

Assessing Microbial Distribution on Food Preparation Surfaces in Old Age Home Kitchen Surfaces: A Quantitative Analysis

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Abstract: Amidst the global challenge posed by foodborne illnesses, this study underscores the vital necessity of implementing robust food safety measures within the unique context of old age homes. Older adults, being more susceptible to severe consequences due to compromised immune systems and pre-existing health conditions, heighten the urgency of addressing food safety in this demographic. Acknowledging surfaces as potential carriers of foodborne pathogens, the primary aim of this research was to evaluate the cleanliness of food preparatory surfaces in old age home kitchens. Microbiological samples were gathered from these surfaces using contact plates featuring various agar types. The analysis focused on quantifying and identifying microorganisms, utilizing Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) for genus and species level identification. The results were assessed according to the criteria outlined in the British Columbia Guide for Environmental Health Officers, categorizing surfaces as satisfactory, acceptable, or unsatisfactory based on the Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972.

The findings indicated that 66.667% of surfaces displayed low total viable counts, while 34.884% exhibited unsatisfactory enumeration with numerous colonies per plate. This implies a potential lack of adherence to proper food handling practices, raising concerns about premises hygiene and the risk of contamination. The study emphasizes the pressing need to implement robust food safety practices in old age homes, to protect the health of the immunocompromised elderly population. The outcomes stress the imperative for increased vigilance and adherence to food safety protocols within the distinctive environment of old age homes. The prevalence of unsatisfactory enumeration highlights the potential risk of contamination, emphasizing the need to prioritize and ensure the safety of food consumed by older adults in these settings. The research additionally emphasizes the importance of addressing food safety in the context of old age homes, urging heightened attention to prevent foodborne illnesses and their severe consequences in this vulnerable population. Highlighting the importance of addressing food safety in this context, where the increased vulnerability of residents requires heightened focus, the research supports the implementation of comprehensive measures. It is crucial not only to identify non-compliance areas but also to implement targeted interventions that enhance food safety practices. This involves thorough training for food handlers, regular monitoring, and the establishment of stringent protocols to minimize the risk of contamination and subsequent foodborne illnesses. The research outcomes contribute valuable insights to a broader discussion on elderly health, emphasizing the complex relationship connection between food safety and the well-being of older adults in institutional settings. The prevalence of unsatisfactory enumeration serves as a compelling call to action for institutional administrators, policymakers, and healthcare professionals to collaboratively develop and implement tailored strategies, ensuring the safety of food consumed by the vulnerable elderly population in old age homes.

Keywords: Food safety, food hygiene, kitchen surfaces, microbial quantification, microbial identification.

Introduction

The microbiological environment and handling of food within food preparation areas plays an important role in ensuring the safety and quality of the food served, particularly in sensitive settings such as old age homes. According to Carstens *et al.* [1], understanding the distribution of microorganisms on surfaces within kitchen facilities is essential for assessing potential health risks to elderly residents, who may be more susceptible to foodborne illnesses.

The main health hazard associated with preparatory surfaces is microbial contamination. Stein and Chirila [2], state that microbial contamination in the kitchen environment can occur through various pathways, posing a potential threat to food safety and human health. One primary source of contamination is through human contact. A study by Carstens *et al.* [1], revealed that food handlers and individuals in the kitchen may inadvertently transfer bacteria, viruses, or other microorganisms from their hands to surfaces, utensils, or directly onto food during preparation. Failure to practice proper hand hygiene, such as washing hands thoroughly and regularly, significantly increases the risk of microbial transfer.

Cross-contamination is another prevalent mechanism for microbial contamination in the kitchen [3]. Cross-contamination (transfer of microbes from a raw product to another surface or product) occurs in food service in numerous ways [4]. This occurs when pathogens from raw food, especially meat and poultry, come into contact with ready-to-eat foods, surfaces, or utensils. For instance, food preparatory surfaces, such as tables, are crucial in food handling and preparation, making them susceptible to microbial contamination. Contamination can occur through various pathways, including direct contact with raw ingredients, contaminated utensils, or improper cleaning and sanitation practices, leading to potential foodborne illnesses [3, 5].

Kitchen basins are susceptible to microbial contamination due to their frequent use in food preparation and dishwashing activities [6]. According to Griffith [7], the presence of organic matter, food residues, and moisture can create an optimal setting for microbial growth, leading to potential contamination. Cross-contamination may occur when basin surfaces come into contact with raw food items or when contaminated water splashes onto adjacent areas [8]. Moreover, inadequate cleaning practices, such as using contaminated sponges or towels, can lead to the spread of microorganisms from kitchen basins to preparatory surfaces. This contamination may extend to holding areas when food handlers wipe surfaces during the cleaning process [7].

Contamination in kitchen basins may additionally occur through various means. For instance, food residues left in the basin can provide a nutrient-rich environment for microorganisms to proliferate. Cross-contamination may also occur when raw food items, such as meats and vegetables, come into contact with the basin surface. Inadequate cleaning practices, including insufficient rinsing or the use of contaminated cleaning tools, can contribute to the build-up of microbial populations [9]. Additionally, a study by Qiu *et al.* [10] stated that the presence of moisture and warmth in the basin creates favourable conditions for microbial growth. By assessing the total viable counts (TVC) on a kitchen basin, researchers aim to quantify and analyze the overall microbial load, providing insights into the hygiene status of the basin and, by extension, the potential risk of foodborne contamination in the kitchen environment; thereby reflecting the overall cleanliness condition of the kitchens.

As holding areas are crucial in a kitchen environment as they are used to store utensils, equipment, and even prepared food items temporarily. Microbial contamination in holding areas can occur through various means. Firstly, inadequate cleaning practices or irregular cleaning schedules may lead to the accumulation of food residues, creating a conducive environment for microbial growth. Additionally, cross-contamination from hands, utensils, or contaminated surfaces during the handling of food items can introduce bacteria to holding areas. Moreover, airborne microorganisms can settle on these surfaces over time [11].

Previous studies conducted to evaluate microbial safety of kitchens utensils and its environment showed that bacterial profiles of hand towels, dishcloths, sponges, tea towels, steel sinks, and working surfaces are significant and contributed to food contamination [1, 12]. Foodborne illnesses linked to foods prepared in unhygienic kitchens are frequently associated with *Salmonella*. Additionally, various bacterial infections related to contaminated kitchen environments include *Listeria*, *Campylobacter*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli* [13, 14]. To prevent microbial contamination in the kitchen, it is important to follow and enforce appropriate food safety measures. This involves consistently washing hands, handling raw and ready-to-eat foods separately, correctly storing perishables, ensuring thorough cooking, and keeping the kitchen environment clean and sanitized. Being aware of these practices and consistently applying them is essential in preventing foodborne illnesses linked to microbial contamination in the kitchen. Therefore, sampling and analysing these surfaces with contact plates provide valuable

insights into the microbial load, aiding in understanding hygiene levels, identifying potential sources of contamination, and implementing effective control measures to ensure the safety and quality of food preparation.

Materials and Methods

Sampling site

The study was conducted at the Mangaung and Lejweleputswa old age homes (Figure 1). These homes are in the Free State Province, one of South Africa's provinces situated in the central part of the country with a population of approximately 2.1 million inhabitants. Free State is divided into the Mangaung Metropolitan Municipality and four other district municipalities, which are then broken down into 18 local municipalities, as illustrated in Figure 1 below:

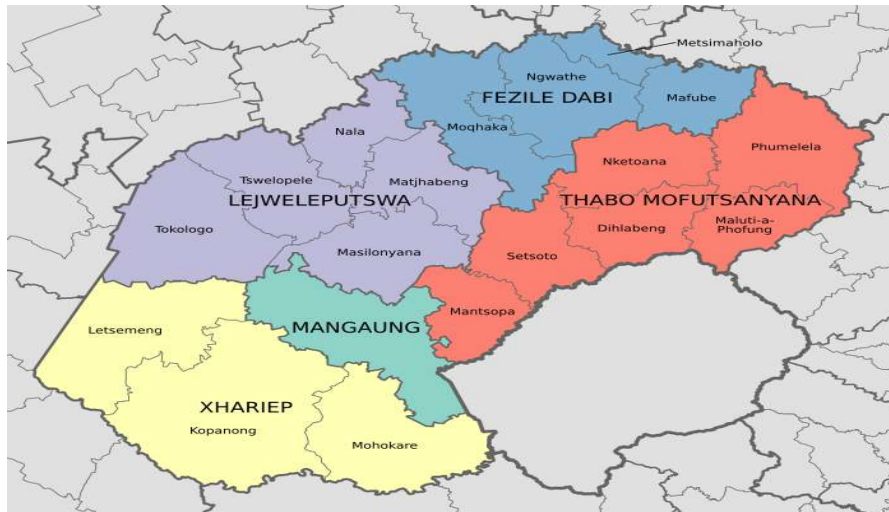


Figure 1: A map illustrating the Free State province, with Mangaung marked in green and Lejweleputswa in purple. The latitude and longitude coordinates for Mangaung Metropolitan Municipality are 29.1303° S, 26.2358° E, while those for Lejweleputswa District Municipality are 28.3991° S, 26.2305° E.

Sample Collection

Samples were collected in the morning using different agar types (Nutrient, Mannitol Salt, and Plate Count Agar [NA, MSA, and PCA]). Two samples were taken for each agar type in every sampling area (holding area, basin, and holding shelf), resulting in a total of 84 representative samples. Following that, 42 plates, representing half of the total (84), were phenotypically examined to assess the appearance, colour, and texture of microbial colonies, specifically focusing on those with better growth, as duplicates were taken for this purpose.

Study Design and Statistical Analysis

In this project, we used descriptive cross-sectional study approaches to assess the cleanliness of kitchen surfaces in elderly care facilities, aiming to prevent food contamination. All surface samples were collected and analysed in duplicate. Plate Count Agar (PCA) (Merck, South Africa) were used for the quantification of total microbial counts. Importantly, each plate was subjected to incubation according to standardized and appropriate procedures, which included positioning them in an upside-down position at specified temperatures (37°C) for a designated incubation period of 24-48 hours.

Analysis of Data

After distinguishing microbial colonies based on their appearance, color and texture, the count was conducted using a Symbiosis aCOLade colony counter (Vacutec, Johannesburg, South Africa) and reported as colony-forming units (CFU) per square centimetre (cm). Evaluation of the results was done following the guidelines provided in the British Columbia Centre for Disease Control (BCCDC) Environmental Health Officers Guide [15].

The results were then categorized into three groups: satisfactory (less than 5 CFU/cm²), acceptable (5-10 CFU/cm²), and unsatisfactory (more than 100 CFU/cm²), based on the Seed and Kurrein (2019) guidelines. The BCCDC guide aligned well with our study's units and assumptions, covering more categories than the South African R638 regulations (Regulations Governing General Hygiene Requirements for Food Premises, the Transport of Food and Related

Matters) [16], which recommend a surface guideline of 100 CFU/cm². However, in this study, when the number of colonies exceeded 300, making individual counting impossible, it was labelled as 'too numerous to count' (TNTC).

Microbial Identification using MALDI TOF MS/ Fingerprinting

Following the phenotypical analysis, 42 separate colonies were isolated with the aim of obtaining pure cultures from the initial plates. The objective was to conduct additional identification of these surface samples, establishing their genus and species through the application of matrix-assisted laser desorption/ionization [MALDI-TOF-MS] [17, 18]; refer to Figure 2 for an illustration of the MALDI-TOF MS process. Briefly, cells (individual colonies) from biological material were collected by scraping the plate and transferring them into an Eppendorf tube with 300 μ L of Ultrapur water (Merck, SA). After thorough mixing, 900 μ L of absolute ethanol was added, mixed well, and then centrifuged at maximum speed (13200 rpm) for 2 minutes at room temperature. The liquid above the sediment was poured off, and the sediment was left to air-dry at room temperature. The dried sediments were well-blended using a vortex mixer with 50 μ L of formic acid (70%) (Merck, SA), followed by the addition of 50 μ L of pure acetonitrile (Merck, USA), and thorough mixing. The mixture underwent centrifugation at maximum speed (13200 rpm) for 2 minutes, and approximately 1 μ L of the resulting liquid was placed onto a Micro Scout Plate (MSP) 96 polished steel target plate (Bruker Daltoniks, Germany) and allowed to dry at room temperature.

Afterward, each sample received a coating of 1 μ L HCCA matrix solution, a saturated mixture of α -cyano-4-hydroxycinnamic acid (Sigma, USA) in 50% acetonitrile-2.5% trifluoroacetic acid (Bruker Daltonics, Germany), and was left to air dry at room temperature. The analysis of all strains used a Microflex LT mass spectrometer (Bruker Daltonics, Germany) with Flex Control software (Version 3.0, Bruker Daltonics, Germany). Spectra were recorded in the linear positive mode (laser frequency of 20 Hz; ion source 1 voltage, 20kV; ion source 2 voltage, 18.6 kV; lens voltage, 7.5 kV; mass range, 2000 to 20,000 Da). Each spectrum involved 240 shots in 40-shot sequences from different positions of the BTS spot (manual mode) and was subsequently analyzed.

The spectra underwent internal calibration using *Escherichia coli* ribosomal proteins as the standard. Raw spectra were imported into the BioTyper software (version 3.0, Bruker Daltonics, Germany), processed through standard pattern matching with default settings, and results were presented in a ranking table with color codes. Outcomes of the pattern-matching process adhered to the MALDI-TOF biotyper (MT) manufacturer's guidelines, expressing identity (ID) scores from 0 to 3. Scores below 1.70 were deemed unreliable, $1.7 < ID < 1.9$ indicated identification at the genus level, and scores exceeding 1.9 were considered reliable for species identification.

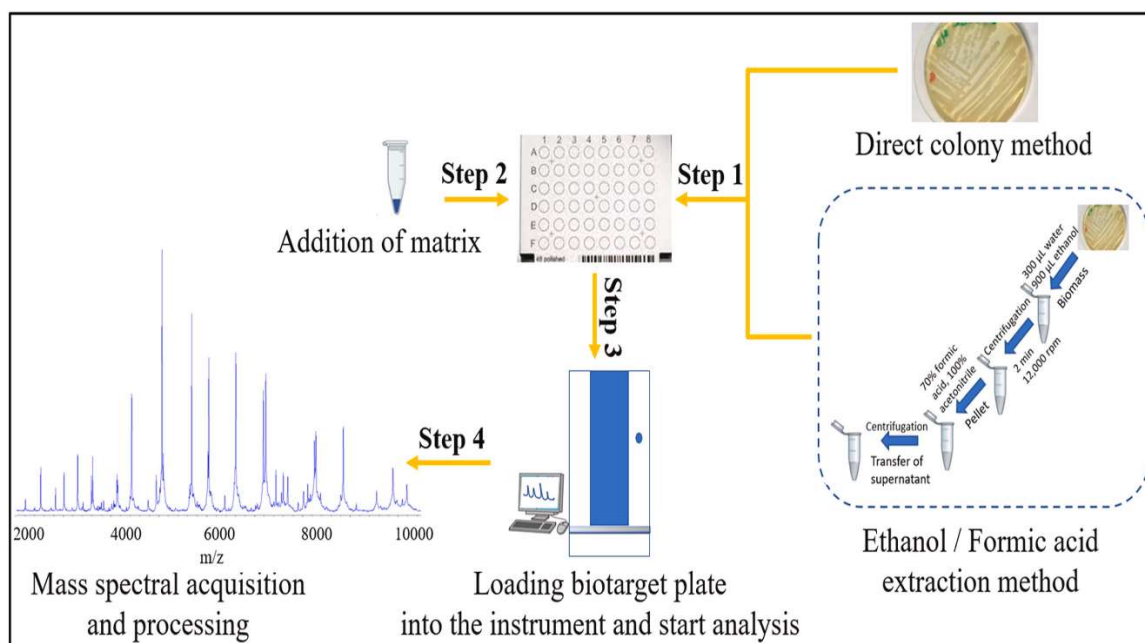


Figure 2: Maldi ToF Ms Step procedure. The figure shows a step-by-step process of how the analysis is performed.

Analysis takes about twenty minutes to conclude, and it includes isolating a colony from the initial petri dish containing microorganisms, mix it with the matrix solution and placing it on a steel biotarget plate in the second step;

third step involves inserting the steel biotarget plate into the machine and start the analysis. Step four indicates the bacterial detection readings from the machine.

Results and Discussion

This study was mainly focused on food safety practices, cleanliness, and hygiene of old age home kitchen surfaces in the Mangaung Metropolitan and Lejweleputswa District Municipalities, in the Free State area. The sample collection was carried for a period of 2 months i.e. from March 2023 till April 2023.

Food Preparatory Surface Bacterial Counts

As shown in Table 1, regarding total viable counts (TVC) obtained from 14 PCA plates for basins using plate count agar, 78.571% (calculated as 11 out of 14 multiplied by 100) of the samples showed counts that were too numerous to count (TNTC). Although NA and MSA were not specifically designed for assessing total viable counts, the colonies were still enumerated before isolating pure colonies for subsequent identification of genus and species names using MALDI-TOF MS. The results from the plates revealed that, for NA representing food preparatory surfaces, 71.429% (calculated as 10 out of 14 multiplied by 100) of the colonies, meanwhile for MSA plates representing holding area surfaces, 57.143% (calculated as 8 out of 14 multiplied by 100) of the observed colonies.

All in all, the cleanliness levels on food preparation surfaces did not meet the standards set by the BCCDC guidelines, as all surfaces were deemed unsatisfactory. Specifically, for preparation surfaces, 78.571% of the counts were unsatisfactory, with 21.429% deemed satisfactory. In the case of food preparatory surfaces, 71.429% were unsatisfactory, and 28.571% were satisfactory. Similarly, for holding areas in old age homes, 57.143% of the counts were unsatisfactory, while 42.857% were satisfactory (refer to Table 1). Among the three surfaces examined, basins exhibited the highest total coliform counts.

According to the Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972 [19] under Standards and Requirements for Food Premises, Section 5 (sub-regulation 2) states that “a food premises must be of such location, design, construction, and finish and must be so equipped and maintained in the condition for which it was intended, that it can be used at all times for the purpose for which it was designed, constructed and equipped without creating a health hazard and that food;

- (a) can be handled hygienically on the food premises and facilities thereon; and
- (b) can be protected effectively by the best available method against contamination or spoilage by poisonous or offensive gases, vapours, odours, smoke, soot deposits, dust, moisture, insects, or other vectors, or by any other physical, chemical (including unintended allergens) or biological contamination or pollution or by any other agent whatsoever.” And Section 6 (sub-regulation 4) which states that “a surface referred to in sub-regulation (1) (Kitchen surfaces) and an item referred to in sub-regulation (2) must be-

(a) cleaned and washed before food comes into direct contact with it for the first time: and (b) cleaned and washed. as and when necessary, during or immediately after the processing of food, so that contamination of the food that comes into contact with any such surface or item is prevented, and any such surface or item must not, before food comes into direct contact with it, contain –

- (i) more than 100 viable microorganisms per centimetre squared (cm^2) upon analysis, conducted in accordance with acknowledged scientific microbiological methods of analysis, of a sample taken in accordance with the swab technique prescribed in the Efficacy of Cleaning Plant, Equipment and Utensils and
- (ii) the remains of cleaning materials or disinfectants which may contaminate the food.”

The observed distinction indicates that the standard plate, which has identified bacteria, deviates from the permissible microbial limits outlined in the regulations. According to the guidelines, there should be no microorganisms on food preparation surfaces, and the count should not exceed 100 viable microorganisms per cm^2 . This suggests that the cleaning practices and hygiene standards at the old age homes did not meet the criteria established by the Act (54 of 1972).

Overall, the results were similar to those of Bukhari *et al.* [20], emphasizing a higher risk of food contamination from preparation surfaces than holding areas. Diverse bacterial colonies on preparation surfaces suggest a lack of cleanliness in old age homes. The many colonies on NA (Preparation surface) suggest it might be a potential contamination source. Different colonies on various agar media indicate the presence of various bacterial species in old age homes.

Table 1: Quantification of Microorganisms on Food Contact Surfaces in Old Age Homes of Mangaung and Lejweleputswa.

Old Age Home	Agar Media	Standard Plate	Duplicate Plate
1	Nutrient Agar (Prep. surface)	Five colonies were seen on top of a white film.	100 colonies
2	Plate Count Agar (Basin)	Large yellow and white colonies that are aggregated and cannot be enumerated.	200 colonies
3	Mannitol Salt Agar (Holding area)	115 colonies	A film with a coarse, textured surface.
4	Nutrient Agar (Prep. surface)	TNTC colonies	TNTC colonies
5	Plate Count Agar (Basin)	Yellow and white separated films.	300 colonies
6	Mannitol Salt Agar (Holding area)	0 colonies	0 colonies
7	Nutrient Agar (Prep. surface)	White separated film.	White film sections that could not be counted.
8	Plate Count Agar (Basin)	White film formed	Patches of white films.
9	Mannitol Salt Agar (Holding area)	Separated film sections.	Yellow and white patches of films.
10	Nutrient Agar (Prep. surface)	Patches of white films.	White film.
11	Plate Count Agar (Basin)	Patches of white film.	White film.
12	Mannitol Salt Agar (Holding area)	Yellow and white patches of films.	Yellow and white patches of films.

13	Nutrient Agar (Prep. surface)	White film with TNCT small colonies.	TNCT small white colonies.
14	Plate Count Agar (Basin)	TNTC white colonies.	TNCT small white colonies.
15	Mannitol Salt Agar (Holding area)	300+ yellow, white, and reddish colonies.	Coarse structure of white and reddish patches of film.
16	Nutrient Agar (Prep. surface)	TNTC white colonies.	TNTC white colonies.
17	Plate Count Agar (Basin)	TNTC white and yellow colonies.	White film with small TNTC yellow colonies.
18	Mannitol Salt Agar (Holding area)	50 coarse structured yellow colonies.	56 coarse structured yellow colonies.
19	Nutrient Agar (Prep. surface)	TNTC white colonies.	TNTC white colonies.
20	Plate Count Agar (Basin)	TNTC white colonies.	TNTC white colonies.
21	Mannitol Salt Agar (Holding area)	300 white colonies.	Yellow and white 400 colonies.
22	Nutrient Agar (Prep. surface)	100 white colonies.	115 white colonies.
23	Plate Count Agar (Basin)	White film.	White film.
24	Mannitol Salt Agar (Holding area)	100 white colonies.	95 white colonies.
25	Nutrient Agar (Prep. surface)	White film.	White film with small TNTC colonies.
26	Plate Count Agar (Basin)	White coarse structured film.	White coarse structured film.

27	Mannitol Salt Agar (Holding area)	TNTC yellow and white colonies.	TNTC yellow and white colonies.
28	Nutrient Agar (Prep. surface)	6 big white colonies.	5 big white colonies.
29	Plate Count Agar (Basin)	49 white colonies.	56 white colonies.
30	Mannitol Salt Agar (Holding area)	0	0
31	Nutrient Agar (Prep. surface)	TNTC white colonies.	TNTC white colonies.
32	Plate Count Agar (Basin)	370 yellow and white colonies.	400+ yellow and white colonies.
33	Mannitol Salt Agar (Holding area)	TNTC yellow and white colonies.	TNTC yellow and white colonies.
34	Nutrient Agar (Prep. surface)	300+ white colonies.	300+ white colonies.
35	Plate Count Agar (Basin)	400+ white colonies.	400+ white colonies.
36	Mannitol Salt Agar (Holding area)	200 white colonies.	115 white colonies.
37	Nutrient Agar (Prep. surface)	38 white colonies.	42 white colonies.
38	Plate Count Agar (Basin)	40 white colonies.	46 white colonies.
39	Mannitol Salt Agar (Holding area)	10 white colonies.	18 white colonies.
40	Nutrient Agar (Prep. surface)	63 white colonies.	66 white colonies.
41	Plate Count Agar (Basin)	55 white colonies.	60+ white colonies.

42	Mannitol Salt Agar (Holding area)	40 white colonies.	38 white colonies.
<p>Summary: This research examined the number of microorganisms on food contact surfaces in old age homes in Mangaung and Lejweleputswa. The goal was to evaluate hygiene and potential health risks linked to these surfaces in settings that serve the elderly by measuring microbial levels.</p>			

MALDI TOF MS/ Microbial Fingerprinting Results

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is often used to analyze unknown bacterial colonies and identify microorganisms [21, 22]. This advanced analytical technique enables the rapid and accurate identification of bacterial species based on their unique mass spectral fingerprints. MALDI-TOF MS works by ionizing microbial proteins directly from bacterial colonies, creating distinctive mass spectra that serve as molecular signatures for different species. According to Tarfeen *et al.* [23], this method is highly efficient, allowing for the identification of a wide range of microorganisms in a short time frame.

Because MALDI-TOF MS is efficient in bacterial identification, it was used in this investigation. Considering that surfaces in old age home kitchens can harbour diverse bacterial species, a swift identification of these microorganisms was crucial. This rapid identification assisted in evaluating potential risks for the elderly residents, who may be more vulnerable to foodborne illnesses.

In this study, a total of 42 clinical bacterial culture isolates were analyzed by MALDI-TOF MS. Out of the 42 samples, 33 were identified as members of the Bacillaceae family, including *Exiguobacterium artemiae*, *B. cereus*, *B. subtilis*, *B. altitudinis*, *B. licheniformis*, *B. pumilus*, *Peribacillus mutalis*, and *Paenibacillus alvei*. One isolate was categorized under the Enterococcaceae family as *Enterococcus faecium*, while two belonged to the Enterobacteriaceae family as *Enterobacter cloacae* and *Proteus mirabilis*. Additionally, four isolates were identified within the Staphylococcaceae family, namely *S. cohnii*, *S. epidermis*, *S. hominis*, and *S. sciuri*. One isolate was classified as *Pseudomonas (P. stutzeri)*, and another belonged to the Micrococcaceae family, specifically *Kocuria rosea*.

As shown in Table 2, the microbial values are clear: the highest value recorded was 2.44, with a corresponding value of 2.18 on the duplicate plate, both associated with *Kocuria rosea*. The lowest value observed was 1.71, and its duplicate had a value of 1.58, with *Bacillus pumilus* identified in the standard plate and no identifiable organisms in the duplicate. Differences in hygiene and kitchen cleanliness likely contribute to the variation in microbial counts between the standard and duplicate plates. Kitchen microbial count regulations emphasize the importance of maintaining low bacterial levels for food safety.

Table 2: MALDI-TOF MS Identified Microorganisms.

Place and area		Bacterial counts in CFU/cm ²			
Old Age Home	Media	Organisms Detected (Best Match)	Score Value	Organisms Detected (Second Best Match)	Score Value
1	NA	<i>Bacillus pumilus</i>	1.90	<i>Bacillus pumilus</i>	1.83
2	PCA	<i>Bacillus pumilus</i>	1.85	<i>Bacillus pumilus</i>	1.82
3	MSA	<i>Kocuria rosea</i>	2.44	<i>Kocuria rosea</i>	2.18

4	NA	<i>Bacillus pumilus</i>	1.89	<i>Bacillus pumilus</i>	1.81
5	PCA	<i>Bacillus pumilus</i>	1.97	<i>Bacillus pumilus</i>	1.92
6	MSA	<i>Bacillus pumilus</i>	1.89	<i>Bacillus pumilus</i>	1.70
7	NA	<i>Bacillus subtilis</i>	2.06	<i>Bacillus subtilis</i>	1.96
8	PCA	<i>Bacillus pumilus</i>	1.93	<i>Bacillus pumilus</i>	1.47
9	MSA	<i>Bacillus subtilis</i>	1.80	No Organism Identification Possible	1.69
10	NA	<i>Bacillus pumilus</i>	1.75	No Organism Identification Possible	1.62
11	PCA	<i>Enterococcus faecium</i>	2.10	<i>Enterococcus faecium</i>	2.02
12	MSA	<i>Exiguobacterium artemiae</i>	2.24	No Organism Identification Possible	1.42
13	NA	<i>Bacillus cereus</i>	2.01	<i>Bacillus cereus</i>	1.93
14	PCA	<i>Bacillus licheniformis</i>	1.80	No Organism Identification Possible	1.67
15	MSA	<i>Bacillus subtilis</i>	1.91	<i>Bacillus subtilis</i>	1.85
16	NA	<i>Bacillus licheniformis</i>	1.78	No Organism Identification Possible	1.55
17	PCA	<i>Bacillus altitudinis</i>	1.70	No Organism Identification Possible	1.64
18	MSA	<i>Staphylococcus sciuri</i>	2.16	<i>Staphylococcus sciuri</i>	1.80
19	NA	<i>Bacillus subtilis</i>	1.95	<i>Bacillus subtilis</i>	1.95
20	PCA	<i>Bacillus pumilus</i>	1.71	No Organism Identification Possible	1.57
21	MSA	<i>Staphylococcus hominis</i>	2.17	<i>Staphylococcus hominis</i>	2.04
22	NA	<i>Peribacillus muralis</i>	1.86	No Organism Identification Possible	1.69

23	PCA	<i>Bacillus pumilus</i>	1.80	No Organism Identification Possible	1.58
24	MSA	<i>Bacillus subtilis</i>	2.09	<i>Bacillus subtilis</i>	1.89
25	NA	<i>Paenibacillus alvei</i>	1.73	No Organism Identification Possible	1.45
26	PCA	<i>Enterobacter cloacae</i>	2.39	<i>Enterobacter cloacae</i>	2.38
27	MSA	<i>Proteus mirabilis</i>	2.39	<i>Proteus mirabilis</i>	2.37
28	NA	<i>Bacillus pumilus</i>	1.87	<i>Bacillus altitudinis</i>	1.83
29	PCA	<i>Staphylococcus cohnii</i>	1.85	No Organism Identification Possible	1.69
30	MSA	<i>Bacillus altitudinis</i>	1.78	No Organism Identification Possible	1.67
31	NA	<i>Bacillus cereus</i>	1.86	<i>Bacillus cereus</i>	1.81
32	PCA	<i>Bacillus pumilus</i>	1.71	No Organism Identification Possible	1.58
33	MSA	<i>Bacillus pumilus</i>	1.77	<i>Bacillus pumilus</i>	1.77
34	NA	<i>Bacillus altitudinis</i>	1.89	<i>Bacillus pumilus</i>	1.86
35	PCA	<i>Pseudomonas stutzeri</i>	2.10	<i>Pseudomonas stutzeri</i>	2.07
36	MSA	<i>Staphylococcus epidermidis</i>	1.81	<i>Staphylococcus epidermidis</i>	1.70
37	NA	<i>Bacillus subtilis</i>	1.84	<i>Bacillus subtilis</i>	1.75
38	PCA	<i>Bacillus pumilus</i>	1.90	<i>Bacillus pumilus</i>	1.84
39	MSA	<i>Bacillus subtilis</i>	1.92	<i>Bacillus subtilis</i>	1.87
40	NA	<i>Bacillus altitudinis</i>	1.86	No Organism Identification Possible	1.60
41	PCA	<i>Bacillus pumilus</i>	1.88	<i>Bacillus pumilus</i>	1.80
42	MSA	<i>Bacillus subtilis</i>	1.95	<i>Bacillus subtilis</i>	1.95

Summary: The highest value on the table was 2.44, and the duplicate plate showed a value of 2.18. The bacteria identified in both cases was *Kocuria rosea*.

The lowest value recorded was 1.71, while its duplicate yielded a value of 1.58. The bacteria identified in the standard plate was *Bacillus pumilus*, whereas no identifiable organism was detected in the duplicate plate.

The existence of various bacterial families implies numerous potential origins of contamination on kitchen surfaces. Some of the bacteria identified in this study are commonly associated with foodborne illnesses and are often isolated from soil and water contamination, such as *Proteus mirabilis*, *P. stutzeri*, *Kocuria rosea*, and *Exiguobacterium artemiae*. Additionally, inadequate cleaning of the milking parlour and insufficient personal hygiene of the individuals handling the milk and the cows contribute to contamination, including *Bacillus* and *Staphylococcus* species on various body parts. Another source of contamination arises from faecal contamination, specifically from *Enterococcus faecium* and *Enterobacter cloacae*. Refer to Table 3 for information about bacterial species isolated from kitchen surfaces using MALDI-TOF MS, including their typical sources, associated implications, and symptoms.

Table 3: Sources of Bacterial Species Isolated Using MALDI-TOF MS in Kitchen Surfaces.

ISOLATED SPECIES	COMMON SOURCE	IMPLICATIONS	PREDOMINANT SYMPTOMS
<i>Bacillus altitudinis</i>	Maize, rice, wheat, sometimes fish.	Food poisoning.	Nausea, vomiting, and diarrhoea.
<i>Bacillus cereus</i>	Fish, dairy, meat, sauces, soups and stews, vegetables.	Food poisoning.	Intestinal illnesses with nausea, vomiting, and diarrhoea.
<i>Bacillus pumilus</i>	Dairy milk, pre-cooked rice.	Food poisoning.	severe abdominal cramps, fever with chills, diarrhoea, dizziness, and loss of appetite
<i>Bacillus subtilis</i>	Chicken intestines, beef tripe.	Food spoilage such as ropy bread, and incidents of food-borne gastroenteritis.	Diarrhoea and/or nausea; abdominal pain.
<i>Bacillus licheniformis</i>	Raw milk, cooked meats, and vegetables; wastewater.	Food poisoning.	Diarrhoea and/or nausea; abdominal pain.
<i>Peribacillus mutalis</i>	Soil and plants (fruits and vegetables).	Food poisoning.	Nausea, vomiting, and diarrhoea.
<i>Paenibacillus alvei</i>	Soil, often associated with plant roots - Fruits and vegetables.	Food poisoning.	Nausea, vomiting, and diarrhoea.
<i>Enterobacterium artemiae</i>	Raw milk, cheeses, and meat products.	Gastrointestinal illnesses.	Nausea, vomiting, diarrhoea, abdominal cramps, and other agent-specific symptoms.
<i>Enterococcus faecium</i>	Raw milk, cheese products, and processed foods.	Severe nosocomial infections.	Fever, chills, shortness of breath, abdominal pain, and diarrhoea.

<i>Exiguobacterium artemiae</i>	Seafood, soil, seawater.	Food poisoning.	Nausea, vomiting, and diarrhoea.
<i>Kocuria rosea</i>	Sausages and cheeses.	Fermentation of foods, food poisoning.	Nausea, vomiting, and diarrhoea.
<i>Proteus mirabilis</i>	Water, soil, and human intestinal environments.	Food poisoning, peritonitis, and meningitis.	Gastrointestinal illnesses (Inflammation, infection, or dysfunction of various components of the digestive system, such as the stomach, intestines, liver, and pancreas).
<i>Pseudomonas stutzeri</i>	Chilled chicken, and red meat.	Spoilage bacteria - Food poisoning.	Gastrointestinal illnesses (Inflammation, infection, or dysfunction of various components of the digestive system, such as the stomach, intestines, liver, and pancreas).
<i>Staphylococcus cohnii</i>	Milk products (cheese, yoghurt, cheese) derived from dairy cows' milk.	Food poisoning.	Human and animal infections, including toxin-mediated foodborne diseases.
<i>Staphylococcus epidermis</i>	At several parts of the skin constituting 90% of microflora - Foods that are not cooked after handling, such as sliced meats, puddings, pastries, and sandwiches.	Food poisoning.	Nausea, vomiting; and stomach cramps.
<i>Staphylococcus hominis</i>	Milk, various cheeses, fermented fish products.	Staphylococcal food poisoning.	Severe nausea and vomiting. Abdominal cramps, diarrhoea, and severe fluid and electrolyte loss (weakness) and very low blood pressure (shock).
<i>Staphylococcus sciuri</i>	Milk, French and Italian cheeses, fish sauces; and fermented sausages.	Food poisoning.	Severe nausea and vomiting.

Microorganisms have the potential to cause foodborne illnesses, especially in susceptible groups like the elderly [14]. Therefore, it is important to maintain cleanliness and disinfection of food preparation surfaces consistently to avoid cross-contamination. The findings from this study indicate that the hygiene and food safety practices on kitchen surfaces are not satisfactory, potentially due to inadequate handling by food handlers. Thus, it is important to consistently remind food handlers about the importance of following safe food handling practices and maintaining good personal hygiene.

Jevsnik *et al.* [24] conducted a study that yielded comparable findings. The research study involved microbiological examination of kitchen hygiene using contact agar plates and assessment of cleaning effectiveness through Adenosine Triphosphate (ATP) bioluminescence measurements. The research results revealed a deficiency in knowledge about certain aspects of food safety, with individuals aged 36 to 55 and women generally displaying the highest levels of

awareness. In terms of cross-contamination, fourteen consumers failed to use separate boards and knives for meat and vegetables, with only two male consumers correctly implementing the separation. Ten consumers successfully distinguished raw poultry meat from other food types, including five individuals over 65 years. However, the rest of the participants did not consistently separate items. Majority of consumers (No. 14) followed the practice of keeping raw foods apart from ready-made foods; however, two younger male consumers were inconsistent in their separation. Additionally, many of the consumers observed (No. 14 out of 16) did not wash poultry meat before preparing it. The findings strongly suggest that the kitchen cleanliness and hygiene standards were not met, as the kitchen cleanliness and hygiene failed to meet the necessary standards. This raises concerns about the potential compromise of food prepared in the kitchen environment.

Using the total coliform count (TCC) values, each surface was categorized into three groups: adequately cleaned, acceptable, and inadequately cleaned. The accepted TCC level for kitchen utensils, as per guidelines (Guidelines for the Microbiological Safety of Food Intended for the Final Consumer) [25], is 100 CFU/20 cm² or 2.0 log CFU/20 cm². Therefore, contamination levels exceeding these standards were considered inadequate. Surfaces with TCC levels below log 1.0 CFU/20 cm² were considered adequately cleaned, while those with values between log 1.1 and log 2.0 CFU/20 cm² were classified as acceptably cleaned. Two cutting boards (12.5%) showed TCC levels above log 2.0 CFU/20 cm², while all consumer plates were either adequately cleaned (n = 12, 75%) or acceptably cleaned (4, 25%). Based on the overall TCC limits of the two tested utensils, individual consumers were classified into four groups: 1st class, where both cutting board and plate were adequately cleaned; 2nd class, where one surface was adequate and the other was acceptable; 3rd class, where both surfaces were acceptable; and 4th class, where one or both tested surfaces were inadequately cleaned.

These results were similar to those obtained by Flores *et al.* [26], who found that the identified 34 bacterial and two archaeal phyla, with the overwhelming majority of sequences ($\approx 98\%$ of all sequences) belonging to only four bacterial phyla: *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. Hassan and El-Bagoury [27] also found that the microbiological quality of samples taken from the sink drain, kitchen sponge and draining rack had high total viable counts (TVC). Furthermore, they harboured the highest number of heterotrophs, pseudomonads, enteric species, staphylococci, and micrococci.

As mentioned earlier, when compared to the criteria set in the British Columbia Centre for Disease Control (BCCDC) Environmental Health Officers Guide and Act 54 of 1972 (Foodstuffs, Cosmetics, and Disinfectants Act 54 of 1972), the bacterial counts in this study did not meet the established standards. Based on the study findings, most of the old age homes did not meet the specified standards. The counts exceeded 100, and in some cases, there were 'too numerous to count' instances, accompanied by the formation of white or yellowish films on the agar mediums, indicating an excess of viable microorganisms beyond the set limit of 100 per cm².

The high counts obtained indicate that there could be several factors for it, such as not handling food properly, bad management, incorrect cleaning in old age kitchens, and workers not practicing good hygiene.

Conclusion

The present investigation revealed that the cleanliness and hygiene conditions in the fifteen (15) old age kitchens in Mangaung and Lejweleputswa were below the required standard, posing a potential health risk to the elderly residents. Nevertheless, some of the old age homes adhered to the Foodstuffs, Cosmetics, and Disinfectants Act 54 of 1972. A limitation was that the study did not assess the knowledge, attitudes, and behaviors of farmers and their workers regarding food safety and foodborne illnesses; it solely focused on quantifying and identifying the distribution of microorganisms on food preparatory surfaces to assess kitchen cleanliness and sanitation. The findings of this study emphasize a notable presence of bacterial contamination in the old age kitchen areas of the study, emphasizing the importance of implementing improved hygiene measures. This is important for reducing the risk of cross-contamination and subsequently lowering the chances of foodborne illnesses. The detection of bacterial pathogens in all collected samples indicates insufficient personal hygiene practices and inadequate cleaning and disinfection of surfaces. Additional factors contributing to the issue may include ineffective disinfection methods or the use of detergents that cannot effectively eliminate the common bacteria found on kitchen surfaces. Moreover, if kitchen cloths are not changed between tasks, such as cleaning raw chicken blood and subsequently wiping a knife before cutting fresh fruits or ready-to-eat vegetables, it could contribute to bacterial contamination.

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