

**ASSESSMENT OF FOOD SAFETY OF STREET-VENDED FOODS
AND NUTRIENT KNOWLEDGE OF STREET FOOD VENDORS
WITHIN THE MANGAUNG METRO MUNICIPALITY, FREE STATE,
SOUTH AFRICA**

BY

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DECLARATION OF OWN WORK

I, PONT'S O LETLOTLO JOYCE LETUKA, declare that the work hereby submitted is my own original work. Where other people's work has been used (either from a printed source, the internet or any other sources), these sources were properly acknowledged and cited accordingly.

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This work is a dedication to my daughter, Baatseba Precious Molapo.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Street-vended foods are ready-to-eat foods and beverages that are prepared and sold on the streets and in other public places by vendors. Such food has no need for further processing (Arifen *et al.*, 2025). Street foods are often a reflection of the traditional cultures of diverse ethnic groups and local communities and present a significant diversity in raw materials and preparation methods (Parikh *et al.*, 2022). A wide body of literature highlights street food vending as a tourist attraction, thereby affirming its contribution towards a portion of the nation's economy (May and Latip, 2021; Mohamad *et al.*, 2022; Abdullah *et al.*, 2023).

However, even though street food vending has been a global practice since ancient times, it has unfortunately remained unregulated in some areas (Imathiu, 2017). This regulation gap associated with street food vending renders it difficult to hold these vendors accountable in the unfortunate event of foodborne disease outbreaks or food-related complaints by consumers. As a result, the dominant mode of governance in some countries has been to eradicate this form of trade (Kazembe *et al.*, 2019). However, this ideal poses challenges because even though street food vending is informal in nature, it is used mostly in third-world countries to solve socio-economic issues through the provision of nutritious, inexpensive meals to the urban poor and to create and sustain employment for others.

Despite the obvious advantages that the street food vending industry presents, it has some disadvantages that are of global concern. Over the years, street-vended foods have often been associated with poor nutritional components, unhealthy diets, and physical, chemical and biological contaminants, resulting in a global concern regarding their consumption (Eke and Elechi, 2021; Nkesi *et al.*, 2023). The general perception that street food is unsafe still prevails, and this is arguably due to the environment in which these foods are prepared and consumed (Rakha *et al.*, 2022). Street food vendors

transport their cooked and/or uncooked food to places characterised by high traffic movement, such as bus and taxi ranks, industrial sites, marketplaces, and busy street corners (Mjoka and Selepe, 2017). These places, however, do not meet food safety requirements as they are highly exposed to motor vehicle exhaust fumes, dust, rodents, and flies. Furthermore, the utensils used by vendors are sometimes of dubious quality, hygiene and may also contribute to toxic metal contamination due to leaching or microbial contamination as a result of infrequent and improper cleaning (Islam *et al.*, 2024). If not addressed, these issues could negatively affect the profitability of the street food vending sector and its ability to contribute towards reducing unemployment and enhancing food security.

Food safety requires a collaborative effort by the government, food producers, and consumers regarding the handling, processing, and storage of food in a manner that prevents foodborne illnesses (WHO, 2024). A widely accepted definition of food security was devised at the World Food Summit in 1996, stating that it is when all people, at all times, have physical and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life. When access to nutritionally adequate and safe food is limited, it is referred to as food insecurity (Drewnowski, 2022). Food insecurity is commonly high among individuals presenting with non-communicable diseases such as diabetes, obesity, heart disease, and arthritis (Garcia-Herrero *et al.*, 2018). Because street-vended foods are affordable, they are able to meet such dietary needs. *The State of Food Security and Nutrition in the World* report that was published in 2024 (FAO, IFAD, UNICEF, WFP and WHO, 2024) indicated that 1 out of 11 people in the world and 1 out of 5 people in Africa experienced food insecurity in 2023. Therefore, street food vending, which is an affordable means of access to food, is one of the mechanisms that could help ease this burden on global populations. Additionally, the affordability and ease of access of street-vended foods could potentially help address SDG target 2.1 of ending hunger and food insecurity as well as SDG target 2.2 of eliminating all forms of malnutrition.

Nutrition is described by the World Health Organization (WHO) (2004) as the intake of food to support the dietary needs of the body. Moreover, nutrition defined as the

scientific study of how organisms ingest, digest, absorb, transport, and utilize nutrients from food to maintain growth, repair tissues, and support overall health (LibreText, n.d.; Temple, 2022). A study conducted in Cape Town, South Africa, found that street-vended foods were energy-dense and contained high levels of saturated fats, trans fats, salt, and sugar, thereby posing a public health risk (Hill *et al.*, 2019). Consumers are often driven towards street food consumption due to factors such as a sedentary lifestyle, lack of time to cook homemade meals, and a low socioeconomic status (Sousa *et al.*, 2022). Sousa *et al.* (2021) further contends that there has been a simultaneous increase in the prevalence of non-communicable diseases (NCD) and mortality, which they partly ascribe to the modern lifestyle and dietary modifications. Despite the relevance of street foods in the foodscape of urban areas in many developing societies, the insufficient knowledge about nutrition, unhealthy ingredients and the poor choice of cooking methods by vendors exacerbate food safety risks associated with street-vended foods (Wentzel-Viljoen *et al.*, 2013; Sousa *et al.*, 2021). Therefore, addressing these challenges requires for targeted education on good food safety practices and basic nutrition knowledge.

Over and above food security, another troubling aspect associated with street-vended foods is contamination. Contamination of street foods often occurs for a number of reasons, such as poor preparation methods, poor packaging, vehicular exhaust emissions, poor sanitation, indiscriminate waste disposal, industrial emissions, and the prevalence of many other pollutants in the environment where street foods are sold (Birgen *et al.*, 2020; Koumassa *et al.*, 2025). Microbial contamination is among the major health hazards associated with street food (Rene, 2011). The use of vending carts and stands that are commonly crude and poorly constructed, the presence of vectors and vermin, and the lack of access to clean running water, amplify the poor sanitary practices in this sector (Raedani, 2023; Gichunge *et al.*, 2023). Among the common foodborne pathogens that can contaminate street food are bacteria such as *Salmonella* spp., *Listeria*, *Escherichia coli* and *Staphylococcus*, or viruses such as norovirus and hepatitis A (FDA, 2021). The WHO recognises that low- and middle-income countries have a disproportionate burden of foodborne illnesses. Therefore, much emphasis must be put on coordinated prevention strategies such as capacity building in member states,

promoting safe food handling practices and surveillance of foodborne illnesses (WHO, 2024;WHO, n.d.) In South Africa, a gradual increase in listeriosis cases starting mid-June 2017 led to the largest listeriosis outbreak worldwide, with 1,060 reported cases from 01 January to 17 July 2018 (Smith *et al.*, 2019; Moloji *et al.*, 2021). This outbreak, marked by its unprecedented scale, highlighted the critical need for robust food safety practices and rapid response mechanisms to prevent similarly widespread health crises in the future. Similarly, an outbreak of *Staphylococcus enterotoxin A* was reported in Tshwane in May of 2015 which resulted from the consumption of contaminated chicken (Ntshiqha *et al.*, 2016). Although there might be even more cases of foodborne illnesses in South Africa, their burden is not well known, possibly as a result of under reporting.

Food may also be contaminated by chemical contaminants such as heavy metals, and in research centered around street food vending, this is often not reported. Ankar-Brewoo *et al.* (2020) suggest that the release of chemical contaminants into food may be prompted by the use of improper cookware and unclean utensils and food containers. Food handling practices expose street foods to environmental contaminants such as vehicle exhaust fumes, dust particles, as well as raw materials that are all potential contributors to foodborne diseases and chemical hazards that pose a risk to the quality of street-vended foods (Sarker *et al.*, 2022). Some of the toxic metals that can be found in street foods include lead (Pb), cadmium (Cd), mercury (Hg), antimony (Sb), manganese (Mn) and aluminium (Al) (Ekhator *et al.*, 2017). The consumption of toxic metal-borne food may not only lead to a series of serious health issues but could also cause the depletion of essential nutrients in the body (Ekhator *et al.*, 2017; Zakaria *et al.*, 2021). This could be as a result of their chemical similarity (ionic size) to essential metals, or by damaging the gut and thereby reducing absorption of nutrients. Additionally, food that is contaminated with adulterants such as chalk powder and metanil yellow also causes diseases such as cancer or brain damage due to their toxicity (Haji *et al.*, 2023).

1.2 PROBLEM STATEMENT

Street foods provide both urban and rural consumers with inexpensive, convenient, culturally acceptable and often tasty meals (Nonato *et al.*, 2016). Furthermore, they are a source of energy and nutrients (macronutrients), thereby helping people to satisfy their

dietary needs (Imathiu, 2017). Moreover, due to industrialization and migration, this trade has significantly grown globally. Street food vending has thus become a source of employment for many (mostly women) in urban and rural areas, thereby alleviating poverty and improving food security. People who engage in street food vending are often from households with low income per capita; therefore, as they have to maximize profits, they commonly prioritise cheaper raw ingredients that are of dubious quality over good quality ingredients (Salamandane *et al.*, 2023; Koumasa *et al.*, 2025). This practice is part of the emerging street food culture that unfortunately increases the risk of the chemical, and microbiological contamination of street-vended food (Choudhury *et al.*, 2011; Nonato *et al.*, 2016).

Street food vending stalls are often situated at roadsides in densely populated or industrial areas (Mjoka and Selepe, 2017), thereby exposing the vended food to adulteration with pollutants such as heavy metals from automobile exhausts, poor packaging, industrial emissions, indiscriminate waste disposal, and other pollutants in the environment around the vending area (Mafuyai *et al.*, 2015). Some of these heavy metals can be carcinogenic, thereby putting the health of consumers at risk (Ugya *et al.*, 2019). Prolonged exposure to these heavy metals, particularly lead, can lead to irreversible brain damage, anaemia, coma, and even death if not immediately treated (WHO, 2024). In addition to contamination with heavy metals, street-vended foods may lack the nutritional components of a healthy diet (Nonato *et al.*, 2012). The lack of healthy nutrients in this food most likely causes overweight, obesity, and chronic and non-communicable diseases (Imathiu, 2017). This is contributed to by disrupted metabolism and hormone regulation, excess caloric intake resulting from low density nutrients and inflammation from low quality diets

Unsafe handling, poor hygiene and improper storage of food are major causes of foodborne diseases. Most cases of severe foodborne illnesses and fatalities are due to bacterial pathogens (Rane, 2011). Of the thousands of bacterial species, more than 90% of foodborne illnesses are caused by species such as *Staphylococcus*, *Salmonella*, *Clostridium*, *Listeria*, *Vibrio*, *Bacillus* and Enteropathogenic *Escherichia coli* (Centers for Disease Control and Prevention [CDC], 2016). The WHO (n.d.) states that as a result of

the complexities in the global food chains, the risk of contamination and cross border spread of these pathogens is increased. Foodborne pathogens such as *Bacillus* spp. are the most predominant in ready-to-eat street food due to holding conditions that are favourable for these pathogens to survive and germinate (Rane, 2011). South Africa currently holds the highest recorded listeriosis outbreak cases reported worldwide, thereby emphasising the need for improved enforcement of food safety management efforts to protect public health (Smith *et al.*, 2019). In September 2024, 900 foodborne illness incidences were reported across South Africa, of which 22 were said to be among children (Ramaphosa, 2024). Moreover, six children died after they had consumed food contaminated with terbufos, a hazardous chemical used as a pesticide in the agricultural industry (Ramaphosa, 2024). These implicated food items had been sold to the children by informal traders at spaza shops. Against this backdrop, stricter regulations on food safety and the enforcement of good practices are essential to prevent the improper handling of hazardous substances and unsafe food practices, which have long been shown to harm human health.

In response to this problem, this study investigated the nutritional value of street-vended food in Bloemfontein. Furthermore, the prevalence of heavy metals and common foodborne pathogens in the street-vended foods was investigated to determine their potential health risks and cancer risk for both adults and children.

1.3 AIMS AND OBJECTIVES OF THE STUDY

1.3.1 Aims

This study aimed to evaluate the safety of street-vended foods and the nutrition knowledge of vendors within the Mangaung Metro Municipality (MMM) in the Free State province.

1.3.2 Objectives

The objectives of the study were to determine the following in the Mangaung Metro Municipality:

- Assess the nutrition knowledge and practices of street food vendors in Mangaung Metro Municipality by the use of an adapted peer reviewed questionnaire.
- Assess the potential for microbial contamination of street-vended foods Mangaung Metro Municipality following ISO methods
- Explore the potential threat of *Staphylococcus aureus* in ready-to-eat (RTE) foods by examining its virulence and antimicrobial properties of
- Identify the heavy metals (if any) present in street-vended foods using Inductively Coupled Plasma Atomic Absorption Spectroscopy (ICP-AAS); and
- Determine the cancer and/or non-cancer risk posed by heavy metals in street-vended foods for both adults and children who consume such foods using multiple established human health assessment models.

1.4 EXPECTED OUTCOMES

- This study will contribute to the body of knowledge on issues affecting street-food vending in Mangaung and South Africa at large, thereby influencing consumer buying patterns.
- The findings will raise awareness of the need to educate street food vendors on preparation methods that preserve the good nutrients in the food they sell.
- The findings established the sources of heavy metal contamination and proposed risk mitigation strategies to reduce this type of contamination in street food.
- The emerging information will be disseminated as training material for public health and food safety officials; and
- Should role-players in street food vending heed the findings and recommendations, future interventions will significantly improve the quality and hygiene of street-vended foods within the MMM area in the Free State province.

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CHAPTER 2

NUTRITION KNOWLEDGE AND ATTITUDES OF STREET FOOD VENDORS

2.1 ABSTRACT

Nutritious and safe food is essential for good health. However, not all food handlers, especially in the street food trade, know how or are willing to prepare nutrient-balanced meals. Many non-communicable diseases are associated with a poor diet, which implies that habitual consumers of street-vended foods could be at risk. Against this background, the current study aimed to analyse the nutrition knowledge and attitudes of street food vendors and the nutritional value of the food types they sell. The study adopted a cross-sectional, quantitative design using a peer-reviewed questionnaire to obtain street food handler demographics, nutrition knowledge, attitudes, and food types sold. Copies of the questionnaire were administered to 100 food vendors (June–August 2022). More females (57%) than males (43%) were recruited for the sample. The nutrition knowledge responses of the vendors indicated a satisfactory knowledge level, as none of the participants scored below 33% (i.e., $\leq 5/15$). The vendors also had a positive attitude regarding the nutritional value of food. However, the meals they sold, such as fat cakes (bread dough fried in plant oil) and processed meat, were mostly fat and energy-dense, indicating the potential to cause health issues such as obesity and cardiovascular disease. Studies on nutrition knowledge, especially among this demographic group, are significantly few, and it was determined that no other study of this kind had been conducted in the Free State prior to this investigation.

2.2 INTRODUCTION

Street food has significant nutritional implications for habitual consumers who are mostly people from the low- and middle-income population (FAO, 1997). The nutritional value of these foods is dependent on the ingredients used, methods of preparation, and portioning at the time of consumption (Sousa *et al.*, 2020). To attain optimum nutrition, a person must consume a combination of foods that provide sufficient amounts of carbohydrates, protein, fat, fibre, vitamins, minerals, and water (FAO, n.d.). Consumption of foods such as vegetables, fruits, lean meats, fish, whole grains,

legumes, nuts, seeds, and dairy products can satisfy individuals' nutritional needs and help reduce the risk of some chronic illnesses (Cesare *et al.*, 2022).

The ingredients used in street foods are area-specific and often undocumented (Namugumya and Muyanja, 2011), but it is commonly accepted that street-vended foods are heterogeneous regarding ingredients (Abrahale *et al.*, 2019). Some of the factors that contribute to the choice of ingredients include culture, affordability, and familiarity. However, it is vital that nutrition should also be taken into consideration when selecting raw ingredients or preparing a meal (Chen and Antonelli, 2020; Maina *et al.*, 2024).

In this context, street food vendors are the key role players in ensuring that safe and nutritious foods are sold to consumers. Their involvement as the preparers of street food makes it paramount that they are knowledgeable about good nutrition as well as food safety. Some concerns raised by Steyn *et al.* (2001) and Steyn *et al.* (2013) are that street foods are generally high in salt and are energy-dense. This imbalance may contribute significantly to weight gain and exposure to health risks associated with overconsumption of total fats, saturated fats, salt, and refined sugar (Steyn *et al.*, 2013). Moreover, street foods sometimes provide insufficient nutrition. For instance, van't Reit (2002) found that total energy provided by street-vended foods in low- to middle-income households and slums was below the daily recommended intakes of 2,300 kcal/day for men and 1,900 kcal/day for women (NRC, 1989). Insufficient access to nutritious food is a major public health issue in developing countries, where the most affected people are vulnerable groups such as children, the elderly, pregnant women, and people with pre-existing health conditions.

Rapid urbanization and poor economic growth have resulted in increasing poverty in many developing countries. As most people in cities have to buy their food and have little to no farming experience in the use of native and adaptive vegetation, food security in urban areas is currently determined mostly by citizens' purchasing power (Garrett, 2000). This has led to the proliferation of street foods, which, to some extent, ensure food security for both consumers and vendors.

2.2.1 Nutritional issues associated with street foods

Street-vended foods, by nature, have the potential to contribute greatly to the nutritional needs of urban dwellers all over the world (Chavez *et al.*, 2025). However, due to a number of contributing factors, such as the choice of cheaper ingredients, which are often of dubious quality, poor preparation like overcooking or under cooking, inappropriate handling of food practices, and a lack of nutrition knowledge by the vendors, street foods are often of poor chemical composition. This can be exemplified by high levels of added sugar, fats, carbohydrates, and salt in such foods (Steyn *et al.*, 2013; Sousa *et al.*, 2021). One of the studies that investigated the nutritional contribution of street foods among low- to middle-income populations was developed in Maputo, Mozambique (Sousa *et al.*, 2019). The investigators found that the vendors sold a number of fried snacks as well as fried cakes, which were the most energy-dense and highest in fats as well as carbohydrates. Dishes like stewed liver had the highest sodium/potassium ratio. As a result, it was recommended by the authors that policies should be formulated for the promotion of nutrient-dense foods and reduced salt intake.

In another study by Koodagi *et al.* (2014) that assessed the same issue in another setting, the mean energy available from street foods was 250 kcal, ranging between 239 to 311 kcal. The mean protein content in the analysed street foods was 7.10 g, ranging between 4.60 and 11.44 g. Evidently, the highest protein content was obtained from non-vegetarian foods, while fried foods offered lower protein content. According to a report by Pendick (2022), the recommended dietary allowance (RDA) for protein is 0.8 g per kilogram of body weight. This means that a person weighing 80 kgs should consume 64 g of protein per day. Having analysed the RDA for protein, Koodaga *et al.* (2013) conclude that, based on the findings, the analysed street foods did not always meet the dietary requirements of habitual consumers.

2.2.2 Street foods and non-communicable chronic illnesses

Ervin (2017) refers to broad changes in the patterns of human diet that have occurred across time and space. The rapid epidemiological transition (a gradual increase in non-communicable chronic diseases) has been attributed to both nutrition transition and urbanization (Kroll *et al.*, 2014; Nonato *et al.*, 2016). In some parts of the world where

extreme poverty is prevalent, as well as in most developing countries, public health challenges in the recent past have centred around protein-energy malnutrition, other micronutrient deficiencies, and infectious diseases (WHO, 2002). However, more recently, the scope of public health issues has been widening to include the prevalence of diseases caused by overconsumption of fats, salt, and added sugar (Cano *et al.*, 2014). Regrettably, street-vended foods have frequently been identified as having excessive amounts of these components (Cano *et al.*, 2014).

In recent studies, alarmingly high levels of salt content have been found in street-vended foods across the globe (WHO, 2017; Marais *et al.*, 2018; Sousa *et al.*, 2021). The WHO (2017) reported that, in their 2016 survey in Ashgabat, the salt concentration found in local street foods was on average 6.6 g per 100 g of food. This value far exceeds the WHO recommendations for a salt intake of less than 5 g per day. Another area of concern is the frequent consumption of foods with high concentrations of trans fats, such as fried potatoes, fried foods, cakes, and biscuits. Mossoba *et al.* (2007, page 87) define trans fats as “the sum of all unsaturated fatty acids that contain one or more isolated, nonconjugated, double bonds in a trans geometric configuration”. The consumption of trans fats has been linked directly to health risks such as cardiovascular disease, some types of cancer, obesity, overweight, and shortening of the pregnancy period, among others (Dhaka *et al.*, 2011; WHO, 2017; Li *et al.*, 2019). Gupta *et al.* (2016) conducted a study in low socioeconomic settings in India and found that the street foods and snacks sold there contained high levels of saturated fats (25% to 69% of total fatty acids) as well as trans fats (0.1% to 30% of total fatty acids). Such undesirable rates warrant tougher regulations for the manufacturers of oil and fats used in food preparation in order to improve food quality. Furthermore, the education of vendors regarding nutrition could influence their practices and encourage them to select wholesome ingredients.

2.3 METHODOLOGY

2.3.1 Study area and design

A descriptive survey design was used in this study and the nutrition knowledge, attitudes, and demographic characteristics were obtained using a questionnaire survey. The study

area comprised 10 main areas of food vendor congestion in the Mangaung Metro Municipality.



Figure 2.1 Map of the Free State province indicating the study area (*Red indicates the location of Mangaung Metro Municipality where samples were collected*)

2.3.2 Sample collection

The data were collected in the period June to August of 2022 from street vendors at taxi ranks, Bloemfontein CBD and near industrial areas of the Mangaung Metro Municipality. In total, 100 street vendors were included in the study.

2.3.3 Nutrition knowledge, attitudes, and demographic characteristics of food vendors

A questionnaire was used to test the nutritional knowledge and attitudes of food vendors. To assess each of the components, the questionnaire was divided into two sections (one assessing knowledge and the other assessing attitudes), with an additional four sections

that elicited the demographic characteristics of the vendors, food items sold, available facilities, and their day-to-day operational activities. All the questionnaires were administered by the researcher and a trained assistant who was proficient in Sesotho as it is the most predominantly spoken language in the Free State province.

The demographic characteristics obtained from the vendors included their age, marital status, gender, country of origin, ethnicity, and highest level of education. The nutrition knowledge questionnaire consisted of 15 questions that elicited the street vendors' knowledge about salt and sugar content, legumes and nuts, milk and meat, starchy foods, fats and oils, as well as fruits and vegetables. For each correct response provided, 1 point was allocated, and no points were allocated for incorrect responses. For a person to get a 100% score, all 15 questions had to be accurately answered. Food vendors with a score between 0 and 5 were considered to have poor nutritional knowledge, those with a score ranging from 6 to 10 were considered to have average nutritional knowledge, and those with scores of 11 to 15 were deemed to have good nutritional knowledge. A Likert-scale questionnaire consisting of 20 statements was used to assess the respondents' attitudes towards nutrition. In order to minimize bias, simple random sampling was used for the selection of the questionnaire respondents, as there was no officially documented number of street vendors in the study area.

2.4 RESULTS AND DISCUSSION

The study was designed to assess the nutrition knowledge and food attitudes of street food vendors in Mangaung Metro Municipality. Questionnaires were administered to a sample of 100 food vendors (June to August 2022). More females (57%) than males (43%) participated as respondents, and this gender ratio is common in street food vendor studies (Hill *et al.*, 2019; Yahaya *et al.*, 2018; Letuka *et al.*, 2021; Oladipo and Tabit, 2021). The predominance of female food vendors in African countries is not surprising, considering that, in African households, women from a young age are involved in the preparation and serving of food. However, as a result of modernization and high unemployment rates, more men have become involved in culinary practices. Biographically, most vendors were South African (54%), Black African (81%), married (28%), and had a high school education (37%). Vendors from other countries constituted

46% of the respondents and most vendors fell within the age range of 25-44 years (59%). According to the by-laws governing street trade in Mangaung, foreign nationals are permitted to work in this trade with the provision that they hold an original copy of their asylum documents or a temporary residence permit issued by the Department of Home Affairs. The by-laws further state that persons below the age of 18 are not permitted to engage in street trading (Mangaung Local Municipality, 2006). Interestingly, one female participant who was employed by one of the stall owners indicated that she was below 18 years of age. This exposes the challenges of enforcing laws relating to street vending as well as the dire economic challenges faced by the South African youth.

Table 2.1 Operational characteristics of food vendors in MMM (*n*=100)

Operational characteristics	Number (N)	Percentage (%)
Days of work/week		
Monday- Friday	100	100
Monday- Saturday	85	85
Monday- Sunday	15	15
Hours worked/day		
≤8	12	12
>8	88	88
Stall ownership		
Owner	83	83
Joint owner	3	3
Non-owner	14	14
Average weekly income (R)		
≤500	14	14
501-1000	35	35
1001-2000	36	36
2001-3000	11	11
3001-4000	4	4
4001-5000	0	0
>5000	0	0
Facilities available		
Water	11	11

Electricity	3	3
Gas	40	40
Stove	41	41
Fridge	2	2
Waste disposal	75	75
Hand washing	40	40

As depicted in Table 2.1 above, most vendors worked over 8-hour shifts (88%) and most worked from Monday to Saturday (85%). According to the South African Labour Guide (2022), employees earning below the earnings threshold (R18 673.37 per month) should work a maximum of 9 hours/day (excluding a lunch hour) for a 5-day work week and 8 hours per day for those working for more than 5 days/week. Most of the vendors in this study (71%) said they made between R501.00 and R3 000.00 per week, implying that their earnings were significantly below the earnings threshold. However, they worked long hours. Only 40% of the participants indicated that they had access to hand washing facilities and 11% had access to clean water onsite. The rest of the vendors had to fetch water using buckets and containers. Young et al. (2021) argue that water security (a term used to refer to a range of water challenges) is essential in ensuring food security, good nutrition, and general well-being. The relationship between clean water, hygiene, and sanitation has been extensively explored. Many international organizations have argued that access to clean water and good hygiene facilities significantly protects people against diarrheal and other diseases (UN, 2016; UNICEF, 2022; WHO, 2022).

Table 2.2 below summarises the food items sold by the vendors. Of the cooked street food, pap (55.2%) was the predominantly sold food item. Pap (stiff porridge) is a South African staple food that is energy dense and a good source of carbohydrates (79.47-85.2g/100g) (Ponka *et al.*, 2015). Other commonly cooked foods that are sold are sausages/wors (40.3%), grilled chicken/chicken stew (37.3%), chicken offal (28.4%), fat cakes (29.9%), French fries (26.7%), and grilled pork/pork stew (22.4%). Read meat in South Africa has become increasingly expensive since 2021, thereby making it a somewhat undesirable product for street food vendors who need to keep costs as low as possible. Although the foods sold by vendors are affordable and filling, they are often

considered unhealthy because they normally have high levels of trans fats, saturated fats, salt, and added sugar (Gupta *et al.*, 2016). Street-vended foods are considered by scholars as the precursors of non-communicable chronic illnesses as well as a cause of overweightness and obesity (Nonato *et al.*, 2016; Hill *et al.*, 2018). Taking into consideration that the habitual consumers of street-vended foods are people of low socioeconomic status, the risks associated with their eating habits could prove injurious to their health (Charity *et al.*, 2015).

Table 2.2 Food items sold by vendors in MMM

Food item	N	% of Total Food (n=74)	% of food cooked by vendors (n=28)
Beans	3	2.4	4.5
Grilled beef and beef stew	6	4.8	9.0
Beef offal	8	6.3	11.9
Boiled eggs	3	2.4	4.5
Bread	11	8.7	16.4
Cabbage stir-fried	5	4.0	7.5
Grilled chicken/chicken stew	25	19.8	37.3
Chicken offal	19	15.1	28.4
Fat cakes	20	15.9	29.9
Fish	6	4.8	9.0
French fries	18	14.3	26.8
Ginger drink	7	5.6	10.4
Gravy	2	1.6	3.0
Kale	12	9.5	17.9
Kota	8	6.3	11.9
Mince meat	5	4.0	7.5
Muffins	5	4.0	7.5
Mutton	1	0.8	1.5
Nyekoe	1	0.8	1.5
Pap/porridge	37	29.3	55.2

Grilled pork/pork stew	15	11.9	22.4
Rice	3	2.4	4.5
Salads	3	2.4	4.5
Sandwiches	2	1.6	3.0
Sausages/ wors	27	21.4	40.3
Sheep trotters and offal	12	9.5	17.9
Tea	6	4.8	9.0
Scones	12	9.5	17.9
Fruits	83	65.9	
Vegetables	65	51.6	
Atchar	1	0.8	
Biscuits	9	7.1	
Store-bought bread	2	1.6	
Candy	16	12.7	
Chillies	4	3.2	
Cooking oil	1	0.8	
Corn chips	10	7.9	
Energy drinks	3	2.4	
Fizzy drinks	18	14.3	
Ginger drink	2	1.6	
Bubble gum	9	7.1	
Milk	1	0.8	
Salt	1	0.8	
Soup sachets	1	0.	
Spices	1	0.8	
Sugar	1	0.8	
Tea bags	1	0.8	
Water	2	1.6	

2.4.1 Nutrition knowledge of vendors

Table 2.3 and Table 2.4 depict the nutrition knowledge of the vendors. The average results indicate a satisfactory knowledge level. More than 75% of the vendors correctly responded to questions relating to fats and over 90% responded correctly to questions

relating to fibre. Because fibre is a dietary component that plays, among other health benefits, a significant role in immune regulation and maintaining bowel health and weight management, it is highly recommended that people consume high volumes of vegetables, fruits, and whole grain cereals (Ventor *et al.*, 2022). About 92% of the vendors had knowledge of the negative health effects of a high salt intake, but fewer respondents (53%) could tell which food seasoning had the least salt content. This finding suggests that food vendors are likely to involuntarily prepare food with a high salt content. In an effort to reduce salt intake by South African consumers, the South African Health Ministry enacted legislation in 2013 that mandates salt reduction in the food production industry (World Action Africa/South Africa, 2013). Through public health campaigns and food regulations, it was envisaged that the mean intake of salt by the population should be reduced from 8-10 g per day in 2011 to 5 g per day by 2020. This new recommendation coincided with the daily recommended intake of salt of 5 g per day per person by the World Health Organization (Heart and Stroke Foundation, South Africa, 2018). Street food vendors, therefore, have to comply with regulations to minimize salt intake by consumers.

Table 2.3 Nutrition knowledge scores of street food vendors (in tertiles)

	Nutrition knowledge			
	Score out of 15			
	<u>Total</u>	<u>0-5 low</u>	<u>6-10 average</u>	<u>11-15 good</u>
Demographics	<i>N</i>	<i>N (%)</i>	<i>N (%)</i>	<i>N (%)</i>
Nationality				
South African	54	0	34 (54.84)	20 (52.63)
Non-South African	46	0	28 (45.16)	18 (47.37)
Race				
Black African	81	0	49 (79.03)	32 (84.21)
Coloured	4	0	3 (4.84)	1 (2.63)
Other	15	0	10 (16.13)	5 (13.16)
Gender				
Male	43	0	29 (46.77)	14 (36.84)

Female	57	0	33 (53.23)	24 (63.16)
Age group				
<18	1	0	1 (1.61)	0 (0)
18-24	16	0	11 (17.74)	5 (13.16)
25-34	36	0	23 (37.10)	13 (34.21)
35-44	23	0	12 (19.35)	11 (28.95)
45-54	18	0	10 (16.13)	8 (21.05)
55-64	6	0	5 (8.06)	1 (2.63)
Education				
Primary school	21	0	17 (27.42)	4 (10.53)
High school	37	0	22 (35.48)	15 (39.47)
Matric	24	0	13 (20.97)	11 (28.95)
Diploma	10	0	5 (8.06)	5 (13.16)
Degree	8	0	5 (8.06)	3 (7.89)

Table 2.4 Nutrition knowledge questions answered correctly by vendors (n=100)

Question	Correct answer %
1. Which vegetable will help with good eyesight? Butternut, cabbage, lettuce, cucumber	10
2. Which fruit will help the body fight colds? Apple, mango, nartjie, peach	89
3. Which vegetable has the most fibre (roughage)? Cabbage, cauliflower, green beans, lettuce	47
4. Which type of potatoes has the least fat? Mashed potatoes, fried potatoes, boiled potatoes, roasted potatoes	77
5. Which food normally has the most fat? Atjar, mayonnaise, mustard, chakalaka	87
6. Why are starchy foods important to eat?	70

	They are easy to digest, they build muscle, they are a source of energy, they fight disease	
7.	When will starchy foods make one gain weight?	55
	When eaten with meat, when eaten in large amounts, when eaten in the mornings, when eaten with vegetables	
8.	How often should oily fish like pilchards and tuna at least be eaten?	32
	Every day, once a week, twice a week, twice a month	
9.	Which food is better for a healthy heart?	28
	Fried chicken, grilled fish, roast beef, boiled sheep brains	
10.	Which food has more fibre (roughage)?	96
	Eggs, nuts, fish, chicken	
11.	Why can legumes like dried beans and lentils be eaten instead of meat?	84
	They have protein, they have vitamins, they have fat, they have fibre	
12.	Which food does not have added sugar?	95
	Canned apricots, apricot jam, apricot juice, fresh apricots	
13.	Which health problem can be caused by drinking sugary cool drinks every day?	60
	Heart disease, TB, liver disease, and weight gain	
14.	Which health problem can one get from consuming too much salt?	92
	High blood pressure, liver failure, lung disease, high blood sugar	
15.	Which has the least salt?	53
	Braai salt, stock cubes, soup powder, dry herbs	

2.4.2 Vendors' attitudes towards nutrition

The assessment of vendors' attitudes towards nutrition indicated that the majority agreed with a reduction in salt intake. WHO (2014) warns that high salt intake could lead to cardiovascular diseases (among other health problems), therefore, it is crucial that food handlers are cautious about the amount of salt they add to the food they sell. In this study, 80% of the vendors strongly agreed that food can still taste good with just a little salt added, while 83% indicated that they took caution when adding salt to food. Moreover, the vendors were positive about the consumption of fruit and vegetables. According to Amao (2018), the daily consumption of fruit and vegetables is important because they are a good source of micronutrients and dietary fibre. The majority of the

participants (92%) indicated that they strongly agreed that fruit and vegetables should be consumed daily. It was also noted that 82% of the vendors agreed that roughage is important in starchy foods.

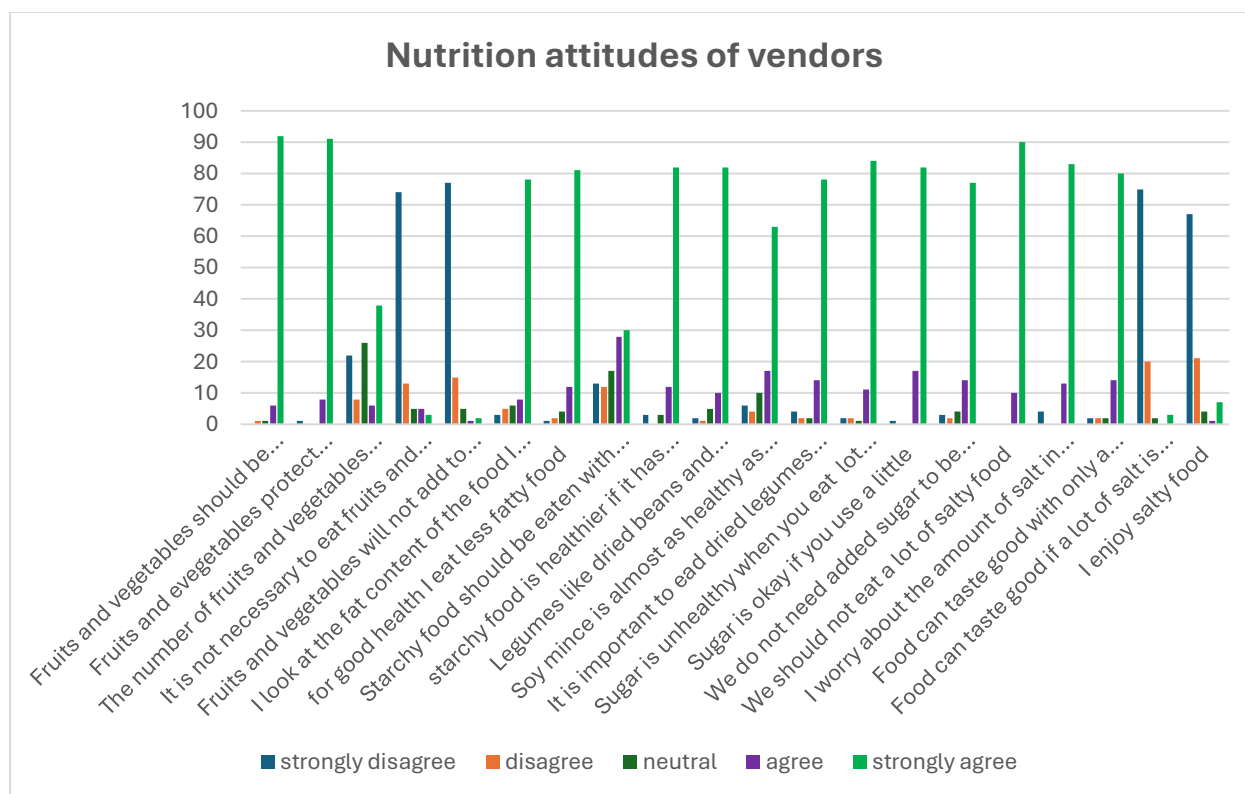


Figure 2.1 Street food vendors' attitude towards food nutrition

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CHAPTER 3

MICROBIAL ANALYSIS OF STREET-VENDED FOODS

3.1 ABSTRACT

Foodborne pathogens pose considerable risks in terms of public health, and this is a particular threat in areas with inadequate or non-existent food safety measures and regulations. This chapter provides data on the microbiological quality and safety of street-vended foods. The chapter also discusses key contaminants, namely *Staphylococcus* spp., *E. coli*, and *Pseudomonas* spp., with particular emphasis on the health risks they pose. In total, 168 food samples were collected from 42 vendors who complied with the criteria of stationary vendors selling food prepared onsite in Mangaung Metro Municipality. The collected samples were analysed to determine the microbial load of the ready-to-eat street-vended foods sold in this region. The samples were subjected to microbial analyses, including Total Viable Count (TVC) and identification of various presumptive bacterial species. The results yielded varying levels of contamination, with *E. coli* isolated from all samples, which is suggestive of faecal-oral contamination and neglect of proper hygiene practices. *Pseudomonas* spp. was present in 78.5% of the samples, further suggesting cross-contamination risks. The TVC indicated that 57% of the sampled RTE foods was satisfactory, while 29% was marginal and 14% was unsatisfactory. These findings amplify the need for targeted interventions that must include food handler/vendor training, strict enforcement of regulations, and monitoring to ensure the safety of street-vended foods and the protection of public health.

3.2 INTRODUCTION

The Codex Alimentarius Commission (2020) defines food as any substance, whether processed, semi-processed or raw, that is intended for human consumption and that contains any substance used for preparation, treatment, or preservation of such food. However, food can be a vehicle for pathogenic microorganisms, resulting in undesirable health outcomes (Alum *et al.*, 2016). According to WHO (2024) and Owusu-Apenten and Vieira (2022), the global survey conducted circa 2010 showed that contaminated food

contributed to an estimated 600 million cases of illnesses caused by foodborne pathogens each year, while it also caused in the region of 420 000 deaths worldwide. Of these fatalities, 126 000 were children under the age of five. In response to these unfortunate events, coordinated global efforts and interventions to circumvent the burden of foodborne illnesses have been established (WHO, 2024; Pontello and Gori, 2023; Pires and Devleesschauwer, 2021). These initiatives include promoting investment in food safety systems and fostering coordinated action across different sectors to strengthen national food safety systems. The goal is to build resilient communities that are aware of the risks associated with foodborne diseases and are motivated to make safe food choices (WHO, 2024).

3.3 THE BURDEN OF FOODBORNE PATHOGENS IN THE SUB-SAHARAN REGION

3.3.1 The scope of health risks associated with foodborne diseases

The COVID-19 pandemic emphasised the crucial need for strong food systems by exposing vulnerabilities in how food is produced, distributed, and accessed. The need to build resilient and well-regulated food supply chains has been highlighted as it is essential to prevent foodborne illnesses during global and regional crises (Dasgupta and Robinson, 2021; Rivera-Ferre *et al.*, 2021). According to Cudjoe (2022), WHO's 2015 report reveals that around 98% of the burden of food safety issues arises in underdeveloped countries, with sub-Saharan Africa being the most affected. Surveys conducted in the latter region reported high rates of deaths and hospitalisation linked to foodborne pathogens. Additionally, about one billion people in sub-Saharan Africa (SSA) and South Asia are at higher risk of food-related health threats emanating from micronutrient deficiency than people in other parts of the world (Wudil *et al.*, 2022). Globally, the African continent experiences the highest per capita burden of foodborne diseases, with microbial and chemical contamination of food causing an estimated 137 000 deaths and 91 million cases of acute illness each year (Cudjoe, 2022). This public health issue is compounded by the economic challenges experienced in these countries. Moreover, the immeasurable cost of loss of life makes food safety a pertinent and an urgent public health issue in this region.

3.3.2 Sources of microbial contamination of street-vended foods

Rakha *et al.* (2022, p. 212) define street-vended foods as “food cooked and served by vendors in streets or local public places that are consumed directly without further cooking”. Similarly, Letuka (2021, p. 303) describes street-vended foods as “ready-to-eat food and beverages prepared and sold on the streets or other similar public places”. These foods are a common source of nutrition for many people, especially in low- and middle-income countries, and among those who are working and need ready-to-eat foods (Akhter and Cameron, 2023).

Concerns over hygiene, freshness, and the nutritious value of street foods can be discouraging for consumers. Moreover, food vendors’ food knowledge, attitudes, and practices can either increase or reduce the risk of the contamination of food and food preparation surfaces by microorganisms (Ncube *et al.*, 2020; Putri and Susanna, 2021). For example, stalls are often crude structures where running water, toilets, and hand washing facilities are rarely available (Rosales *et al.*, 2023; Nkosi and Tabit, 2021). Therefore, a lack of these facilities may lead to the ineffective washing of hands, utensils, and dishes as vendors commonly attempt to maximise profits, thereby limiting the use of such necessary resources (Alealign *et al.*, 2023; Meki *et al.*, 2021; Rosales *et al.*, 2023).

Inappropriate food handling practices, such as storage of food at improper temperatures, encourages the proliferation of microorganisms, while dirty or uncovered food containers have often been observed in the street-food vending market (Eke-Ejiofor *et al.*, 2021; Mwove *et al.*, 2020). Serving food without sufficiently preheating it to destroy some microorganisms also poses a threat. Such food carries the risk of harbouring pathogens such as *E. coli*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Salmonella* spp. that have been found prevalent in street foods sold to customers (Birgen *et al.*, 2020). Some microorganisms that have been found prevalent in street foods, such as those of the *Enterobacteriaceae* family, are a result of cross contamination (Ndlovu, 2020; Shodikin *et al.*, 2023). The transfer of microorganisms from one food to another can either occur through direct human contact (food vendors harbour microbes in their hair, nails, or on their hands), contact surfaces, or through the air (Dawson, 2020; Sharma *et al.*, 2021).

3.3.3 Common foodborne pathogens found in street-vended foods

3.3.3.1 Salmonella

The *Salmonella* species are part of the *Enterobacteriaceae* family. They are Gram-negative, rod-shaped bacteria that cause illnesses ranging from gastrointestinal infection, diarrhoea, abdominal pain, nausea, vomiting, to fever (CDC, 2019; Kgoale *et al.*, 2024). The bacteria are frequently spread by those who handle food and do not follow proper hygiene practices such as adequate handwashing using sanitizing agents, working in decontaminated food processing environments, and properly cleaning utensils. Those who eat raw and undercooked foods are also at risk (Ehuwa *et al.*, 2021). It is estimated by CDC (2024) that this microorganism causes 1.35 million infections, 26 500 hospitalisations, and 420 deaths in the United States annually. Furthermore, salmonellosis is the most commonly reported disease in European Union countries where it causes around 91 000 cases of salmonellosis per annum, while the economic burden of human salmonellosis is estimated at 3 billion Euros per annum.

3.3.3.2 Staphylococcus aureus

Staphylococcus aureus is one of the most prolific foodborne pathogens that is notorious for poisoning food through the production of enterotoxins (CDC, 2024). It is highly prevalent in improperly handled and stored foods, especially those with high protein content such as dairy products, meat, and creamy dishes (Bencardino *et al.*, 2021). When food is contaminated with *S. aureus*, the bacteria can multiply and produce toxins if left at room temperature for a long time. Also, if consumed they can cause symptoms such as nausea, vomiting, abdominal pain, and diarrhoea (CDC, 2024). Globally, *S. aureus* stands out as a prominent foodborne pathogen with a notable impact on sub-Saharan Africa, where countries like the Republic of South Africa, Ghana, Nigeria, Kenya, and Zambia have reported a significant number of cases (Egyir *et al.*, 2022; Freitas *et al.*, 2024; Govender *et al.*, 2019). Challenges in maintaining hygienic conditions during food preparation contribute to high incidence rates, underscoring the critical need for effective sanitation measures (Hachem *et al.*, 2019).

3.3.3.3 *Escherichia coli*

Escherichia coli (*E. coli*) is a significant foodborne pathogen and an indicator of faecal contamination that may result in severe gastrointestinal illness. Several *E. coli* strains are harmless, but strains such as Shiga toxin-producing *E. coli* (STEC) serotypes can produce harmful toxins that can lead to food poisoning (WHO, 2018; Asime *et al.*, 2020). Ruminant animals have been identified as natural reservoirs of STEC strains, but monogastric animals such as pigs and chickens can also act as spill-over hosts (Sajeena and Kalyanikutty, 2024). Human exposure to various strains of *E. coli* may occur through faecal-oral transmission due to undercooked meat products such as ground beef, unpasteurized dairy products, contaminated fruits and vegetables, and poor hygiene practices of food handlers (Bolívar *et al.*, 2023; Wang *et al.*, 2024). Common symptoms associated with *E. coli* infection are abdominal cramps, diarrhoea, nausea, and vomiting (WHO, 2018).

3.4 FOOD SAFETY CONCEPTUAL FRAMEWORK

Figure 3.1 depicts the factors that can compromise the safety and quality of street-vended foods. It also indicates the interconnectedness of the variables at play. The framework implies that addressing the environmental, personal, and physiological factors that cause food contamination can result in improved safety and quality of street-vended foods. Environmental factors such as the materials used for food preparation and storage could affect safety and quality if they are of poor quality. Also, a lack of knowledge about and poor attitudes towards food safety and nutrition could lead to poor food handling practices and/or poor meal planning, resulting in food that is unhealthy and unsafe for consumers.

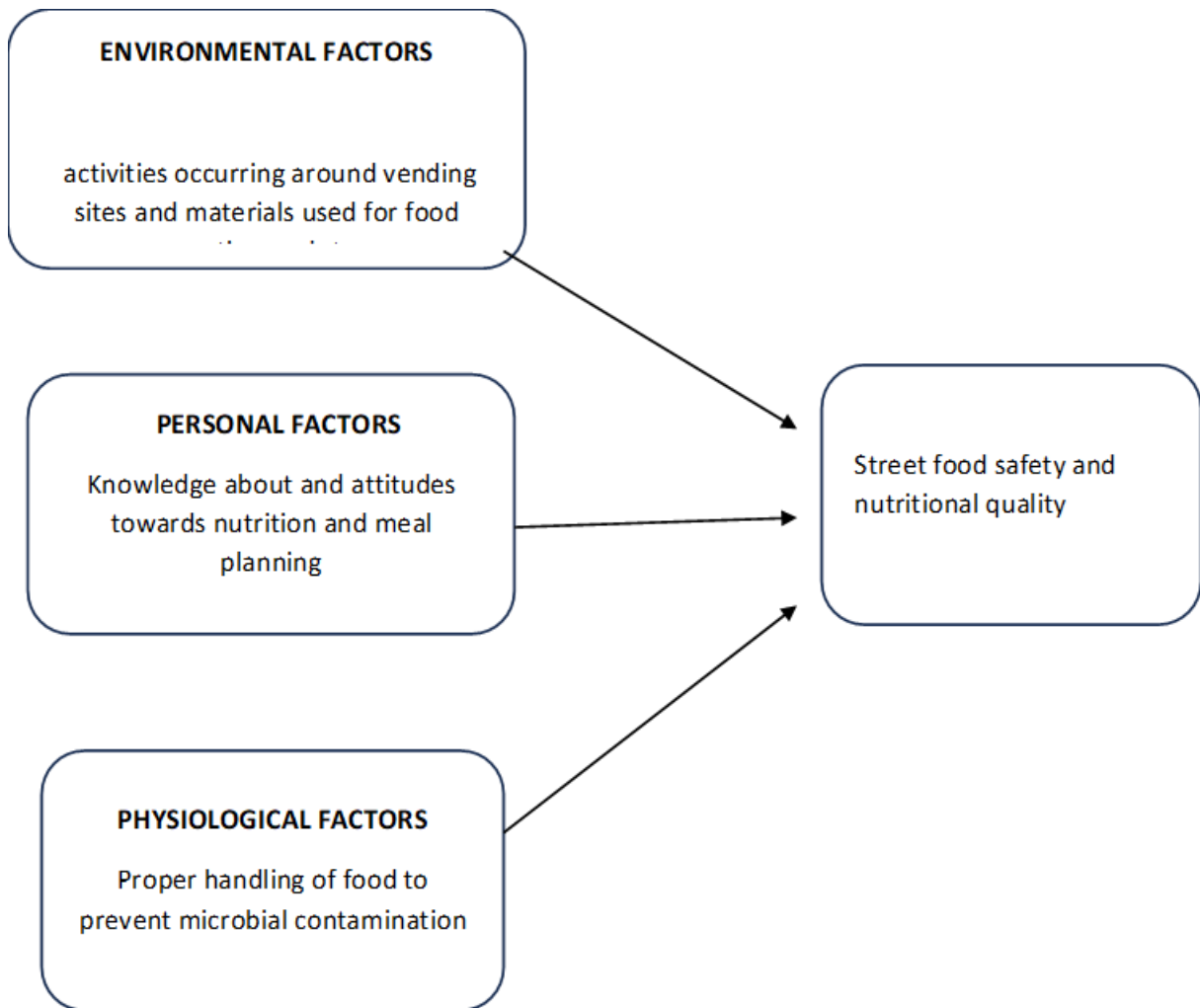


Figure 3.1 Conceptual framework of street food safety and nutrition

3.5 METHODOLOGY

3.5.1 Study site and sample collection

The current study was conducted in seven main areas in the Mangaung Metro Municipality. These areas were: Bloemfontein, Botshabelo, Thaba Nchu, Wepener, Dewetsdorp, Soutpan, and Van Stadensrus (Figure 3.2). Mangaung Metro Municipality is a Category A municipality nestled in the central interior of South Africa in the Free State province (29.1303°S, 26.2358°E). In total, 168 samples were collected from four different food types typically found in this study area: pap (42), chicken (42), pork (42), and salad (42). The samples were collected between March and April 2023 (Table 3.1). The samples were aseptically collected using sterile bags and immediately transported to the Centre for Applied Food Sustainability and Biotechnology, Bloemfontein, for analysis.

Transportation of samples was done using cooler boxes with ice to minimise microbial proliferation.

Table 3.1 Number of food samples and areas where they were collected

Sampling area	Number of samples			
	Pap	Chicken	Pork	Moroho/Salad
Thaba Nchu	6	6	6	6
Bloemfontein	6	6	6	6
Botshabelo	6	6	6	6
Wepener	6	6	6	6
Soutpan	6	6	6	6
Dewertsdorp	6	6	6	6
Van Stadensrus	6	6	6	6
TOTAL	42	42	42	42

3.5.2 Enumeration of presumptive isolates

Laboratory-based experiments were conducted to analyse the collected food samples for the following microbial indicators and presumptive isolates: Total Viable Count, *Escherichia coli*, *S. aureus*, and *Pseudomonas* spp. The selection of these microorganisms was guided by the established relevance as key indicators of food hygiene, contaminations sources and public health risk. For the enumeration of these indicators, 10 g of each food sample was homogenised using a Stomacher® 400 at 230 rpm for 1 minute in 90 ml buffered peptone water. Subsequently, 10-fold serial dilutions were done following the International Organization for Standardization (ISO) standards (ISO, 2017). The enumerations of *E. coli*, *S. aureus*, and *Pseudomonas* spp. were conducted following the procedures proposed by ISO 2001 and 1999. This entailed spreading 0.1 ml of each decimal dilution on Plate Count Agar, Biard Parker Agar (with egg yolk emulsion and tellurite solution), Violet Red Bile Agar, and *Pseudomonas* Agar medium agar plates. Incubation was done at 37±1°C for 24-48 hours. Subsequently,

presumptive colonies were identified following characteristic morphology and biochemical tests.

3.6 RESULTS AND DISCUSSION

The 168 food samples (pap, chicken, pork, and moroho/salad) presented with presumptive bacterial isolates as reflected in Table 3.3. *Staphylococcus* spp. and *E. coli* were detected in 100% of the samples, while only 78.5% tested positive for *Pseudomonas* spp. Cultural, morphological, and biochemical characteristics were used to identify the bacterial isolates.

Total Viable Count was performed and used as an indicator of the microbiological quality of the various food samples. Averages of cfu/ml were calculated for each food type obtained from the seven towns where samples were taken. The bacteria count ranged from 1.76×10^3 to 8.1×10^6 in pap, 4.7×10^3 to 1.42×10^6 in chicken, 5.6×10^6 to 9.3×10^5 in pork, and 2.50×10^3 to 9.3×10^5 in moroho/salad. According to the Guideline for the Microbiological Examination of Ready-to-Eat foods (2001), the standard plate count of RTE foods that will require no further processing should be considered as satisfactory if cfu/ml $< 10^4$, marginal if cfu/ml $< 10^5$, and unsatisfactory if cfu/ml $\geq 10^5$. Therefore, the results obtained indicated that 57% of the sampled RTE foods was satisfactory, while 29% was marginal and 14% was unsatisfactory. TVC is indicative of unhygienic practices during handling, processing and storage. However, to establish the significance of these results, further analyses were conducted to identify predominating microorganisms in the food samples.

3.6.1 Presumptive *Escherichia coli*

E. coli was detected in 100% of the examined food samples. This bacterium is part of normal flora in humans and is helpful in digestion. However, *E. coli* can be pathogenic if found outside the intestines and is often used as an indicator of faecal-oral contamination (Mulyati and Raharjo, 2024). It was observed that the contamination levels of *E. coli* exceeded the standards set by the Guidelines for the Microbiological Examination of Ready-to-Eat foods (2001), which state that for RTE foods, a level of < 3 cfu/g is ideal while levels exceeding 100 cfu/g are unacceptable. The contamination

levels detected ranged from 2.37×10^3 to 6.2×10^5 cfu/ml, 2.63×10^2 to 3.8×10^6 cfu/ml, 1.34×10^2 to 3.5×10^6 cfu/ml, and 1.26×10^3 to 2.0×10^7 cfu/ml for pap, chicken, pork, and moroho/salad respectively. Similarly, Moloi et al. (2021) found *E. coli* to be one of the predominantly isolated organisms in the Mangaung Metro with levels exceeding set standards. The exceedingly high microbial counts are cause for concern because they indicated poor food handling and preparation practices by the food vendors.

3.6.2 Presumptive *Pseudomonas* spp.

The street-vended food samples yielded a lower *Pseudomonas* spp. prevalence compared to the other bacteria species (78.5%). Consistent with the results of this study are those by Okhuebor and Izevbuwa (2022), who found a significantly high number of RTE foods that tested positive for *Pseudomonas aureginosa*. The lowest occurrence of *Pseudomonas* spp. was in Thaba Nchu, where it was only isolated from the salad (2.33×10^1 cfu/ml). However, it was highest in Dewetsdorp in pork samples (9.4×10^3 cfu/ml). *Pseudomonas* spp. is characterised as a Gram-negative rod-shaped bacterium that is motile and aerobic. Its classification falls under the family Pseudomonadaceae, and its genus has 191 officially recognised species (Okhuebor and Izevbuwa, 2022). According to Fakhar et al. (2022), *Pseudomonas* spp. is commonly found in water, soil, humans, and surfaces if animals are prevalent. Therefore, the intricacies involved in the handling and processing of street-vended foods can lead to cross-contamination that can cause problematic issues. As it is a foodborne pathogen, the detection of *Pseudomonas* spp. is especially concerning because it is a biofilm-forming organism that can grow on abiotic surfaces such as preparation surfaces and utensils used in the food industry (Kovalchuk et al., 2021; Zhang et al., 2025).

Isolates of interest were biochemically identified following their morphological characterisation. The biochemical test results that were obtained are presented in Table 3.2. Characteristic colonies were successfully tested for their confirmation. *S. aureus*, *E. coli*, and *Pseudomonas* spp. were identified by means of an indole test, methyl red test, citrate test, oxidase test, and catalase test. For presumed *Pseudomonas* isolates, characteristic blue-green fluorescent colonies were tested, while presumptive *S. aureus* was characterised by black and shiny colonies with a white border. Presumed *E. coli*

colonies that were subjected to testing were characterised by red colonies encircled by reddish precipitation zones (Microbe Notes, 2022).

Table 3.2 Biochemical results of bacteria obtained from different food samples

Bacteria isolate	Indole test	Methyl red test	Citrate test	Oxidase test	Catalase test
<i>Staphylococcus aureus</i>	-ve	+ve	+ve	-ve	+ve
<i>Escherichia coli</i>	+ve	+ve	-ve	-ve	+ve
<i>Pseudomonas spp.</i>	-ve	-ve	+ve	+ve	+ve

Table 3.3 Presumptive microbial counts of bacteria obtained from various food samples

Area		Bloemfontein				Botshabelo				Thaba Nchu			
Presumptive CFU/ml	Food Item	Pap	Chicken	Pork	Salad	Pap	Chicken	Pork	Salad	Pap	Chicken	Pork	Salad
	TVC	8.1x10 ⁶	1.42x10 ⁶	2.41x10 ⁴	1.79x10 ⁵	1.76x10 ³	5.3x10 ³	4.0x10 ⁴	1.82x10 ⁴	2.59x10 ⁴	8.1x10 ⁴	9.3x10 ⁵	7.0x10 ⁴
	BPA	7.2x10 ⁵	1.25x10 ⁵	1.73x10 ⁴	1.23x10 ⁴	1.83x10 ³	1.27x10 ³	3.7x10 ⁴	2.11x10 ⁵	7.5x10 ⁴	1.32x10 ⁵	7.8x10 ⁵	4.9x10 ⁴
	PA	7.6x10 ³	2.51 x10 ³	5.4 x10 ³	6.3 x10 ³	3.5 x10 ³	4.8 x10 ³	9.5 x10 ³	8.2 x10 ³	ND	ND	ND	2.33 x10 ¹
	VRB	6.2x10 ⁵	3.0x10 ⁵	6.5x10 ⁶	3.9x10 ⁵	1.92x10 ⁵	2.63x10 ²	1.34x10 ²	3.1x10 ⁴	2.74x10 ⁴	3.8 x10 ⁶	7.6 x10 ⁴	8.0 x10 ⁷
Area		Wepener				Dewetsdorp				Soutpan			
Presumptive CFU/ml	Food Item	Pap	Chicken	Pork	Salad	Pap	Chicken	Pork	Salad	Pap	Chicken	Pork	Salad
	TVC	6.0x10 ³	5.65x10 ³	5.3x10 ³	4.9x10 ³	3.2x10 ³	4.7x10 ³	1.13x10 ⁴	2.50x10 ³	9.8x10 ³	6.3x10 ³	7.4x10 ³	5.6x10 ³
	BPA	2.09x10 ³	3.9x10 ³	3.4x10 ³	4.6x10 ³	1.56 x 10 ⁴	1.72 x 10 ⁴	2.40 x10 ⁴	1.44x10 ⁴	2.34x10 ³	1.92x10 ³	1.40x10 ²	4.6x10 ⁴
	PA	2.31 x10 ¹	5.7 x10 ¹	8.9x10 ¹	1.24 x10 ³	7.1 x10 ¹	3.8 x10 ¹	9.4 x10 ³	6.2 x10 ³	ND	ND	4.5 x10 ¹	1.30 x10 ²
	VRB	1.20 x10 ⁵	3.4 x10 ⁴	5.6 x10 ⁵	2.19 x10 ⁴	4.8 x10 ⁵	7.3 x10 ⁴	6.5 x10 ⁵	9.1 x10 ⁴	1.84 x10 ⁵	2.29 x10 ⁴	5.2 x10 ⁵	4.3 x10 ⁴
Area		Van Stadensrus											
Presumptive CFU/ml	Food Item	Pap			Chicken			Pork			Salad		
	TVC	1.50 x10 ⁴			2.28 x10 ⁴			4.3 x10 ⁴			3.7 x10 ⁴		
	BPA	6.1x10 ²			3.9x10 ²			4.7x10 ²			1.48x10 ³		
	PA	ND			2.53 x10 ²			7.8 x10 ²			4.3 x10 ²		
	VRB	2.37 x10 ³			5.6 x10 ⁴			8.9 x10 ⁴			1.26 x10 ³		

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CHAPTER 4

EXPLORING THE THREAT OF *STAPHYLOCOCCUS AUREUS* IN READY-TO-EAT FOODS

4.1 ABSTRACT

Human staphylococcal food poisoning is caused by a range of heat-stable staphylococcal enterotoxins that are released into food by *Staphylococcus aureus*. Additionally, antimicrobial resistance has steadily grown into a significant global issue that endangers food safety and human health. Therefore, the frequency of *S. aureus* in foods sold on the streets of Mangaung Metropolitan Municipality, South Africa, was determined. The isolated strains' genetic traits, virulence, and antibiotic resistance profiles were thoroughly examined. All samples 168/168 (100%) analysed in this study displayed the growth of *Staphylococcus* species. Furthermore, 36/168 (21.42%) and 50/168 (29.76%) isolates were subsequently identified by the RapID STAPH Plus system and MALDI-TOF MS as *S. aureus*. Moreover, the antimicrobial susceptibility testing of 50 *S. aureus* isolates showed that 26/50 (52%) were resistant to penicillin, followed by cefoxitin at 23/50 (46%) and ciprofloxacin 22/50 (44%). The multidrug-resistant (MDR) profile revealed that 21/50 (52%) of the isolates were resistant to three or more classes of antibiotics. Additionally, four sequenced isolates were identified by in silico MLST as having sequence types (STs) 243, although strain SVF3 contained a unique ST designated as * ff2b. All four isolates were identified as belonging to *spa-type* t21 by whole genome sequencing. All sequenced isolates exhibited a total of nine antibiotic resistance genes and 63 virulence genes. The current study showed the importance of consistent retail food monitoring for high virulence potential and antimicrobial resistance of *S. aureus* and for increased awareness of the potential risk posed by such strains. Furthermore, infection control measures, antimicrobial stewardship, and periodic One Health epidemiological surveillance studies are needed to monitor and contain the threat of increasing antibiotic resistance in Africa.

Keywords: *Staphylococcus aureus*; whole genome sequencing; antibiotic resistance; ready-to-eat food

4.2 INTRODUCTION

Street food vending has recently expanded as a result of many socio-economic factors. The need to provide employment, boost household income, and provide affordable, convenient varieties of meals has contributed to a boom in the street food industry (Salamandane *et al.*, 2023). However, street food vending is mainly unregulated and managed informally (Desye *et al.*, 2023). As a result, this trade has become synonymous with unhygienic and unsanitary practices, lack of sanitary facilities, and the use of poor-quality equipment (Werknen *et al.*, 2023; Verma *et al.*, 2023; Akhter and Cameron, 2023). This has had a domino effect on the safety of the foods produced, with many concerns being raised about the health risks they pose for consumers. The World Health Organization (2022) estimates that one in ten people worldwide develops an illness due to consuming contaminated food. Among the most prevalent foodborne pathogens detected in food is *S. aureus* (Thaha *et al.*, 2024).

Staphylococcus aureus is responsible for causing foodborne illnesses on a global scale. Its ability to produce toxins in contaminated food can lead to severe food intoxication that occurs in individuals and communities worldwide (Fetsch and Johler 2018). *S. aureus* and other *Staphylococcus* spp. exist as part of normal human flora and are commonly found in the nose and the perineum (Taylor and Unakal, 2017). Therefore, when food vendors fail to practise good hygiene, such as handwashing with running water and soap, they can contaminate the food they touch. Previous studies have indicated that *S. aureus* can survive and proliferate in food and on contact surfaces, creating perfect cross-contamination opportunities (Castro *et al.*, 2018; Bai *et al.*, 2023).

In the food industry, control measures of *S. aureus* and other pathogens focus primarily on hygiene strategies. However, because *S. aureus* is a biofilm-forming organism and hygiene is poor in street food vending, it can be challenging to curb or eliminate it (Saad *et al.*, 2023; Letuka *et al.*, 2021). Moreover, its virulence factors and antibiotic resistance make it a

particularly worrisome pathogen that poses a real threat to food safety (Fernandes *et al.*, 2022). To obtain the ecological and epidemiological characteristics of *S. aureus* implicated in food poisoning, numerous molecular techniques such as coagulase typing, staphylococcal protein A gene (*spa*) typing, and pulsed-field gel electrophoresis (PFGE) have commonly been employed (Suzuki *et al.*, 2014; Roussel *et al.*, 2015). While these techniques have provided valuable insights into the spread, origin, and genetic analysis of *S. aureus*, they have often lacked the resolution necessary to detect genetic differences between strains (Khasapane *et al.*, 2024). Moreover, whole genome sequencing has emerged as the preferred method due to its superior capability to enhance understanding of phylogenomics and intraspecies variations (Khasapane *et al.*, 2024; Lekota *et al.*, 2024).

Given the global context and the variety of *S. aureus* strains and virulent antibiotic-resistant lineages present in South Africa, there is a significant need for large-scale genomic analyses of local *S. aureus* isolates (Ndhlovu *et al.*, 2024). Such studies will enhance our understanding of the regional pangenome, revealing unique genetic elements and potential public health concerns. Ongoing genomic surveillance is crucial for tracking the emergence and spread of virulent or resistant strains, which can inform infection control strategies and treatment guidelines (Singh-Moodley *et al.*, 2019; Founou *et al.*, 2019; Piper *et al.*, 2024). While findings from specific pan-genomic studies on *S. aureus* in South Africa are limited, recent research has highlighted this pathogen's genetic diversity and adaptability in this region. Comprehensive local studies are essential to fully understand the pangenome landscape and its implications for public health.

Staphylococcus aureus displays considerable genetic variation, which enhances its capacity to inhabit a wide range of hosts and environments (Wang *et al.*, 2024). This variation is evident in its pangenome and contains many genes related to antibiotic resistance (Moller *et al.*, 2022). Street-vended foods are frequently prepared and sold in conditions that may not adhere to rigorous hygiene standards. Hence, lack of proper sanitation can result in contamination with pathogenic bacteria, such as *S. aureus*, that are known to cause foodborne illnesses (Amare *et al.*, 2019). Very few studies have been done to assess the

microbial profile of street-vended foods in the Mangaung Metropolitan Municipality, and none have characterised and/or defined the genetic variations of food microorganisms found in ready-to-eat foods in this area. Therefore, the purpose of this study was to rigorously assess the prevalence of *S. aureus* in street-vended foods, along with a comprehensive analysis of the genomic characteristics and antimicrobial resistance of the isolated strains.

4.3 MATERIALS AND METHODS

4.3.1 Study site and collection of samples

The study site and sampling procedure described in this chapter were the same as those described in Chapter 3.

4.3.2 Bacteriological examination

Collected samples were transported to the lab in cooler boxes with ice in order to minimize microbial proliferation. Upon arrival in the laboratory, samples (10g) were weighed and homogenised in 90 ml buffered peptone water (Thermo Fischer Scientific). To determine *S. aureus*, counting and isolation were done following ISO 6888-1:1999: A1:2003. Tenfold serial dilutions were conducted using buffered peptone water, and 0.1 ml aliquots were cultured on Baird Parker agar supplemented with egg yolk tellurite emulsion (Thermo Fischer Scientific, South Africa). Incubation was done at 35°C for 24-48 hours. The isolated samples were purified on nutrient agar (Merck, Wadeville, South Africa) and then incubated at 37°C for 24 hours (Ntelekwane *et al.*, 2024). After phenotypic identification, the isolates were confirmed as putatively pathogenic *S. aureus* isolates and were subjected to Gram-staining and coagulase activity (Alksne *et al.*, 2020; Ntelekwane *et al.*, 2024). All the purified isolates, which were obtained through the standard streak plate method, were stored in 15% (v/v) glycerol at -80°C for further analysis (Fernandes *et al.*, 2022).

4.3.3 Detection of *Staphylococcus* species using the RapID STAPH

The following protocol for identifying *S. aureus* by RapID STAPH PLUS was followed according to the manufacturer's instructions (Thermo Fisher Scientific, South Africa): Test organisms were grown in pure cultures and Gram-staining was conducted. Subsequently, the microorganisms were tested for catalase production before inoculating the RapID STAPH PLUS System tray with the test organism and incubating it at 35°C for 24 hours. The following day, the tray was examined for colour changes in the wells and then compared to a database or Code Compendium to identify the organism.

4.3.4 Detection of *Staphylococcus* species using the MALDI-TOF method

Utilising the Biotyper 3.1 tool (Bruker, Johannesburg 2191, South Africa), staphylococcal species or genera were identified. The Autoflex Speed apparatus (Bruker Daltonics, Billerica, MA) was calibrated using the *Escherichia coli* DH5α bacterial test standard (BTS). As advised by Cameron *et al.* (2018), every bacterial isolate was also identified. In short, sterile 1.0-μL disposable plastic inoculating loops were used to transfer the pure colony to individual 1.5 mL microcentrifuge Safe-Lock tubes (Sigma-Aldrich, Brøndbyvester, Denmark) that contained 300 μL of ultra-HPLC grade water (Merck, Hellerup, Denmark). After a quick vortex to produce a uniform suspension, 900 μL of 100% ethanol (Merck, South Africa) was added, and the tubes were vortexed for 15 seconds. After centrifuging the tubes for three minutes at 12,200 × g at room temperature (RT), the supernatants were drained out and disposed of, and the tubes were centrifuged once more for three minutes at RT. A micropipette was used to aspirate the leftover ethanol/water properly. After allowing the cell pellets to air dry for three minutes, a suitable volume of 70% formic acid (15–50 μL) was added. By visually sizing the pellet, the ideal formic acid volume was identified. Each sample was thoroughly mixed with an equal volume of 100% acetonitrile for up to three minutes. At room temperature, samples were centrifuged at 12,200 × g for three minutes. After carefully placing eight 1 μL volumes of supernatant on a ground steel target plate and letting it air dry, 1.0 μL of cyano-4-hydroxycinnamic acid (HCCA) matrix (Bruker Daltonics) diluted in 50% acetonitrile with 2.5% trifluoroacetic acid (Sigma-Aldrich) was applied to each spot. Every isolate's analysis

was performed twice. If isolates were not resolved after two rounds of MALDI-TOF MS analysis, they were deemed unidentified. A cut-off score of ≥ 1.7 was employed as a threshold for detecting bacteria to ensure the credibility of the analyses.

4.3.5 Antimicrobial susceptibility testing

For phenotypic susceptibility testing of the isolate, disk diffusion was performed with four antibiotics on a 90 mm plate (Mekhloufi *et al.*, 2021). Thereafter, tests for antibiotic sensitivity were conducted using newly propagated overnight cultures. Using a sterile cotton swab, 100 μL aliquots from the suspensions were spread-plated on Mueller Hinton agar (MH), and the plates were incubated at 37°C for 24 hours. The single disk diffusion technique was utilised to evaluate the susceptibility of *Staphylococcus* isolates to widely used antimicrobial drugs. Antibiotic discs (ThermoFisher, South Africa) comprising Tetracycline (TE, 30 μg), Penicillin (P, 10 μg), Erythromycin (E, 15 μg), Cefoxitin (FOX, 30 μg), Gentamicin (GEN, 10 μg), and Ciprofloxacin (CIP, 10 μg) were utilised in this investigation. The Clinical Laboratory Standards Institute (CLSI) guidelines (2024), which are interpreted as intermediate (I), susceptible (S), and resistant (R) (Table 4.1), were used to evaluate the antimicrobial profile of isolated staphylococci against various antibiotics using quality control strain *Staphylococcus aureus* ATCC 25923 (CLSI, 2024). Multidrug-resistant (MDR) isolates were defined as those that exhibited resistance to at least three or more different classes of antibiotics (Khasapane *et al.*, 2024).

4.3.6 Molecular characterisation

Following the manufacturer's instructions, the Quick-DNA Microbe Mini-prep DNA kit was used to extract gDNA (ZYMO Research, USA). The concentration and purity of the extracted DNA were determined using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The DNA was sent to an outsourced company for genome sequencing after using the PacBio Sequel II platform at Inqaba Biotechnical Industries (Pty) Ltd (525 Justice Mahomed Street, Muckleneuk, Pretoria, 0002).

4.3.7 Bioinformatics analysis

The quality of sequenced reads generated by the PacBio platform was evaluated using FastQC software version 0.10.1 (Andrew *et al.*, 2010). Paired-end trimmed reads were de novo assembled using Flye version 2.3.3 (Kolmogorov *et al.*, 2019). CheckM (Parks *et al.*, 2015) was utilised to identify potential contaminants in individual assembled genomes. Quast version 2.3 (Gurevich *et al.*, 2013) was employed to evaluate the assembled genomes. Subsequently, the assembled contigs were annotated using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) (Tatusova *et al.*, 2016).

The taxonomic classification of bacterial strains was conducted in silico using multilocus sequence typing (MLST) as proposed by Carattoli *et al.* (2014) and the Pasteur database implemented in PathogenWatch (<https://pathogen.watch/>). GTDBtk v1.7.0 (Chaumeil *et al.*, 2020) was employed to identify *Staphylococcus* species following the methodology proposed by Chaumeil *et al.* (2020). To determine the taxonomic positions of the *Staphylococcus* strains, DNA-DNA hybridization values were calculated, and a phylogenomic tree based on whole genome sequencing (WGS) was reconstructed using the Type Strain Genome Server (TYGS), as described by Meier-Kolthoff and Göker (2019). Average nucleotide identity (ANI) was used to determine the genetic relatedness of the strains using IPGA (Varghese *et al.*, 2015). Spa types were identified using the Centre for Genomic Epidemiology online tool (<https://www.genomicepidemiology.org/>).

Pangenome analysis of the dispensable genomes was conducted using Roary v3.6.8 (Page *et al.*, 2015) and Anvio-7.1 (Eren *et al.*, 2015). Eighty-four *S. aureus* genomes from South Africa were downloaded from GenBank and compared with the four sequenced isolates in this study (see Supplementary Table 6). The genomes were annotated using Prokka version 1.14 (Seemann, 2014) and Prodigal (Hyatt *et al.*, 2010). Pairwise BLASTp and the Markov Cluster Algorithm (MCL) were employed for similarity searches between the coding domain sequences (CDSs) of the assembled genomes, following the methods proposed by Magome *et al.* (2024).

The ABRicate pipeline (assessed on 25 July 2024) and AMRFinderplus (Feldgarden *et al.*, 2022) were utilised to identify antibiotic resistance and virulence genes in the genome of *S. aureus*. Antimicrobial resistance determinants were detected in the assembled genome using the CARD database (Hackenberger *et al.*, 2024), applying minimum identity and coverage thresholds of 75% (`-minid 75`) and 50% (`-mincov 50`), respectively. Moreover, the Comprehensive Antibiotic Resistance Database (CARD) was employed to identify antibiotic resistance genes. ABRicate was also used to analyse efflux pump coding genes and virulence factors in the sequenced genome, utilising the Virulence Factor Database (Liu *et al.*, 2022) with minimum identity and coverage thresholds of 70% (`-minid 70`) and 50% (`-mincov 50`), respectively. Additionally, plasmid replicons were identified in the sequenced genomes by ABRicate using the Plasmid Finder database (<https://www.genomicepidemiology.org/>). The profiles were visualised using circos plots (Krzywinski *et al.*, 2009).

4.4 RESULTS

4.4.1 Identification of *Staphylococcus* species isolates

The findings revealed that, of the 168 samples of pap, chicken, pork, and salad, every single one showed growth of *Staphylococcus* species when examined morphologically. Thereafter, one colony was picked from each of the samples for further identification by the RapID STAPH Plus system and MALDI-TOF MS. The results showed that 36/168 (21.42%) and 50/168 (29.76%) isolates were then identified as *S. aureus* by the RapID STAPH Plus system and Maldi-TOF MS, respectively (Table 4.1). Moreover, the results showed that most isolates were recovered from pork at 34, followed by chicken at 26 isolates. The least were recovered from pap at 14 and salad at 12 isolates using both identification methods.

Table 4.1 Number of collected samples resulting in positive *Staphylococcus* spp. isolates

Samples	Number of Samples	Identification methods	
		RapID STAPH	MALDI-TOF MS
Pap	42	3	11
Chicken	42	10	16
Pork	42	16	18
Salad	42	7	5
Total:	168	36	50

4.4.2 Antimicrobial resistance profiles of the *Staphylococcus aureus* isolates

Furthermore, phenotypic antimicrobial susceptibility testing showed that 26/50 (52%) of the isolates were resistant to penicillin, followed by cefoxitin at 23/50 (46%) and ciprofloxacin at 22/50 (44%). The isolates were less resistant to gentamicin at 12/50 (24%), followed by erythromycin and tetracycline at 7/50 (14%), respectively (**Figure 4.1**).

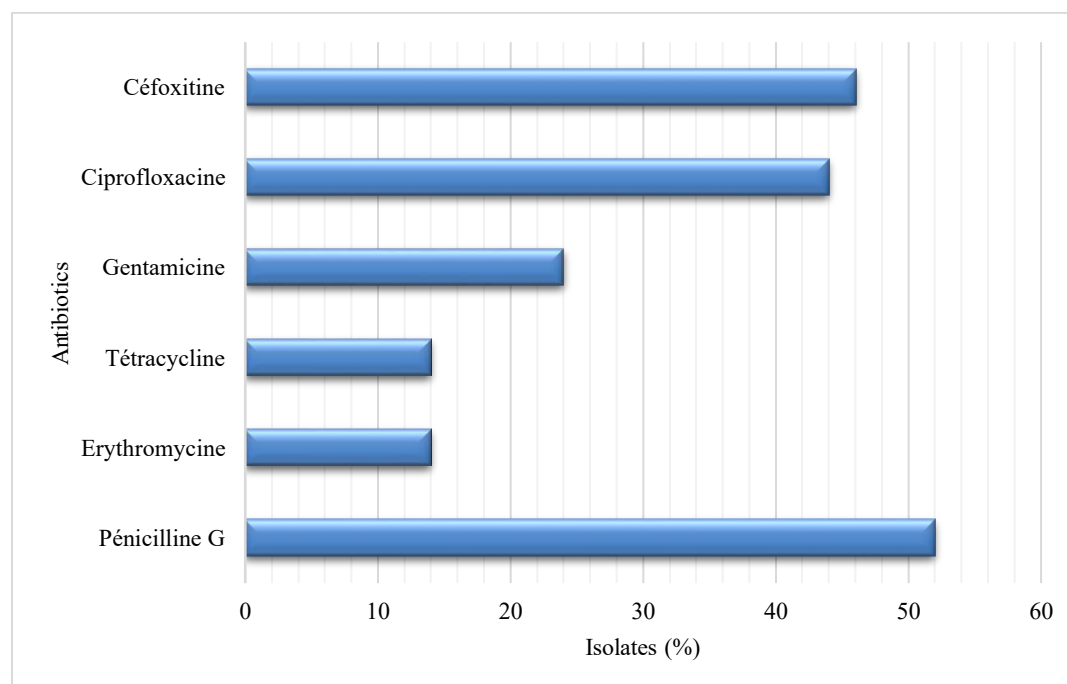


Figure 4.1 Antibiotic resistance patterns of *S. aureus* isolated from the ready-to-eat food

The multidrug-resistant results revealed that 21/50 (52%) of the isolates were resistant to three or more classes of antibiotics. Table 4.2 shows that 6/21 (12%) of the isolates were resistant to three antibiotics, namely P, CIP, and FOX. Moreover, one isolate (2%) was resistant to 6 antibiotics, whereas seven isolates were resistant to at least four antibiotics. The results indicated that chicken had the most MDR isolates at 11 (52.3%), followed by pork, pap and salad at 5 (23.8%), 4 (19%), and 3 (14.2%), respectively.

Table 4.2 Antibiotic resistance patterns of *S. aureus* isolated from the street-vended food

	Sample ID	Beta-lactam		Macrolide	Tetracycline	Aminoglycoside	Fluoroquinolones
		Penicillin	Cefoxitin	Erythromycin	Tetracycline	Gentamycin	Ciprofloxacin
Pap	P1	+	+	+	+	-	+
	P2	+	-	-	-	-	+
	P3	-	-	+	-	+	+
	P4	+	-	-	-	+	+
	P5	+	-	-	-	+	+
	P6	+	+	-	+	+	-
	P7	+	-	+	-	-	+
	P8	+	+	-	-	-	-
	P9	-	+	-	+	-	+
Chicken	C10	-	-	+	-	-	+
	C11	+	-	-	-	+	+
	C12	+	+	-	-	-	+
	C13	+	-	-	-	-	+
	C14	+	-	-	-	+	+
	C15	+	-	-	+	+	+
	C16	+	+	-	-	+	+

	C17	+	+	-	-	-	-
	C18	+	+	-	-	-	+
	C19	+	+	-	+	-	+
	C20	+	+	-	-	-	-
	C21	+	-	-	+	-	+
	C22	+	-	-	-	-	+
	C23	+	-	-	-	+	-
	C24	+	-	-	-	-	+
	C25	+	-	-	+	-	+
	C26	+	-	-	-	-	+
	C27	+	-	-	-	-	-
	C28	+	+	+	-	-	-
	C29	+	-	-	-	+	+
	C30	+	+	-	-	+	+
	C31	+	-	-	-	+	+
	C32	+	-	-	-	-	-
Pork	PK33	+	-	-	-	-	+
	PK34	+	-	-	-	+	+
	PK35	+	+	-	+	-	-
	PK36	+	-	-	-	+	+
	PK37	+	+	+	+	-	+
	PK38	+	+	-	-	+	+
	PK39	+	+	-	-	-	+
	PK40	+	+	-	-	+	+
	PK41	+	+	-	-	-	+
Salad	S42	-	+	+	+	-	-
	S43	+	+	-	-	-	+
	S44	-	+	-	-	+	+
	S45	-	+	-	-	-	-

S46	+	-	-	-	+	+
S47	+	+	+	-	+	+
S48	-	+	+	-	-	+
S49	+	+	-	-	-	-
S50	+	+	-	-	-	+

4.4.3 Genomic features and *in silico* identification

The genomes for four isolates (P1, C15, PK37, and S47) were sequenced based on their MDR patterns (Table 3.3). The characteristics of these genomes are presented in Table 4.3. The sequenced strains' genome sizes ranged from 2.6 Mb to 2.8 Mb, with a GC content of 32.9%. The use of TYGS groups showed these isolates of *S. aureus* clustering with *S. aureus* DSM 20231. The number of coding sequences ranged from 2,626,607 to 2,855,328 sequences.

Table 4.3 Genomic features of four *S. aureus* strains isolated from street-vended food

Strain name	BioSample accession no.	GenBank accession no.	Completeness	No. of raw reads	No. of Contigs	Coverage Depth	N ₅₀ Value (bp)	Genome Length (bp)	Total no. of Genes
SVF1 (P1)	SAMN45157884	SUB14878302	97.37	70 158	5	140x	2489803	2855949	2,673
SVF2 (C15)	SAMN45158357	SUB14908207	98.72	91 766	3	140x	2237147	2833403	2,668
SVF3 (PK37)	SAMN45158359	SUB14908216	91.28	130 620	4	140x	2196342	2627141	2,445
SVF4 (S47)	SAMN45158360	SUB14908224	99.51	115 379	3	140x	2227546	2836470	2,650

Moreover, in silico MLST using the Pasteur database clustered all sequenced isolates as sequence types (STs) 243, while strain SVF3 had a novel ST assigned as * ff2b (**Figure 4.2**). The whole genome sequencing analysis revealed that all four isolates belonged to spa-type t21. The four genomes were compared with 81 South African genomes reportedly present in bovine milk (n = 38) and human samples (n = 39). Comparative genomics showed that ST8500 and ST97 were prominent in bovine milk samples from Bloemfontein (Khasapane *et al.*, 2024). Human isolates harboured ST-5 (n =11), ST-152, and other variant sequence types. The four sequenced genomes in this study were 99,99% similar based on ANI analysis. Furthermore, the cluster mentioned above had grouped with human isolates (i.e., strain DRKM31 and DRKM28) from a cutaneous abscess in Gauteng, South Africa (ANI <96.8%) (**Figure 4.3**). Three clade groupings catered for most of the *S. aureus* sampled from humans, while most bovine isolates formed sub-clonal significant clades based on the ANI profile.

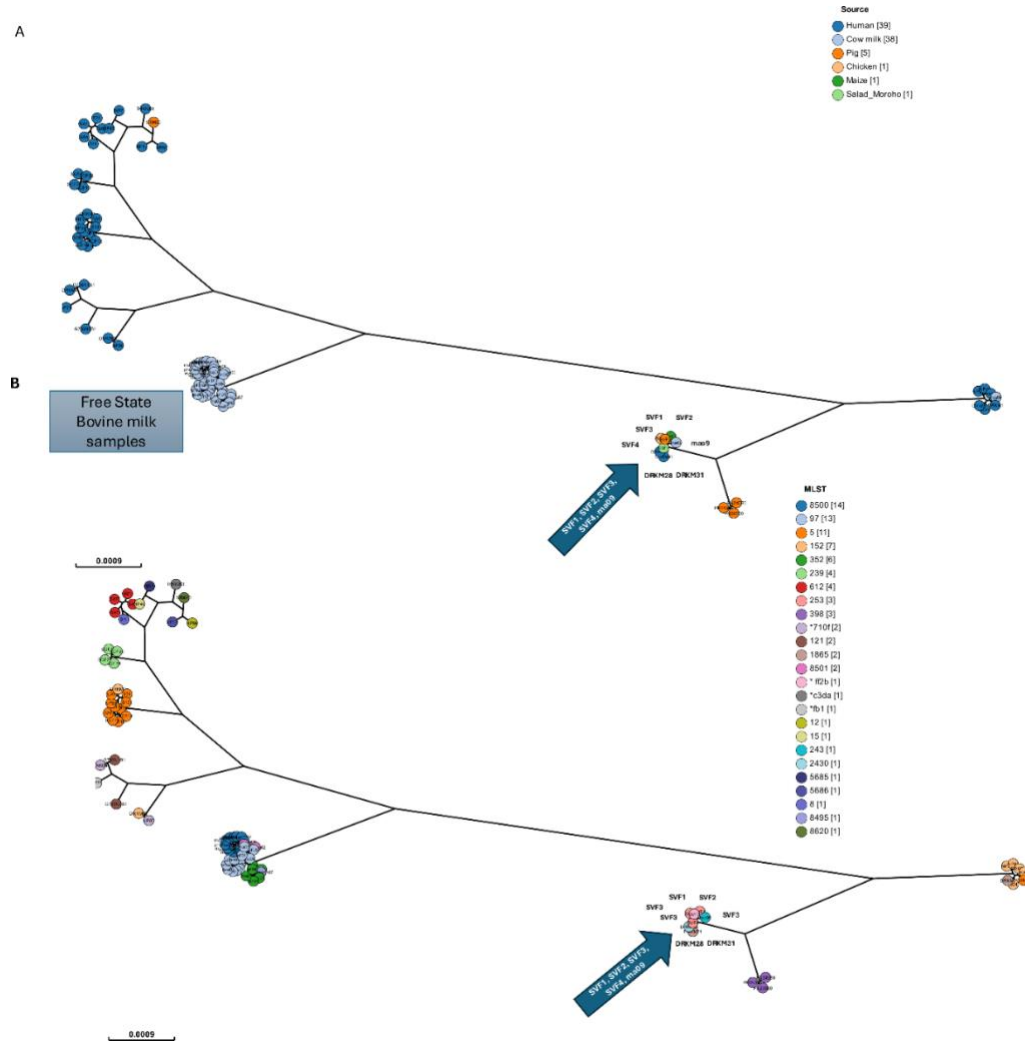


Figure 4.2 Multi Locus Sequence Typing indicating the clustering of the 85 *Staphylococcus aureus* strains in South Africa. (A) The colour of the strains is grouped according to sources, while (B) uses sequence types identified using the PubMLST in PathogenWatch. The phylogenetic tree was computed using the maximum likelihood of whole genome core single nucleotide polymorphisms.

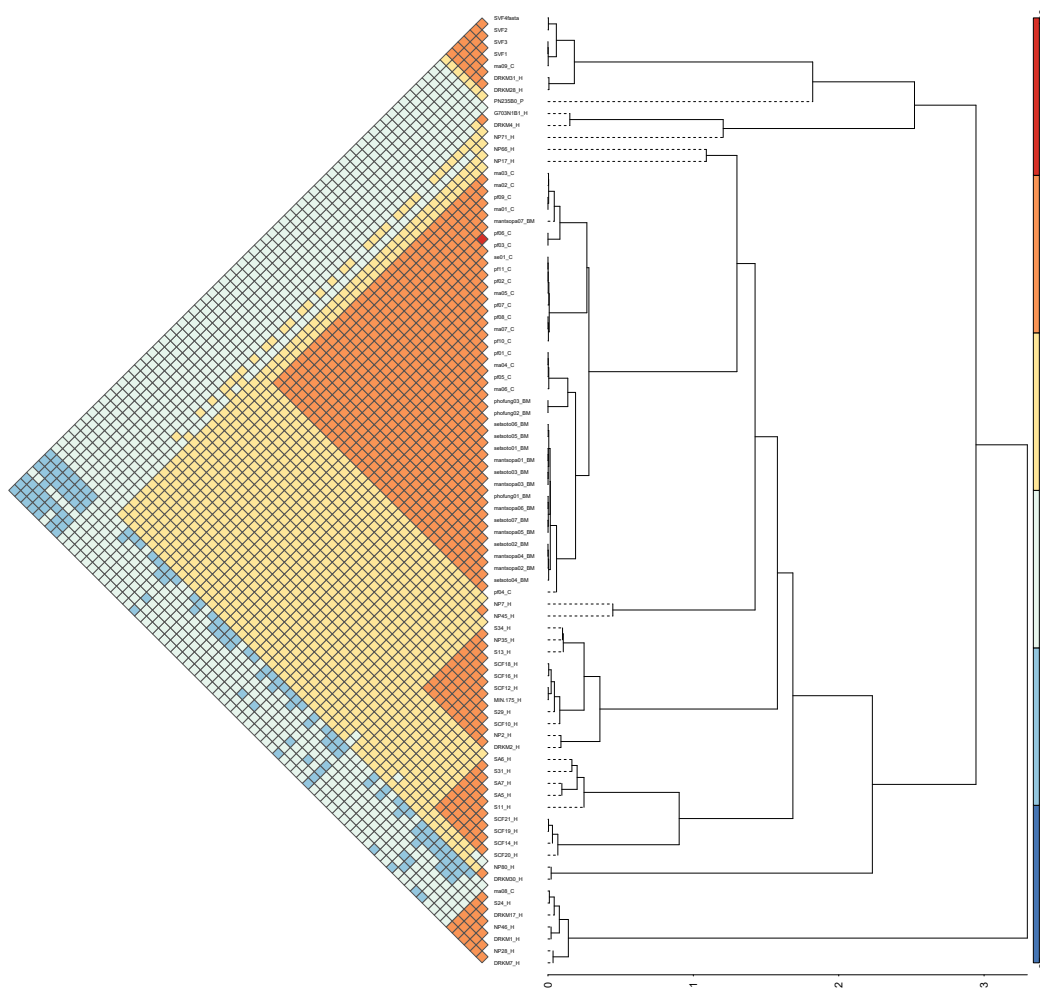


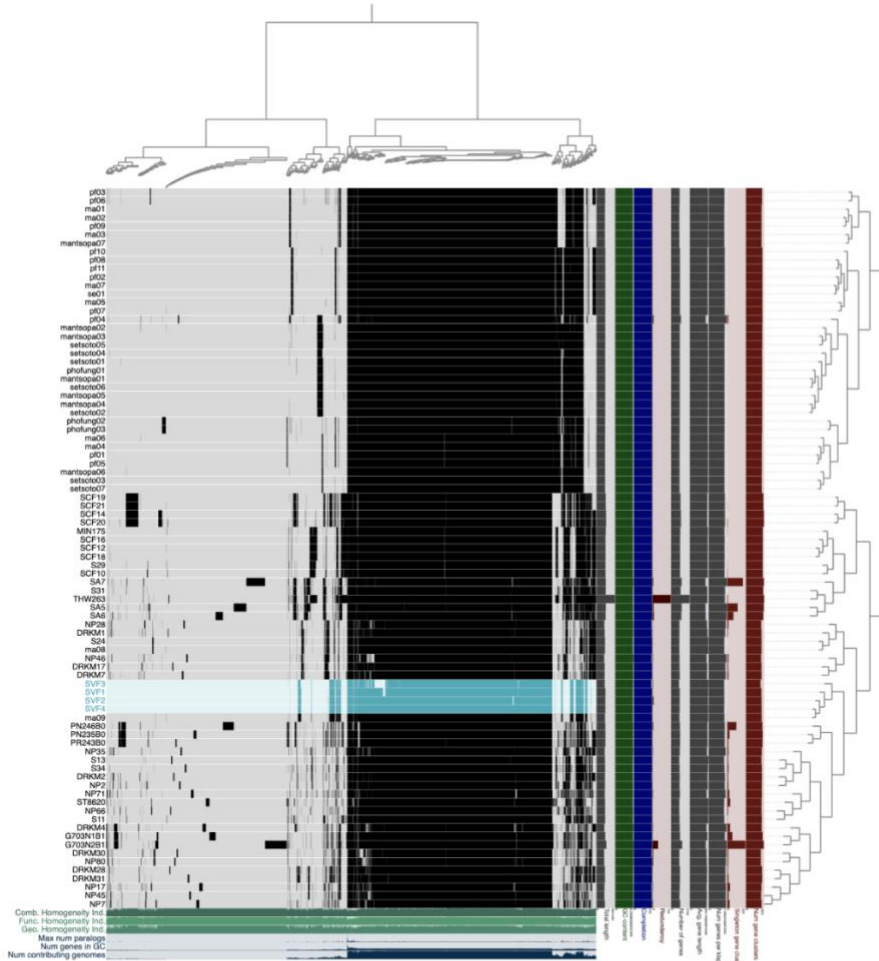
Figure 4.3 Average nucleotide identity of the 85 *Staphylococcus aureus*, including the four sequenced genomes

4.4.4 Pan-genomics analysis

Pan-genomic analysis of 85 South African *S. aureus* genomes comprised the four sequenced genomes and 81 genomes, retrieved (**Figure 4.4**). We identified 200693 genes across the strains using Roary, establishing approximately 1,144 core genes among the genomes analysed. Notably, these sequenced genomes were closely related to the ma09 strain isolated from bovine milk in Mofutsanyana, Free State, thus strongly indicating the spread of closely related species within the Free State province. About 2,293 core genes defined this cluster, and we found no upset clusters among the four sequenced genomes in this study (Figure 4.5A). Furthermore, Anvio pangenome analysis identified 5,098 gene

clusters across all 86 genomes. No upset gene clusters were detected among the four sequenced genomes. About 77.72% (181522 / 233570) of sequences were classified into the COG functional category (Figure 4.5B). The COG functional classification revealed that 10.43% and 10.17% of the genes corresponded to translation, ribosomal structure biogenesis (J), and amino acid transport and metabolism (E), respectively (Figure 4.5B).

A



B

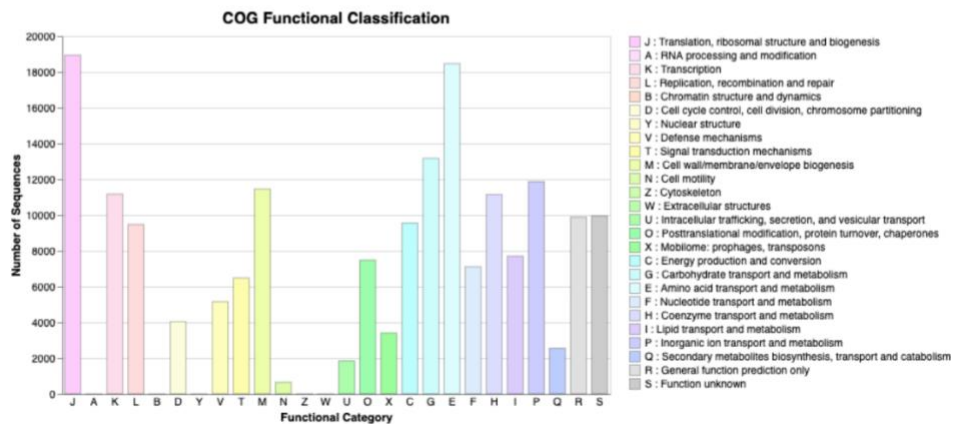


Figure 4.4 Pangenome visualization of the *Staphylococcus aureus* (n = 85) genomes, including the four sequenced strains in this study. **A:** Phylogeny of the *S. aureus* indicating the core and accessory genes placing the sequenced genomes highlighted in blue. Visualization of pangenome analyses using Anvio. Central dendrogram clustering of samples is ordered by gene cluster presence/absence. Items order presence absence (D,

Euclidean; L, Ward). **B:** COG functional classification of the core genome of the analysed genomes.

4.4.5 Antibiotic resistance and virulence gene profiles

The antimicrobial resistance profile confidently identified nine genes across four sequenced *S. aureus* strains (**Figure 4.5**). These genes included those responsible for lincomycin resistance (*LmrS*), a tetracycline efflux pump (*tet-38*), fosfomycin resistance (*fosB*), and the more regulon, which is critical for virulence, biofilm formation, and antibiotic resistance. Additionally, two multidrug binding transcription regulators (*mepR* and *mepA*) and two regulators of oxacillin resistance (*arlS* and *arlR*) were also present. Notably, the fluoroquinolone resistance gene (*norA*) was exclusively detected in strains SVF 2 and SVF 4. This study unequivocally confirms that none of the isolates exhibited detectable Plasmid Inc types.

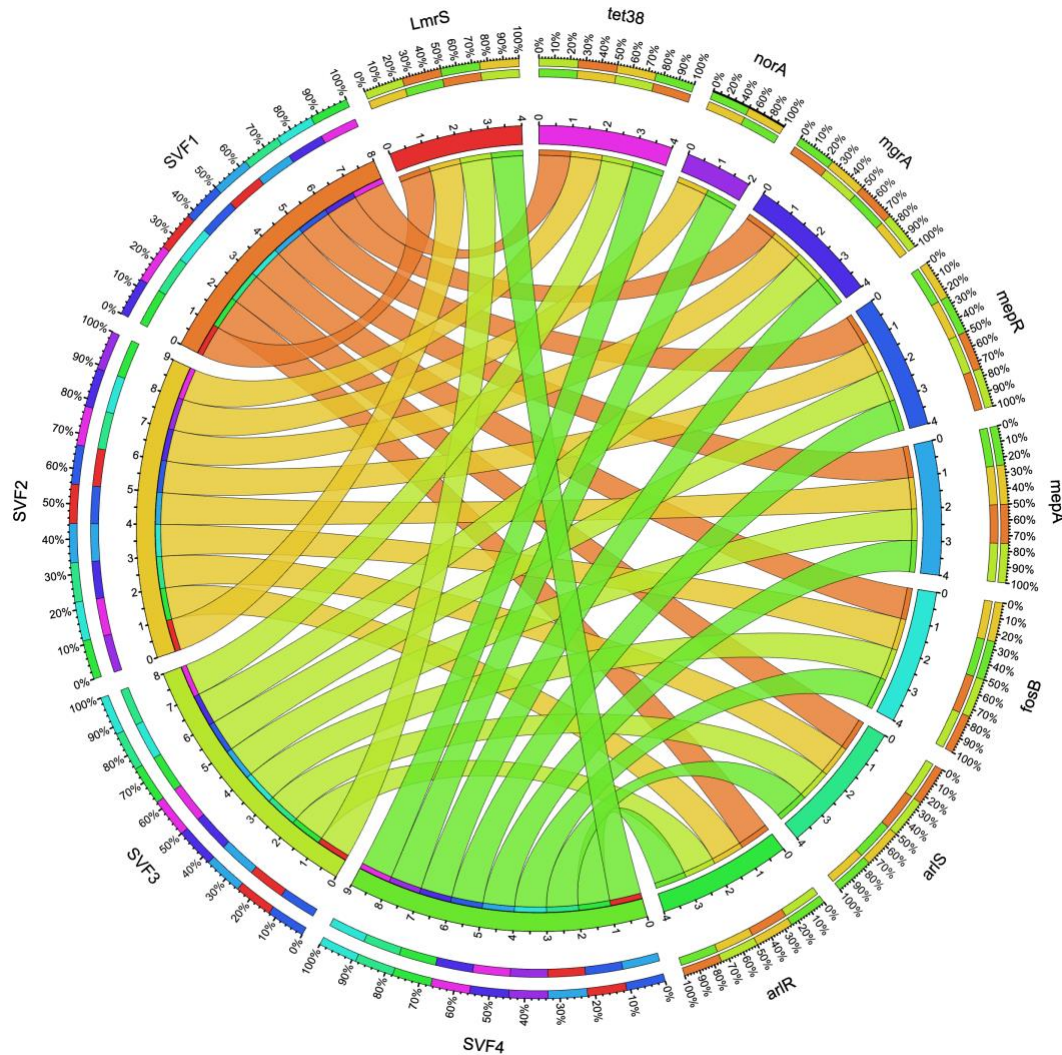


Figure 4.5 Antibiotic resistance gene profiles of the four sequenced *Staphylococcus aureus* isolated from street-vended food determined using the CARD database. The study further predicted 63 virulence genes from the sequenced genomes (Figure 4.6). *S. aureus* displayed a diverse range of virulence-associated genes in the genomes examined. These included adhesion molecules such as *clfA* and *spa*, immune evasion proteins like *sbi* and *spa*, hemolysins such as *hlgA* and *hla*, genes responsible for biofilm formation (*icaA-D*), iron acquisition systems (*isdA-G*), and various proteases (*sspA-C*). Collectively, these factors enhance the pathogenic potential and adaptability of *S. aureus* within host environments. The genome SVF3 lacked Willebrand factor-binding protein (*vWbp*) and clumping factor A (*clfA*) genes.

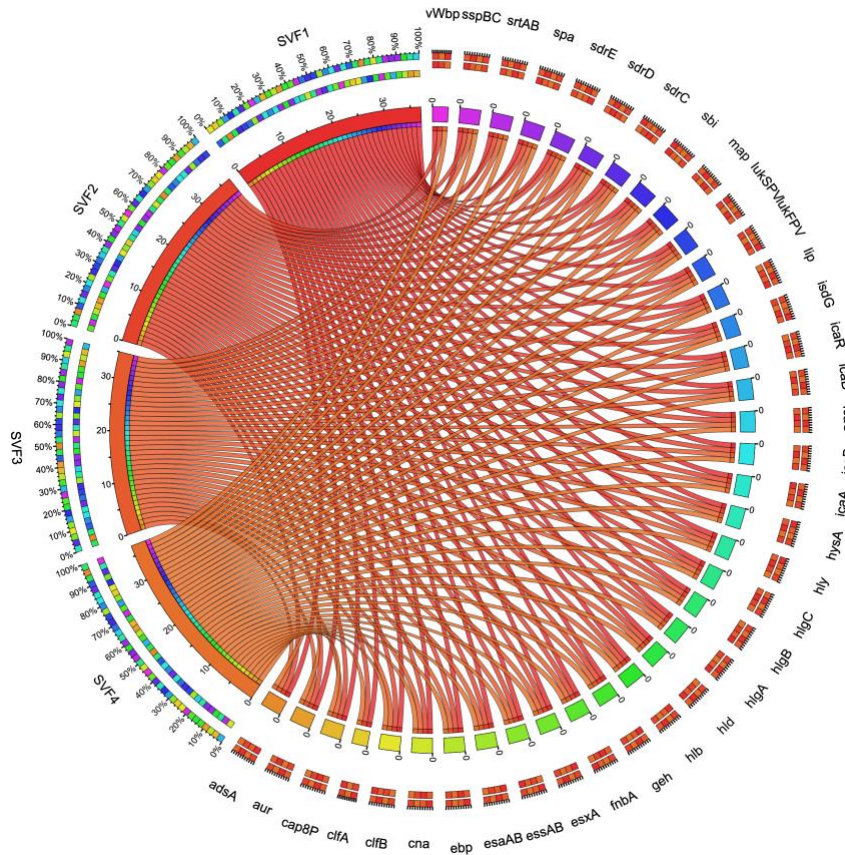


Figure 4.6 Virulence gene profiles of the four sequenced *Staphylococcus aureus* strains isolated from the analysed food samples using the virulence factor database

4.5 DISCUSSION

Foodborne pathogens pose a significant public health concern in South Africa, particularly in food derived from informal food markets where food safety regulations are often inadequate. *Staphylococcus aureus* is a well-documented foodborne pathogen with high prevalence. This is especially concerning for public health because of its ability to develop antimicrobial resistance, thereby complicating treatment and control strategies. In South Africa, street-vended foods are widely consumed due to their affordability and accessibility. Yet, they are often prepared in unhygienic conditions, creating an ideal environment for bacterial contamination and foodborne outbreaks. Despite growing concerns regarding this phenomenon, limited data exist on the prevalence and antimicrobial resistance profiles of *S. aureus* in such food sources. This study addressed this gap by investigating the

prevalence, genetic diversity, virulence factors, and antimicrobial resistance profiles of *S. aureus* isolated from street-vended foods in the Mangaung Metropolitan Municipality.

To describe the transmission of pathogens, it is crucial to comprehend the interactions among strains. The present study's results surpass those reported in previous research on RTE meat products linked to foodborne illnesses. Those investigations found that *S. aureus* isolation rates were as high as 9.5% in southern Taiwan (Wei *et al.*, 2006), 11.8% in China (Yang *et al.*, 2016), and 15.4% in Taiwan (Fang *et al.*, 2003). This study found that *S. aureus* was present in 100% of Shanghai's ready-to-eat (RTE) vegetable (salad) samples. The prevalence that this study observed was above the rates for RTE fruits and vegetables reported in the Chinese province of Sichuan (20.5%), Saudi Arabia (11.8%), and Korea (1.8%) (Hassan *et al.*, 2011; Wang *et al.*, 2019; Lin *et al.*, 2019; Tango *et al.*, 2018).

The study revealed high antimicrobial resistance among isolates, with resistance rates of 52% for penicillin, 46% for cefoxitin, and 44% for ciprofloxacin, while lower resistance was observed for gentamicin (24%), erythromycin (14%), and tetracycline (14%). Whole genome sequencing identified all isolates as ST243, except one novel ST, and showed a close genetic relationship (99.99% ANI) with bovine and human *S. aureus* strains as had been detected earlier in the study area. These findings highlight potential cross-host transmission dynamics. The findings of the current study also highlight an alarming presence of *S. aureus* in all tested food samples, indicating pervasive contamination throughout food handling or processing. A substantial amount of these isolates exhibited multidrug-resistance (MDR), which markedly elevates the public health risk since treatment options may be limited. For instance, one study found that foodborne *S. aureus* isolates frequently carried both virulence determinants and antimicrobial-resistance genes, including those conferring resistance to penicillins and macrolides (Yu *et al.*, 2023). Moreover, the presence of virulence-associated genes suggests that these contaminated foods are not only likely to transmit *S. aureus* but also to do so in a manner capable of causing severe illness. Globally, meta-analyses and reviews of ready-to-eat foods have implicated MDR *S. aureus* as an

emergent risk in food safety and antimicrobial-resistance surveillance (Onohuean *et al.*, 2025). These results underscore the urgent need for strengthened hygiene controls, rigorous vendor training, and antimicrobial-resistance monitoring within the ready-to-eat food sector.

Antimicrobial susceptibility testing showed that the isolates were more resistant to ceftiofur, ciprofloxacin, and penicillin than to gentamicin, erythromycin, and tetracycline, in that order. Although the study's findings are not comparable to those of a study by Wang *et al.* (2014) that demonstrated higher resistance to penicillin, tetracycline, and erythromycin, they are comparable to other research studies on raw food products in China and other nations (Rodríguez-Lázaro *et al.*, 2015). However, because RTE foods are ingested without additional cooking, which could eliminate or lessen the microbial load, this study's findings are even more concerning from a public health standpoint. Therefore, it is evident that the presence of *S. aureus* and MRSA in RTE street food, as well as the proliferation of antibiotic-resistant strains, poses a risk to human health.

Many antibiotics are also commonly used in veterinary medicine, particularly β -lactams (penicillin), macrolides (erythromycin), and lincosamide (clindamycin) (Cháfer-Pericás, *et al.*, 2010). In Bangladesh, 87 ready-to-eat meals yielded a total of 128 *S. aureus* isolates. Of these, 100 (78.1%) and 52 (40.6%) isolates, respectively, showed resistance to erythromycin and tetracycline (Xing *et al.*, 2014), which are findings that are comparable to those of this investigation.

However, the findings of the current study are nearly identical to those of a study conducted by Chajęcka-Wierzchowska *et al.* (2014), who discovered that 40 of the isolates could be categorised as MDR and 49.6% of the isolates were resistant to ceftiofur, while tetracycline (17.9%) and erythromycin (8.5%) were more susceptible to *S. aureus* isolates. Therefore, RTE foods may be a significant source of antibiotic resistance phenotypes, as evidenced by the high percentages of MDR staphylococci recovered from them. In the case of strains

isolated from RTE food, the majority of isolates showed resistance to ceftazidime, which identifies methicillin-resistant strains that are phenotypically resistant to all beta-lactam antibiotics that have been used thus far in treatment, including carbapenems, penicillin, aminopenicillins, isoxazolyl penicillins (oxacillin, cloxacillin, dicloxacillin, and flucloxacillin), nafcillin, cephalosporins, and cephalosporins with inhibitors (Gajic *et al.*, 2022).

Notably, the four sequenced genomes were closely related to the ma09 strain isolated from bovine milk in Mofutsanyana, Free State, and DRKM31. In contaminated agricultural settings, *S. aureus* isolates are primarily found in and transmitted to humans through farm animals, including cattle, pigs, and poultry (Kozytska *et al.*, 2023). Meat and other animal products, such as dairy products or eggs, may become contaminated during slaughtering and processing (Zhang *et al.*, 2024; Zenu and Bekele, 2024). Consequently, individuals may unknowingly consume contaminated RTE products that contain *S. aureus*. Therefore, it is crucial to understand the genetic diversity of *Staphylococcus aureus* in a 'One Health' context from sources such as RTE. This means that its adaptability and potential for antimicrobial resistance across human, animal, and environmental reservoirs must be understood.

Pan-genomic analysis of *S. aureus* revealed that 10.43% of the genes were associated with translation, ribosomal structure, and biogenesis (classified under COG J), while 10.17% were linked to amino acid transport and metabolism (classified under COG E). As a rapidly growing bacterium, *S. aureus* requires an efficient protein synthesis machinery to thrive, particularly in nutrient-rich environments or host tissues (Balasubramanian *et al.*, 2017; Bhattacharya *et al.*, 2024). The notable percentage of genes within COG J emphasises the bacterium's commitment to maintain robust ribosomal and translational functions, which are vital for growth, adaptation, and survival during stressful conditions, including host immune responses (Shen *et al.*, 2024). *S. aureus* relies significantly on effective nutrient acquisition systems, particularly on amino acids, to satisfy its metabolic needs during

infections when resources may be scarce (Richardson, 2019). These genes allow the bacterium to utilise amino acids for protein synthesis and as energy sources and precursors for various biosynthetic pathways (Halsey *et al.*, 2017). The prominence of these functional classifications reflects *S. aureus*'s capacity to adapt to diverse environments, ensuring its survival and competitive edge in niches such as human hosts (Giulieri *et al.*, 2022). These functions are essential for its pathogenesis, biofilm formation, and resistance mechanisms (Giulieri *et al.*, 2022).

The ability of *S. aureus* to attach to host cells or extracellular matrix is a defining feature of its pathogenicity (Bhattacharya and Horswill, 2024). Adhesion is the initial stage of biofilm formation or host cell invasion, which shields bacteria from the immune system and promotes persistent infection (Mea *et al.*, 2021; Pugazhendhi *et al.*, 2022). Eight genes—*clfA*, *clfB*, *cna*, *fnbA*, *fnbB*, *srdC*, *srdD*, and *srdE*—encode a repertoire of surface proteins known as microbial surface components that recognise adhesive matrix molecules (MSCRAMMs) that are necessary for adhesion, as detected in this study. The release of biofilm-related proteins depends on these genes' expression (Foster *et al.*, 2017). Bacterial pathogens' capacity to form biofilms is thought to be antibiotic-resistant and thus they are linked to chronic infections in humans and animals (Dufour, Leung, and Lévesque, 2010; Singh *et al.*, 2017). When *S. aureus* isolates sequenced in this study were analysed, nearly every gene in the *ica* operon was detected. All sequenced *S. aureus* isolates included the *hly*, *hld*, *hlgA*, *hlgB*, *hlgC*, and *hly* genes, which encode various hemolysins and leukocidins. The latter are virulence factors that contribute to the bacterium's pathogenicity by lysing red blood cells and immune cells (Zecconi and Scali, 2013). *Staphylococcus aureus* utilises a sophisticated array of hemolysins and leukocidins to enhance its virulence by lysing host cells and evading immune responses. These factors are tightly regulated and play specific roles in different stages of infection, making them critical targets for potential therapeutic interventions. Understanding these mechanisms is essential for developing strategies to combat *S. aureus* infections (Schmidt *et al.*, 2017; Zhang *et al.*, 2018). Leukotoxin-encoding *luk* genes were present in all sequenced genomes in the studies conducted by Budd *et al.*

(2015) and Khasapane *et al.* (2024). The *luk* genes encode these leukotoxins, and their presence and expression vary among different *S. aureus* strains (Von Eiff *et al.*, 2004). However, human-derived isolates have also been reported to have *luk* genes (Enwuru *et al.*, 2018).

Moreover, genomic analysis showed that selected *S. aureus* isolates had MDR efflux pumps. Nonetheless, these components must be elevated to be connected to resistance (Papkou *et al.*, 2020). It is noteworthy that the existence of genes that encode MDR efflux pumps, like *NorA*, has been linked to multiple virulence genes (Gaetano *et al.*, 2023). Specific virulence genes have frequently been found in *S. aureus* isolates with these MDR efflux pumps, while others are less common or completely absent in that subset of isolates. It is still being determined whether these patterns indicate a gene interaction that affects AMR and virulence against the lack of mechanical research. All known genetic factors related to antibiotic resistance and virulence factor genes and their interactions can be found throughout bacterial genomes. Furthermore, the results of the current study showed that the isolates had a *mepA* gene from the multi-antimicrobial extrusion (MATE) protein family, which is present in the *mepRAB* operon. This may be a multidrug transporter protein, according to a multidrug resistance phenotype seen in eight reported isolates (Costa *et al.*, 2013).

The current study also identified a tetracycline-resistant *tet* (38) gene that is a member of the major facilitator superfamily (MFS) efflux pump genes. The chromosome-encoded Tet38 is a crucial membrane protein in *S. aureus* that helps explain the bacteria's resistance to natural substances, including antibacterial fatty acids and antibacterial medications. Tet38 also helps *S. aureus* to infiltrate epithelial cells by interacting with host cell CD36 and surviving in skin abscesses. Therefore, this study assessed some of the structural factors that influence Tet38's various roles, concentrating on efflux-mediated resistance, epithelial cell invasion, and interaction with CD36 (Truong-Bolduc, Wang and Hooper, 2019).

The resistance of *S. aureus* to erythromycin and gentamicin is attributed to the *LmrS* efflux pump gene, which encodes the lincomycin resistance protein. This study suggests that the *LmrS* gene is possibly resistant to fenicol, oxazolone, and diaminopyrimidine (trimethoprim). Furthermore, Andersen *et al.* (2015) demonstrated that the projected 47 kDa protein product, which consists of 14 transmembrane alpha-helices (TMH), is used by the *LmrS* gene activity to successfully extrude fusidic acid, kanamycin, lincomycin, and linezolid. The expression of the *norA* efflux pump gene, which regulates ferric uptake regulator (*Fur*) and mutations occurring in the *norA* promoter area, were linked to a chromosomally encoded resistance mechanism of ciprofloxacin (quinolone). Therefore, this study showed that 44% of all the *S. aureus* isolates investigated were indeed resistant to the antibiotics in question (Leroy *et al.*, 2019).

Defensin (*mprF*), glycylicycline (*mepA*), multidrug and toxic compound (*mepR*), daptomycin (*clsA*), fluoroquinolone and acridine dye (*arlS* and *arlR*), glycylicycline (*mepA*), and diaminopyrimidine (*dfrC*) were among the genes that were found in the *S. aureus* genomes in the current study. The presence of these genes aligned with findings by Naorem *et al.* (2020, 2021), who also discovered genes linked to antibiotic resistance in *S. aureus* isolated from the food industry, particularly in bovine milk and a clinical sample (Ajose *et al.*, 2024).

4.6 CONCLUSION

This study identified the significant presence of antimicrobial resistance profiles of *S. aureus* in ready-to-eat foods sold by food vendors in Mangaung Metropolitan Municipality. Of concern is that over 50% of the isolates could be associated with MDR. Furthermore, the study also detected the presence of virulence genes, namely leucocidin, hemolysin, and aureolysin, along with antimicrobial resistance (AMR) genes such as *LmrS*, *mepA* and *tet (38)* in the analysed food samples. These results suggest that contaminated food may play a role in disseminating drug-resistant strains of *S. aureus*. To combat this issue, it is crucial to establish a targeted education and awareness campaign, alongside a comprehensive surveillance program for *S. aureus* throughout the entire food production and supply chain,

with particular emphasis on industries that produce RTE foods. Additionally, the findings may hold broader implications for developing nations and other regions where ready-to-eat food is sold and consumed. Gaining a deeper and ongoing understanding of the epidemiology of *S. aureus* genotypes in ready-to-eat (RTE) food is essential for developing effective treatment and control strategies to curb the spread of this pathogen.

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CHAPTER 5

THE PRESENCE OF HEAVY METALS IN STREET-VENDED FOODS

5.1 ABSTRACT

Street food vending contributes to food security and social justice by nourishing communities, empowering vendors, and fostering cultural resilience. However, this sector faces safety challenges mostly due to anthropogenic activities. Chemical and heavy metal contamination from agricultural practices and industrial activities are significant concerns. This study evaluated heavy metal contamination in commonly vended foods in the Free State province, South Africa, and assessed the potential for health risks among consumers using ICP-OES. Shapiro-Wilk's normality test and Kruskal-Wallis's H test were conducted to evaluate differences between means, followed by Pairwise Comparison ($p=0.05$). The results indicated heavy metal contamination in all the samples that were analysed. The overall medians and IQR of in mg/kg were: As, Cd, Cr, Cu, Fe, Pb, and Zn. In descending order, they were Fe 4.365(4.424), Zn 3.605(3.711), As 2.653(2.971), Pb 1.095(1.266), Cu 0.271(0.326), Cr 0.212(0.117), and Zn 0.023(0.027). Multiple pairwise comparisons showed a statistically significant difference ($p < 0.05$) for As among all pairs, while other heavy metals showed some variations without statistical significance. The EDI values for the examined food samples were below the recommended dietary values, suggesting that the detected heavy metals did not pose a public health risk in the study area at the time of the investigation. The health risk assessment using Hazard Index (HI) indicated potential non-carcinogenic health risks from heavy metals in the street-vended foods tested, as $HI > 1$. The prevalence of street-vended foods, especially in developing countries, is unlikely to decline; therefore, more efforts should focus on education and regulation in the sector to protect consumer health. Specifically, guidance about the importance of hygiene and the quality of utensils and vended foods used by vendors should be provided.

5.2 INTRODUCTION

Street-vended foods are classified as those foods that are cooked, prepared, and served by vendors in streets and/or other public places. These foods are directly consumed without further preparation (Rakha *et al.*, 2022). Street foods provide essential nutrients and are a source of energy for millions of consumers worldwide. Moreover, street food vending is also a means of income for many low-income earners around the world (Ekhator *et al.*, 2017). These foods are inexpensive and readily accessible and, as a result, the street-vended food market has experienced an economic boom in the past few years (Rakha *et al.*, 2022). Although street foods offer a great deal of advantages in terms of food security, concerns about their safety and sustainability are far too great to be ignored. Food safety entails the handling, preparation, and processing of food in a manner that ensures that any food consumed does not cause illness or pose any health risks to consumers (Ekhator *et al.*, 2017). However, more often than not, food safety has been threatened by the intended or unintended introduction of contaminants. This is problematic as street foods are susceptible to microbial, chemical, and physical contaminants. Moreover, due to their ubiquitous existence in the environment, heavy metals are among food-associated contaminants, thus posing a threat to human health as they are believed to have carcinogenic properties (Bamuwanye *et al.*, 2015). This threat presents a great challenge that goes beyond the control of extrinsic and intrinsic conditions as undesirable food handling and preparation practices must be avoided.

5.3 FOOD ADULTERATION

5.3.1 Contextualising food adulteration

Efforts to contain food adulteration have gained momentum in recent years, as unhealthy or contaminated foods pose not only an economic problem, but a dire health risk for consumers (Schieber, 2019). Food adulteration entails the use of prohibited substances that can depreciate or injuriously affect the health of consumers (WHO, 2023). The adulteration process entails the partial or entire substitution of a food's original nature by the abstraction of a portion of vital substance from it or by presenting a food substance as

an imitation of another (Public Health Notes, 2018; Sadiku, 2019). Food adulteration occurs for various reasons, which include food insecurity, increased profitability, illiteracy among the general public, increased food demand, and changing trends in the informal food chain (Bansal *et al.*, 2017).

A range of non-nutritional substances can be found in food, be it those that have been tested for safety-in-use or those of which little is known, particularly contaminants. It is within these contaminants that carcinogens may unexpectedly lurk (Pascal, 2019). Cancer is globally recognized as a major cause of death, yet it is sometimes ironically attributed to the consumption of a particular food type, such as red or barbequed meat, that is consumed to sustain life (National Cancer Institute, 2023). According to the Cancer Council NSW (n.d.), N-nitroso chemicals are formed in the gut as a result of the breakdown of a haem, and these chemicals are damaging to the lining of the bowel, which could result in bowel cancer. Food is often manipulated through the addition of substances called food additives that have technological importance or needs (Codex Alimentarius Commission, n.d.). Technological needs include efforts to maintain or improve the safety, freshness, taste, texture, and appearance of food (WHO, 2020). While food security remains a major concern, researchers have increasingly studied the health risks posed by food additives, including their potential to disrupt the gut microbiome, contribute to metabolic disease, and impair long-term health (ERC, 2023; FAO, 2025; Hikamah *et al.*, 2025). Regardless of these efforts, much about food additives is still open for debate and further investigation (Bashir *et al.*, 2018).

Anthropogenic stressors have contributed to the overuse or misuse of food additives in some parts of the world, and this has further raised the alarm about food additives and food adulteration (Zhong *et al.*, 2018). The prevalence of food adulteration practices in modern times has undoubtedly contributed to abating public health and, to a larger extent, the widespread use of contaminants containing toxic trace elements (Brevett and Cox, 2024). The use of adulterants such as metanil yellow, chalk powder, and brick powder to enhance food products such as turmeric, coffee, and wheat flour has caused illnesses such as

anaemia, brain damage, paralysis, stomach disorders, and cancer (Gizaw, 2019; Sharma *et al.*, 2017).

5.3.2 Heavy metal contamination of street-vended foods

Studies have warned against the threat of the contamination of various food substances and street-vended foods with heavy metals and have highlighted the various conditions leading to this phenomenon (Ekhtor *et al.*, 2017; Soliman, 2016; Goswami and Mazumdar, 2016). Some of the metals reportedly found in street-vended foods include lead (Pb), Cadmium (Cd), Mercury (Hg), antimony (Sb), manganese (Mn) and aluminium (Al). Although consumers are advised to visually inspect food before purchasing it, contamination of food with heavy metals, which are present in parts per million (ppm), cannot be detected with the naked eye (Mainotra, 2016). Food may thus be intentionally (through adulteration) or unintentionally contaminated with trace metals before, during, or after processing.

Throughout the preparation and vending process, food can be unintentionally contaminated with toxic metals due to numerous factors. These include environmental conditions during growth, inappropriate post-harvest handling, incorrect storage and meal preparation, poor selection of raw materials, and unhygienic vending sites (Birgen *et al.*, 2020). Heavy metals contamination may also result from poor packaging and vehicular exhaust emissions, and indiscriminate waste disposal (Ankar-Brewoo *et al.*, 2020). Ankar-Brewoo *et al.* (2020) claims further that industrial pollutants and other environmental contaminants near vending sites can still contribute to food contamination.

Conversely, street-vended foods also have the potential to greatly contribute to the daily intake of essential trace elements, otherwise known as essential minerals (Mchiza *et al.*, 2018). However, data on the bioavailability of these essential minerals is not widely available. Sufficient intake of essential minerals by humans is necessary (Razzaque and Wimalawansa, 2025). People should take in about 1 000 to 2 500 mg per day, depending on the mineral. This intake is essential for immune functions and biochemical processes that

facilitate growth and prevent chronic diseases (Silva *et al.*, 2019). The essential minerals are divided into two classes, namely macrominerals and microminerals. Macrominerals are those that are required in greater quantities and include calcium, magnesium, potassium, and sulphur (Lere *et al.*, 2021). Conversely, micronutrients are those that are only necessary in smaller amounts, such as zinc, iron, selenium, manganese, copper, chromium, and boron (Lere *et al.*, 2021). It has been demonstrated that the insufficient intake of macronutrients or the overconsumption of micronutrients could be problematic to human health and development (Espinosa-Salas and Gonzalez-Arias, 2023). One objective of this study was to assess the level of heavy metals in ready-to-eat street-vended foods in the MMM in the Free State, South Africa.

5.4 METHODOLOGY

5.4.1 Study area and sample collection

The study was conducted in the Mangaung Metro Municipality, popularly known as “The home of the Cheetahs”. Mangaung Metro Municipality (MMM) is a Category A municipality nestled in the central interior of South Africa in the Free State province (29.1303°S, 26.2358°E). It has a population of around 861,651 people, with 51.5% being female and 48.5% being male (Cooperative Governance & Traditional Affairs, 2020). With reference to the lower bound poverty line, 36.6% of the population lives in poverty. This makes MMM the perfect hub for small-, medium-, and micro-enterprises (SMMEs), such as street food vending. A total of 168 food samples of four different food types (pap, chicken, pork, and moroho/salad) were purchased from 42 street food vendors in the MMM area (6 vendors were targeted per area). The samples were aseptically placed in sterile bags and sealed using the same utensils that the vendors used to serve the food. These samples were transported in a cooler box on ice packs to the laboratory for analysis. This was done to preserve the food matrix and minimize volatilization or redistribution of metals that are temperature sensitive.

5.4.2 Sample preparation

The concentration of heavy metals in the street-vended samples was determined using ICPE (ICPE-9820, Shimadzu, Japan) and sample preparation was done following the method proposed by Kaushik *et al.* (2023). All reagent solutions were prepared using analytical-grade reagents dissolved in deionized water. The samples (168) were dried at 105°C for 3–4 h in a hot-air oven, followed by manual crushing using a mortar and pestle and sieving. A sample of 0.5 g was then digested using a closed vessel microwave digestion method with a mixture of 65% HNO₃, 36% HCl, and 30% H₂O₂. The digestion parameters were as follows: 130°C for 10 min, 150°C for 5 min, 180°C for 5 min, and 210°C for 15 min, at a power of 400 W. After digestion, the samples were filtered and the volume was adjusted to 50mL without further dilution.

5.4.3 Determination of heavy metals using ICP-OES

The amounts of arsenic, cadmium, chromium, lead, iron, copper and zinc in the prepared solutions were measured using an inductively coupled plasma optical emission spectrometer (ICP-OES), IRIS Interpid II XSP Model and five standard solutions (10, 50, 100, 500 and 1000 µg L⁻¹) for each heavy metal. The operational parameters for determining the elements by ICP-OES are presented in Table 5.1 (Mostafidi *et al.*, 2021).

Table 5.1 Performance parameters of determining the elements by ICP-OES

Method	Parameters
RF power	1400
Plasma generator	Argon
Plasma gas flow rate (L/min)	9.99
Auxiliary gas flow rate (L/min)	0.60
Nebulizer gas flow rate (L/min)	0.70
Sample uptake time (s)	240 total

Rinse time of (s)	45
Initial stabilization time (s)	Preflush:45
Pump rate (rpm)	15
Measurement replicates	3
Frequency of RF generator (MHz)	Resonance frequency: 27.12
Type of detector solid state	CCD
Type of spray chamber cyclonic	Modified Lichte

5.4.4 Health risk assessment

To determine the health risk rate, concentrations of carcinogenic and non-carcinogenic metals obtained from various food samples were used. Oral reference doses (RfD) of heavy metals obtained from the United States Environmental Protection Agency (US EPA) and the Agency for Toxic Substances and Disease Registry, as well as methodologies prescribed by Muhammed *et al.* (2011), Bamuwanye *et al.* (2015), and Onyele and Anyanwu (2018) were used in this study. The presumption was that the amount of contaminant ingested would equal the dose absorbed, and cooking was considered not to have had an impact on the toxicity of the heavy metals in the street-vended foods.

Table 5.2 Oral reference doses of heavy metals

Metal	RfD (mg/kg/day)
Arsenic	0.0003
Cadmium	0.0005
Chromium	0.0003
Copper	0.04
Lead	0.0035
Iron	0.007
Zinc	0.3

Source: USEPA IRIS (2011) and ATSDR (2024)

5.4.4.1 Estimated daily intake

The estimated daily intake depends on the metal concentration, ingestion/consumption rate, and body weight. The EDI was determined using equation 5.1:

$$EDI = \frac{C_{metal} \times D_{food\ intake}}{BW_{average}} \quad (5.1)$$

Where C is the metal concentration in food (mg/kg) and D represents the daily intake of food in kg per person, and BW is the average body weight in kg (70 kg for adults).

5.4.4.2 Carcinogenic risks

To assess both the cancer and non-cancer risk presented by exposure to heavy metals, the Chronic Daily Intake was calculated. However, the probable cancer risk associated with exposure to a measured dose of a heavy metal was estimated using the Incremental Lifetime Cancer Risk (ILCR). The ICLR was calculated using the Cancer Slope Factor (CSF), which is considered as the risk produced by a lifetime average dose of 1 mgkg⁻¹ BWday⁻¹ and is contaminant specific. ICLR was calculated for As, Cd, Pb and Cr using respective slope factors (mgkg⁻¹day⁻¹) following equation 5.3 (Bamuwanye *et al.*, 2015; ATSDR, 2019; Qing *et al.*, 2020):

$$CDI = \frac{C_w \times IR \times EF \times ED}{B_w \times AT} \quad (5.2)$$

$$ICLR = CDI \times CSF \quad (5.3)$$

Table 5.3 Parameters used for calculating CDI and cancer risk

Factor/parameter	Symbol	Units	Adult
Exposure duration	ED	Years	30
Lifetime		Years	70

Exposure frequency	EF	days/year	365
Average time	AT: Lifetime x 365 or ED x 365	Days	25 550 or 10 950
Body weight	B _w	Kg	70
Oral Cancer Slope Factor	CSF	(mg/kg/day) ⁻¹	As- 1.5; Cd- 0.38; Cr- 0.5; Pb-0.0085
Ingestion rate	IR:	kg/day	
	Pap		0.366
	Chicken		0.105
	Pork		0.105
	Moroho/salad		0.252

5.4.4.3 Non carcinogenic risk

Hazard quotient (HQ) values were used to determine the potential for non-carcinogenic health risk to occur as a result of heavy metal oral exposure. The HQ was determined as the human exposure to a metal of interest (mg/kg/day) divided by its reference dose (mg/kg/day) (Benson, 2014).

$$HQ = \frac{CDI}{RfDo} \quad (5.4)$$

The chronic hazard index (HI) was calculated as a sum of all hazard quotients (HQ) calculated for individual heavy metals for a particular exposure pathway (Badeenezhad *et al.*, 2023). This was to allow for the evaluation of potential risk to consumer health as a result of exposure to more than one heavy metal. The HI was determined using equation 5.5:

$$HI = HQ_1 + HQ_2 + \dots + HQ_n \quad (5.5)$$

It is assumed that the overall impact on human health increases in direct proportion to the sum of different heavy metals one is exposed to (Sultana *et al.*, 2017). According to guidelines by the Agency for Toxic Substances and Disease Registry, a population is

considered safe when $HI < 1$ and at risk when $HI > 1$ (Agency for Toxic Substances and Disease Registry, 2022).

5.4.5 Statistical analyses

Quantitative data were captured on Excel in preparation for statistical analyses. Various statistical analyses were carried out on the quantitative data using computer-based SPSS software version 26. A normality test was performed for the continuous variables in order to ascertain which methods would be appropriate for analysing the variables. Visual inspection (i.e., looking at the histogram and Q-Q plots) was used to investigate the normality of the data. However, as the visual inspection might not have been reliable, the Shapiro-Wilk normality test was also applied to establish whether the data followed a normal distribution. The Kruskal-Wallis H test was conducted to evaluate differences in the meaning of different heavy metals in different food types and sampling areas. Subsequently, the Pairwise Comparison with significance values having been adjusted for the Bonferroni correction for multiple tests was done for both the food types and the sampling areas. The significance level was 0.05. Data did not have a normal distribution and the assumption of equality of variance was not fulfilled, hence the need to use a non-parametric test.

5.5 RESULTS

The test for normality, as shown in Table 5.4, indicated that the data sets were not normally distributed ($p < 0.01$). Therefore, the median and interquartile range are reported. The results indicated that there was heavy metal contamination in all the analysed samples. The overall median and IQR of As, Cd, Cr, Cu, Fe, Pb, and Zn were 2.653(2.971), 0.023(0.027), 0.212(0.117), 0.271(0.326), 4.365(4.424), 1.095(1.266), and 3.605(3.711) mg/kg, respectively. Iron had the highest overall concentration at 4.365(4.424) mg/kg, while cadmium had the lowest concentration at 0.023(0.027) mg/kg. Furthermore, a Kruskal-Wallis H test was done, and the results are depicted in Figure 5.1 A-G. The test showed that there was a statistically significant difference in the amounts of As, Cd and Cu where $p < 0.001$ among the different food types. However, no statistically significant differences were observed among the different food types for Cr, Fe, Pb and Zn.

5.5.1 Heavy metal contamination

Table 5.4 Test for normality

Metal	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Arsenic	0.231	168	< 0.001	0.928	168	< 0.001
Cadmium	0.366	168	< 0.001	0.418	168	< 0.001
Chromium	0.137	168	< 0.001	0.789	168	< 0.001
Copper	0.196	168	< 0.001	0.896	168	< 0.001
Iron	0.077	168	0.016	0.945	168	< 0.001
Lead	0.098	168	< 0.001	0.933	168	< 0.001
Zinc	0.083	168	0.007	0.940	168	< 0.001

a. Lilliefors Significance Correction

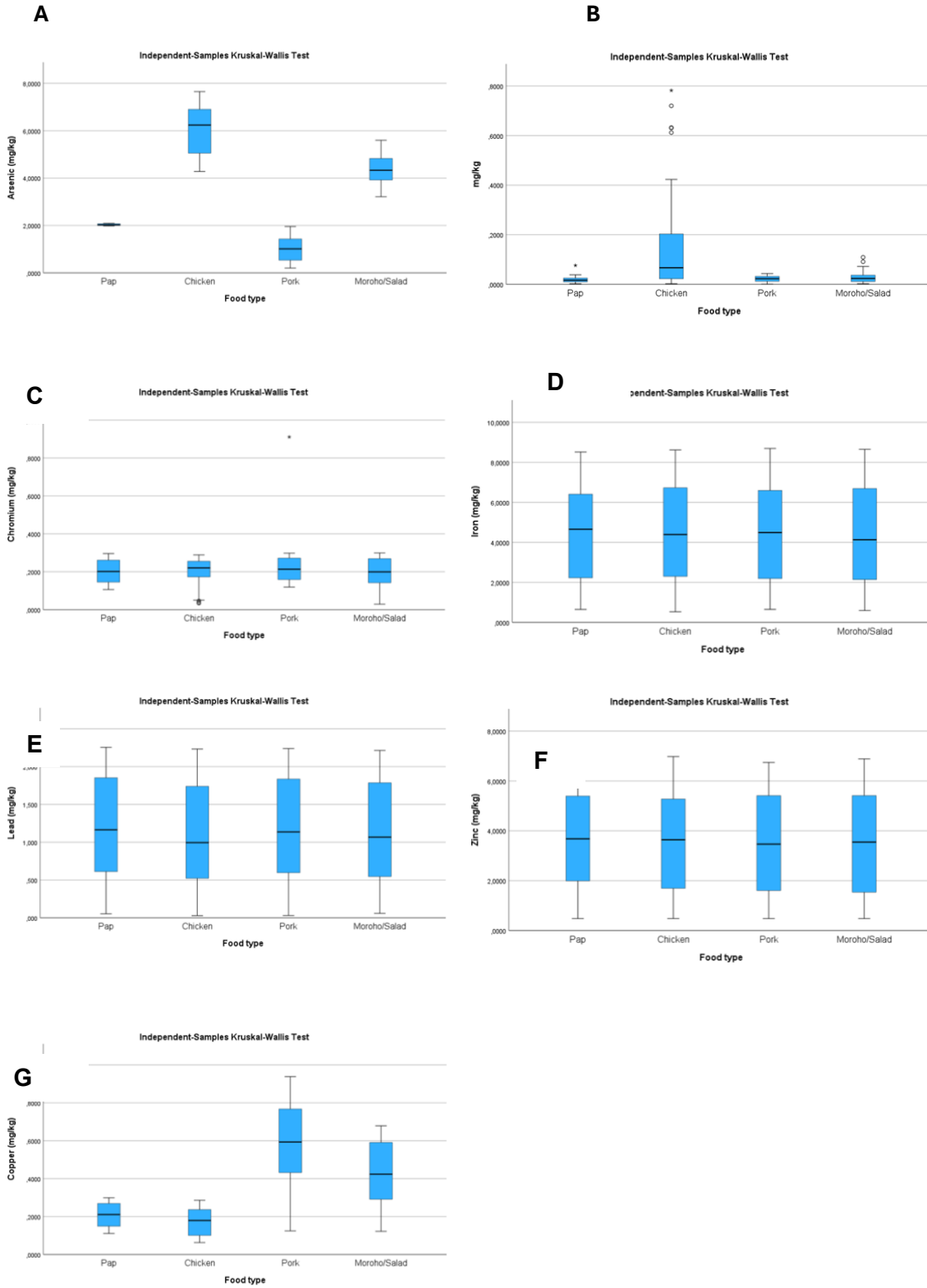


Figure 5.1 Differences in mg/kg of heavy metals among the different food types

The qualitative results and mean concentrations of the heavy metals are depicted in Table 5.5 and Table 5.6 below. The Kruskal-Wallis H test showed that there was a statistically significant difference in the amount of As (mg/kg) among the different food types ($H=151.637$, $p<0.001$), with a mean rank pain score of 63.59 for pap, 143.88 for chicken, 21.50 for pork, and 109.12 for moroho/salad. Across the different food types, As, which is considered one of the most toxic heavy metals (Ogunkunle *et al.*, 2014), was found to be the highest in chicken samples (6.117 ± 1.011 mg/kg) and lowest in pap (2.034 ± 0.037 mg/kg). Compared to the results reported by other similar studies, the results found in this study for the meat samples (chicken and pork) were significantly higher. Zhang *et al.* (2020) obtained 0.051 ± 0.024 mg/kg of As in a barbequed meat sample. In a study conducted in Kampala, Uganda, on street roasted and vended meat, As contamination was also high. The South African Foodstuffs, Cosmetics and Disinfectants Act of 1972 recommends a maximum limit of 1.0 mg/kg for As in meat and processed meat. According to the Collaborative for Health and Environment (2016), a lethal dose of arsenic is 2-20 mg/kg. Although resistance varies from one individual to the next, prolonged consumption of highly contaminated street-vended foods that exceed permissible limits as exemplified in this study could have negative effects on consumers' health. Because coal is predominantly used in street food vending in South Africa for grilling meat, it could be highly attributed to the alarming levels of As contamination in the meat samples. Moreover, As can be introduced to crops through contaminated irrigation water and soil. Therefore, it is imperative, as a mitigation measure, to adequately wash vegetables before preparation. As a result of lack of access to running water in the study areas, this might prove quite challenging.

The toxicities associated with Cd and its resultant deleterious impact on humans and animals on a global scale cannot be underestimated. Direct links between exposure to cadmium and male reproductive complications have been studied and established around the globe (Arzuaga *et al.*, 2019; Ikokide *et al.*, 2022). These studies have indicated that Cd can induce testicular inflammation, spermatogenic cell apoptosis, decreased androgenic and sperm cell function, and decreases sperm count. In the current study, Cd was detected

in all the food samples, with mean concentrations ranging from 0.018 ± 0.013 to 0.16 ± 0.218 mg/kg. It was gratifying to note that in three of the four sampled food types, contamination levels were below the maximum permissible limits as recommended by the Foodstuffs, Cosmetics and Disinfectants Act of 1972. These counts were 0.2 mg/kg for leafy vegetables and 0.1 mg/kg for cereal grains.

The current study detected chromium in all the food samples, but it was notable that there was no significant difference in the contamination levels among the food types ($p > 0.765$). Chromium is commonly present in many food types such as meat, grain, and vegetables, which has been attributed to agricultural and manufacturing practices (National Institutes of Health, 2022). In the current study, the mean concentrations of Cr ranged from 0.196 ± 0.076 to 0.227 ± 0.124 mg/kg. The independent Kruskal-Wallis H test indicated that there was a significant difference in the contamination levels across the different food types ($p < 0.001$). Chromium has biological functions such as enhancing the action of insulin, and therefore it is recommended that at least 0.05mg/day should be consumed (Lewicki *et al.*, 2014), while the permissible limit set by Codex is 0.025-0.2 mg/kg. Compared to these standards, the levels of Cr in the moroho/salad and chicken samples were below the maximum permissible limit, while pap was relatively equal to the highest permissible limit (0.2 ± 0.061 mg/kg). On the other hand, pork slightly exceeded this limit (0.227 ± 0.124 mg/kg). Chromium III, which was also found in the food samples, is less toxic and is an essential nutrient. Nonetheless, it is paramount that it is consumed in trace amounts (Department of Climate, Energy, the Environment and Water, 2022). Therefore, the high levels in which it was found in the pork samples in the current study is matter of concern, and it is advised that such foods should be consumed with great caution.

Lead is a well-known noxious heavy metal and paediatric toxin that has injurious effects on organs such as the brain, thyroid glands, kidneys, and bones. The levels of Pb quantified in the street-vended foods sampled ranged from 1.108 ± 0.693 to 1.2 ± 0.707 mg/kg. The highest concentration was found in pap (1.2 ± 0.707 mg/kg) while the lowest concentration was found in chicken (1.108 ± 0.693 mg/kg). The lead (Pb) content in all the food samples

exceeded the CODEX permissible limit of 0.01 mg/kg. Meat sold by street vendors in MMM is mostly grilled or barbecued over an open charcoal or wood flame. It is therefore argued that the combustion of wood/charcoal coals results in the production of toxicants such as carbon monoxide, the formation of trace elements such as lead, and an increase in particulate matter that settles on the food (Niu *et al.*, 2021, Soeroso *et al.*, 2021 and Onopiuk *et al.*, 2022). Exposure to any such substances could result in detrimental health effects in humans. Ali *et al.* (2022) similarly found that the Pb level in barbecued chicken was higher than the permissible limit. In the current study, the lowest concentration in one sampled area was 1.90 mg/kg, while the highest concentration obtained was 3.70 mg/kg.

Copper (Cu), Iron (Fe), and Zinc (Zn) are considered essential trace elements as they are needed for body growth and development. However, when consumed in exceedingly high amounts, they can become deleterious to health (Mehri, 2020). In this study, the results indicated that there was more copper in pork (0.577 ± 0.223) than in the other food samples. According to a review study by Taylor *et al.* (2020), the oral RfD that is protective of both acute and chronic toxicity in adults and children is 0.04 mg Cu/kg/day. The European Food Safety Authority (2022) and Food Safety Africa (2023) recommend a daily dose of Cu of not more than 0.7 mg/kg of body weight for an adult to prevent chronic copper toxicity. The results of this study found that the mean concentrations of Cu were lower than the recommended daily limit, therefore consumers may not be at risk of Cu-related health issues in the study area.

Iron offers health benefits such as oxygen transport, cellular energy metabolism, and various enzymatic reactions, and the deficiency of this metal can result in an array of health issues such as anaemia, cognitive development impairment, and reduced ability to do physical work (Lal, 2020; Benso *et al.*, 2021). The recommended daily (RDA) allowance of Fe varies among groups of people, taking into consideration their gender, age, pregnancy status, breastfeeding, or menstruating (Bathla and Arora, 2022; Healthline, 2023).

According to Moustarah (2022), the RDA for Fe ranges from 0.27 to 27 mg/d. In this study, the mean concentration of iron ranged from 4.325 ± 2.568 to 4.529 ± 2.518 mg/kg.

Zinc is a ubiquitous essential trace element that exists naturally in numerous environments, biological systems, and organisms. The highest concentration of Zn was detected in the pap samples (3.678 ± 1.972 mg/kg), while the lowest concentration (3.458 ± 2.021 mg/kg) was detected in the moroho/salad samples. All the street-vended food samples tested had Zn levels that were below the WHO recommended maximum limit of 60 mg/kg, but they were consistent with results reported by Bamuwanye *et al.* (2015) and Hassan *et al.* (2022). Oyet and Samuels (2020) and Kubala (2022) state that the need for Zn in the human body changes over time, and the deficiency thereof could lead to various health issues, such as compromised immune function, poor skin health, and a compromised olfactory sense.

Table 5.5 Qualitative results of heavy metals found in street-vended foods

Food item	As	Cd	Cr	Cu	Fe	Pb	Zn
	193.759	214.438	267.716	324.754	238.204	220.353	202.548
Pap	+	+	+	+	+	+	+
Chicken	+	+	+	+	+	+	+
Pork	+	+	+	+	+	+	+
Moroho/salad	+	+	+	+	+	+	+

Positive/Detected (+), Negative/Not Detected (-)

Table 5.6 Means of heavy metal concentrations (mg/kg) in MMM street-vended foods

Heavy Metals	Food type			
	Pap	Chicken	Pork	Moroho/salad
As	2.034 ± 0.037	6.117 ± 1.011	1.022 ± 0.529	4.367 ± 0.645
Cd	0.018 ± 0.013	0.168 ± 0.218	0.022 ± 0.012	0.028 ± 0.023

Cr	0.2±0.061	0.197±0.079	0.227±0.124	0.196±0.076
Cu	0.208±0.062	0.172±0.072	0.577±0.223	0.423±0.174
Fe	4.467±2.402	4.477±2.454	4.529±2.518	4.325±2.568
Pb	1.2±0.707	1.108±0.693	1.192±0.699	1.141±0.673
Zn	3.678±1.972	3.574±2.072	3.519±1.987	3.458±2.021

A Kruskal-Wallis H test (one-way non-parametric ANOVA) was conducted to determine the concentration of heavy metals present in the sampled food types. For this test, a comparison between groups based on the mean rank of the dependent variable (heavy metal concentration) was done. The null hypothesis stated that concentration distributions across the various food types would be the same. The multiple pairwise comparisons among the different food types are presented in Table 5.7. For As, there was a statistically significant difference ($p < 0.05$) between all pairs. However, for Cd, a significant difference was observed only among the following pairs: pap and chicken, pork and chicken, and moroho/salad and chicken. Copper concentrations were significantly different only among the following pairs: chicken and moroho/salad, chicken and pork, pap and moroho/salad, and pap and pork. Notably, no significant differences ($p > 0.05$) were observed among all pairs for Cr, Fe, Pb and Zn concentrations.

The results of these analyses suggest that, for some heavy metals, there was a real and meaningful difference in the levels of contamination among the compared food samples. Considering the location of the sampling areas (urban areas with busy traffic), this difference can be attributed to various anthropogenic activities such as industrial activities, motor vehicle gas emissions, choice of cooking utensils, as well as some undetermined environmental factors that were beyond the scope of this study (Abas *et al.*, 2019).

Table 5.7 Pairwise comparisons of food types for the various heavy metals

Pairwise Comparisons of Food type						
	Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
Arsenic	Pork-Pap	42,000	10,614	3,957	<.001	,000
	Pork-Morocho/Salad	-87,619	10,614	-8,255	<.001	,000
	Pork-Chicken	122,381	10,614	11,530	<.001	,000
	Pap-Morocho/Salad	-45,619	10,614	-4,298	<.001	,000
	Pap-Chicken	-80,381	10,614	-7,573	<.001	,000
	Morocho/Salad-Chicken	34,762	10,614	3,275	,001	,006
	Cadmium	Pap-Pork	-14,548	10,612	-1,371	,170
Pap-Morocho/Salad		-19,262	10,612	-1,815	,070	,417
Pap-Chicken		-59,381	10,612	-5,595	<.001	,000
Pork-Morocho/Salad		-4,714	10,612	-,444	,657	1,000
Pork-Chicken		44,833	10,612	4,225	<.001	,000
Morocho/Salad-Chicken		40,119	10,612	3,780	<.001	,001
Chromium	Pap-Morocho/Salad	-,690	10,614	-,065	,948	1,000
	Pap-Chicken	-4,190	10,614	-,395	,693	1,000
	Pap-Pork	-10,167	10,614	-,958	,338	1,000
	Morocho/Salad-Chicken	3,500	10,614	,330	,742	1,000

	Morocho/Salad-Pork	9,476	10,614	,893	,372	1,000
	Chicken-Pork	-5,976	10,614	-,563	,573	1,000
Copper	Chicken-Pap	14,881	10,614	1,402	,161	,966
	Chicken-Morocho/Salad	-64,369	10,614	-6,064	<.001	,000
	Chicken-Pork	-86,988	10,614	-8,195	<.001	,000
	Pap-Morocho/Salad	-49,488	10,614	-4,662	<.001	,000
	Pap-Pork	-72,107	10,614	-6,793	<.001	,000
	Morocho/Salad-Pork	22,619	10,614	2,131	,033	,199
Iron	Morocho/Salad-Pap	2,476	10,614	,233	,816	1,000
	Morocho/Salad-Chicken	2,500	10,614	,236	,814	1,000
	Morocho/Salad-Pork	3,500	10,614	,330	,742	1,000
	Pap-Chicken	-,024	10,614	-,002	,998	1,000
	Pap-Pork	-1,024	10,614	-,096	,923	1,000
	Chicken-Pork	-1,000	10,614	-,094	,925	1,000
Lead	Chicken-Morocho/Salad	-2,571	10,614	-,242	,809	1,000
	Chicken-Pork	-6,643	10,614	-,626	,531	1,000
	Chicken-Pap	7,405	10,614	,698	,485	1,000
	Morocho/Salad-Pork	4,071	10,614	,384	,701	1,000
	Morocho/Salad-Pap	4,833	10,614	,455	,649	1,000
	Pork-Pap	,762	10,614	,072	,943	1,000

Zinc	Morocho/Salad-Pork	1,298	10,614	,122	,903	1,000
	Morocho/Salad-Chicken	2,702	10,614	,255	,799	1,000
	Morocho/Salad-Pap	5,238	10,614	,493	,622	1,000
	Pork-Chicken	1,405	10,614	,132	,895	1,000
	Pork-Pap	3,940	10,614	,371	,710	1,000
	Chicken-Pap	2,536	10,614	,239	,811	1,000

Each row tests the null hypothesis that Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is 0.050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

5.5.2 Cancer and non-cancer risk

To quantitatively evaluate the likely health risks posed by street vended food for consumers in the Mangaung Metro Municipality, a health risk assessment was conducted. The results of EDI, CDI, and HQ are presented in Tables 5.8 to 5.10 below.

Table 5.8 shows the estimated daily intake (EDI) of heavy metals for adults with a body weight of 70 kg. The EDI of As ranged between 0.0002 and 0.02 mg/kg/day, with the highest value being obtained from moroho/salad. The EDI of Cr and Cd from the street foods was highest in pork (0.003) mg/kg/day). Among the other food types, moroho/salad had the highest EDI of both Cu and Fe at 0.002 and 0.02 mg/kg/day, respectively. The daily intake rates of Pb and Zn ranged from 0.002 to 0.006 mg/kg/day and 0.005 to 0.02 g/kg/day, respectively. These results indicate that the EDI values for the examined food samples were below the recommended dietary values. Therefore, it was concluded that the prevalence of the heavy metals in the food items assessed in this study did not pose a public health risk.

Table 5.8 EDI of heavy metals in street-vended foods consumed by adults (mg/kg/day)

Food type	As	Cd	Cr	Cu	Fe	Pb	Zn
Pap	0.01	0.0007	0.001	0.001	0.02	0.006	0.02
Chicken	0.0002	0.0002	0.001	0.0003	0.007	0.002	0.005
Pork	0.002	0.003	0.003	0.0009	0.007	0.002	0.005
Moroho/salad	0.02	0.0000	0.0007	0.002	0.02	0.004	0.01
Recommended daily dose (mg/kg/day)	0.13 (Ullah et al., 2017)	0.062 (Sataru et al., 2017)	0.035 (Havard School of Public Health, 2023)	2-3 (FAO/WHO, 1995)	18 (National Research Council, 2001)	0.21 (Ullah et al., 2017)	14-20 (FAO/WHO, 1995)

Table 5.9 The Chronic Daily Intake of heavy metals by adults (mg/kg/day)

Heavy Metal	Food type			
	Pap	Chicken	Pork	Morocho/salad
Copper	0.001	0.0003	0.0001	0.002
Iron	0.023	0.007	0.007	0.016
Zinc	0.019	0.005	0.005	0.013
Arsenic	0.011	0.009	0.002	0.016
Cadmium	<0.0000	0.0003	<0.0000	0.0001
Chromium	0.001	0.0003	0.0003	0.0007
Lead	0.006	0.002	0.002	0.004

According to USEPA, a one in a million chance of contracting cancer over a 70-year lifetime period (ICLR= 10^{-6}) is considered acceptable, while ICLR of/or greater than 10^{-3} (one in a thousand) is said to be serious and is a cause for remediation (Li *et al.*, 2013). In this study, cancer risk was computed as 2.44×10^{-2} for highest and 3.17×10^{-3} for lowest risk for morocho/salad and pork, respectively. Cumulatively, all ICLRs were found to be equal to or greater than what is considered to be an increased risk of cancer (10^{-3}). The chronic exposure to low concentrations of heavy metals such as Pb, As and Cd has the potential to result in many forms of cancers, while these metals also pose the risk of other toxic effects (Bhattacharjee *et al.*, 2016; Flora and Agrawal, 2017). The toxicity of chromium is dependent on its chemical composition. The health effects of chromium exposure can include, but are not limited to, respiratory problems, lung cancer, and skin effects (Teklay, 2016).

Table 5.10 ICLR for adults in MMM through the consumption of street-vended foods

Heavy Metal	Food type			
	Pap	Chicken	Pork	Morocho/salad
Arsenic	1.65×10^{-2}	1.35×10^{-2}	3.00×10^{-3}	2.40×10^{-2}
Cadmium	0	1.14×10^{-4}	0	3.80×10^{-5}
Chromium	5.00×10^{-5}	1.50×10^{-4}	1.50×10^{-4}	3.50×10^{-4}
Lead	5.10×10^{-5}	1.70×10^{-5}	1.70×10^{-5}	3.4×10^{-5}
Σ ICLR	1.66×10^{-2}	1.38×10^{-2}	3.17×10^{-3}	2.44×10^{-2}

Table 5.11 The hazard quotient of heavy metal for oral ingestion in adults

Heavy Metal	Food type			
	Pap	Chicken	Pork	Morocho/salad
Copper	0.025	0.008	0.003	0.05
Iron	3.286	1	1	2.286
Zinc	0.063	0.017	0.017	0.043
Arsenic	36.67	30	6.67	53
Cadmium	<0.001	0.6	<0.001	0.2
Chromium	0.33	1	1	2.33
Lead	1.71	0.57	0.57	1.14
$HI = \Sigma HQ$	42.085	33.195	9.261	59.019

Aina *et al.* (2023) describe the hazard quotient as “a proportion of the probable exposure to an element or chemical at such a level with no expected negative impacts when the quotient is less than 1, but an indication of potential health risks resulting from exposure, when it is greater than 1”. The hazard quotients (HQs) were calculated to determine the non-cancer risk for all metals evaluated in this study. The results are presented in Table 5.11. The HQs

in decreasing order are: As, Fe, Pb, Cr, Zn, Cu and Cd for pap; As, Fe, Cr, Cd, Pb, Zn and Cu for chicken; As, Fe, Cr, Pb, Zn, Cu and Cd for pork; and As, Cr, Fe, Pb, Cd, Cu, and Zn for moroho/salad. The HQs were less than 1 in 86% (6 out of 7) of the heavy metals in chicken and pork, with As being the only heavy metal with an HQ >1. Similar observations were reported by Bamuwanye *et al.* (2015).

The hazard index (HI) for individual food types was calculated and the results in descending order were as follows: $HI_{\text{moroho/salad}}$ 50.019, HI_{pap} 42.085, HI_{chicken} 33.195 and HI_{pork} 9.261. The HI of all the sampled food types as per their daily ingestion rate indicated that consumers were at a significant non-carcinogenic health risk. The results in Table 5.11 show that As THQs were the major contributors to the very high HI in all the food types. Contrary to the findings of this study, Kaushik *et al.* (2023) reported a target hazard quotient and hazard index of <1 for all analysed metals, which was indicative of health risks being lower.

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CHAPTER 6

CONCLUSIONS, LIMITATIONS, AND RECOMMENDATIONS

6.1 CONCLUSIONS

This chapter reflects on the key findings and the overall conclusions related to the study objectives. The primary aim of this study was to evaluate the safety of street-vended foods and the nutritional knowledge of vendors operating in the Mangaung Metro Municipality in the Free State province. Pursuant to this aim, the following objectives that related to street food vending in the study area were to:

- Assess street food vendors' nutrition knowledge and practices;
- Assess the microbial contamination of street-vended foods;
- Explore the extent of the threat of *Staphylococcus aureus* contamination in RTE foods;
- Identify the extent of heavy metals present in street-vended foods using Inductively Coupled Plasma Atomic Absorption Spectroscopy (ICP-AAS); and
- Determine/calculate the cancer and non-cancer risk posed by heavy metals in both adults and children who may consume street-vended foods in the study area.

In Chapter 2, the assessment of street food vendor nutrition knowledge and practices revealed that, although these vendors in the study are had some good knowledge and positive attitudes towards food nutrition, the food types and food portions they served might compromise consumers' health. Key findings were that the vended foods were often energy-dense, high in salt, high in saturated fats, and comprised mostly unbalanced meals in terms of nutritional value. Therefore, in consideration of the many benefits of street-vended foods such as cultural preservation and combating food insecurity, changing the food menu or food preparation methods could threaten the profitability and in turn the livelihoods of the street food vendors.

For nutritional assessment, the microbiological quality of the vended foods was also assessed. The findings of this investigation were presented in Chapter 3. These findings revealed valuable insights into the microbiological quality and safety of ready-to-eat street-vended foods sampled from various locations within the Mangaung Metro Municipality. The presence of bacterial isolates such as *Staphylococcus* spp., *E. coli*, and *Pseudomonas* spp. highlighted significant public health concerns related to street food handling and vendors' hygiene practices.

Findings related to the total viable counts (TVC) of the analysed food samples indicated that a substantial proportion of the street-vended foods did not meet acceptable microbial safety standards and could pose a risk to consumers. Additionally, the detection of *E. coli* in 100% of the samples, with contamination levels exceeding permissible limits in RTE foods, further underscores the potential for faecal-oral contamination and the need for improved food preparation and handling by street food vendors. Moreover, the high prevalence of *Pseudomonas* spp. (78.5%) in the food samples posed risks associated with biofilm formation on cooking surfaces, which may potentially contribute to persistent cross contamination now and in the future.

In light of the above conclusions, the study highlights the need for stricter enforcement of hygiene regulations, mandated training of food handlers, and regular microbial assessments of street-vended foods. Extensive and ongoing research that releases statistical analyses should be conducted to provide in-depth insights into the significance of contamination patterns across different food types and locations. Such data are currently lacking in the research setting. Addressing these concerns is crucial to safeguard public health and ensure the safety of RTE foods across the board.

Expanding on the microbiological findings, Chapter 4 enhanced understanding of *S. aureus* genotypes found in the street-vended foods. These findings should aid in the development and management of effective strategies to better control street food vending and

consequently protect public health. Multidrug-resistant (MDR) strains were detected in high frequency in this study in over 50% of isolates. In addition, critical virulence determinants, including genes encoding hemolysins, leukocidins, and biofilm-associated MSCRAMMs, were detected. The prevalence of these strains is suggestive of the public health threat posed by these pathogens that abounded in the informal food markets under study. Furthermore, the importance of the One Health approach in understanding the ecology and transmission of *S. aureus* is highlighted by the genomic characterization that revealed the close phylogenetic relatedness between food-derived isolates and those of human and animal origins. Antimicrobial resistance genes, such as *tet (38)*, *mepA*, *lmrS*, and *norA* that are often associated with efflux-related resistance mechanisms, were found in the foods that were analysed. These genes are indicative of the adaptive potential of this pathogen under antimicrobial pressure. According to the findings, RTE are implicated not only as potential reservoirs facilitating the horizontal transfer of resistance determinants, but also as vehicles for direct human exposure to virulent and drug-resistant *S. aureus* strains. As a result, remedial interventions such as intensified surveillance, stricter enforcement of food safety regulations, and comprehensive risk communication strategies are urgent. These interventions should be contextually tailored to specific settings in order to ensure efficiency and implementation.

To conclude the food safety assessments of the street foods vended in the MMM, analyses of heavy metal contamination were conducted. All the food samples that were analysed tested positive for the range of heavy metals tested. Although some of the metals are essential trace elements, exposure to them in concentrations exceeding safety standards can be detrimental to the health of consumers. The estimated daily intake obtained for all the heavy metals that were tested was below the recommended limits, thereby indicating no real risk to public health. However, the results of the incremental lifetime cancer risk indicated the necessity for attention and remediation. Moreover, the hazard index of the various food types was disconcertingly high ($HI > 1$). This result implies that habitual consumers are at risk of contracting non-carcinogenic health conditions. The high HI

observed could be attributed to various reasons, such as low RfD and the cumulative exposure to multiple heavy metals. With the aim of addressing the health and safety issues posed by the consumption of street-vended foods, food safety education and regulatory support need to be prioritised. This will help reinforce and cement the value of street food vending to ensure food security and address the socio-economic challenges associated with street food vending in a safe and appropriate manner.

6.2 STUDY LIMITATIONS

Due to limited funds available for the study, genetic data for other isolates could not be extracted, therefore *S. aureus* was prioritized. Moreover, the study focused on the MMM in particular, which limited its scope and thus prevent the findings from being generalised.

6.3 RECOMMENDATIONS

In line with the findings of the study, the following recommendations are offered:

- Targeted training programs that improve the knowledge of vendors, inform them of nutrition requirements, advise them of the quality of the value-for-money food that they sell, and equip them with health-related knowledge should be implemented.
- Mandatory hygiene and food safety training should be implemented for food vendors to promote and enhance safe food handling practices.
- Collaborative efforts towards providing access to clean water, waste disposal facilities, and adequate vending stalls should be explored to help curb the spread of foodborne pathogens.
- One Health efforts should be implemented for interdisciplinary collaboration among the food safety, human health, and animal health sectors to address the transmission of pathways of resistant organisms.
- Future studies should expand the scope of this study by conducting similar investigations into street food vending in other human-congested areas in South Africa.