilution were inoculated onto plates containing plate count agar (Oxoid) and incubated at 30 ± 1°C for 72 ± 3 h of dilutions with the colony number counted between 30 and 300 CFU. As for EB, after serial dilutions, 1 mL aliquots of the dilutions were inoculated into Petrifilm® TM EB (3M) and incubated at 36°C ± 1°C for 48 ± 2 h. Plates of dilutions with counts less than or equal to 100 colonies were counted. In addition, 130 swabs were collected after the cleaning procedures and tested for LM. The swab tips were placed in a tube containing 10 mL of Listeria enrichment broth (Oxoid) and incubated at 30°C ± 1°C for 24 ± 2 h. Microbiological criteria and analytical methods of swabs, food, beverages, hands, and water followed Brazilian normatives and international methods (Suppl. Table 1). Hand swabs were taken before and after hand washing and during the work shift (Suppl. Data B). Brazilian microbiological limits for antimicrobial soaps were used to classify hand washing as satisfactory. The LM isolates were subjected to molecular confirmation (*prs, lmo0737, lmo1118,*

**Table 1.** Number of samples collected before and after the intervention to assess the effectiveness of the GMP in the hospital kitchen.

| Sampling criteria | Swab of surfaces | | Foods | Water | Hand |
|---|---|---|---|---|---|
| | AMC and EB | LM | | | |
| Total | [233/233][1] | [130/130] | [28/21] | [19/19] | [32/43] |
| **By area** | | | | | |
| Butchery | 42/42 | 28/28 | 5/6 | 3/3 | 4/5 |
| Cold storage | 10/10 | 9/9 | 3/2 | - | - |
| Kitchen | 45/45 | 25/25 | 7/5 | 1/4 | 8/12 |
| Distribution | 08/08 | 4/4 | 1/1 | - | - |
| Snacks | 48/48 | 24/24 | 2/2 | 3/1 | 6/5 |
| Pre-preparation | 36/36 | 18/18 | 6/4 | 5/4 | 10/9 |
| Dessert | 44/44 | 22/22 | 4/2 | 4/6 | 4/12 |
| Other non-production area | - | - | - | 3/1 | - |
| **By moment** | | | | | |
| AC | 130/130 | 130/130 | - | V | - |
| DS | 103/103 | 0/0 | - | V | - |
| **By type of surface** | | | | | |
| FC | 128/128 | 74/74 | - | - | - |
| WFC | 105/105 | 56/56 | - | - | - |
| **By food group** | | | | | |
| A – Raw materials | - | - | 1/4 | - | - |
| B – Pre-prepared and prepared | - | - | 8/9 | - | - |
| C – Prepared and ready-to-eat | - | - | 3/1 | - | - |
| D – Cooked | - | - | 15/7 | - | - |
| E – Ready-to-eat | - | - | 1/0 | - | - |
| **By water source** | | | | | |
| Tap | - | - | - | 14/14 | - |
| Filter | - | - | - | 5/5 | - |
| **By work moment** | | | | | |
| Before hand wash | - | - | - | - | 16/14 |
| After hand wash | - | - | - | - | 0/15 |
| During food handling | - | - | - | - | 16/14 |

[1]Number of samples (before intervention/after intervention); AMC: aerobic mesophilic count; EB: *Enterobacteriaceae* count; LM: *Listeria monocytogenes*; AC: after cleaning; DS: during shift; FC: surface with food contact (wash with neutral detergent followed by a 15-min immersion in 1% chlorine solution or 70% alcohol spray); WFC: surface without food contact (rinsed with water); v: sampling moment varied between AC and DS.

*ORF2819,* and *ORF2110*), virulence (*inlA, inlB, inlC, hlyA,* and *actA*), biofilm formation, and tolerance to stressful conditions (*prfA*, *flaA, agrA*, *agrB*, *agrC*, *agrD*, and *luxS*) (Suppl. Table 2).

### 2.4 Action plan

The findings were reported to the hospital's board of directors. An action plan was implemented by the kitchen quality assurance staff to address the deviations. Due to the large number of noncompliances at the first round, the deviations were classified as urgent, high, medium, and low priority. Serious structural and equipment failures were considered urgent because they presented high potential for food unsafety. It took 6 months for the deviations to be corrected. Within this 6-month time frame, the checklist and microbiological samples were re-evaluated to determine the extent of microbial improvement.

### 2.5 Second good manufacturing practices' checklist

The effectiveness of implementing the action plan was assessed through observation of the teams' routines, reapplication of the checklist (Suppl. Data A), collection of samples of equipment and utensils, raw materials, and ready-to-eat foods, and assessment of hand hygiene handlers, aiming to observe the correct implementation of GMP.

### 2.6 Second microbiological assessment

To verify the reduction in microbial levels on surfaces, hands, water, and food, all previously sampled areas were retested using the same sampling criteria and analytical methods as previously described. The exchange and replacement of surfaces such as equipment and utensils was a limiting factor. In order to know the microbiological result of the same surface before and after the action plan, results of surfaces that were sampled in the first microbiological assessment but not in the second were excluded from the study.

### 2.7 Statistical analysis

Statistical analyses were performed with the SAS 3.81 software. Qualitative outcomes were presented as frequencies. The Fisher exact test was used to compare before and after the intervention. Normality of counts was assessed with the Shapiro–Wilk test and considered nonparametric. The results were presented as median log CFU/cm². The Kruskal–Wallis test followed by Dunn's test was used to access differences in median values before and after the intervention and between areas.

## 3 RESULTS

### 3.1 Good manufacturing practices' compliances on the checklist

Before the intervention, 55.55% (75/136) of the criteria were in compliance with GMPs, and after the intervention, 69.63% (94/136) were in compliance, so an overall improvement was observed, but no statistical difference was found between the pre- and post-intervention results individually ($p > 0.05$). Pest control and water supply did not show any improvement (Figure 1).

### 3.2 Surface microbial count

By area, most median counts (CFU/cm²) of AMC and EB showed significant reductions ($p < 0.05$) (Figure 2). Median counts of AMC in the butchery were 556.50 CFU/cm² before intervention and decreased to 1.92 CFU/cm² ($p = 0.0003$) after intervention, cold storage was 299.19 CFU/cm² and decreased to 1.27 CFU/cm² ($p = 0.0341$), kitchen 48.09 CFU/cm² to 0.08 CFU/cm² ($p = < 0.0001$), distribution 14.23 CFU/cm² to 0.20 CFU/cm² ($p = 0.2072$), snacks 15.25 CFU/cm² to 0.02 CFU/cm² ($p = < 0.0001$), pre-preparation 545.30 CFU/cm² to 0.44 CFU/cm² ($p < 0.0001$), and dessert 12.90 CFU/cm² to 0.16 CFU/cm² ($p = 0.0116$). EB counts decreased significantly ($p < 0.05$) in the kitchen, snack, and pre-preparation areas (Figure 3). Median EB

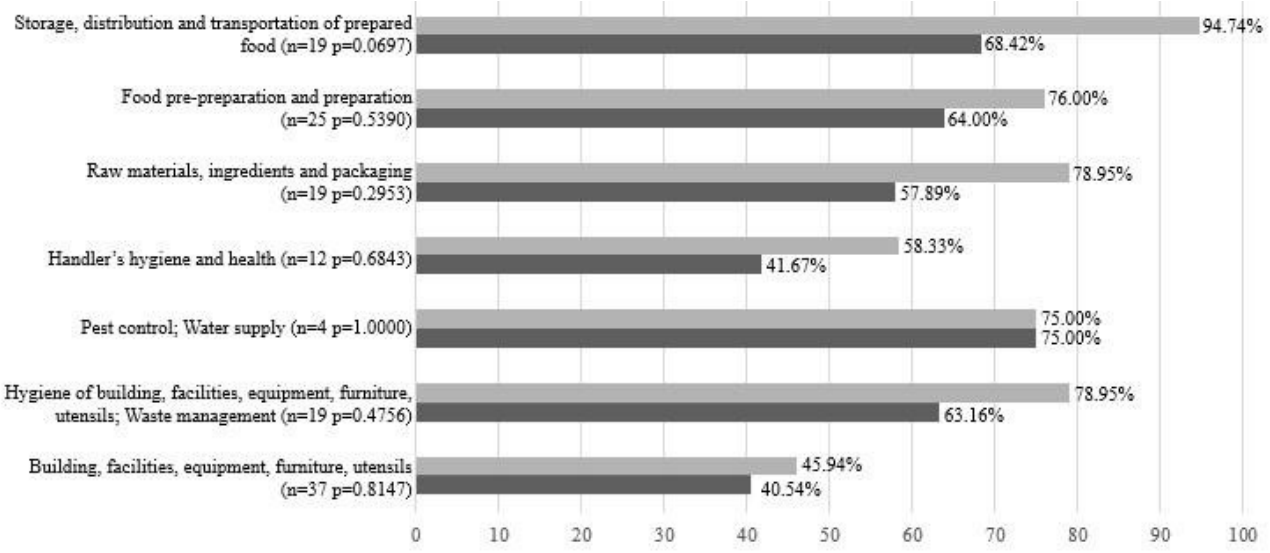



**Figure 1**. Compliance frequency of the GMP criteria in the hospital kitchen before and after the intervention. The dark gray bars mean pre-intervention. The light gray bars mean post-intervention.

counts in the butchery were 2.03 CFU/cm² pre-intervention and decreased to 0.12 CFU/cm² ($p = 0.3031$) post-intervention, cold storage was 0.39 CFU/cm² and increased to 3.12 CFU/cm² ($p = 0.8575$), kitchen 0.59 CFU/cm² to 0.06 CFU/cm² ($p = 0.1554$), distribution 0.00 CFU/cm² to 0.00 CFU/cm² ($p = 1.000$), snacks 0.07 CFU/cm² to 0.00 CFU/cm² ($p = 0.0380$), pre-preparation 3.12 CFU/cm² to 0.13 CFU/cm² ($p = 0.0662$), and dessert 0.00 CFU/cm² to 0.09 CFU/cm² ($p = 0.6424$).

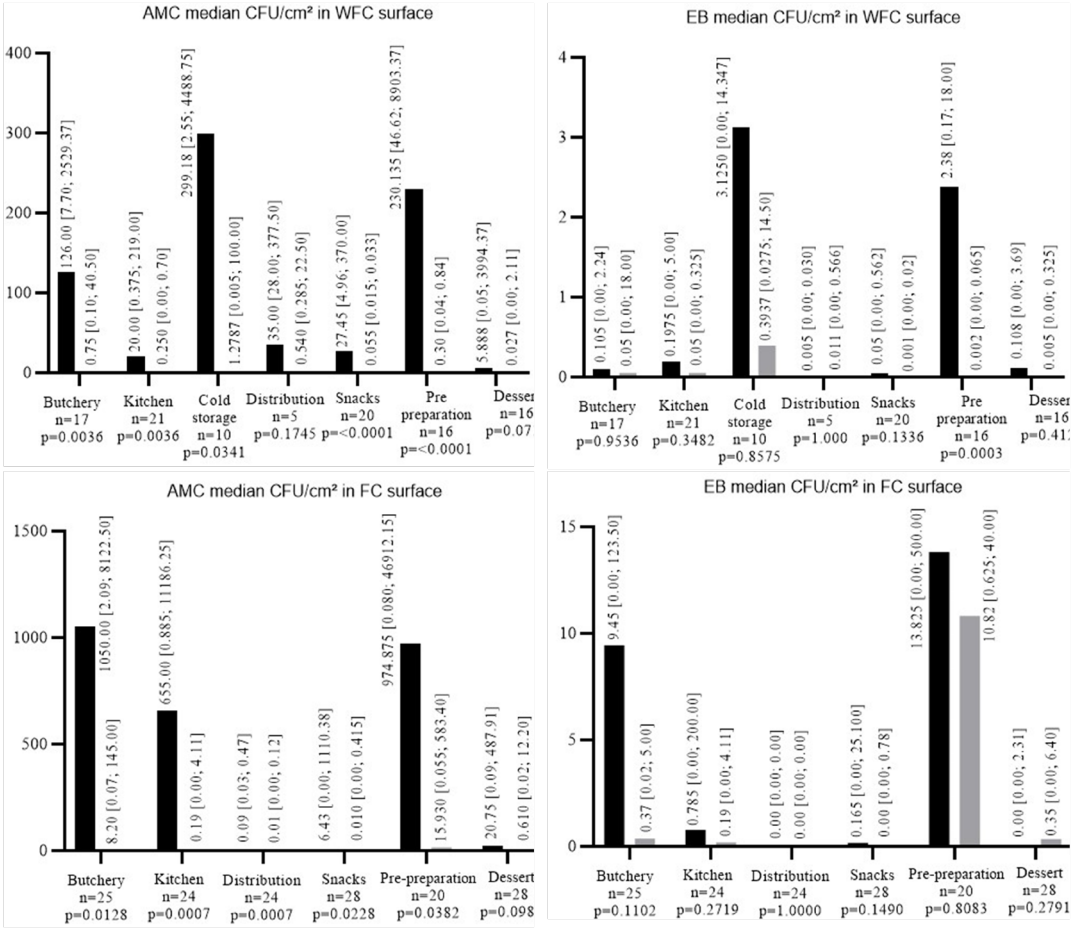
When comparing AMC before and after the intervention by surfaces with (FC) and without (WFC) food contact (Figure 4), almost all were statistically different ($p < 0.05$), as for EB, most of the results were not statistically different ($p > 0.05$). It is expected that FC surfaces will have lower counts compared to WFC surfaces. This difference was observed in the snack area, which showed a significant difference in AMC counts before ($p = 0.0140$) and after the action plan ($p = 0.0147$). As for the EB counts, the surfaces of the prep area showed a significant difference before ($p = 0.0240$) and after ($p = 0.0175$).

When comparing AMC and EB before and after the intervention by sampling time, it was expected that the counts would be lower after the cleaning procedures (AC) than during the work shift (DS). The data showed no statistical difference in the distribution and cold storage areas (Figure 4), regardless of time, study round, or bacteria ($p > 0.05$). Except for these areas, the AC and DS samples for AMC before and after the intervention showed a statistically significant reduction ($p < 0.05$). However, the same results were not shown for EB, where almost all samples were not statistically significant ($p > 0.05$).

When comparing the AMC of the sampling moments by area, before the intervention, the numbers at AC were higher in the kitchen and dessert area, and the number was reduced in the DS. After the intervention, the kitchen showed a significant reduction in both AC and DS. This improvement can also be seen in the pre-preparation area: pre-intervention counts were high, and post--intervention counts were significantly reduced in both periods ($p < 0.05$). In the cold store, before the samples taken, AC was lower than DS, and after, the counts decreased DS. As for EB, the snacks showed a significant difference before the intervention ($p = 0.0218$).

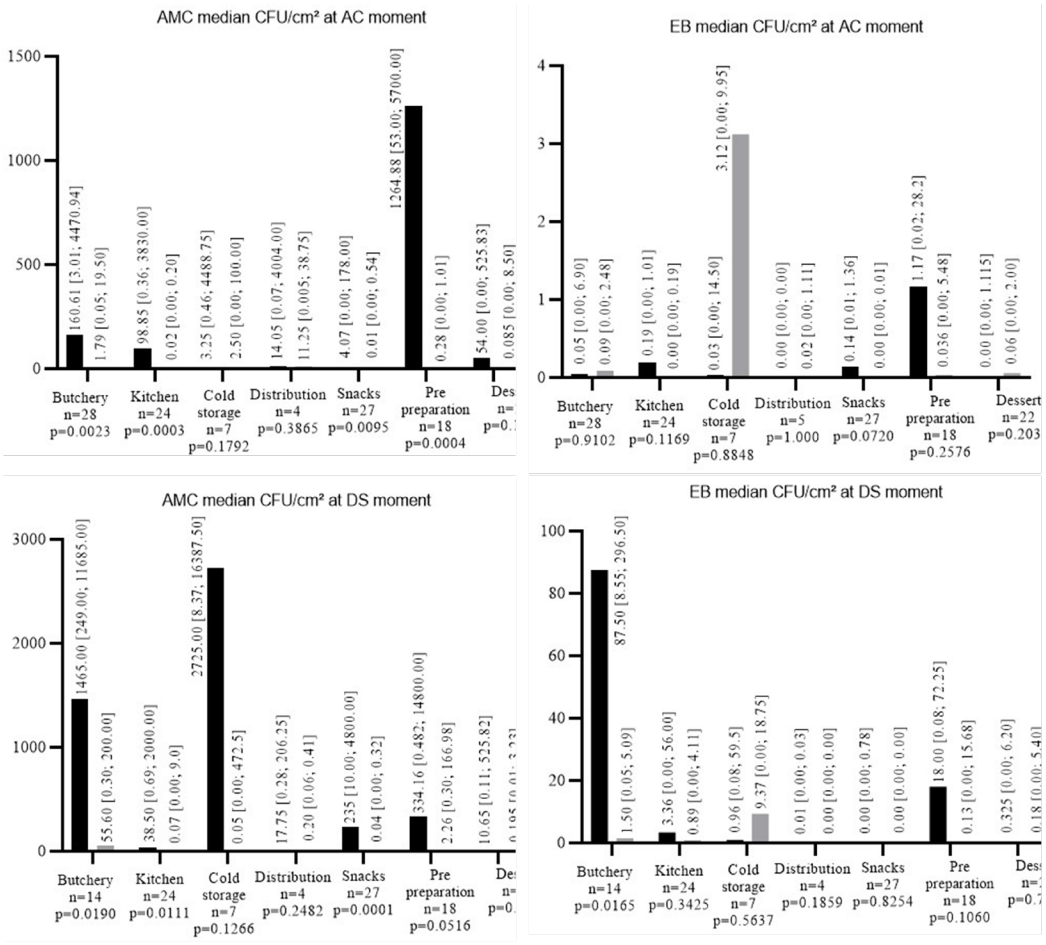
### 3.3 Detection of *Listeria monocytogenes* on the surfaces and food and its genetic characterization

All positive surfaces, regardless of study round, came from the butchery, 25.00% (4/16) FC and 8.34% (1/12) WFC. Pre--intervention, 2.30% (3/130) of surfaces and 10.71% (3/28) of




AMC: aerobic mesophilic count; EB: Enterobacteriaceae count; FC: surface with food contact; WFC: surface without food contact. The black columns mean pre-intervention. The gray columns mean post-intervention.

**Figure 2**. Pre- and post-intervention comparison of hygiene indicator microorganisms (median [Q1;Q3]) by the type of surfaces in the areas.




AMC: aerobic mesophilic count; EB: Enterobacteriaceae count; AC: after cleaning; DS: during shift. The black columns mean pre-intervention. The gray columns mean post-intervention.
**Figure 3**. Pre-and post-intervention comparison of hygiene indicator microorganisms (median [Q1;Q3]) by sampling moment.

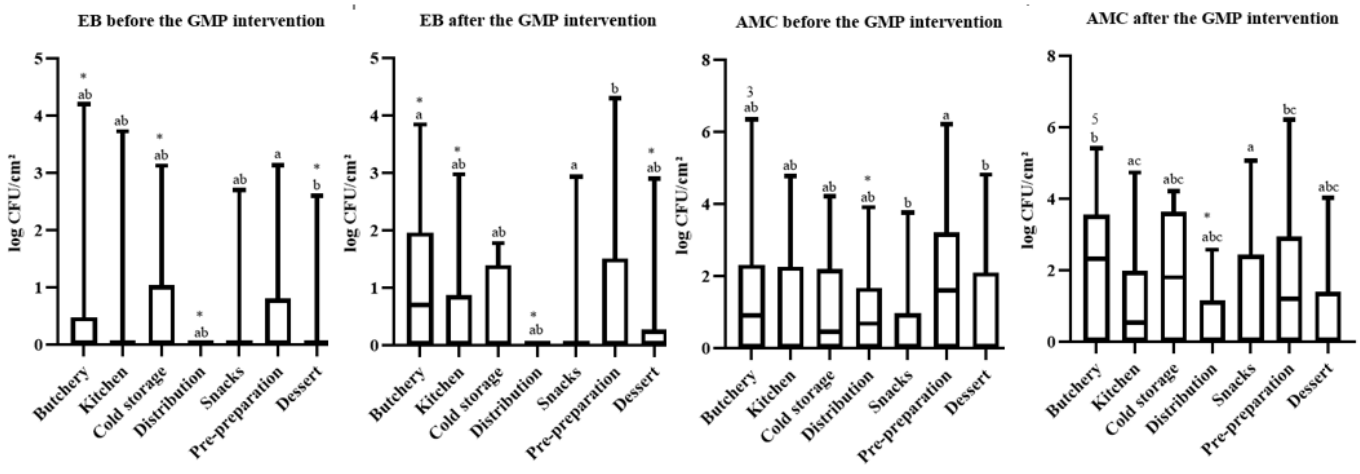



**Figure 4**. AMC and EB (log CFU/cm²) by production area (a) before and (b) after the intervention in the hospital kitchen to assess the GMP improvement. The bars sharing the same superscript are not significantly different from each other ($p > 0.05$). Asterisks: no significant reduction before and after the intervention ($p > 0.05$). Numbers above bars: number of samples positive for LM by area.

food samples tested positive. Post-intervention positivity was 3.84% (5/130) on surfaces. There were no statistical differences ($p > 0.05$) between the pre- and post-intervention comparisons.

Associating the LM presence with AMC, after the action plan, AMC was lower and LM was higher than before. Before the action plan, one cutting board (2.32 log CFU/cm²) was positive for LM, and no package ($n = 15$, median 1.57 log CFU/cm²) nor stainless steel cart ($n = 1$, 4.63 CFU/cm²) presented the pathogen. After, two samples of cutting board (median log 1.08 CFU/cm²), two samples of packaging (median log 0.81 CFU/cm²), and one sample of stainless-steel cart (1.60 log CFU/cm²) tested positive. Before the intervention, one LM-positive sample of cutting board ($n = 15$) presented 2.32 log CFU/cm². After intervention, two cutting boards showed the pathogen (median log 1.08 CFU /cm²). LM isolates were serotyped and analyzed for virulence genes (*inlA, inlB, inlC, hlyA,* and *actA*) and for genes related to biofilm formation and tolerance to stressful conditions (*prfA*, *flaA, agrA*, *agrB*, *agrC*, *agrD*, and *luxS*) (Figure 5). Before the intervention, strains from the butchery were serotype 4b, and chicken and celery 1/2a. After the intervention, the chicken cutting board retained serotype 4b, while other surfaces showed serotype 1/2a.

### 3.4 Microbiological characteristics of the food samples

*Bacillus cereus* and *Salmonella* sp. were not detected in the food categories before or after the intervention. Before the intervention, one sample of unsterilized celery of the pre-preparation area, one chicken, and one beef from the butchery was positive for LM. Yeasts and molds were found in ready-to-eat bread (0.30 log CFU/g) and coagulase-positive staphylococci (CPS) in a sample of fresh fish (1.36 log CFU /g). No LM was found after the intervention, but a sample of minced meat exceeded the EC limit (6.09 log CFU/g). Other food categories met the normative standards (Suppl. Table 3).

### 3.5 Microbiological results of the water samples

The water results were compared according to the Brazilian microbiological standards for drinking water (Brasil, 2021; 2022). According to the regulations, only one sample of 100 mL of water collected aseptically at the point of consumption each week may be positive for total coliforms within the sampling schedule established by the monitoring system. In the present study, before the intervention, three presences were observed for both coliforms at 36°C and coliforms at 45°C in the 4 consecutive weeks of sampling days (Suppl. Table 3).

| Round | Area | Source | Serotype | orf 2819 | orf 2110 | lmo 0737 | lmo 1118 | inl A | inl B | inl C | hly A | act A | prf A | fla A | agr A | agr B | agr C | agr D | lux S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1st | | Celery | 1/2a | | | p | | p | p | p | p | | p | p | p | p | p | p | |
| 1st | | Chiken cutting board | 4b | p | p | | | p | p | p | p | | p | p | p | p | p | p | p |
| 1st | | Chicken strips | 1/2a | | | p | | p | p | p | p | | p | p | p | p | p | p | |
| 1st | | Refrigerator | 4b | p | p | | | p | p | p | p | p | p | p | p | p | p | p | p |
| 1st | | Beef strips | 4b | p | p | | | p | p | p | p | p | p | p | p | p | p | p | p |
| 1st | | Refrigerator | 4b | p | p | | | p | p | p | p | p | p | p | p | p | p | p | p |
| 2nd | | Chicken cuttin board | 4b | p | p | | | p | | | p | p | | p | p | p | p | p | |
| 2nd | | Beef cutting board | 1/2a | p | | p | | | | | p | p | | | | p | p | | |
| 2nd | | Package | 1/2a | p | | p | | p | | | p | p | | | | p | p | | |
| 2nd | | Package | 1/2a | p | | p | | p | | | p | p | | | | p | p | | |
| 2nd | | Stailess steel cart | 1/2a | p | | p | | p | p | | p | p | | | | p | p | p | |

Source of the LM isolates:
Pre-preparation area
Butchery area
p presence

**Figure 5**. Molecular characterization of Listeria monocytogenes isolated before (first round) and after (second round) the intervention for the microbiological assessment of surfaces and foods from the hospital kitchen.

The coliform average counts at 36°C from the taps of the pre-preparation were 0.32 log MPN/mL ($n = 3$), dessert 3.04 log MPN/mL ($n = 1$), and snack area 1.01 log MPN/mL ($n = 3$), the last one also having counts 3.04 log MPN/mL ($n = 1$) for EC. The filter in the prep area, used for storage by immersion of sliced vegetables, was also noncompliant for coliforms with two positives in the 4-week survey (median 2.00 log MPN/mL, $n = 2$).

Post-intervention results showed that the dessert area filter used to make beverages tested positive for coliforms (median 0.32 log MPN/mL, $n = 3$). Taps from the dessert and butchery areas repeatedly showed coliforms over the 4 weeks of sampling days (median 1.01 log MPN/mL, $n = 3$, and 2.57 log MPN/mL, $n = 3$, respectively). CPS was found in the butchery's tap water (0.84 log CFU/mL), above the limits established in the Brazilian normative (absence/250 mL).

### 3.6 Microbiological results of hand samples

No significant AMC differences were found before and after the intervention ($p > 0.05$). The hand of one kitchen worker showed CPS before hand washing (0.90 log CFU/hand), and the hand of one pre-preparation worker showed CPS during food handling (0.60 log CFU/hand). The bacteria were not found after the action plan. No EC was found in either period (Suppl. Table 3). Initial results showed that 3.13% (3/32) of the hands had AMC counts above 3.69 log CFU/hand, which was unsatisfactory, but after the action plan, 98.45% of the hands (32/43) showed satisfactory hygiene (< 3.69 log CFU/hand).

## 4 DISCUSSION

Knowing that foodborne pathogens can worsen a hospitalized patient's clinical condition, hospital kitchens must ensure food safety by identifying and controlling food hazards. This study was designed to determine whether GMP can identify and prevent potential sources of bacteria that pose a risk to patient meals. To accomplish this, we conducted two GMP checklists followed by microbiological sampling of potential sources of contamination 6 months apart to assess the facility's ability to reduce bacterial counts and ensure food safety. The high-throughput analysis identified the raw material handling areas as the most concerning in terms of characteristics that could cause food contamination. All areas had visible deficiencies in cleaning and operator hygiene, and microbiological analysis confirmed the findings. Therefore, the GMP guideline and microbiological characterizations were instrumental in understanding and addressing the bacterial contaminants in each area.

The food operator plays the most important role in implementing Hazard Analysis and Critical Control Point (HACCP) and complying with its requirements (Raadabadi et al., 2012).

The lack of food safety knowledge among food handlers is a serious threat to food safety in service establishments (Teffo & Tabit, 2020). Ayçiçek et al. (2004) collected 180 hand samples from hospital kitchen workers before and during food preparation. Of 60 samples, 57 (95%) hands sampled before the work shift tested positive for 16 different types of bacteria. The most common were *Staphylococcus aureus* (70%), coagulase-negative *Staphylococcus* sp. (56.7%), *Bacillus* spp. (10.5%), and *Escherichia coli* (7.8%). In this study, although the hygiene classification of almost 90% of the hands was considered satisfactory, the decrease in AMCs between the two times of each sampling day was not significant.

Areas more likely to favor bacterial growth on surfaces and food contamination by cross contamination were the butchery and prep areas. Although lower in the second visit, all areas in both periods had visible food residues immediately after cleaning, associated with high AMC and EB counts, demonstrating shortcomings in this procedure, and explaining the detection of LM, a serotype associated with listeriosis outbreaks (Pan et al., 2009). Associating LM with AMC, after the action plan, AMC was lower and LM was higher than before. According to Townsend et al. (2022), certain individual factors such as material, porosity, whether a sampling site is cleaned or not, and the frequency of cleaning may be important with respect to the persistence of *Listeria* spp. in food facilities. The LM is capable to coexist and interact with multiple bacterial species found in food processing environments. Heir et al. (2018) demonstrated that strains with strong and weak competitive abilities to grow and compete in diverse bacterial environments have a selective advantage, increasing their potential for survival, growth, and persistence in food-related conditions. This is particularly relevant for LM, which is known to be a difficult foodborne pathogen to control in many food processing facilities.

A lack of standardization of surface cleaning methods was observed before and after the intervention, even after staff training on the procedure. Pieniz et al. (2019) reported similar results to the present study on surfaces. Before the intervention, the meat cutting board had an AMC of 4.64 log CFU/cm², the vegetable cutting board had 4.08 log CFU/cm², and the refrigerator had 4.14 log CFU/cm², and after the intervention, the counts were reduced to 4.11, 4.78, and 2.76 log CFU/cm², respectively. The authors considered cleaning counts of mesophilic aerobic microorganisms above 20 CFU/cm² for surfaces and 100 CFU/cm² for equipment to be unsatisfactory. Scanning electron microscopy of a plastic cutting board used for meat handling showed biofilm formation after daily cleaning, indicating that special attention should be given to more efficient disinfection strategies (Pieniz et al., 2019).

Raw foods are a source of pathogens in processing areas. In this study, LM were found in celery prior to disinfection. Gaul et al. (2013) reported an outbreak of LM originating from diced celery. Therefore, the risk of listeriosis should be considered when selecting fresh produce for immunocompromised patients.

Thermal transformation of raw and pre-prepared materials has been shown to significantly reduce AMC, but these results reflect the microbiological quality of the foods sampled and tested immediately after preparation. During both GMP checklist assessments, a lack of food temperature verification and a gap of more than 6 hours between food packaging and distribution were observed, and all equipment designed to maintain temperatures at safe levels failed to achieve ≥ 60°C during the first visit. To assess the quality of the meals during transport from the kitchens to the patients. Réglier-Poupet et al. (2005) analyzed the delays at each stage of the transport process and measured the temperature inside the food trolley and the meals. The internal temperature of the meals was below 10°C in 91.7% of cases. It is essential to control food storage and delivery time and temperature to ensure food quality and safety in hospitals. The kitchen water source may contain microbial contamination and substances that interfere with the microbicidal activity of antiseptics and disinfectants (WHO, 2009). The microbiological levels of the water found in this study may also explain the high AMC and EB counts on the recently cleaned surfaces at both visits, as well as the low decrease in AMC in the post-intervention after hand washing. Puga et al. (2018) linked the ability of LM to colonize pre-established *Pseudomonas* biofilms; although we did not evaluate the ability of LM to form biofilms in pure or associated cultures, this may partly explain why this microorganism can persist in food processing environments.

## 5 CONCLUSION

In conclusion, the study emphasized the importance of implementing GMPs and ensuring food safety in hospital kitchens as this has been shown to reduce microbiological counts on surfaces. It highlighted areas of concern such as manipulation of raw materials and hygiene of food handlers, and cleaning and sanitizing procedures for the surfaces of equipment, utensils, and facilities. Establishing routine microbiological surveillance and monitoring HACCP requirements and critical control points are essential in this process. Addressing these issues will help prevent foodborne illness and protect the health of hospitalized patients.

## ACKNOWLEDGMENTS

The authors are grateful for the kitchen staff support and the Meal Production Centre of the Hospital of the Universidade Estadual Paulista "Júlio de Mesquita Filho" Medical School. This study was carried out with funding from the Public Health Food Service Laboratory of the Universidade Estadual Paulista "Júlio de Mesquita Filho", Unesp, and partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001.

## REFERENCES

Al-Abri, S. S., Al-Jardani, A. K., Al-Hosni, M. S., Kurup, P. J., Al-Busaidi, S., & Beeching, N. J. (2011). A hospital acquired outbreak of Bacillus cereus gastroenteritis, Oman. *Journal of Infection and Public Health*, *4*(4), 180-186. https://doi.org/10.1016/J.JIPH.2011.05.003

Altekruse, S. F., & Swerdlow, D. L. (1996). The changing epidemiology of foodborne diseases. *American Journal of the Medical Sciences*, *311*(1), 23-29. https://doi.org/10.1097/00000441-199601000-00005

Argaw, S., & Addis, M. (2015). A review on staphylococcal food poisoning. *Food Science and Quality Management*, *40*(2015), 59-72.

Assanasen, S., & Bearman, G. M. L. (2018). *Guide to infection control in the hospital setting. Food: considerations for hospital infection control*.

Ayçiçek, H., Aydoğan, H., Küçükkaraaslan, A., Baysallar, M., & Başustaoğlu, A. C. (2004). Assessment of the bacterial contamination on hands of hospital food handlers. *Food Control*, *15*(4), 253-259. https://doi.org/10.1016/S0956-7135(03)00064-1

Banna, M. H. Al, Khan, M. S. I., Rezyona, H., Seidu, A. A., Abid, M. T., Ara, T., Kundu, S., Ahinkorah, B. O., Hagan, J. E., Tareq, M. A., Begum, M. R., Chowdhury, M. F. T., & Schack, T. (2022). Assessment of food safety knowledge, attitudes and practices of food service staff in Bangladeshi hospitals: a cross-sectional study. *Nutrients*, *14*(12), 2540. https://doi.org/10.3390/NU14122540

Boone, I., Rosner, B., Lachmann, R., D'Errico, M. L., Iannetti, L., Van der Stede, Y., Boelaert, F., Ethelberg, S., Eckmanns, T., Stark, K., Haller, S., & Wilking, H. (2021). Healthcare-associated foodborne outbreaks in high-income countries: a literature review and surveillance study, 16 OECD countries, 2001 to 2019. *Eurosurveillance*, *26*(41). https://doi.org/10.2807/1560-7917.ES.2021.26.41.2001278

Brasil (1976). Presidência da República. Casa Civil. *Decreto no 77.052, de 19 de janeiro de 1976*. Dispõe sobre a fiscalização sanitária das condições de exercício de profissões e ocupações técnicas e auxiliares, relacionadas diretamente com a saúde.

Brasil (1990). Presidência da República. Casa Civil. *Lei no 8.080, de 19 de setembro de 1990*. Dispõe sobre as condições para a promoção, proteção e recuperação da saúde, a organização e o funcionamento dos serviços correspondentes e dá outras providências.

Brasil (1997). Presidência da República. Casa Civil. *Lei no 9.431, de 6 de janeiro de 1997*. Dispõe sobre a obrigatoriedade da manutenção de programa de controle de infecções hospitalares pelos hospitais do País. Retrieved from https://www.planalto.gov.br/ccivil_03/leis/l9431.htm#:~:text = LEI

Brasil (2004). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. *Resolução n° 216, de 15 de setembro de 2004*. Dispõe sobre Regulamento Técnico de Boas Práticas para Serviços de Alimentação.

Brasil (2020). Agência Nacional de Vigilância Sanitária. *Nota técnica NT 18.2020 - COVID-19 e as Boas Práticas de Fabricação e Manipulação de Alimentos*. Agência Nacional de Vigilância Sanitária.

Brasil (2020). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. *Instrução normativa - IN no 82, de 17 de dezembro de 2020*. Dispõe sobre Boas Práticas de Fabricação de fórmulas dietoterápicas para erros inatos do metabolismo.

Brasil (2020). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. *Resolução da Diretoria Colegiada - RDC no 460, de 21 de dezembro de 2020*. Dispõe sobre os requisitos sanitários das fórmulas dietoterápicas para erros inatos do metabolismo.

Brasil (2021). Ministério da Saúde. *Portaria GM/MS no 888, de 4 de maio de 2021*. Altera o Anexo XX da Portaria de Consolidação GM/MS nº 5, de 28 de setembro de 2017, para dispor sobre os procedimentos de controle e de vigilância da qualidade da água para consumo humano e seu padrão de potabilidade.

Brasil (2022). Ministério da Saúde. Agência Nacional de Vigilância Sanitária. *Instrução normativa - IN no 161, de 1o de julho de 2022*. Estabelece os padrões microbiológicos dos alimentos.

Cokes, C., France, A. M., Reddy, V., Hanson, H., Lee, L., Kornstein, L., Stavinsky, F., & Balter, S. (2011). Serving high-risk foods in a high-risk setting: survey of hospital food service practices after an outbreak of listeriosis in a hospital. *Infection Control & Hospital Epidemiology*, *32*(4), 380-386. https://doi.org/10.1086/658943

Cordero, N., Maza, F., Navea-Perez, H., Aravena, A., Marquez-Fontt, B., Navarrete, P., Figueroa, G., González, M., Latorre, M., & Reyes-Jara, A. (2016). Different transcriptional responses from slow and fast growth rate strains of listeria monocytogenes adapted to low temperature. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00229

Evans, M. R., Hutchings, P. G., Ribeiro, C. D., & Westmoreland, D. (1996). A hospital outbreak of salmonella food poisoning due to inadequate deep-fat frying. *Epidemiology and Infection*, *116*(2), 155. https://doi.org/10.1017/S0950268800052389

FAO (2022). *Food safety and quality - Background: FAO's work on food safety and quality*. FAO. Retrieved from http://www.fao.org/food-safety/background/en

Food Standards Australia New Zealand (2002). *Food Safety: Temperature control of potentially hazardous foods. Guidance on the temperature control requirements of Standard 3.2.2 Food Safety Practices and General Requirements*. Food Standards Australia New Zealand.

Gaul, L. K., Farag, N. H., Shim, T., Kingsley, M. A., Silk, B. J., & Hyytia-Trees, E. (2013). Hospital-acquired listeriosis outbreak caused by contaminated diced celery-texas, 2010. *Clinical Infectious Diseases*, *56*(1), 20-26. https://doi.org/10.1093/cid/cis817

Governo do Estado de São Paulo (2013). Secretaria de Estado da Saúde. Coordenadoria de Controle de Doenças. Centro de Vigilância Sanitária. *Portaria CVS 5, de 09 de abril de 2013*. Aprova o regulamento técnico sobre boas práticas para estabelecimentos comerciais de alimentos e para serviços de alimentação, e o roteiro de inspeção, anexo. Governo do Estado de São Paulo.

Heir, E., Møretrø, T., Simensen, A., & Langsrud, S. (2018). Listeria monocytogenes strains show large variations in competitive growth in mixed culture biofilms and suspensions with bacteria from food processing environments. *International Journal of Food Microbiology*, *275*, 46-55. https://doi.org/10.1016/j.ijfoodmicro.2018.03.026

Hobbs, J. L., Lee, C., Thompson, B., Andrew, A., Navarro, C., Dubey, V., Maki, A., Kong, A., Griffin, M., Chau, K., Murphy, A. M., Lombos, M., Majury, A. L., Gerrie, M., Szidonya, E., Chung, J., Ozaldin, O., Patel, T., Brandon, N., & Warshawsky, B. (2023). Two Listeria monocytogenes outbreaks in a cancer centre: onsite food premises and their potential health risk to patients. *BMC Public Health*, *23*(1), 1443. https://doi.org/10.1186/s12889-023-16371-7

Khuri-Bulos, N. A., Khalaf, M. A., Shehabi, A., & Shami, K. (1994). Foodhandler-associated salmonella outbreak in a university hospital despite routine surveillance cultures of kitchen employees. *Infection Control and Hospital Epidemiology*, *15*(5), 311-314. https://doi.org/10.1086/646918

Lacey, S. (1993). Isolation of Salmonella enteritidis from cook-chill food distributed to hospital patients. *Journal of Hospital Infection*, *25*(2), 133-136. https://doi.org/10.1016/0195-6701(93)90104-8

Lahou, E., Jacxsens, L., Verbunt, E., & Uyttendaele, M. (2015). Evaluation of the food safety management system in a hospital food service operation toward Listeria monocytogenes. *Food Control*, *49*, 75-84. https://doi.org/10.1016/j.foodcont.2013.10.020

Lund, B. M., & O'Brien, S. J. (2011). The occurrence and prevention of foodborne disease in vulnerable people. *Foodborne Pathogens and Disease*, *8*(9), 961-973. https://doi.org/10.1089/fpd.2011.0860

McKillop, E. J. (1959). Bacterial contamination of hospital food with special reference to clostridium welchii food poisoning. *Journal of Hygiene*, *57*(1), 31-46. https://doi.org/10.1017/S0022172400019872

Najjar, Z., Gupta, L., Sintchenko, V., Shadbolt, C., Wang, Q., & Bansal, N. (2015). Listeriosis cluster in sydney linked to hospital food. *Medical Journal of Australia*, *202*(8), 448-449. https://doi.org/10.5694/mja14.00913

Naranjo, M., Denayer, S., Botteldoorn, N., Delbrassinne, L., Veys, J., Waegenaere, J., Sirtaine, N., Driesen, R. B., Sipido, K. R., Mahillon, J., & Dierick, K. (2011). Sudden death of a young adult associated with Bacillus cereus food poisoning. *Journal of Clinical Microbiology*, *49*(12), 4379-4381. https://doi.org/10.1128/JCM.05129-11

Pan, Y., Breidt, F., & Kathariou, S. (2009). Competition of Listeria monocytogenes Serotype 1/2a and 4b Strains in Mixed-Culture Biofilms. *Applied and Environmental Microbiology*, *75*(18), 5846. https://doi.org/10.1128/AEM.00816-09

Pieniz, S., Rodrigues, D. F., Arndt, R. M., Mello, J. F., Rodrigues, K. L., Andreazza, R., Camargo, F. A. O., & Brandelli, A. (2019). Molecular identification and microbiological evaluation of isolates from equipments and food contact surfaces in a hospital food and nutrition unit. *Brazilian Journal of Biology*, *79*(2), 191-200. https://doi.org/10.1590/1519-6984.175350

Pinto, U. M., Cardoso, R. R., & Vanetti, M. C. D. (2004). Detecção de Listeria, Salmonella e Klebsiella em serviço de alimentação hospitalar. *Revista de Nutrição*, *17*(3), 319-326. https://doi.org/10.1590/S1415-52732004000300005

Public Health England (2020). *Investigation into an outbreak of* Listeria monocytogenes *infections associated with hospital-provided pre-prepared sandwiches, UK May to July 2019*. Retrieved from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/937907/2019-05-Listeria--CC8-Outbreak-Report.pdf

Puga, C. H., Dahdouh, E., SanJose, C., & Orgaz, B. (2018). Listeria monocytogenes colonizes pseudomonas fluorescens biofilms and induces matrix over-production. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01706

Raadabadi, M., Sadeghifar, J., Dargahi, H., Bahadori, M., & Nejati, M. (2012). Investigating the level of meeting HACCP standard requirements in the nutrition department of the selected hospitals affiliated to Tehran University of Medical Sciences. *Archives of Pharmacy Practice*, *3*(2), 159-165.

Regan, C. M., Syedt, Q., & Tunstall$, P. J. (1995). A hospital outbreak of Clostridium perfringens food poisoning-implications for food hygiene review in hospitals. *Journal of Hospital Infection*, *29*(1), 69-73. https://doi.org/10.1016/0195-6701(95)90295-3

Réglier-Poupet, H., Parain, C., Beauvais, R., Descamps, P., Gillet, H., Le Peron, J. Y., Berche, P., & Ferroni, A. (2005). Evaluation of the quality of hospital food from the kitchen to the patient. *Journal of Hospital Infection*, *59*(2), 131-137. https://doi.org/10.1016/j.jhin.2004.07.023

Roy, S., Rigal, M., Doit, C., Fontan, J. E., Machinot, S., Bingen, E., Cezard, J. P., Brion, F., & Hankard, R. (2005). Bacterial contamination of enteral nutrition in a paediatric hospital. *Journal of Hospital Infection*, *59*(4), 311-316. https://doi.org/10.1016/j.jhin.2004.09.026

Russini, V., Spaziante, M., Zottola, T., Fermani, A. G., Di Giampietro, G., Blanco, G., Fabietti, P., Marrone, R., Parisella, R., Parrocchia, S., Bossù, T., Bilei, S., & De Marchis, M. L. (2021). A nosocomial outbreak of invasive listeriosis in an Italian hospital: Epidemiological and genomic features. *Pathogens*, *10*(5), 591. https://doi.org/10.3390/pathogens10050591

Sharp, J. C. M., Collier, P. W., & Gilbert, R. J. (1979). Food poisoning in hospitals in Scotland. *Journal of Hygiene*, *83*(2), 231-236. https://doi.org/10.1017/S0022172400026012

Shetty, A., McLauchlin, J., Grant, K., O'Brien, D., Howard, T., & Davies, E. M. (2009). Outbreak of Listeria monocytogenes in an oncology unit associated with sandwiches consumed in hospital. *Journal of Hospital Infection*, *72*(4), 332-336. https://doi.org/10.1016/j.jhin.2009.01.012

Steere, A. C., Hall, W. J., Wells, J. G., Craven, P. J., Leotsakis, N., Farmer, J. J., & Gangarosa, E. J. (1975). Person-to-person spread of salmonella typhimurium after a hospital common-source outbreak. *Lancet*, *305*(7902), 319-322. https://doi.org/10.1016/S0140-6736(75)91221-0

Teffo, L. A., & Tabit, F. T. (2020). An assessment of the food safety knowledge and attitudes of food handlers in hospitals. *BMC Public Health*, *20*(1). https://doi.org/10.1186/S12889-020-8430-5

Townsend, A., Strawn, L. K., Chapman, B. J., Yavelak, M., Mishra, A., & Dunn, L. L. (2022). Factors that predict *Listeria* prevalence in distribution centers handling fresh produce. *Food Microbiology*, *107*, 104065. https://doi.org/10.1016/j.fm.2022.104065

Wiktorczyk-Kapischke, N., Skowron, K., Grudlewska-Buda, K., Wałecka-Zacharska, E., Korkus, J., & Gospodarek-Komkowska, E. (2021). Adaptive response of listeria monocytogenes to the stress factors in the food processing environment. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.710085

World Health Organization (WHO) (2002). *Prevention of hospital-acquired infections: a practical guide*. WHO. Retrieved from http://www.who.int/emc

World Health Organization (WHO) (2009). Patient Safety. *WHO guidelines on hand hygiene in health care: first global patient safety challenge clean care is safer care*. WHO.

Wu, Y., Liu, X., Chen, Q., Liu, H., Dai, Y., Zhou, Y., Wen, J., Tang, Z., & Chen, Y. (2018). Surveillance for foodborne disease outbreaks in China, 2003 to 2008. *Food Control*, *84*, 382-388. https://doi.org/10.1016/j.foodcont.2017.08.010